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A MANUAL OF ANALYTICAL METHODS FOR WASTEWATERS
Oil Shale Retort Waters

May 1984

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A MANUAL OF ANALYTICAL METHODS FOR WASTEWATERS

(Oil Shale Retort Waters)

Second Edition*
Revised and Expanded

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PREFACE

This manual of methods was developed for the routine chemical analysis of various water-quality criteria. Each method is specifically adapted for application to the highly complex sample matrices of aqueous wastes that are generated by the pyrolytic production of shale oil. These methods have evolved from specific needs of the LBL-SEEHRL Oil Shale Project for the study of waste treatment. Although the methods have been developed specifically for oil shale wastewaters, the stringent requirements imposed by these sample matrices would probably allow for the successful direct application of these methods to other aqueous waste samples; the major limitation would be that of insufficient lower detection limits, because oil shale wastewaters commonly require methods with wide linear dynamic ranges. Although these methods appear to give excellent results for the waters analyzed during this study, it cannot be overemphasized that these methods must be validated for each new water under investigation.

We hope that this manual will be useful to regulatory agencies for setting monitoring standards, to the synfuels industry for routine monitoring, and to researchers involved in the study of synfuel waste treatment processes.

Discussions of theory, literature review, methods comparisons, validation and precision data, and detailed operator protocols are presented for each of the methods, including: quantitation of organic and inorganic carbon, ammonia, organic nitrogen, total nitrogen, chemical oxygen demand, and microbial biomass. Methods are also presented for simple and rapid fractionation of organic carbon (also used for quantifying oil and grease) and for separating ammonia from organic nitrogen (for organic nitrogen analysis). Some of the protocols are routine standard methodologies that have been validated for oil shale process wastewaters, while others are modified standard methods or totally new approaches. The question of accuracy has not been fully addressed in these chapters because it is a tremendously complex issue. The origins of the wastewaters that are discussed in each chapter are fully described in Appendix I, and the data obtained from each of the methods is summarized for all of the waters in Appendix II.

This edition was revised and expanded from the First Edition ("Quantitation of Oil Shale Wastewater Quality: A Manual of Analytical Methods," December 1982). The title has been changed to emphasize that these methods should also be useful for other wastewaters. Chapters II and V are new, and Chapter I has been extensively rewritten. Portions of Chapter III have been published (J. Test. Eval. **1984**, 12[4]). Portions of Chapters I, II, and V will be published at a later date. The development of other methodologies will be described in future supplements or subsequent editions to this manual. Concurrent with publication of the first edition, another report that specifically addresses the chemical analysis of oil shale wastewaters had been issued by the Denver Research Institute ("Methods of Chemical Analysis for Oil Shale Wastes," John R. Wallace et al., October 1982); the methodology discussed in that report is complementary to the methods described in this Second Edition.

The mention of trade names or commercial products in this manual is for illustrative purposes and does not constitute endorsement or recommendation for use by the U.S. Department of Energy.

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ACRONYMS

BOD	biochemical oxygen demand; usually refers to 5-day test
C/CL	combustion/chemiluminescence
COD	chemical oxygen demand
DIC	dissolved inorganic carbon
DOC	dissolved organic carbon; synonymous with soluble organic carbon (SOC)
HpF	hydrophilic fraction from RPF
HpF-TN	total nitrogen in HpF
IC	inorganic carbon (nonspecific)
LpF	lipophilic fraction from RPF
LpF-TN	total nitrogen in LpF
NOGD	nonosmotic dissolved-gas dialysis
NPON	nonpolar organic nitrogen (LpF-TN)
NVON	nonvolatile organic nitrogen (NOGD-TN)
OC	organic carbon (nonspecific)
OKN	organic Kjeldahl nitrogen
PN	polar nitrogen (HpF-TN)
POC	polar organic carbon (HpF-DOC)
RPF	reverse-phase fractionation
SCOD	soluble chemical oxygen demand
TC	total carbon; includes TIC and TOC
TDC	total dissolved carbon; includes DIC and DOC
TIC	total inorganic carbon; includes particulate and dissolved forms
TKN	total Kjeldahl nitrogen
TN	total nitrogen (by C/CL)
TOC	total organic carbon; includes particulate and dissolved forms

Chapter I

RAPID FRACTIONATION OF OIL SHALE WASTEWATERS BY REVERSE-PHASE PARTITIONING

C.G. Daughton, B.M. Jones, and R.H. Sakaji

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ABSTRACT

A simple and rapid method is described for quantifying polar and nonpolar organic solutes as bulk, colligative properties of complex oil shale process wastewaters. These two classes are separated by reverse-phase chromatographic partitioning, using a stationary phase of octadecylsilyl-bonded silica. Unretained organic solutes in the fractionated, aqueous effluent are classified as belonging to the hydrophilic fraction (HpF); these solutes contain polar functional groups. Those that are retained belong to the lipophilic fraction (LpF); these solutes contain few polar functionalities and are elutable with organic solvents. Nonspecific, colligative measurements such as total organic carbon or chemical oxygen demand can be used to quantitate the organic solutes directly (in the HpF) or indirectly, by difference (in the LpF). For nine wastewaters from oil shale retorting processes, the proportion of organic carbon in the HpF ranged from less than 20% to more than 80%.

This reverse-phase fractionation (RPF) method also can be applied to the quantitation of "oil and grease" and aliphatic (true) oil in aqueous wastes. The compounds in the retained LpF can be eluted with Freon 113, and the infrared (IR) absorbance of the asymmetric methylene C-H stretch at 2930 cm^{-1} can be determined and compared with that of oil standards as a measure of "oil and grease"; if the Freon eluent is passed over normal-phase silica, the "greases" (i.e., certain nonpolar compounds containing few polar functionalities) are removed, and the true oil in the effluent can be quantified. Values for oil and grease (dissolved) ranged from 56 to 448 mg/L for seven waters when quantified as mineral oil by IR.

INTRODUCTION

Pyrolysis of oil shale, which contains an organic polymer known as kerogen, yields a petroleum-like crude oil and by-products, including retorted (spent) shale, gases, and wastewaters. Depending on the retorting process and mode, the wastewaters can result from combustion, mineral dehydration, retort input steam, and groundwater intrusion. At the elevated temperatures of the retorting process (e.g., >500°C), these waters extract hydrophobic and amphipathic (i.e., containing both nonpolar and polar functionalities) compounds from the shale oil during the continual intermixing, condensation, and vaporization of these two streams downstream of the combustion/pyrolysis retorting zones. Most of the process water is condensed with the product oil (retort water) from which it then must be physically separated; the remaining water (gas condensate) is condensed from a gaseous stream. Materials in these two aqueous streams include: (i) inorganic species such as ammonia and carbonate salts, (ii) polar or amphipathic organic compounds such as aliphatic carboxylic acids up to C_{14} (Rogers et al. 1981), and (iii) certain nonpolar organic compounds (e.g., oils, tarry material, nitriles, phenols, and aromatic heterocycles and amines); the more volatile, neutral species, predominantly inorganic gases and low-molecular-weight organic compounds are primarily associated with the gas condensate.

The partial characterization of organic compounds in wastewaters from only a few retort processes has been reported (Farrier, Poulson, and Fox 1980; Hawthorne 1984; Leenheer, Noyes, and Stuber 1982; Pellizzari et al. 1979; Raphaelian and Harrison 1981; Sievers, Conditt, and Stanley 1981); at most, only half of the total organic carbon has been identified for an individual water.

Although these studies have not been exhaustive characterizations, they have required the application of numerous analytical methods and have shown that retort waters are highly complex mixtures of dissolved and suspended (e.g., emulsified or particulate) inorganic and organic compounds. Investigation of waste treatment schemes for upgrading these waters is hampered by the lack of rapid methods for quantifying treatment performance.

Although the number of individual compounds in synfuel wastewaters is large, these compounds possess relatively few of the large number of possible functional groups. Methods that can determine functional group content (Petrakis et al. 1983) would be better suited to quantifying waste treatment performance than would methods that are selective for individual compounds. Methods for functional group analysis, however, are complex and not amenable to routine use. Two basic approaches are available to the analyst for the routine quantitation of chemical classes or individual compounds in aqueous, heterogeneous mixtures. The first quantifies solutes by methods of detection that are specific for individual chemical classes or particular compounds; many of these methods involve functional group derivatizations or colorimetric reactions that enable the compounds of interest ("analytes") to be distinguished from myriads of other solutes. Each analyte, however, requires a specific method of reaction and detection. The alternative approach employs a preliminary step that effects a sufficient degree of separation of the analyte from the mixture and thereby enables the use of routine, nonspecific, bulk-property methods as means of detection.

Tremendous qualitative differences can exist between quantitatively identical values obtained by any bulk- or colligative-property method such as chemical oxygen demand (COD), dissolved organic carbon (DOC), biological oxygen demand (BOD), or organic nitrogen. In oil shale wastewater treatment, for example, the removal of a large percentage of DOC may be irrelevant if the toxicity, color, and odor of the wastewater are associated strictly with the remaining small percentage. Identical values for any colligative property obviously can result from solutions of different compounds. The information from the nonspecific quantitative data yielded by colligative properties can be greatly amplified, however, by the physical separation or "fractionation" of the sample matrix prior to the detection step.

The physical separation of chemical classes is usually accomplished by (i) "isolation" processes such as liquid-liquid partitioning, solvent extraction (i.e., leaching), ion exchange, crystallization, preparative chromatography, purge-trap, or sorption (e.g., onto graphitized carbon, charcoal, or macroreticular resins) and (ii) physical "concentration" methods such as lyophilization, dialysis, and ultrafiltration (Jolley 1981; Karasek, Clement, and Sweetman 1981). The isolation schemes usually depend on various sequences of pH adjustment in the acidic, neutral, and basic ranges followed by extraction with water-immiscible solvents and back-extraction into aqueous phases or passage of the sample through series of exchange resins or sorbents. The fractionated classes are combinations of acidic, neutral, or basic hydrophobic and hydrophilic compounds. The analytes are then recovered from the various fractions by selectively removing the water or organic solvent. Most of these procedures risk the chemical or physical alteration or contamination of the sample by introduction of solvents, acids, bases, and heat. Although these methods are generally capable of effecting extensive separations of many different chemical classes, they are too time consuming for use in routine

experiments, where numerous parallel treatments are followed, or for routine monitoring of waste treatment performance. A detailed fractionation scheme for oil shale wastewater using macroreticular resins has been developed by Leenheer (1981); recently, a detailed examination of macroreticular resins for sorption of aromatic bases, major constituents of retort waters, was reported by Stuber and Leenheer (1983).

We have developed a simple fractionation protocol that is also applicable to other types of aqueous wastes. The method essentially effects a crude, but rapid, separation of compounds based on polarity, which is dictated by the types and contents of functional groups. It is therefore an extremely rapid means of crude, functional-group analysis. Wastewater solutes are separated into polar (hydrophilic) and nonpolar (hydrophobic or lipophilic) fractions by passing the sample through a disposable reverse-phase chromatographic cartridge. In a manner analogous to reverse-phase liquid chromatography (RPLC), we call this approach reverse-phase fractionation (RPF). Although these cartridges have found wide use in chromatography for sample preparation, cleanup, and trace enrichment (e.g., Puyear et al. 1981; Wolkoff and Creed 1981), they have not been applied for use in a rapid and simple fractionation scheme. This crude fractionation step increases the information that can be derived from the subsequent application of colligative analytical detection methods. A major advantage is that the hydrophilic fraction is not contaminated with organic solvent, thus allowing the application of methods such as carbon analysis and chemical oxygen demand. Moreover, the fractions themselves (especially the hydrophilic fraction) can be directly subjected to experimental treatments (such as biooxidation or physicochemical treatment) for comparison with parallel treatments of the raw, unfractionated water.

It is crucial to recognize that the fractions generated by any separation scheme are arbitrarily defined, being strictly dependent on the idiosyncrasies of the scheme. With RPF, compounds are separated according to "polarity". Here, "polarity" refers to the ability of a molecule to interact on a molecular or atomic level via forces generated by dispersive or permanent dipoles, hydrogen bonding, and ionization; operationally, polarity is a relative characteristic, but "relative polarity" can be determined by summing all of these interactions (Snyder and Kirkland 1979, chapter 6).

THEORY

Polarity-Class Separation by Reverse-Phase Fractionation

RPLC utilizes a stationary phase that is less polar than the mobile phase, which is commonly water modified with organic solvents. After sample solutes are introduced to the mobile phase, and if they are subsequently retained by the stationary phase, they can be eluted by further modification of the mobile phase with organic solvent. With RPLC, in contrast to normal-phase chromatography, solvent "strength" or eluotropic strength (i.e., capacity to elute sorbates or retained compounds) increases with increasing hydrophobicity of the solvent. A typical bonded-phase packing material for use in RPLC is composed of silica particles whose inherently polar surface silanol groups are covalently bonded with organochlorosilane reagents to yield siloxane groups that contain aliphatic moieties (e.g., octadecylsilyl, C_{18} groups). This yields an immobilized hydrophobic layer around each silica particle.

Numerous models have been proposed for the molecular retention mechanism of bonded, reverse-phase silica. One of these (see: Gilpin 1982) fits our observations with the RPF system. The aliphatic bonded moieties of the stationary phase can exist in two conformational extremes. At one extreme, the alkyl moieties project fully extended from the silica surface like bristles on a brush. At the other extreme, the alkyl moieties lie folded against the surface. The former conformation ("brush" or "up" state) is favored by hydrophobic mobile phases (i.e., organic), which tend to solvate the stationary phase; solvent becomes entrapped in the stationary phase. The latter is induced by totally aqueous mobile phases, since interactions between the two totally dissimilar phases are minimized. For reverse-phase retention, the "up" state is more retentive because it can interact with the mobile phase and because it is more hydrophobic. The "down" state can be converted to the "up" state by several methods, one of which is exposure to organic solvents such as methanol. If a water-miscible organic solvent is used to rearrange the alkyl groups to the "up" state, they tend to remain fully extended even in the presence of a totally aqueous mobile phase. Extensive exposure to water, however, will eventually extract the solvent from the stationary phase, which will collapse to the "down" state. For purposes of further discussion, pretreatment of the stationary phase with a water-miscible organic solvent (e.g., methanol) will be referred to as "wetting" or "activation."

If an aqueous sample containing organic solutes is passed through a bed of reverse-phase silica, the composition of the effluent generally remains unchanged from the influent. If the stationary phase is activated, however, the less polar solutes are retained by the hydrophobic stationary phase; the more polar solutes remain in the aqueous effluent. The solutes retained by the activated stationary phase from the mobile aqueous phase can be eluted with an organic solvent of sufficient strength. We refer to the retained and nonretained compounds as the lipophilic fraction (LpF) and hydrophilic fraction (HpF), respectively.

The general principle of RPF can be demonstrated by the following experiment. An aqueous mixture of bromophenol blue and *p*-nitrophenoxide ion is prepared. Bromophenol blue is a relatively hydrophobic, intensely purple sulfonphthalein dye substituted with two dibromophenyl groups. *p*-Nitrophenoxide, in contrast, is a relatively polar, bright-yellow ionized phenol. The mixture of these two compounds is deep violet, and when it is applied to unactivated C_{18} material, the effluent is also deep violet; no color is retained by the stationary phase. In contrast, when the cartridge is activated, the LpF is retained and the HpF appears in the aqueous effluent; the effluent is bright yellow and the stationary phase becomes deep purple. A water-miscible organic solvent can easily elute the bromophenol blue from the stationary phase. Complete physical separation of the two solutes is thereby effected.

It must be emphasized that RPF is not analogous to RPLC. In RPLC, the sample is applied as a plug, and a mobile phase carries the components through a highly efficient analytical column. In RPF, which is analogous to trace-enrichment, the sample and mobile phase are synonymous, and the mobile phase is therefore as weak as possible; the miniature chromatographic cartridges actually serve as extraction devices. Many solutes will concentrate on the packing material at the inlet because their capacity factors (k' values, or distribution coefficients) will be maximized in the weakest possible reverse-phase solvent, water; the strength of the water can be further decreased

by the addition of salts. Since the column has been miniaturized and packed with less efficient chromatographic material, it is therefore essential to ascertain the volume of sample that can be applied before the stationary phase becomes saturated and solutes that normally would be retained begin to elute from the column. The analyst also should be aware that the polarity of the stationary phase can be altered during fractionation simply by the accumulation of partitioned solutes; this is referred to as the "mutual zone of solubility effect" (Saner, Jadamec, and Sager 1979). With mixtures of solutes comprising a wide range of k' values, the initial sorption of many solutes with high k' values shifts the selectivity away from solutes with lower values (Ram and Morris 1982).

PROTOCOL SUMMARY

Polarity-Class Fractionation

Miniature reverse-phase cartridges are available from several manufacturers (e.g., C_{18} SEP PAKs from Waters Assoc., Inc., Milford, MA; Disposable Extraction Columns from J.T. Baker Chemical Co., Phillipsburg, NJ; Bond Elut extraction columns from Analytichem International, Harbor City, CA). We have extensively investigated C_{18} SEP PAK cartridges because of their ease of use; other cartridges should give comparable results, although modifications to the protocol would be required. The C_{18} SEP PAK (Sample Enrichment Purification) cartridges are made from virgin polyethylene tubes that contain, under compression, about 350-400 mg of C_{18}-bonded Porasil A silica (80-μm diam. particle size); the bonded silica is held in place by fritted polypropylene discs (Wolkoff and Creed 1981). The cartridges have interchangeable influent and effluent female Luer-slip ends to which male-Luer syringes can be attached for introduction of solvents and samples.

The fractionation procedure is summarized in Figure 1. Activation is achieved by applying 5 mL of methanol to the cartridge followed by rinsing with 20 mL of water; when used as a reagent, "water" refers to ASTM Type I quality. Sufficient rinsing is required for removal of "free" residual methanol and minimization of background interferences. Excess, unretained water is expelled from the cartridge with about 20 cm^3 of air. The syringe always should be disconnected from the cartridge before the plunger is removed or pulled back for filling the barrel; this prevents pulling liquid back through the cartridge. The sample is then passed through the cartridge at a sufficiently slow rate (e.g., 5 to 10 mL/min for retort waters; 100 mL/min can be used successfully on dilute waters). The maximum quantity of sample applied must be predetermined from breakthrough experiments; these volumes are commonly 2.5 to 10 mL for retort waters, and up to several liters for cleaner waters. With larger sample sizes, however, the octadecyl surface may become deactivated; addition of methanol to the sample (e.g., up to several percent) may prevent deactivation (Andrews and Good 1982), but this would preclude the subsequent application of bulk measurements such as COD or DOC, because of contamination of the HpF. The cartridge should be held vertically to prevent channeling during sample application and elution. The initial milliliter of aqueous effluent (HpF) is discarded because of its dilution by the 400 μL of bound water that remains from activation/rinsing of the cartridge. Subsequent HpF can be collected for analysis (or subjected to waste treatment studies); as an alternative method of analysis, if the exact volume of applied sample is known, all of the effluent can be collected in a volumetric flask and brought to volume. The collection of

HpF by pulling the sample through the cartridge with a vacuum is not recommended because of loss of volatile organic species and because of foaming, which is caused by outgassing of CO_2 and NH_3.

For the work reported here, dissolved organic carbon (DOC) was determined following the method in Chapter III that uses direct analysis of a CO_2-free sample by UV/peroxydisulfate oxidation and coulometric titration of the evolved CO_2.

The retained organic compounds (LpF) can be eluted after the residual, unpartitioned 400-μL aqueous sample is rinsed from the cartridge with water. Since the LpF concentrates at the top of the cartridge, it is best to elute in the direction opposite to sample application; if the sample was not prefiltered, however, particulates will also be dislodged. The solvent used for initial elution must be mutually miscible in water and in whatever strong solvent may be subsequently used. Methanol, however, will elute nearly all retort water compounds that have not been irreversibly bound to the stationary phase; less polar solvents will not elute the more polar compounds. Compounds of different polarities can be eluted depending on the strength of the organic solvent. For other types of samples, tetrahydrofuran or dichloromethane may be needed for elution.

The particle size of the C_{18} stationary phase and fine porosity of the fritted polypropylene discs impart the cartridges with depth-filters characteristics. The particulates in retort water are effectively retained by both the inlet disc and by the silica particles; when the organic eluant is passed through the cartridge, the tarry and oily residues that coat many of these particulates will be dissolved. The concentration of suspended hydrophobic solutes can be determined as the difference between the LpF concentrations yielded by raw and filtered samples.

Determination of "Oil and Grease" by RPF

Oil and grease is a broad classification of organic compounds that is arbitrarily defined by the analytical method applied. As specified by APHA (1980), it is the group of substances that can be determined quantitatively on the basis of their common solubility in Freon 113. Oil and grease includes aliphatic hydrocarbons (e.g., paraffins, waxes, and oils), lipids, fatty acids, and soaps. This class of compounds should be analogous to the group of lipophilic solutes that compose the LpF.

Liquid-liquid partitioning followed by gravimetric quantitation is the standard method for determining the dissolved or suspended "oil and grease" concentrations of a wastewater sample. This method is fraught with difficulties, however, when applied to oil shale process waters. A stable emulsion often forms between the aqueous and organic extraction phases. The high concentrations of carbonate and ammonia in retort water require the addition of large amounts of salt and acid to destabilize the emulsion. This can lead to CO_2 outgassing (which causes excessive foam production), protonation of aliphatic carboxylic acids, and precipitation of elemental sulfur. The large quantity of additional salts would "salt-out" some of the less hydrophilic solutes that are not oils (i.e., decrease their solubilities in the aqueous phase). In addition, the partitioning process itself is not selective and leads to the loss of volatile solutes into the headspace. Polar compounds can be

co-extracted as organic ligands or ion-pairs, and many polar and nonpolar species tend to concentrate at the organic/aqueous phase interface when surface-active agents, such as fatty acids, are present; when the organic phase is separated from the aqueous phase, the compounds in the interface are lost. During removal of the organic solvent, compounds are lost continuously via volatilization; standardization of these losses is impossible because the volatilization rate is substantial and not constant. Finally, quantitation by gravimetric detection is notoriously inaccurate and totally nonselective; it simply measures the mass of dissolved solids that remain after applying a standardized drying step. Some of these problems are discussed further by Wallace and Bonomo (1984).

We have adapted the general RPF procedure to the determination of dissolved "oil and grease" (Fig. 2). The hydrophobic analytes (LpF) are retained by the stationary phase, and the effluent (HpF) is discarded. At this point in the general RPF protocol, the procedure is modified, because the method of detection precludes use of a "switchover" solvent of mutual miscibility. Although it has been reported that hydrophobic sorbates can be directly "extracted" from a reverse-phase cartridge that contains an aqueous residuum by using a water-immiscible solvent (Pallante et al. 1982), Freon does not efficiently extract the aliphatic compounds from the retained LpF; furthermore, any water in the Freon eluate will interfere with the subsequent infrared (IR) analysis. The aqueous residuum in the cartridge must therefore be removed by mechanical means; this is best achieved by lyophilization. The nonpolar compounds can then be eluted with Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane), the eluent diluted to known volume, and the concentration quantified via IR spectroscopy by measuring the absorbance of the asymmetric methylene C-H stretch at 2930 cm^{-1} and interpolating from a standard curve of absorbance versus concentration of oil (e.g., standards prepared from mineral oil or other suitable oil). The absorbance was determined by scanning from 3200 to 2800 cm^{-1} and using the tangent/base-line method (Fig. 3). The concentration of oil is derived as:

$$(mg/L) = (m) \times (\log P_o/P_{sample} - \log P_o/P_{blank}) + (b)$$

where P_o = incident energy, P = transmitted energy, and "m" and "b" are the slope and intercept of the standard curve, respectively. Blanks were carried through the entire RPF procedure, substituting distilled water for the sample. The scans were done with a Perkin-Elmer model 298 IR spectrophotometer at 4 min/full-range scan and medium slit width. Samples were placed in 1-cm path length quartz cuvettes and scanned against Freon 113. Peak heights were kept below 80% of full-scale by appropriate dilution.

True aliphatic oil was determined by passing the C_{18} Freon eluate directly through an activated (i.e., dried) normal-phase silica cartridge (Si SEP PAK, Waters Assoc.). This step removed LpF solutes that contained basic and acidic functional groups and allowed aliphatic hydrocarbons to pass through in the effluent for quantitation after dilution to a known volume. Absorbance values for blanks depend on the final diluted volume; they were typically 0.010 for "oil and grease" and 0.023 for true oil when the final volumes were 5 to 10 mL.

APPLICATION OF RPF

Oil Shale Process Waters

The origins of the oil shale wastewaters used in this study are described in Appendix I. Values obtained for other water quality parameters described in the manual are summarized in Appendix II.

Reverse-Phase Activation: Retort Water Fractionation

Application of retort water to C_{18} cartridges that had not been activated always yielded effluents that were unchanged. In contrast, activation of the cartridge with methanol permitted the subsequent retention of a large percentage of the organic solutes. Raw retort water generally is dark brown and has a pungent tarry odor; these organoleptic characteristics are common to nitrogenous heterocycles. The aqueous effluent from activated C_{18} cartridges, however, smelled strongly of ammonia, whose odor would ordinarily have been totally obscured by the intense odor of the heterocycles. If the water was removed from the HpF by drying, the ammonia also was removed. The absence of ammonia then permitted the intense odor of fatty acids to be recognized. The aqueous effluent (HpF) was generally colorless or pale yellow; the stationary phase retained nearly all of the color. A large percentage of the retained compounds could be eluted with organic solvent (e.g., methanol). If the organic solvent was removed from the eluate, the residuum (i.e., LpF compounds) possessed all of the characteristic odor and color of raw retort water.

A portion of the soluble LpF was irreversibly retained by the stationary phase. This was probably a result of ligand formation by contaminative metals in the silica particles and of sorption of solutes containing basic functionalities by surface silanol groups that remained unreacted after bonding of the silica; the particular cartridges used in the protocol reported here were packed with material whose residual surface groups had not been endcapped (e.g., with chlorotrimethylsilane). Variability among reverse-phase silicas depends on whether the silica has been endcapped and whether the bonded phase has been synthesized with monofunctional silanes (which yield a more uniform, thin "brush" layer) or with bi- or tri-functional silanes (which may yield a thicker, partially polymerized coating) (Snyder and Kirkland 1979, chapter 7).

Quantitation of HpF as DOC

The nine process wastewaters were fractionated according to the RPF procedure. The unfractionated raw sample and the HpF were analyzed for DOC. Three separate sets of samples were analyzed over a one-year period. In Table I, the waters are ranked in decreasing order of average percentage of HpF-DOC content. The only differences between the three sets were the relative concentrations of the samples before they were fractionated; the final values were corrected by the relevant dilution factors. The discrepancies between the values for some of the waters resulted from time-dependent storage effects and relative concentrations. The average HpF-DOC contents ranged from a high of 82% for Paraho retort water, which has very high concentrations of lower-molecular-weight fatty acids (Rogers et al. 1981), to a low of 15% for Oxy-6 gas condensate, which would not be expected to contain polar solutes since it was a gaseous stream. The average HpF-DOC content for Omega-9 retort water (36%) fell

directly between the two values (24% and 51%) reported for total hydrophilic DOC in Omega-9 using the more rigorous and time-consuming method of Leenheer (1981).

A method that was developed for separating mutagenic compounds into polar and nonpolar compounds using C_{18} cartridges was reported by Toste, Sklarew, and Pelroy (1982). Unfortunately, they eluted the retained compounds, removed the eluent methanol, and determined the dry mass of the residuum instead of determining organic carbon; volatilization of organic material inevitably occurred. These results therefore could not be related to the organic carbon values for the raw waters (Paraho, Oxy-6, and Geokinetics). Furthermore, the organic carbon values reported for the raw waters differed greatly from other published values.

The average percentage of the total DOC that resided in the HpF for Oxy-6 gas condensate and retort water (Table I) was 15% and 48%, respectively. Of the organic solutes that have been quantified for these two waters, the major polar chemical classes (i.e., those that would be expected in the HpF) are aliphatic mono- and di-carboxylic acids and low-molecular-weight nitriles, alcohols, ketones, and oxygenated heterocycles. These classes comprise up to a maximum of about 10% and 45% of the DOC for Oxy-6 gas condensate and retort water, respectively (Leenheer et al. 1982); these data show correspondence with the fraction of carbon in the HpF of either water.

<u>Quantitation of LpF as "Oil and Grease" by IR</u>

The RPF procedure, when used for determining oil and grease of a wastewater sample, has distinct advantages over the partition-gravimetric procedure. The RPF procedure obviates the need for liquid-liquid partitioning and gravimetric detection along with the attendant problems of emulsion formation and loss of volatile hydrocarbons during sample concentration. The procedure is simple to perform, minimizes the use of glassware (generally one volumetric flask is the only glassware required per sample), and minimizes solvent consumption (usually about 20 mL of solvent is required per sample, regardless of sample size, compared with 100 mL of solvent for the partition-gravimetric method). Dilute samples are concentrated directly (i.e., via trace enrichment) by the cartridges; the final degree of enrichment is dictated by the minimum volume of organic solvent required to elute the sorbates. This means that the detection limit is restricted only by the sample matrix; large sample sizes can be applied to the cartridges if the total solute concentrations are low. The detection limit for oil that we observed in the Freon eluent was 20 mg/L (as mineral oil). The volumes of retort waters that could be applied were generally in the 5- to 20-mL range; Puyear et al. (1981) also report that for samples containing greater than 1500 mg/L of hydrocarbons, less than 20 mL of sample should be applied. For on-site use, the cartridges can be used to directly fractionate samples and thereby avoid problems associated with collection and storage in containers.

The major advantage of the RPF procedure is throughput. An analyst can easily determine LpF "oil and grease" by the RPF procedure for 25 to 50 samples in an eight-hour working period. The major disadvantage of the method is that it is not known if the compounds in the LpF are the same as the compounds that would be quantified by the partition-gravimetric method because true fortification/validation experiments cannot be performed for either method; oil

cannot be "solubilized" in the water (for standardized aqueous solutions) without addition of other organic agents.

The reproducibility of the LpF method for determining aliphatic oil and "oil and grease" was determined for Oxy-6 and 150-Ton retort waters (Table II). The relative standard deviation values for both parameters were usually less than 10%. The oil and grease concentrations for Oxy-6 retort water (and presumably for other waters as well) varied from sample to sample because of changes in composition over time. Some of these values were obtained for samples withdrawn from different drums over a period of a year.

The aliphatic oil values for unfiltered samples (i.e., total aliphatic oil) represented less than a fifth of the total oil and grease concentration (Table II). In contrast, aliphatic oil for filtered samples (i.e., dissolved aliphatic oil) of both Oxy-6 and 150-Ton retort waters was not detectable. Apparently, the aliphatic "oil" in these waters, in contrast to the total oil and grease, is associated exclusively with the particulate fraction.

The RPF method was used to determine oil and grease in seven oil shale process waters (Table III). The values were determined for both unfiltered raw samples and for filtered samples. The difference between each pair of values represents particulate oil and grease. The samples are ranked in order of decreasing total oil and grease concentrations; this trend is maintained for dissolved oil and grease with the exception of Oxy-6 retort water. The lowest LpF oil and grease concentrations were found for Omega-9 and Oxy-6 GC; these were about an order of magnitude lower than the highest concentration, which was found for 150-Ton. The low values are consistent with the origins of these two waters. Omega-9 was from an in-situ site that had extensive dilution from groundwater, and the gas condensate had not contacted the shale oil during retorting.

The content of oil and grease in oil shale wastewaters depends on (i) the time that the water contacts the oil during retorting and before decantation, (ii) whether heat was used for the separation process, and (iii) the time during which the water was kept in cold storage. These data can be compared with the limited data obtained by the standard partition/gravimetric method: 580 mg/L for Omega-9 (Farrier at al. 1980), and 86, 142, and 109 mg/L for Oxy-6 GC, Oxy-6 RW and 150-Ton, respectively (J.J. Ahern, unpublished data). The above-ground process waters had lower percentages of total oil and grease that was dissolved (i.e., 53% to 70% versus 83% to 101% for in-situ) even though their absolute dissolved values were generally higher. Some of these wastewaters had already been filtered after they were collected; this explains why some of the unfiltered values were the same as the filtered values (e.g., Omega-9).

A major difficulty with development of the LpF oil and grease procedure was a high IR background absorbance that was sporadically encountered. This problem had several possible origins (e.g., leaching of trace quantities of phthalate plasticizers or residual alkylsilyl bonding reagent from the cartridges). We also found substantial lot-to-lot variation in both background absorbance and apparent partitioning efficiency; this same variability was noted by Saner et al. (1979).

Quantitation of LpF as "Oil and Grease" by Carbon Analysis

The RPF method for determining oil and grease has a further, major advantage over the partition-gravimetric method. Routine methods that measure bulk parameters such as DOC or COD can be used to quantify the nonpolar class of compounds that resides in the retained fraction. This can be done indirectly by calculating the difference in values for the unfractionated sample and for the HpF (aqueous effluent).

LpF oil and grease was quantified as DOC for the seven oil shale process waters (Table IV; these values are complementary to the HpF values for replicate set #3, Table I). The relative rankings of the waters in Tables III and IV were about the same. By dividing the LpF oil and grease values (i.e., those obtained by the IR procedure) by their respective LpF-DOC values, qualitative information can be obtained about the composition of the LpF. This unitless value reflects the degree of saturation of the carbon. All of the waters appeared to contain about the same types of LpF constituents (i.e., degrees of saturation ranged from 0.10 to 0.14) except for 150-Ton, whose degree of saturation was 0.22.

Variables Affecting the Fractionation Process

The RPF process can be inadvertently changed by altering various parts of the protocol. The volume of sample applied, pH, solvent used for activation, osmolality of the sample (e.g., ionic strength as affected by degree of dilution) and rinse water, and lot-to-lot, manufacturer-to-manufacturer variability of the cartridges can each drastically alter results; lot-to-lot variability can be extreme and is a major problem with reverse-phase materials (see: Wise and May 1983). Alternatively, modification of these variables can be purposefully implemented to redefine the chemical classes that are separated by the fractionation process. For example, adjustment of sample pH prior to fractionation can force compounds with basic functionalities or those with acidic functionalities into the LpF, if the pH of the sample is adjusted upward or downward, respectively. Other modifications to the protocol that would change the selectivity of the separation would include: (i) use of different bonded phases (e.g., shorter carbon chain lengths and lower specific carbon content decrease k' values), (ii) use of different activation solvents (e.g., nitriles, ketones, and other alcohols), and (iii) addition of an organic modifier to the sample. We also have observed that some sorbate can be stripped from the stationary phase during the rinsing step. This could have been caused by sample overloading or because the pH and osmolality of the wash water differed from that of the sample.

The reuse of the cartridges has not been investigated, but some color is irreversibly retained; tetrahydrofuran or methylene chloride will not elute this material. Furthermore, the basic pH of retort waters may result in extensive hydrolysis of the siloxanes if a cartridge is reused excessively.

Other Applications of RPF

We have used the RPF procedure routinely for monitoring the performance of our biological and physicochemical treatment research and for generating fractions for further experimental treatment work. Some major conclusions from these data have been summarized (Healy et al. 1983; Jones, Sakaji, and Daughton 1982; Sakaji et al. 1982). The RPF method also has utility in separating the

large quantities of ammonia and polar organic nitrogen from the numerous nitrogenous heterocycles in retort waters, thereby allowing for the subsequent estimation of polar-nitrogen (in the HpF; mainly ammonia) and nonpolar organic nitrogen (in the LpF) by the rapid method of combustion/chemiluminescence (see: Chapter V); this approach circumvents the need for the laborious Kjeldahl method.

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Table I. Percent HpF-DOC Contents[a] for Oil Shale Process Wastewaters

	series of replicate analyses[b]									
	1			2			3			
sample	raw DOC	HpF DOC	%	raw DOC	HpF DOC	%	raw DOC	HpF DOC	%	avg %
Paraho	42460	38440	90.5	43850	32275	73.6	--[c]	--	--	82
Oxy-6 RW	2800	1583	56.5	2534	1194	47.1	3194	1271	39.8	48
S-55	2364	1048	44.3	1824	720	39.5	2263	1009	44.6	43
TOSCO HSP	2986	1411	47.3	2790	1180	42.3	2550	727	28.5	39
150-Ton	3434	1429	41.6	2999	1055	35.2	3054	974	31.9	36
Omega-9	870	346	39.8	845	301	35.6	803	254	31.6	36
Geokinetics	1692	542	32.0	1676	548	32.7	1646	498	30.3	32
Rio Blanco	212	62	29.2	184	52	28.3	--	--	--	29
Oxy-6 GC	752	120	16.0	797	136	17.1	671	74	11.0	15

[a] All DOC values in mg/L; percentages calculated on the basis of the raw DOC values for each of the three series of samples; waters are ranked according to the average percent HpF-DOC content for the three series of samples.

[b] Prior to fractionation, samples for series 1 were all diluted to 50% with phosphate buffer, series 2 were each diluted to yield equivalent DOC values, and series 3 were not diluted.

[c] Values not determined.

Table II. Reproducibility of RPF Method (IR) for Aliphatic Oil and "Oil and Grease" in Oil Shale Process Waters

test	sample	mean (mg/L) oil and grease as mineral oil	n	rsd (%)
oil and grease[a]	Oxy-6 RW	204	8	13.2
oil and grease	Oxy-6 RW	262	8	5.8
oil and grease	Oxy-6 RW (filtered)[b]	273	15	5.2
oil[c]	Oxy-6 RW	47.1	9	4.1
oil	Oxy-6 RW	32.9	5	9.8
oil	Oxy-6 RW	27.2	9	9.5
oil	Oxy-6 RW (filtered)	nil	15	--
oil	150-Ton	121	10	6.0
oil	150-Ton (filtered)	nil	10	--

[a] Oil and grease defined as the compounds in the Freon eluate from a C_{18} cartridge (see: Fig. 2).

[b] Filtered through 0.8-μm pore diameter polycarbonate membrane.

[c] Oil (i.e., aliphatic) defined as the compounds in the Freon effluent from a normal-phase silica cartridge (see: Fig. 2).

Table III. Applicability of RPF "Oil and Grease" Method (IR) to Oil Shale Process Waters

	oil and grease as mineral oil (mg/L)				
	unfiltered		filtered[a]		% dissolved[b]
sample[c]	mean	range	mean	range	oil & grease
150-Ton	641	58	448	37	70
S-55	334	78	178	20	53
TOSCO HSP	276	20	175	18	63
Oxy-6 RW	242	40	219	39	90
Geokinetics	160	24	162	8	101
Oxy-6 GC	86	48	71	35	83
Omega-9	58	33	56	44	97

[a] Filtered through 0.8-μm pore diameter polycarbonate membrane.

[b] (filtered/unfiltered) X 100 (i.e., percent of total oil and grease that is dissolved).

[c] Sample volumes applied to cartridges were 2.5 or 5.0 mL; n=4 for each sample type.

Table IV. Ranking of Retort Waters by LpF Content as Measured by Both Carbon Analysis (DOC) and IR Absorbance (Oil & Grease)

sample[a]	raw DOC[b]	HpF DOC (mg/L)[c]	LpF DOC (mg/L)[d]	LpF oil & grease[e]	degree of saturation[f]
150-Ton	3054	974	2080	448	0.22
Oxy-6 RW	3194	1271	1923	219	0.11
TOSCO HSP	2550	727	1823	175	0.10
S-55	2263	1009	1254	178	0.14
Geokinetics	1646	498	1148	162	0.14
Oxy-6 GC	671	74	597	71	0.12
Omega-9	803	254	549	56	0.10

[a] Sample volumes applied to C_{18} cartridges were 4.0 mL.

[b] DOC of unfractionated water; filtered through 0.8-μm pore diameter polycarbonate membrane.

[c] C_{18} effluent DOC.

[d] Calculated indirectly; difference between DOC values of raw water and HpF.

[e] Values of filtered samples from Table III.

[f] (LpF oil & grease)/(LpF DOC); i.e., extent of hydrogenation.

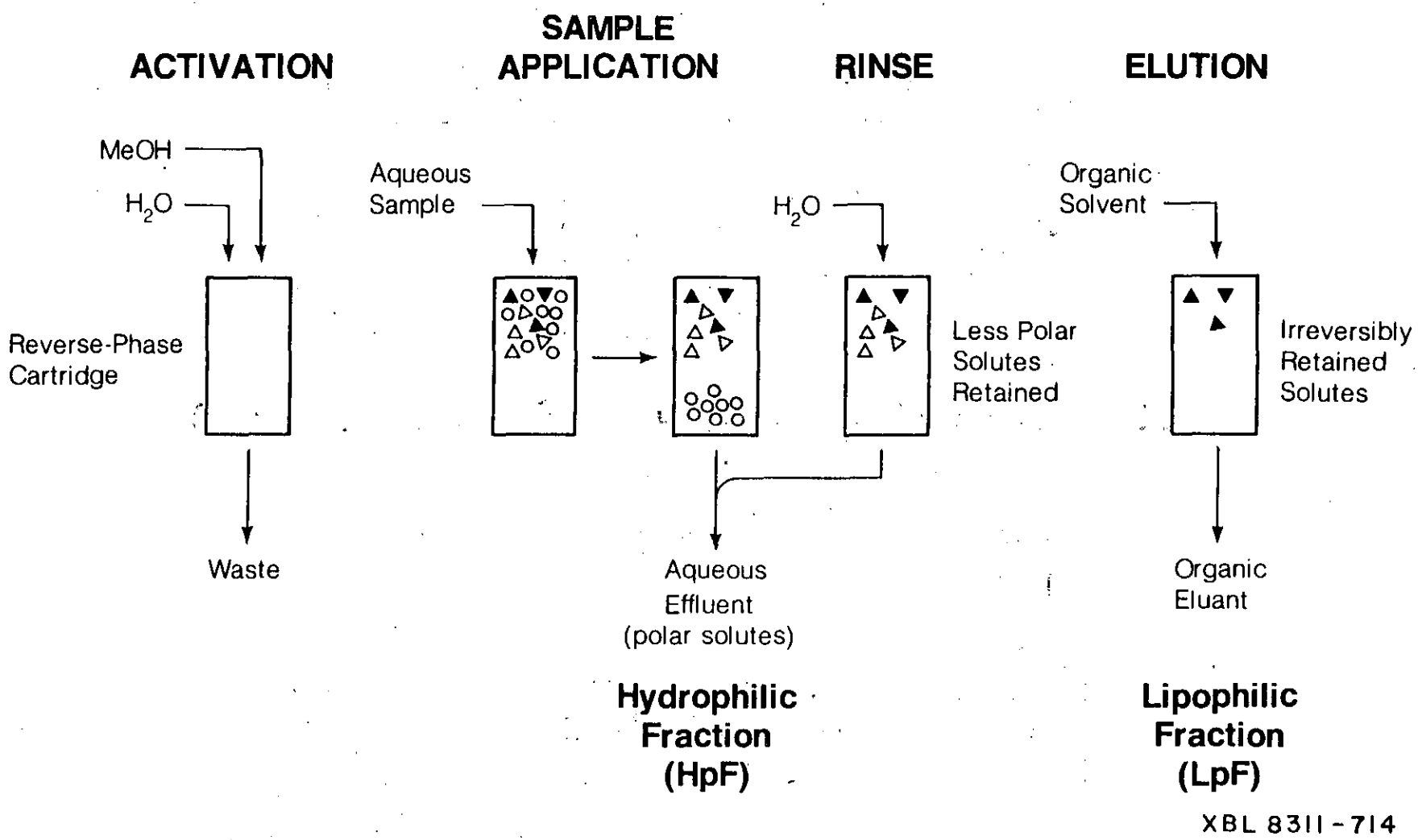

Figure 1. RPF scheme for separation of aqueous-waste solutes into hydrophilic (HpF) and lipophilic (LpF) fractions. Solutes belonging to the HpF are represented by (O). Solutes belonging to the LpF are represented by triangles; those which are irreversibly retained are represented by (▲).

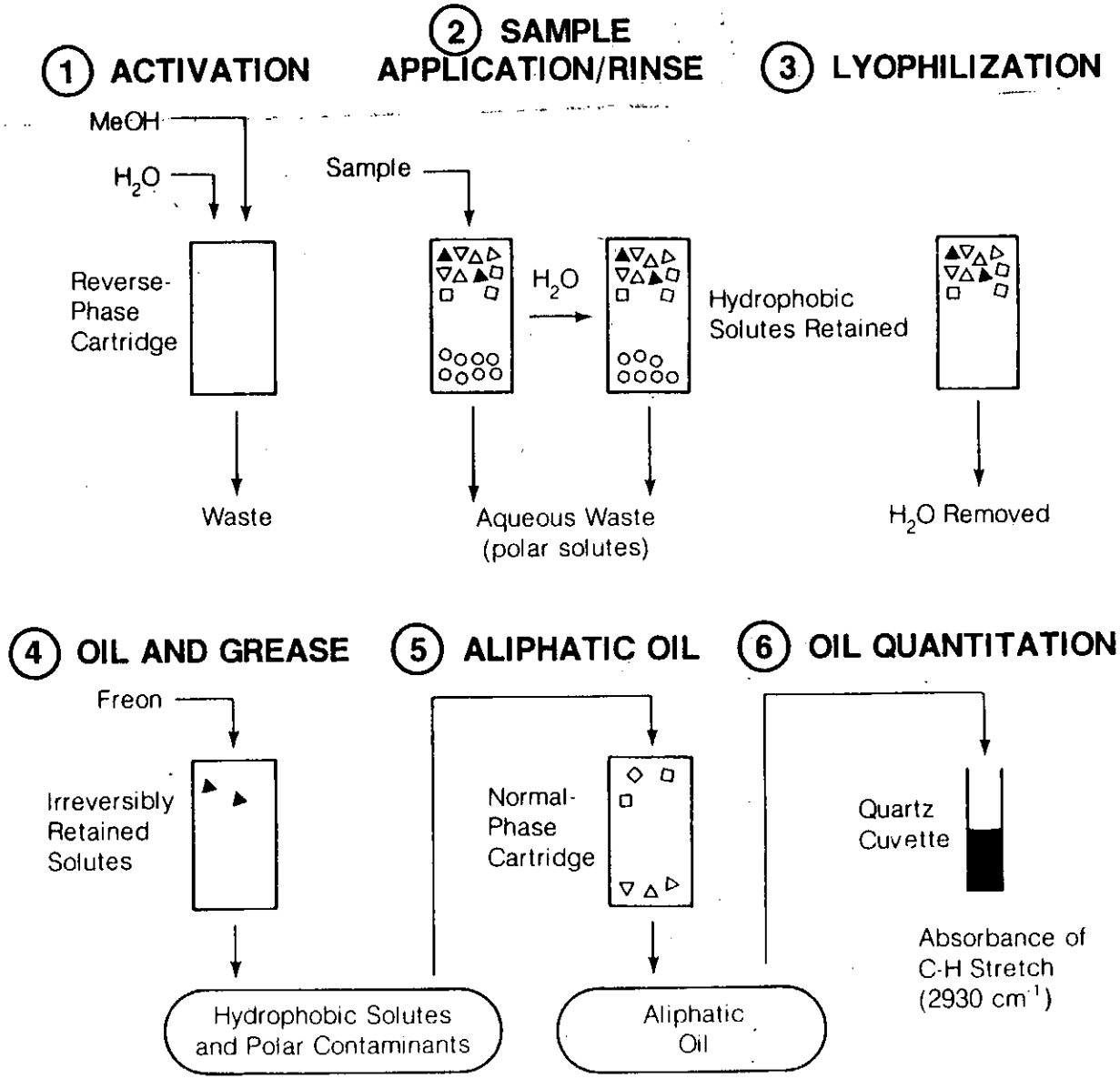

Figure 2. Application of RPF to quantitation of "oil and grease" and aliphatic oil in aqueous wastes. Solutes belonging to the HpF are represented by (○). Solutes belonging to the LpF (i.e., oil and grease) are represented by triangles and open squares; those which are irreversibly retained by the C_{18} cartridge are represented by (▲), and those which are not retained by the normal-phase cartridge (i.e., aliphatic oil) are represented by (△) (XBL 8311-713).

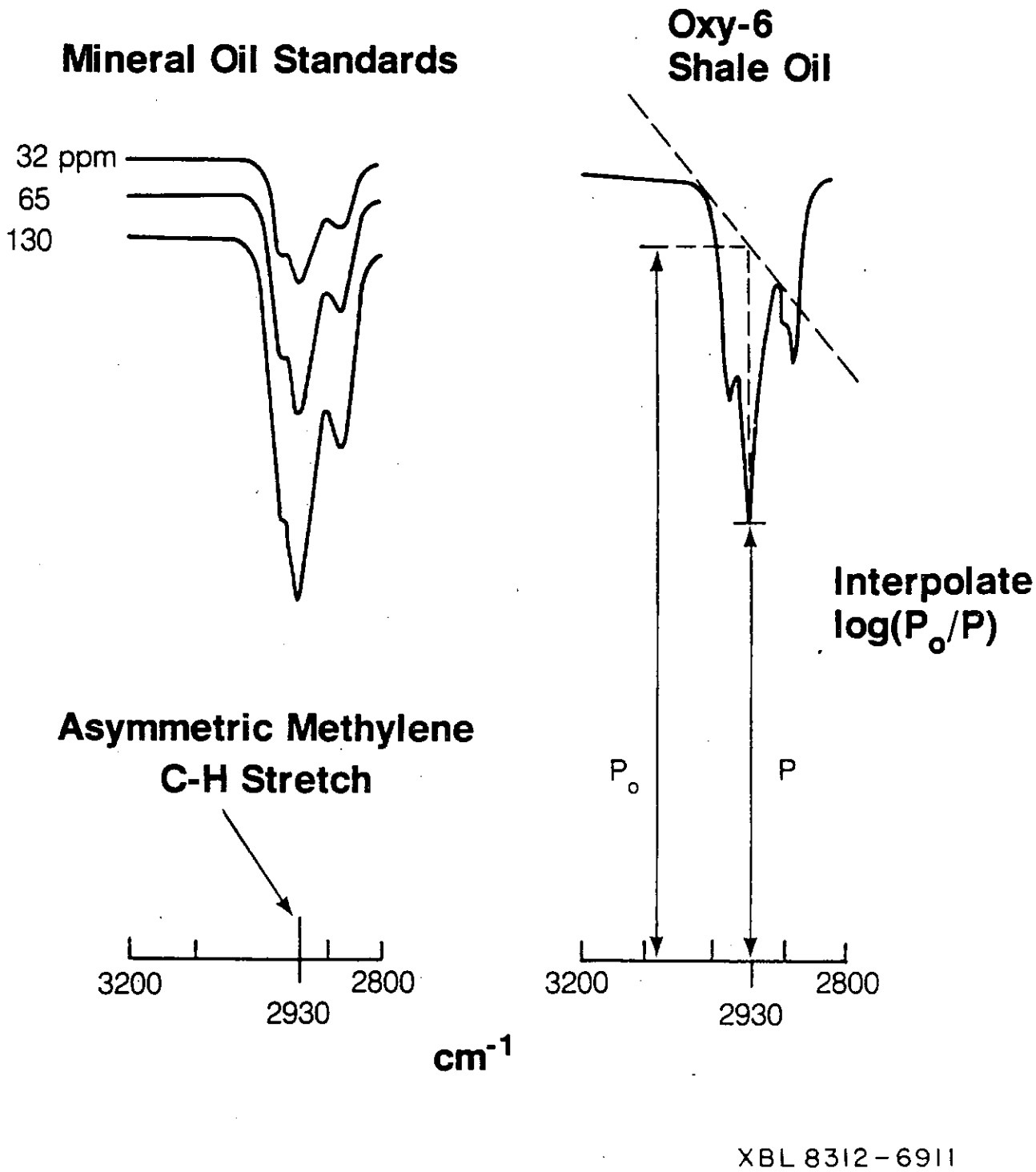

Figure 3. Quantitation of oil by infrared spectroscopy (tangent/base-line method).

PROTOCOL: REVERSE-PHASE FRACTIONATION

I. Apparatus
   A. Glassware
      1. Acid-wash all glassware (soak in 35% nitric acid overnight and rinse with ASTM Type I water)
      2. Volumetric flasks (size and number will depend on organic solute concentration in each sample; generally one per sample). Rinse volumetric flasks with Freon 113.
   B. Reverse-phase C_{18} cartridges (C_{18} SEP-PAKs, Waters and Associates, Milford, MA; Part #51915)
   C. Normal-phase Si cartridges (Si SEP-PAKs, Waters and Associates; Part # 51905)
   D. Positive-displacement pipettes (e.g., 1.0-mL and 2.5-mL from SMI, Inc., Emeryville, CA)
   E. 10-mL gastight glass syringe with Teflon-tipped plunger and male Luer tip (two)
   F. Numbered aluminum tags (e.g., for gas chromatography columns; one per cartridge)
   G. Lyophilization connectors
      a. No. -0- Vikem or silicone solid stopper, 13-gauge stainless-steel tubing with female Luer hub, Teflon male Luer union (Hamilton Co., Reno, NV), and polypropylene male Luer plug (Value Plastics, Inc., Fort Collins, CO) (1 set per cartridge).
      b. To construct lyophilizer connection apparatus, insert 4-cm length of 13-gauge stainless steel tubing with female Luer hub through the narrow end of a No. -0- stopper. Connect the Luer hub to the cartridge end-sleeve with a male Luer union. Seal the open end of the cartridge with a male Luer plug.
   H. Lyophilization apparatus (manifold, vacuum pump, etc.)
   I. Teflon male Luer unions (for coupling C_{18} and Si cartridges)
   J. Infrared scanning spectrophotometer (repetitive scan recommended, e.g., Perkin-Elmer 298; matched quartz cuvettes, 1-cm path length)

II. Reagents
   NOTE: All reagents are made from Analytical Reagent grade chemicals unless otherwise specified. When used as a reagent, "water" refers to ASTM Type I quality.
   A. Tetrahydrofuran (THF)
   B. Methanol (MeOH)
   C. 1,1,2-Trichloro-1,2,2-trifluoroethane (Freon 113) (Fisher Scientific)

III. Protocol
   NOTE: The maximum number of samples and blanks is limited by the capacity of the lyophilizer.
   A. <u>Hydrophilic Fraction (HpF)</u>
      1. C_{18} cartridge preparation
      Note: Use cartridges from same lot for each experiment; record lot #.
         a. To introduce solvents, connect male Luer tip of gastight syringe to the longer end-sleeve of the C_{18} cartridge.
         b. Pre-wash each C_{18} cartridge with 10 mL of THF.
         NOTE: Always disconnect the syringe or pipette from the cartridge prior to withdrawing the plunger.

c. To activate each cartridge, apply 5 mL of MeOH, followed by 20 mL of water, using gastight syringes. Partially remove residual water by forcing approximately 10 cm^3 of air through the cartridge using a gastight syringe.

2. Sample application
   a. Withdraw subsample from aqueous sample with an SMI pipette. To introduce sample, connect male Luer tip of SMI pipette to the longer end-sleeve of the C_{18} cartridge.
   b. Apply sample to the cartridge immediately after activation, at a flow rate of 5 to 10 mL/min. The SMI pipette tip fits directly inside the cartridge end-sleeve. The cartridge should be held vertically during sample application.
3. Collection of HpF

   NOTE: This fraction is discarded if only "oil and grease" values are desired; refer to III.B.
   a. Discard the initial milliliter of effluent; this portion is diluted by the residual water retained by the cartridge.
   b. Collect effluent in appropriate container for subsequent analysis or treatment.

B. <u>Lipophilic Fraction (LpF)</u> -- "Oil & Grease" and "Aliphatic Oil" --

1. Blank preparation
   a. Blanks are prepared by following step III.A.1.
   b. Blanks should be prepared in duplicate for each elution volume for oil & grease and aliphatic oil.
2. Sample application
   a. Follow III.A.1. and III.A.2.
   b. Remove residual sample from cartridge by forcing approximately 10 cm^3 of air through the cartridge using a gastight syringe.
   c. Rinse residual pore-retained sample from cartridge with 1 to 2 mL of water (positive displacement pipette). Partially remove residual rinse-water by forcing approximately 10 cm^3 of air through the cartridge using a gastight syringe.
3. Sample lyophilization
   a. Identify each cartridge with a numbered aluminum tag. Seal both ends of the cartridge with male Luer plugs. Store cartridges at -20°C until lyophilization.

   NOTE: Do not mark cartridges with marking pen; the pigment can contaminate the eluent.
   b. Prepare lyophilizer.
   c. Start the vacuum pump; operate lyophilizer at less than 0.10 torr.
   d. Remove male Luer plug from long end-sleeve of cartridge. Attach each cartridge to lyophilizer manifold (e.g., VirTis 6205-1650, with 0.5-in OD Quickseal valves).
   e. Submerge each cartridge in a MeOH/dry-ice bath for 30 seconds.
   f. Immediately after this freezing step, apply the vacuum; if any loss in vacuum occurs, check for leaks.
   g. Lyophilize for about two hours. Lyophilization is complete when the cartridges are at room temperature and no further condensation forms when they are wiped dry.
   h. When lyophilization is complete, release the vacuum, and turn off the pump.
   i. Remove samples from lyophilizer.

4. Elution of LpF
   a. For the determination of oil & grease, elute the lyophilized cartridges with 5 mL of Freon; force residual Freon through with 10 cm^3 of air. Elute in the same direction as sample application to avoid washing out nonsoluble particulates. Collect eluent and residual in an appropriate size volumetric flask. Bring to volume with Freon.
   b. For the determination of aliphatic oil, prewash the Si cartridges with 10 mL of Freon; force residual Freon through with 10 cm^3 of air and discard. Elute the retained solutes from the C_{18} cartridges directly through the Si cartridges (cartridges coupled with male Luer unions) with 5 mL of Freon; force residual Freon through with 10 cm^3 of air. Collect eluent and residual in an appropriate size volumetric flask. Bring to volume with Freon.
   c. The stoppered volumetric flasks containing the eluates can be stored at 4°C.
5. Quantitation
   a. Prepare a set of standards using mineral oil or appropriate reference material (e.g., shale oil).
   b. For the stock solution, place appropriate volume of oil in a tared 50-mL volumetric flask (if using mineral oil, place 75 µL in flask). Determine the mass of the oil, and bring to volume with Freon.
   c. For working standards, place 100, 250, 500, or 1000 µL of stock solution (positive displacement pipettes) in 5-mL volumetric flasks, and bring to volume with Freon.
   d. Turn on the IR spectrophotometer; allow a 20-minute warm-up period.
   e. For a Perkin-Elmer model 298 IR spectrophotometer, set the repetitive-scan feature for the range 3200 to 2800 cm^{-1}, and set for medium slit width.
   f. Fill one matched quartz cuvette with Freon 113; place in the reference slot.
   g. Place sample in second matched cuvette.
   h. Scan between 3200 and 2800 cm^{-1} at 4 min/full-range scan.
   i. Quantify the absorbance by tangent/base-line measurement of peak height (Fig. 2). Keep peak height below 80% of full-scale by making appropriate dilutions.
   j. Interpolate the "oil & grease" or oil values from the oil standard curve:

      $$\text{mg/L oil} = m(\log P_o/P \text{ sample} - \log P_o/P \text{ blank}) + b$$

      (P_o = incident energy; P = transmitted energy)

   k. Determine slope (m) and intercept (b) of the regression equation.
   l. Calculate the values of the samples from their log Po/P values.
   m. If absorbance peaks are beyond the range of the standard curve, dilute sample, and bring to volume with Freon. Repeat analysis.

n. Report data in the following column format:

A[1]	B	C	D[2]	E[3]
log P_o/P	(log P_o/P) x (dilution factor)[4]	(B) - (log Po/P blank)[5]	oil in volumetric flask (mg/L)	oil in sample (mg/L)

[1] Absorbance determined by tangent/base-line measurement of peak height.

[2] Obtained by interpolation from standard curve for value in column C.

[3] Transform column D by degree of enrichment or dilution that occurred during elution of samples (i.e., if 2.5 mL of sample was applied to the cartridge and the cartridge was eluted into a 10-mL volumetric flask, multiply the resultant value in D by 4 to determine the true sample concentration, i.e., the value for column E).

[4] If a dilution was required to keep peak within 80% of full-scale, multiply by dilution factor (e.g., if sample was diluted 1 volume in 4 volumes total, multiply the values in column A by 4 to obtain value for column B).

[5] blank should be consistent with sample treatment, i.e., if sample was eluted into a 10-mL volumetric flask, blank should be eluted into a 10-mL volumetric flask.

Typical absorbance values for blanks of different final volumes:

oil and grease (10 mL)	0.010
oil and grease (5 mL)	0.015
oil (5 mL)	0.023

Protocol prepared by: B.M. Jones, R.H. Sakaji, and C.G. Daughton

Chapter II

SEPARATION OF AMMONIA FROM ORGANIC NITROGEN USING TUBULAR MICROPOROUS POLYTETRAFLUOROETHENE MEMBRANES: NONOSMOTIC DISSOLVED-GAS DIALYSIS

C.G. Daughton and R.H. Sakaji

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ABSTRACT

A simple and rapid method is described for physically separating dissolved ammonia from organic nitrogen in complex wastewater samples, in particular oil shale process waters. This separation method has utility in directly quantifying organic nitrogen by nonspecific methods that ordinarily can only detect total nitrogen. The sample is buffered with a sodium carbonate solution to a pH of 10.5. This deprotonates the ammonium ion to dissolved ammonia gas, while many nitrogen heterocycles and aromatic and aliphatic amines remain nonvolatile because they either have vapor pressures lower than ammonia, high solubilities in the aqueous phases or remain protonated. The sample is introduced into a tubular microporous polytetrafluoroethene (Teflon) membrane. The ends of the tubing are sealed, and the membrane is immersed in a 1N sulfuric acid bath. The tubular membrane is extremely permeable to gases, but since it is hydrophobic, liquid water and associated nonvolatile solutes cannot permeate. The diffusion of ammonia is driven by the concentration gradient that is maintained across the membrane by absorbing the permeated ammonia into the acid solution, where it is protonated to give ammonium ion. The method is analogous to dialysis, but differs in that osmosis of liquid water does not occur; it is referred to as nonosmotic dissolved-gas dialysis. The dialyzed sample can then be analyzed for total nitrogen by a nonselective, rapid method such as combustion/chemiluminescence. The result is a direct and rapid estimate of organic nitrogen if the sample contains sufficiently low concentrations of nonvolatile inorganic nitrogen.

INTRODUCTION

Three classes of nitrogen can be defined for the purpose of chemical analysis: (1) organic nitrogen, (2) reduced inorganic nitrogen (e.g., ammonia and hydrazines), and (3) anionic inorganic nitrogen (e.g., nitrogen oxide salts and cyanates). Numerous methods have been reported for the analysis of the latter two classes, but a simple and rapid method for quantifying organic nitrogen is not available. For samples that have high concentrations of inorganic nitrogen, the only available methods for determining organic nitrogen involve the reduction of any nitrogen oxides to ammonia, followed by removal of this and any endogenous free ammonia from the sample by distillation; all of the remaining nitrogen is presumably organically bound, and it is then converted to ammonia by time-consuming wet-chemical digestion methods (e.g., Kjeldahl digestion) and quantified (see Chapter V).

Distillation is the most commonly used method for separating ammonia from aqueous samples. The problems with this approach include hydrolysis of primary amines in the distilland (caused by use of high pH and temperature) and concomitant distillation of low-molecular-weight aliphatic amines. The distilland, which contains the major portion of organic nitrogen, must then be acidified for the wet-chemical digestion. This method is extensively discussed in Chapter IV (as applied to analysis of ammonia) and in Chapter V (as applied to Kjeldahl analysis for total and organic nitrogen).

In Chapter I, a method is presented (RPF; reverse phase fractionation) for separating polar from nonpolar compounds by reverse-phase chromatography. This method can be adapted for separating inorganic nitrogen from nonpolar nitrogen (see Chapter V). Another method for separating ammonia from organic nitrogen is presented here. Compared with RPF, this approach is complementary in that it

separates ammonia from nonvolatile nitrogen. It has a disadvantage in not removing inorganic nitrogen anions (e.g., nitrate, nitrite, cyanate, and thiocyanate); for oil shale process wastewaters, however, these anions (with the exception of thiocyanate) are usually present at insignificant concentrations (Wallace et al. 1982). The separation method reported in this chapter makes use of two characteristics of microporous polytetrafluoroethene membranes: (1) selective permeability to gases and (2) impermeability (under isobaric conditions at low pressures) to aqueous liquids and their associated solutes.

THEORY

Synthetic, selectively porous polymeric membranes have been used for numerous types of separation processes. These include micro- and ultra-filtration, reverse osmosis, gas-phase separation, and dialysis. The selective transport of solutes through polymeric membranes is driven by a concentration or pressure gradient normal to the membrane surface. Dialysis, for example, is driven by a concentration gradient; water diffuses from a solution of lower solute concentration to a more concentrated solution, while solutes flow counter. These processes effect separations by one of two mechanisms: (1) selectively passing smaller solutes or particles, while physically retaining those that are larger; the molecular or particle size cut-offs differ for each process depending on the nominal pore size of the membrane (from the nanometer range for dialysis to the micrometer range for microfiltration), or (2) selectively transporting those molecules that have a higher rate of permeation within the matrix of the membrane itself.

None of these membranes is useful, however, for separating dissolved ammonia from an aqueous sample and its associated solutes, because each of them also passes water and low-molecular-weight organic compounds or passes water while retaining most of the solutes. In the application reported here, a new type of membrane is used to segregate and prevent the contact of two miscible solutions, while allowing the selective transport of dissolved ammonia through the membrane.

Homogeneous and Heterogeneous Membranes

Polymeric membranes are arbitrarily classified into two types: heterogeneous (porous) and homogeneous (nonporous). Although both have pores, heterogeneous membranes have pore sizes much larger than the size of solutes. Two processes act to transport molecules (permeants) through these membranes: diffusion and bulk flow. Bulk flow is associated mainly with heterogeneous membranes that are operated under pressure gradients, whereas diffusion can occur through either heterogeneous or homogeneous membranes, under isobaric conditions.

The membrane properties used to describe the transport of a solute through a homogeneous membrane are the permeability coefficient (P), the diffusion coefficient (D), and the solubility (S) of the compound in the membrane material. These properties are related by the following equation (Crank and Park 1968):

$$P = DS$$

Yasuda and Peterlin (1973) resolve the permeability coefficient into two components to describe gas transport in homogeneous and heterogeneous membranes:

the permeabilty coefficient (P') determined under isobaric conditions and the permeability coefficient (K) determined under an applied pressure gradient. Transport in homogeneous membranes occurs only by diffusion, which means that values of P' are nearly identical to values of K (Yasuda and Peterlin 1973). In contrast, with heterogeneous membranes, P' is orders of magnitude lower than K (Yasuda and Peterlin 1973), because permeability under an applied pressure gradient occurs also by bulk flow. Yasuda and Peterlin also point out that P' has an upper limit (the self-diffusion constant; the diffusion within a pure solvent of its component molecules), whereas K theoretically has no upper limit.

The permeation rate or flux through the membrane can be expressed as a product of the permeability coefficient and concentration gradient normal to the membrane surface (Crank and Park 1968; Yasuda and Peterlin 1973):

$$\text{Flux} = P(dc/dx)$$

This equation indicates that the flux of a compound through a membrane also depends on the thickness (x) of the membrane.

Membrane Separation of Gases from Liquids

Nonporous polymeric membranes can be used for the separation of dissolved gases from both a liquid and its associated solutes, if the gases have higher solubilities than the other solutes in the polymer matrix. Gaseous mixtures are separated on this basis using elastomers or polymers such as silicone rubber, polycarbonate, and Teflon. Silicone membranes have been used extensively for gas separation purposes. One example is the enrichment of trace contaminants from inert gas to enhance their detection by mass spectrometry (Greenwalt, Voorhees, and Futrell 1983). For such a gas transport process, a critical variable is temperature. Both the permeability coefficient and the diffusion coefficient follow an Arrhenius type relationship; the natural log of either coefficient is proportional to the reciprocal temperature (Crank and Park 1968; Sacher and Susko 1982). Although increasing the temperature increases diffusivity, it also selectively decreases the solubility of volatile organic compounds in the membrane. Since the permeation coefficient is the product of diffusivity and solubility, the predominance of one term over the other means that the permeation rate or flux will be controlled by the dominant term. According to Greenwalt et al. (1983), the permeation of gases (e.g., neon, krypton, hydrogen, and helium) through a silicone membrane increases with increasing temperature (diffusivity predominates), whereas the permeability of organic compounds, such as alkanes, decreases with increasing temperature (solubility predominates). The selective removal of volatile organic compounds from gases by a silicone membrane would therefore be maximized at a lower temperature. In contrast, data indicate that alkane permeation through a homogeneous Teflon FEP membrane may not behave in a similar manner since the permeability coefficients of gases such as methane, ethane, ethene, and propane increase as temperature increases (Sperati 1975; Yasuda and Stannett 1975). This means that permeation of alkanes through Teflon FEP membranes, and therefore probably PTFE membranes, would increase with temperature.

Reim (1983) reported the use of silicone elastomer tubing for deoxygenating aqueous samples; an analogous solution-transport process could be used for separating ammonia from aqueous samples, but the process would be slow since it depends entirely on diffusive transport in a homogeneous membrane. Hydrophobic, porous membranes (e.g., microporous polytetrafluoroethene) have been used for

removal and subsequent quantitation of ammonia in aqueous samples (Aoki, Uemura, and Munemori 1983). We do not believe that this process has been used, however, so that the total nitrogen remaining in the degassed sample can be quantified as a rapid means of estimating organic nitrogen.

TUBULAR POLYTETRAFLUOROETHENE MEMBRANES

Microporous fluorinated polymeric membranes were originally developed by W.L. Gore and Associates, Inc. (Elkton, MD); fabrication of this material into tubular membranes for commercial use (as vascular grafts) was first accomplished in 1974. These membranes are manufactured by a proprietary process (possibly involving controlled stretching or expanding) of polytetrafluoroethene film (PTFE or polytef; homopolymer of CF_2 units made under a variety of trade names such as Teflon and Fluon) to yield a microporous hydrophobic membrane. The porous structure of the wall of a sample of Gore-Tex microporous PTFE tubing is shown in the scanning electron micrographs in Figure 1; these were obtained with an AMray 1000A scanning electron microscope operated at 20 kV. Strands of PTFE bridge fissures and run parallel to the centerline of the tubing; the fissures circumvent the inner wall of the tubing. As these micrographs show, the pores are not hollow cylinders (such as those in polycarbonate membrane filters) that run through the tubing wall, but rather, the bridges of PTFE themselves act like a sieve through which the permeants must pass.

Although these tubular membranes are no longer manufactured in the United States, because of litigation concerning their misapplication as vascular grafts, they are still manufactured by Japan Gore-Tex, Inc. (42-1 Gotokuji 1-Chome; Setagaya-Ku, Tokyo, 154 Japan). They also can be obtained by special order from W.L. Gore and Associates, Inc. (Biomedical Division, P.O. Drawer 2, Flagstaff, AZ 86002). PTFE tubing, similar to the tubing used in this study, is listed in the catalog of Berghof/America, Inc. (cat # 14030; Raymond, NH).

Degassing Aqueous Samples

Tubular, microporous PTFE membranes have several qualities that lend to their use in degassing aqueous samples for chemical analysis: (i) relatively large pore size (2.0 or 3.5 μm) and porosity (50%), which allow for high permeation rates, (ii) flexibility, which allows for ease of coiling, (iii) availability in a variety of internal diameters, (iv) high ratio of membrane surface area to sample volume, (v) adaptability to continuous-flow applications, (vi) extreme chemical inertness and thermal stability, and (vii) extreme hydrophobicity (water is unable to displace air from the voids in the membrane unless a water-miscible organic solvent is first used to "wet" the surface; the solvent displaces air from the pores and allows water to then enter the pores). Even though these tubular membranes have rather large pore sizes, their hydrophobicity prevents the passage of liquid water (i.e., osmosis cannot occur). Therefore, the passage of nonvolatile solutes is also prevented. Gases, however, can rapidly permeate, because the process involves diffusion through large pores as opposed to solvation in the membrane matrix itself.

Only recently has the use of tubular microporous PTFE membranes been reported in the literature. The only applications reported have been for the chemical analysis of dissolved gases. Aoki and Munemori (1983) report a method for the determination of molecular chlorine in aqueous medium. The sample pH is lowered below 2, and molecular chlorine diffuses through the membrane into a

dialysate solution of pH 12 where it is converted to ClO^- and determined spectrophotometrically. Of several salts tested (including nitrate), none could permeate the membrane. Dichloramine was not found to permeate the membrane, whereas monochloramine could permeate. Aoki et al. (1983) evaluated the use of microporous PTFE tubing for separating ammonia from aqueous samples; the dialysate ammonia was then derivatized and quantified by fluorometry.

In these instances, the analytes (i.e., dissolved gases) are separated from the sample and then quantified. In contrast, for the method reported here, the analytes (i.e., organonitrogen compounds) are those compounds that remain after the dissolved gas (i.e, ammonia) has been removed. The selective removal of ammonia from aqueous samples can be accomplished with microporous PTFE membranes in a process analogous to dialysis in the sense that it is driven by a concentration gradient.

Nonosmotic Dissolved-Gas Dialysis

For analytical purposes, dialysis is best accomplished in a batch process, although a continuous flow process (Daughton and Sakaji 1984) could be easily designed and automated; the limiting factor for an automated application of microporous PTFE tubing is its water entry pressure, which is 12.8 psi for tubing with 2-μm effective pore diameter. A specific length of microporous PTFE tubing is filled with sample that has been buffered to a pH value above the pK_a of ammonia (e.g., 10.5). The two ends are sealed, and the membrane is fully immersed in an acidified water bath. Dissolved ammonia gas diffuses from the sample, into the pore space of the membrane, and out through the membrane, where it becomes protonated by the acid solution yielding ammonium ion. We have termed this process nonosmotic dissolved-gas dialysis (NOGD), since it is a dialysis process without osmosis (see: Brock 1983).

As a tool for quantitatively separating ammonia from organic nitrogen, NOGD relies on the greater "volatility" of ammonia (under the conditions of the dialysis process) compared with organonitrogen compounds. Volatility of a solute in water is not easily quantified, since it is a function of solubility, molecular weight, vapor pressure, and the nature of the gas-liquid interface (Thomas 1982). Increased solubility and molecular weight impede volatilization; increased vapor pressure enhances volatilization. For compounds with ionizable functions (such as many organonitrogen compounds), the relationship of the sample pH to the analyte pK_a is also important, since charged solutes will not be volatile. For compounds with pK_a's greater than ammonia, volatilization will be impeded. The data required for quantifying volatilities are not available for most compounds. Some values are presented in Table I, however, for nitrogenous compounds of representative classes with respect to pK_a, solubility in water, and vapor pressure (or boiling point). Certain compounds, such as the alkyl amines, that have high vapor pressures will not volatilize as fast as ammonia because their pK_a's are higher. Other compounds, such as the pyridines, that have lower pK_a's will not volatilize as fast as ammonia because their vapor pressures are lower (higher boiling points) and their solubilities are higher. For oil shale wastewaters, therefore, the total nitrogen (TN) in a dialyzed sample would be a direct measure of nonvolatile organic nitrogen (NVON), which is an estimator of organic nitrogen.

Selectivity. The classes of organic nitrogen compounds that could permeate the membrane together with ammonia are those which exhibit considerable volatility,

especially mono-alkyl amines. Aoki et al. (1983) determined the ability of several nitrogenous compounds to diffuse through a tubular microporous PTFE membrane and interfere with the determination of ammonia. Amino acids do not permeate. Although methylamine and ethylamine diffuse slowly at pH 9, the rate is rapid at pH 13. Hydrazine was also found to diffuse, but it could not be determined whether secondary alkyl amines or aniline diffused. The concentration of nitrogen that remains in a dialyzed sample would therefore be an underestimator of the true organic nitrogen concentration, provided that nonvolatile inorganic nitrogen is absent. The loss of aliphatic amines can be minimized by proper adjustment of the sample pH (Aoki et al. 1983), since these compounds have higher pK_a values than ammonia (Table I). Equivalent values for dialyzed TN obtained at both pH 9 and 13 would indicate that aliphatic amines are not present.

Optimization of Ammonia Separation. Variables that affect the permeation rate of ammonia through the tubing are (i) temperature and pH of both the sample and dialysate (i.e., the fluid external to the membrane), (ii) thickness of the membrane wall, (iii) internal diameter (as it affects the surface area:volume ratio), (iv) degree of anisotropy (i.e., the gradient of transport properties through the membrane), and (v) pressure across the membrane. Increased temperature lowers the solubility of ammonia in the aqueous phase, decreases the pK_a of the ammonia-ammonium ion reaction (causing a shift in the equilibrium to favor the formation of dissolved ammonia gas), and increases the diffusivity. For a given set of operational conditions, the rate of permeation can be maximized by increasing both temperature and pH. The process can be made more selective for the removal of ammonia, however, if the temperature and pH are kept as low as possible.

Once the operating conditions of pH and temperature are set, the physical process of transporting the solute from the sample to the dialysate becomes important. The resistance to mass transfer in the dialysate can be assumed to be negligible, since the reaction of ammonia to ammonium ion in the dialysate is very fast; there would be a steep gradient of ammonia concentration between the outer membrane wall and the dialysate. Mass transfer would therefore be limited by one of the following steps (Fig. 2): (i) diffusion of ammonia from the bulk phase of the liquid sample to the inner wall of the membrane, (ii) transfer through the liquid-gas interface into the pore space, (iii) diffusion through the gaseous pore space, or (iv) passage through the gas-liquid interface at the external membrane wall.

PROTOCOL SUMMARY

Apparatus

For sample analysis, NOGD is done in batch mode. This requires that the PTFE tubing be sealed during dialysis to prevent evaporation. Since the tubing must be reused, the sealing process must not destroy the tubing. Crimping and the repeated insertion of removable plugs were both found to tear the fragile tubing. Therefore, fittings that could permanently remain in the tubes were designed. Reusable connections could then be made with these fittings.

Each NOGD unit (Fig. 3) is made from a 28.5-cm length of 1-mm i.d. PTFE tubing; this length could contain 200 μL of sample with minimal headspace. Two 1.1-cm pieces of 18-ga stainless steel tubing with chamfered ends are carefully

inserted into both ends of the PTFE tubing so that 0.5 cm of the stainless steel tubing protrudes from either end. These connections are secured by stretching the opening of a 1-cm length of silicone tubing (1-mm i.d.) around both the PTFE tubing and the 18-ga stainless steel tubing. This can be accomplished by first stretching the silicone tubing around a piece of thin-wall, 2.6-mm i.d. 10-ga metal tubing; the stainless steel protruding from the PTFE tubing is then placed within this assembly, and the silicone tubing is gradually pushed off onto the PTFE tubing and then onto the stainless steel tubing. The remaining exposed portion of the 18-ga stainless steel tubing from each end of the PTFE tubing is then inserted into another 1-cm piece of 1-mm i.d. silicone tubing. Each NOGD unit is sealed after sample introduction by connecting the two silicone tubing ends to an 8-cm length of 1.5-mm o.d. stainless steel rod that is bent at right angles into a "U" shape.

Sample Introduction and Dialysis

Wastewater samples are pressure filtered through 0.4-μm pore diameter polycarbonate membranes. Depending on the ammonia concentration of a sample and whether the sample becomes acidic after the ammonia has been removed, a known dilution is then made with at least an equal volume of an aqueous 2M Na_2CO_3 solution; the samples should be diluted to have ammonia-nitrogen concentrations of no more than 1500 mg/L and a pH of 10.5. Samples (200 μL) are placed in the NOGD units with an air-displacement pipette; the plastic tip fits snugly into the silicone tubing end-connector. The unit is immediately sealed with the U-shaped rod, and the resulting loop is immediately submerged in a 1-L beaker containing deionized water (room temperature). This prevents evaporative water loss and solute enrichment during preparation of the remaining samples. The loops are suspended in the water from the stainless steel U-shaped rods that rest on two glass rods placed in parallel across the top of the beaker; the rods are held apart so that the stainless steel U's hang down. Each PTFE loop is identified by affixing a label to the stainless steel rod. When all of the samples have been prepared, the NOGD units are removed from the water bath by lifting the glass rods. The NOGD units are then lowered into a 1-L beaker containing 1N sulfuric acid (technical grade), and a timer is started. The acid solution is filled so that the level fully covers the PTFE tubing. This beaker is submerged in a water bath maintained at 30°C and stirred with a magnetic stir bar (Fig. 3). At the end of 15 minutes of dialysis, the NOGD units are removed from the acid bath and resubmerged in the room-temperature water bath. For each NOGD unit, the stainless-steel U is disconnected, and the contents of the tube are expelled into a suitable container with air from a disposable Pasteur pipette.

Analysis

Dialyzed samples can be analyzed for ammonia by the phenate colorimetric method using a wavelength of 635 nm (Chapter IV) to verify the completion of ammonia removal. Analysis for total nitrogen is most rapidly done by combustion/chemiluminescence (Chapter V).

Reuse of Membranes

Since PTFE tubing is relatively expensive (several dollars per foot), its reuse must be maximized. Only two problems have been encountered with repeated use: (1) deposition of tarry materials (from oil shale wastewaters) on the

membrane inner walls and (2) low physical strength. The tubing must be cleaned between uses with an appropriate organic solvent, such as methanol. Since many organic solvents will "wet" PTFE, making it permeable to water, it is important that the solvent be completely removed from the tubing before reuse. This is most easily accomplished by heating in an oven at about 95°C after preliminary air drying. The low strength of the tubing necessitates care in handling; the ends tend to become overly stretched from insertion of the stainless steel connectors.

APPLICATION OF NOGD FOR DETERMINING ORGANIC NITROGEN

The optimization of NOGD conditions for separating ammonia from organic nitrogen in an aqueous sample is a complex task because of its dependency on temperature, pK_a (which changes with temperature), pH, ammonia concentration, and time. Solubility and pK_a tend to decrease with increasing temperature, whereas vapor pressure increases. Duration of dialysis is partly dictated by the concentration of ammonia that must be removed. Optimization would require a study of all of these variables for each water analyzed. Such a study is very complex and exceeds the scope of this work. Suitable conditions were therefore developed for an ammonium hydroxide solution and for a composite retort water that contained equal volumes of each of the waters listed in Appendix I.

Optimization of pH

For a given temperature, the distribution of ammonia and ammonium ion in an aqueous sample is determined by the sample pH. For example, if the sample pH is 9.3 (25°C), half of the ammoniac-nitrogen exists as ammonium ion. If the pH is increased by one unit to 10.3 (25°C), about 90% exists as dissolved ammonia gas. The permeation rate during dialysis should therefore increase if the pH can be maintained at a higher value during dialysis. This pH-dependent effect was verified in a time-course study using an ammonium hydroxide solution (1213 mg-N/L) (Fig. 4). Samples were dialyzed for times up to 5 min. at 25°C. Ammonium hydroxide solutions were made in two buffers: 500 mL of 0.1M KH_2PO_4 combined with 291 mL of 0.1M NaOH and diluted to 1 L with water (pH 7 buffer); and 500 mL of a mixture of 0.1M KCl and H_3BO_3 combined with 437 mL of 0.1M NaOH and diluted to 1 L with water (pH 10). The y-axis is the percentage of ammoniac-nitrogen remaining in the dialyzed sample, and the x-axis is the duration of dialysis.

The separation of ammonia from wastewater samples by NOGD is a physically complex phenomenon that depends on the liquid-gas partitioning and liquid-phase reactions of ammonia. The extent to which ammonia in a dilute solution will be able to volatilize into the gaseous pores of the PTFE membrane will be governed by Henry's Law. To remove ammonia from a wastewater sample; the reaction:

$$NH_3\ (l) \rightleftharpoons NH_3\ (g) \qquad (1)$$

will proceed only until Henry's Law is satisfied. As ammonia volatilizes (eq. 1) the reaction:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \qquad (2)$$

is forced to the right as the system tries to maintain equilibrium. The extent to which the forward reaction (eq. 2) proceeds depends on the pK_a and pH.

Protons that are generated make the solution more acidic and inhibit subsequent formation of dissolved ammonia gas (eq. 2). To prevent a decrease in pH caused by this reaction, the generated protons must be neutralized. The need for buffering retort water samples was indicated by preliminary work on Oxy-6 and Paraho retort waters. When a neat sample of Oxy-6 retort water was dialyzed, the NVON value was very close to the organic Kjeldahl nitrogen (OKN) values (see Chapter V). In contrast, the NVON values for neat samples of Paraho retort water were up to 3000 mg/L higher than the OKN value. These latter results were believed to be caused by the incomplete degassing of ammonia. The pH values of the dialyzed Paraho samples were between 6.7 and 4.7, whereas the dialyzed samples of Oxy-6 retort water had pH values of 8.5. From this observation, it was concluded that the Paraho samples were insufficiently alkaline to efficiently degas them without the use of a buffer.

The buffer must fulfill three requirements: (1) the pH of the final solution should be about 10.5 so that a majority of the ammoniac-nitrogen is present as dissolved ammonia gas, while a maximum of the low-molecular-weight nitrogenous compounds remain protonated, (2) it cannot contain organic nitrogen, which would interfere with the anlaysis of NVON in the wastewater sample, and (3) the buffering capacity must be sufficiently strong to maintain a pH of 10.5 during the period of dialysis. Since most oil shale process wastewaters are buffered by carbonate systems, a 2M sodium carbonate solution was selected as the buffering agent.

A major problem with using a sodium carbonate solution is the continual plugging of the injection port of the combustion tube that is used for combustion/chemiluminescence nitrogen determination (Chapter V). This problem is believed to result from: (i) deposition of sodium carbonate residue when the aqueous sample is vaporized and (ii) devitrification of the quartz combustion tube caused by the diffusion of sodium into the quartz. Diffusion of sodium into the quartz lowers the melting temperature and increases the coefficent of thermal expansion. Two possible solutions to this problem were considered, but not evaluated: selection of an alternative buffer such as ethylacetoacetate, which has a pK of 10.68, and use of a quartz ladle to introduce the samples into the combustion tube.

Optimization of Temperature

The time of dialysis required for removal of ammonia also depends on temperature, which affects solubility, vapor pressure, diffusivity, and pK_a. Increased permeation rates during NOGD for elevated temperatures were demonstrated in a time-course study using ammonium hydroxide diluted with a pH 7 buffer (Fig. 5).

The rate of ammonia permeation through the PTFE tubing for a given temperature cannot be easily determined (with batch treatment), because the concentration continually decreases during dialysis. This problem is further complicated because total ammoniac nitrogen is a combination of ammonia gas and nonvolatile ammonium ion (eq. 2). As the temperature increases, not only is the solubility of ammonia is decreased, but the pK of the ammonia-ammonium ion reaction decreases. The decrease in pK means that a sample at pH 10 and 60°C has more dissolved ammonia gas present than the same sample at a temperature of 25°C. As a result, the warmer sample will degas at a faster rate (Fig. 5). For the determination of NVON, however, a temperature of 30°C was chosen to minimize

the loss of organonitrogen compounds; this temperature is sufficiently high so that cooling of the dialysate would not be required in a warm laboratory.

Dialysate Solution

The importance of maintaining a concentration gradient of ammonia across the membrane was illustrated by dialyzing an ammonium hydroxide solution (1670 mg-N/L) against an ammonium hydroxide solution of higher concentration (2363 mg-N/L). Essentially no ammonia was removed from the tubing. Although the initial permeation rate would be at maximum if samples are dialyzed against distilled water, the use of an acid dialysate solution is recommended because the cumulative permeation of ammonia from the dialysis of many samples will increase the dialysate pH and thereby lessen the concentration gradient; sulfuric acid is a good choice because it is not volatile.

Samples should not be dialyzed against air because of significant water vaporization; this would necessitate dilution of the dialyzed samples to a known volume before quantitation. This problem was demonstrated by filling dialysis tubes with water and capping the ends. The tubes were then left in quiescent air at room temperature. The permeation of water was quantified by successively weighing the tubes on a semi-micro balance at intervals up to 15 min. The loss rate of mass from three PTFE tubes is plotted in Figure 6. At room temperature, the rate of water loss from the three tubes ranged from 2.21 to 1.55 mg/min, and the average mass flux of water through the membrane wall ranged from 0.12 to 0.15 mg/cm^2-s. The water loss rate data were analyzed using a two-way analysis of variance, which indicated that the losses from the tubes were not uniform, but varied significantly between the tubes. The absence of water loss from samples dialyzed against a liquid is more difficult to verify, because it cannot be done gravimetrically; when a solution of a dye (Rose Bengal) that does not sorb to Teflon (Josefson, Johnston, and Trubey 1984) was dialyzed against acid, there was no detectable change in the absorbance (547 nm) of the dialyzed sample.

Selectivity

To determine the types of organonitrogen compounds that would and would not permeate the membrane during NOGD, individual samples of 27 nitrogen containing compounds of representative chemical classes were evaluated. All of these compounds were obtained from Chem Service, Inc. (Nitrogen Chem Supply Unit, Model On-275, West Chester, PA) with the exception of 3-hydroxypyridine (Aldrich Chemical Co., Milwaukee, WI, 98% purity) and 3-ethyl-4-methylpyridine and 2*n*-propylpyridine (Noah Chemical, Farmingdale, NY; purity not listed). Each compound was dissolved in ASTM Type I water so that the total nitrogen concentration was approximately 160 mg/L. Each solution was diluted with an equal volume of 2M Na_2CO_3. A portion of each diluted sample was stored as the time-zero sample, and 200 μL of the remainder was dialyzed for 20 minutes at 30°C. All samples were analyzed for total nitrogen using the combustion/chemiluminescence technique (Chapter V). The fraction of nitrogen that was lost after NOGD (i.e., the reduction in total nitrogen concentration after dialysis divided by the total nitrogen concentration in the undialyzed sample) is shown in Table I. This fraction is an indicator of the permeability of the membrane to the compound.

This measure of PTFE "permeability" does not elucidate the mechanism by which the organic compound is lost, since a compound can either permeate the membrane or sorb to the membrane; for either mechanism, the compound is lost from the dialyzed sample. For example, quinoline and indole would not be expected to permeate the membrane since they have relatively high boiling points; very little of either was recovered, however, from the dialyzed samples. Since quinoline can sorb to Teflon (Josefson et al. 1984), it was not possible to determine whether the primary mechanism of removal was permeation or sorption. The sorption of aromatic cationic compounds, such as certain dyes, has been reported; this is proposed to occur because of the combined effects of the hydrophobic and electronegative surface of the membrane (Josefson et al. 1984). If sorption were significant for organonitrogen compounds in retort waters, the membranes would have to be eluted with organic solvent to increase the recovery of nonvolatile organic nitrogen.

Representatives of several chemical classes were fully recovered from dialyzed samples. Of these compounds, p-nitrophenol, potassium nitrate, nicotinic acid, 3-hydroxypyridine, and pyrrolidine would have been ionized, which prevented their volatilization into the gaseous pore space. Of the total piperidine concentration, 85% would have been ionized, but only 65% was recovered after dialysis; 35% of the piperidine was lost by either sorption onto the membrane or permeation. Aoki et al. (1983) have shown that low-molecular-weight amines (e.g., ethylamine and methylamine) permeate these membranes; as the degree of alkylation increases or if the amine is arylated (e.g., aniline, dimethylamine, diethylamine, and trimethylamine), permeation decreases. Our results have shown, however, that aniline, triethylamine, and n-dibutylamine were not recovered from dialyzed samples. Since significant portions of both triethylamine and n-dibutylamine should have been ionized at pH 10.5, perhaps they sorbed to the membrane wall.

Several compounds with high boiling points (>200°C) also did not permeate the membrane. These included 2-pyrrolidinone, imidazole, benzimidazole, and pyridazine. Piperazine, which has a moderately high boiling point (146°C), also did not permeate.

Pyridine and its alkylated homologs exhibited extensive losses after NOGD. Altough these compounds are not ionized at pH 10.5, the high boiling points of some of them would suggest that they should not permeate the membrane; perhaps they sorb or wet the membrane surface. If this is true, then it is not surprising that other cyclic compounds such as pyrazine, 2-methylpyrazine, indole, and pyrrole also are not recovered after NOGD. Aoki et al. (1983) found that hydrazine easily permeates PTFE membranes because of its low vapor pressure and low molecular weight. Our data show that alkylation of hydrazine does not prevent its loss during dialysis. For the same reasons, acetonitrile was also lost during dialysis. Finally, a mixture of the 27 nitrogenous compounds was dialyzed. Of the total nitrogen, 62% permeated the membrane during dialysis. This compared with 60% as calculated using the the results from the individual compounds. It should be noted that this mixture was highly biased with those compounds that exhibit rapid permeation (e.g., amines and pyridines).

This study indicates the difficulty in attempting to predict solute-membrane interactions during dialysis. Since there are two possible mechanisms by which organic solutes could be lost during dialysis, it is difficult to predict if a compound will be removed during dialysis.

Oil Shale Retort Waters

In preliminary studies, gas stripping was evaluated as a means for removing ammonia from retort water. A 50-mL sample of composite retort water (diluted 1 in 5) was adjusted to pH 13, heated to 60°C, and purged with helium at 25 mL/min. After 45 minutes, the total nitrogen content of the sample had not reached a constant value, and ammonia was still present. Gas stripping also presented the problem of foaming. For comparison, a solution of ammonium sulfate (124 mg-N/L) was stripped at room temperature and pH 13 using a helium gas flow rate of 25 mL/min. After two hours of stripping, 9% of the ammonia remained. Stripping the ammonia by purging is extremely inefficient. In contrast, a composite sample of retort water can be dialyzed for 15 min at 30°C, and no detectable ammonia will remain.

Dialysis of retort water samples for the determination of organic nitrogen is best done at the lowest possible temperature and pH. This minimizes the loss of organonitrogen compounds. Several time-course experiments with the composite retort water showed that the total nitrogen in the dialyzed sample never stabilized when the temperature was high (85°C) or when the pH was high (>12). The results from a time-course study at 25°C and pH 10.5 are shown in Figure 7. The total nitrogen concentration stabilized between 15 and 20 minutes. At this point, ammonia was not detectable in the dialyzed sample. For the NOGD protocol, the dialysis conditions were standardized at 30°C and pH 10.5 for 15 minutes.

The reproducibility of NOGD was determined with triplicate determinations of 10 dialyzed composite samples. The mean NVON concentration was 763 mg/L, and the rsd was 1.95%. This value agrees remarkably well with the average NVON value obtained for the nine constituent oil shale wastewaters (768 mg/L; Chapter V).

The correlation between NVON and OKN for oil shale wastewaters was demonstrated by correlation analysis performed on the NVON and OKN values listed in Appendix A. The correlation coefficient was 0.991. If the values of NVON and OKN are assumed to be uncorrleated (null hypothesis) the correlation coefficient (0.991) indicates that the null hypothesis can be rejected at the 1% level (Sokal and Rohlf 1973). Linear regression of the NVON (abscissa) and OKN (ordinate) values yielded a line with a slope of 0.89. The high degree of correlation between NVON and OKN indicates that, for these nine oil shale wastewaters, NVON provides a means of rapidly estimating OKN.

The extremely high correlation of NVON and OKN strongly supports one of two possibilities: (1) the major classes of organonitrogen compounds that are lost during dialysis comprise a small portion of the nitrogenous compounds in retort waters, or (2) the same compounds that are lost during dialysis are also lost during the predistillation step in Kjeldahl analysis.

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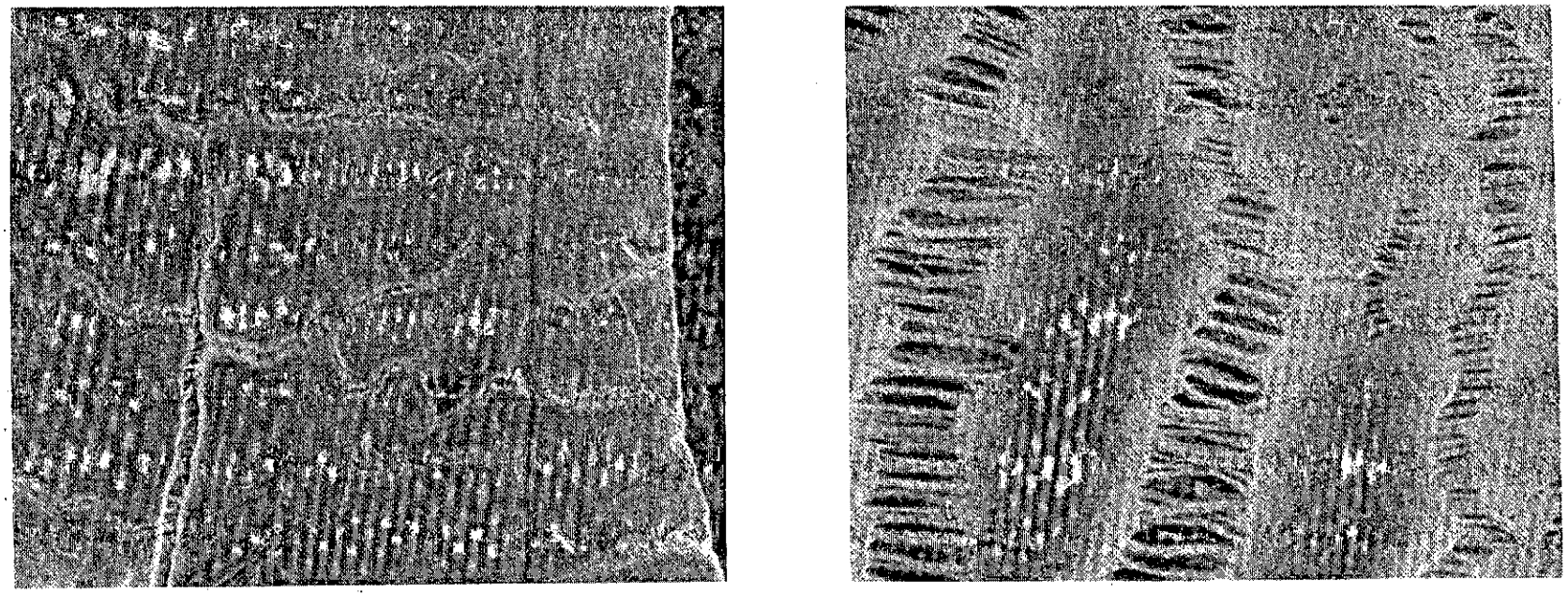
Table I. Correlation of pK_a, Boiling Point, Vapor Pressure, and Solubility with PTFE Membrane Permeability for Nitrogenous Compounds of Representative Classes.

Compound	pK_a [a] $HA = H^+ + A^-$	Boiling Point °C [b]	Vapor Pressure mm Hg, (°C) [b]	Solubility in H_2O mg/L (°C) [b]	PTFE Permeability [c]
Ammonia	9.3 [d]	-33.4	7600 (26)	531 000 (20)	> 0.99
Methylamine	10.66	-6.5	2356 (20)	11 539 (12.5)	nt [e]
Dimethylamine	10.73	7.4	1292 (20)	vs [f,g]	nt
Trimethylamine	9.75	3.5	1444 (20)	sol [f,h]	nt
Ethylamine	10.87	16.6 [f]	912 (20)	misc [f,i]	nt
Propylamine	10.70	49	245 (20)	misc [f]	nt
Butylamine	10.77	78	72 (20)	misc [f]	nt
Triethylamine	10.75	90	50 (20)	15 000 (20)	> 0.87 [j]
n-Dibutylamine	11.25	160 [f]	na [k]	3 100 (unk) [l]	> 0.87
Tributylamine	9.93	213	0.7 (20)	ss [f,m]	nd [n]
Aniline	4.6	185 [f]	1 (63)	34 000 (unk)	> 0.87
1,1-Dimethylhydrazine	7.2 (30°C)	64 [f]	157	misc [f]	0.72
Acetonitrile	4.3	82	74 (20)	misc [f]	> 0.87
Pyrrole	-3.8	130	na	ss [f]	> 0.87
Pyrrolidine	11.27	89 [f]	na	misc [f]	0.03
2-Pyrrolidinone	na	245 [f]	na	misc [f]	0.02
Indole	-2.4	253 [f]	na	sol [f]	> 0.87
Carbazole	-3	na	na	1 (20) [o]	nt
Pyrazole	2.48	187 (757.9) [f]	na	sol [f]	nt
Imidazole	6.95	257 [f]	na	sol [f]	0.04
Benzimidazole	5.5	> 360 [f]	na	ss [f]	0.11
Pyridine	5.21	115	14 (20)	misc [f]	> 0.87
3-Hydroxypyridine	5.10, 8.60	na	na	na	0.08
2-Methylpyridine	5.94	129	8 (20)	sol [f]	> 0.87
2,3-Dimethylpyridine	6.57	na	na	na	nt
2,4-Dimethylpyridine	6.99	na	na	na	nt
2,6-Dimethylpyridine	6.60	144 [f]	na	272 000 (45) [f]	> 0.87
2,4,6-Trimethylpyridine	7.43	171 [f]	na	35 000 (20) [f]	> 0.87
2n-Propylpyridine	na	na	na	na	0.82
3-Ethyl-4-methylpyridine	na	196 [f]	na	ss [f]	> 0.87
Nicotinic acid	2.0, 4.85	na	na	16 666 [f]	0.00
Piperidine	11.12	106 [f]	35 [p]	misc [f]	0.35
Pyridazine	2.2	208 [f]	na	misc [f]	0.05
Pyrazine	1.1	117 [f]	na	sol [f]	> 0.87
2-Methylpyrazine	na	na	na	na	0.83
Piperazine	5.55, 9.8	146 [f]	na	sol [f]	0.00
Quinoline	4.8	238 [f]	1 (60)	6 110 (unk)	> 0.87
Isoquinoline	5.38 (20°C)	242 (743) [f]	< 1 [q]	als [f,q]	nt
p-Nitrophenol	7.2 [d]	279	2.2 (146)	16 000 (25)	0.06
Potassium Nitrate	na	na	na	35 714 (unk) [f]	0.00
average [r]					0.60
Nitrogen composite [s]					0.62

[a] all values from Perrin (1965) for 25°C, unless noted otherwise; [b] all values for boiling point (760 mm Hg), vapor pressure (25°C), and solubility from Verschueren (1977), unless noted otherwise; [c] fraction of initial concentration (approx. 80 mg-N/L) that is lost after NOGD (20 min, 30°C); [d] Dean (1979); [e] not tested; [f] Windholz (1983); [g] very soluble; [h] soluble; [i] miscible; [j] limit of detection is 10 mg-N/L; the value of (1 - 10/80 mg-N/L) = 0.87); [k] not available; [l] unknown; [m] sparingly soluble; [n] compound not detected in either the initial or dialyzed sample; [o] Smith et al. (1978); [p] Weast (1978) (linear interpolation of tabularized data); [q] almost insoluble; [r] average value calculated using values from all the compounds tested, excluding ammonia (values > 0.87 were assumed to be 1.0); [s] sample made from equal volumes of each of the individual compound solutions, excluding ammonia.



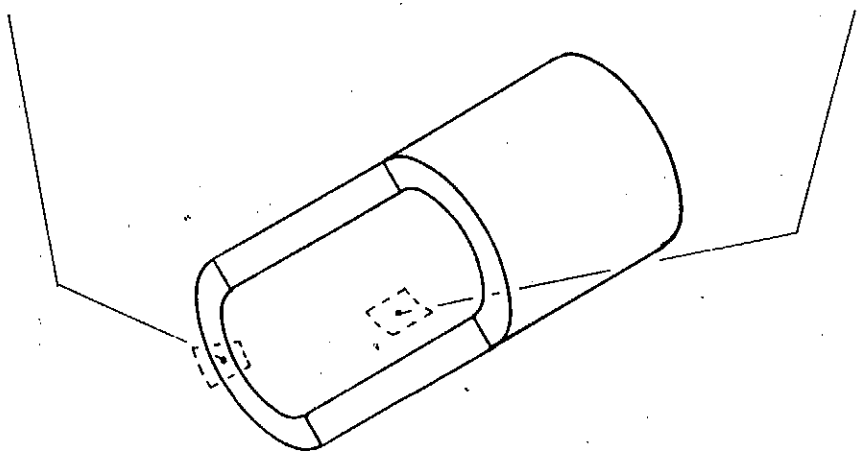


Figure 1. Isometric cutaway of 1-mm i.d. microporous PTFE tubing (0.4-mm wall thickness). Scanning electron micrographs correspond to cross-sectional slice of membrane wall (left SEM) and to the inner wall surface (right SEM); on left-most SEM, the curvature at right edge corresponds to inner membrane wall. Left bar = 100 μm; right bar = 10 μm (XBB 846-4306)

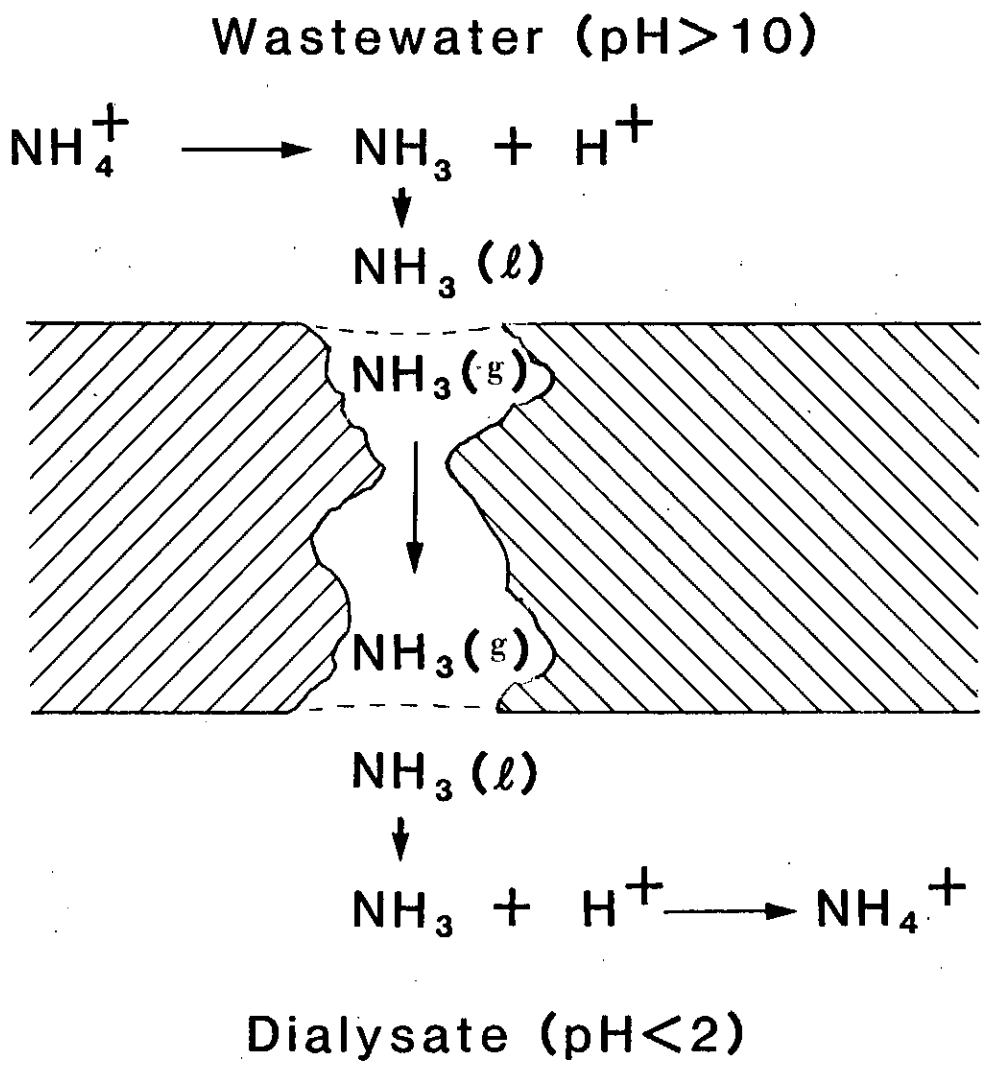


Figure 2. Mechanism of ammonia diffusion through the pore structure in microporous PTFE membrane wall (XBL 846-2296).

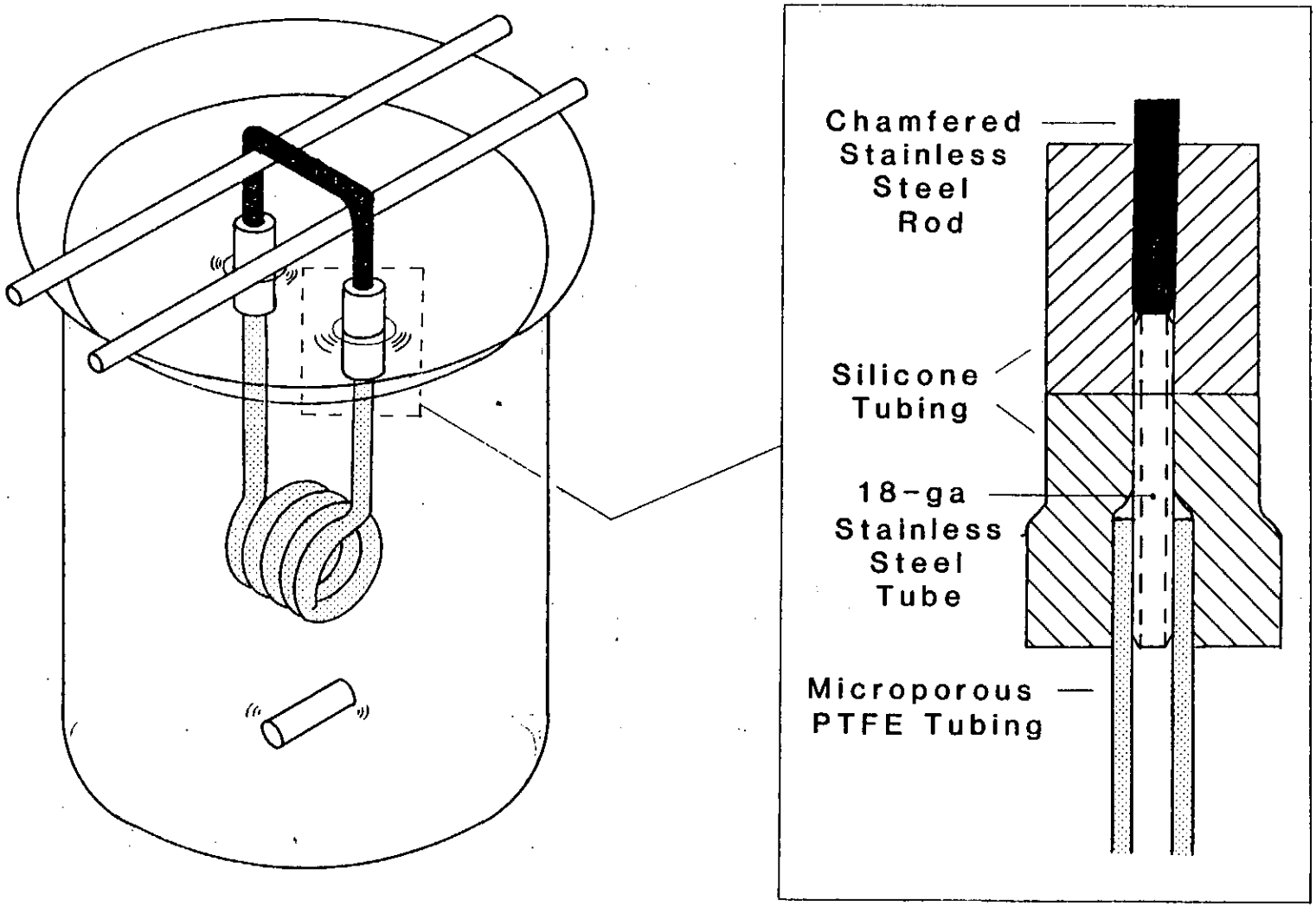


Figure 3. Batch nonosmotic gas dialysis reactor; inset shows how ends of PTFE tubing are sealed (XBL 846-2297).

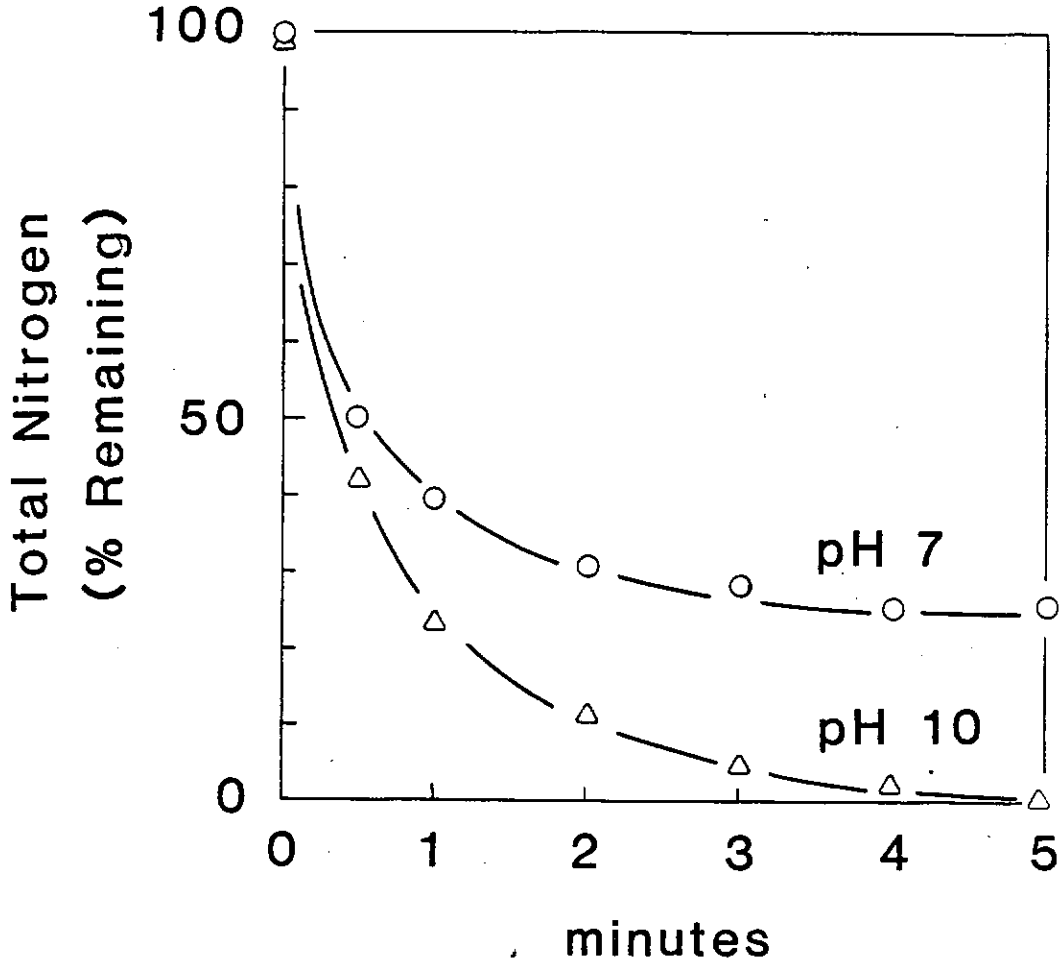


Figure 4. Effect of sample pH on ammonia removal from ammonium hydroxide solution (25°C); time-course NOGD study (XBL 846-2298).

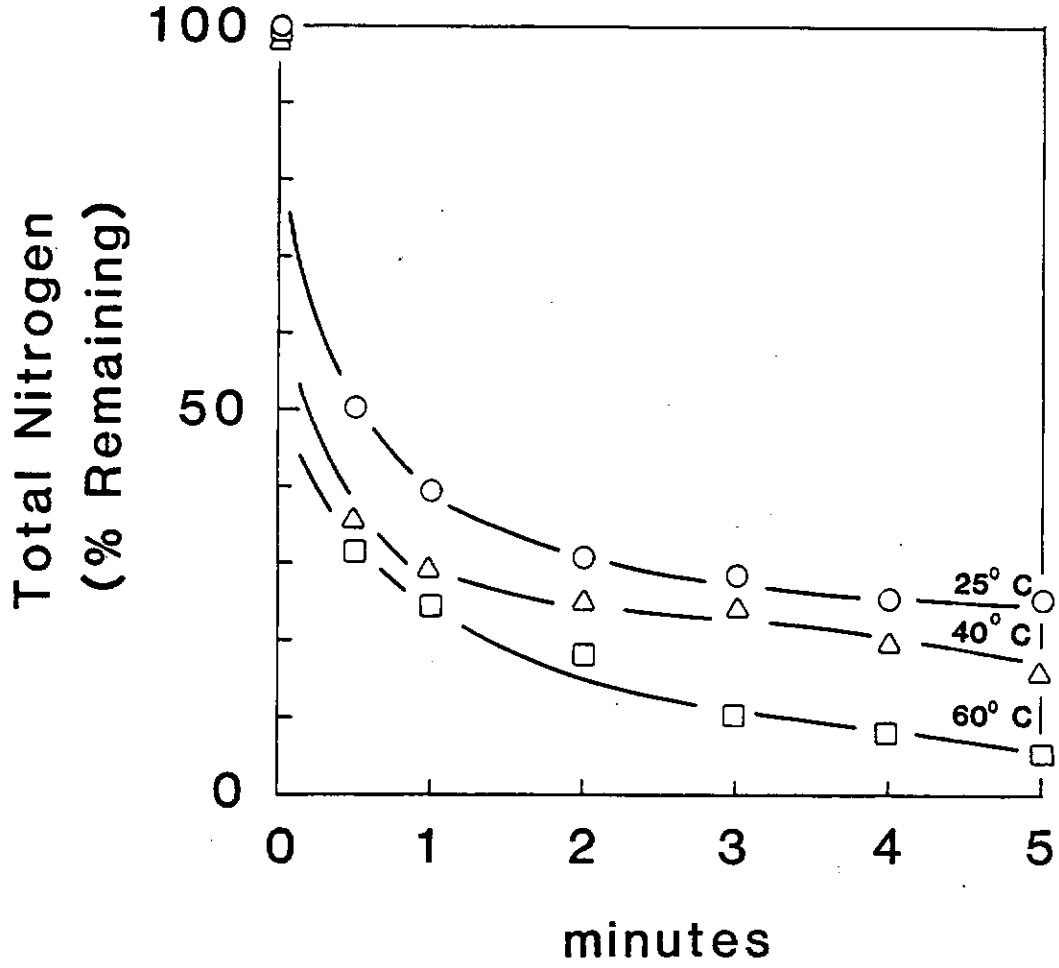


Figure 5. Effect of temperature on ammonia removal from ammonium hydroxide solution (pH 7 buffer); time-course NOGD study (XBL 846-2299).

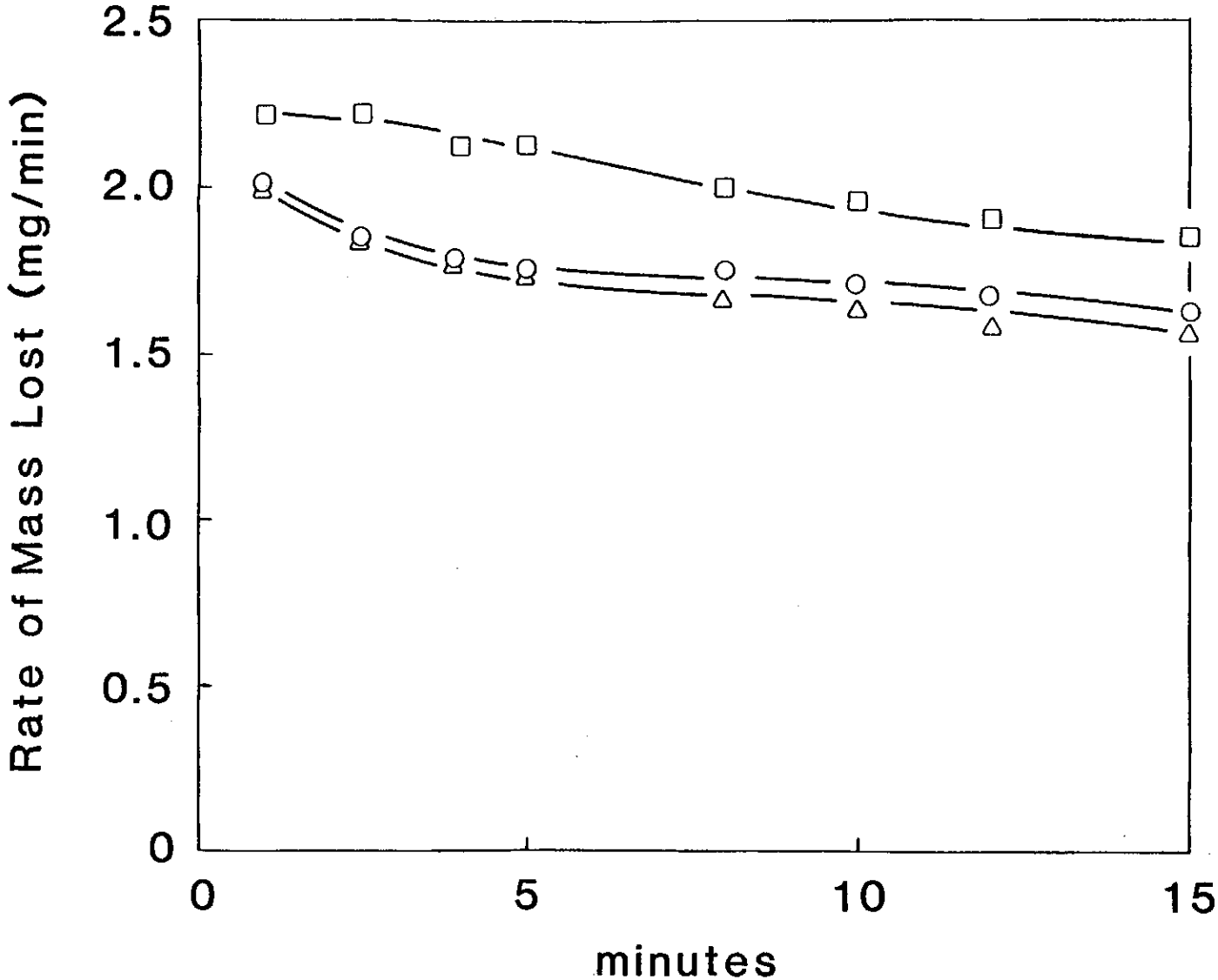


Figure 6. Rate of mass loss during dialysis of water (triplicates) against air; time-course NOGD study (XBL 846-2300).

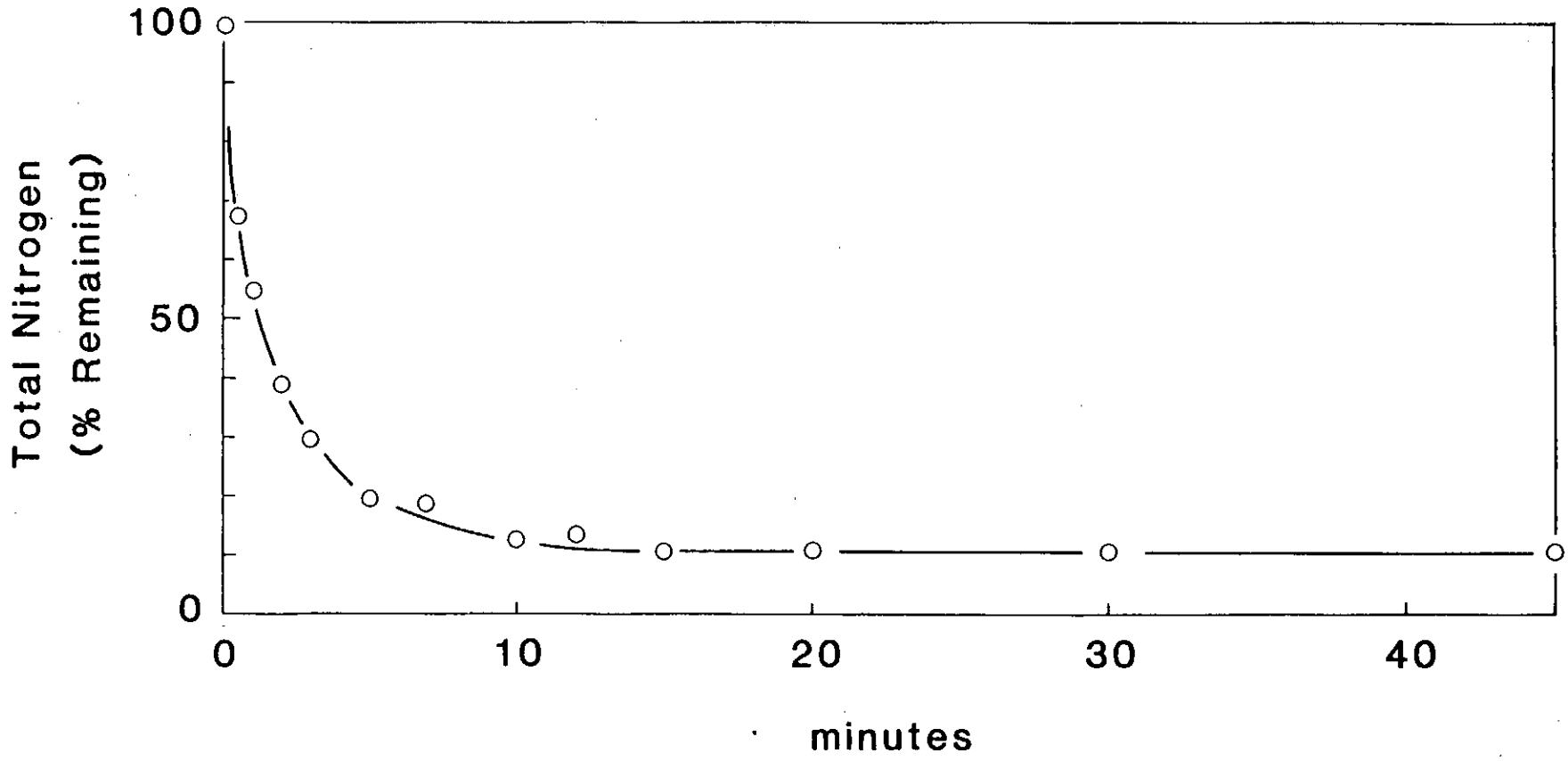


Figure 7. Dialysis of a composite oil shale wastewater; time-course NOGD study (XBL 846-2301).

PROTOCOL: NONOSMOTIC DISSOLVED GAS DIALYSIS

I. Apparatus

A. Glassware

1. Acid-wash all glassware (soak in 35% nitric acid overnight and rinse with ASTM Type I water)
2. 1-mL reaction vials; one each per sample.

B. NOGD units

1. See Chapter II (Protocol Summary) for fabrication of the NOGD assemblies and design of the dialysis bath.
2. Wash the PTFE tubing by passing a small amount of tetrahydrofuran followed by methanol through the tubing; use a Pasteur pipette fitted with a dropper bulb. Air-dry, followed by drying at 95°C for 30 minutes in a convection oven.

NOTE: PTFE tubing is totally permeable to organic solvents; ensure that most of the solvent is evaporated prior to drying in the oven.

3. Ensure that each unit is labelled; affix label to U-shaped rod.

C. Air-displacement pipette, 200 μL (e.g., Gilson Pipetman), and disposable Pasteur pipettes.

D. Haake E2 circulating water heater and bath.

E. 1000-mL griffin beaker (2), to contain the acid dialysate solution and room-temperature water bath.

F. Polypropylene balls, to minimize loss of water from the heated water bath.

II. Reagents

NOTE: All reagents are made from Analytical Reagent grade chemicals unless otherwise specified. When used as a reagent, "water" refers to ASTM Type I quality.

A. 1N sulfuric acid solution; dilute 28 mL of technical grade sulfuric acid to 1 L with water (prepare fresh).

B. 2M Na_2CO_3 solution; dissolve 212 g in 1-L flask, and bring to volume with water.

III. Protocol

NOTE: The maximum number of samples is limited by the capacity of the acid bath and by the time required to prepare each NOGD unit for dialysis. The first sample prepared should not be allowed to sit in the room-temperature water bath for more than 15 min.

A. Turn on the circulating water bath; adjust temperature to 30°C.

B. Preparation of samples.

1. If ammonia concentration exceeds 3,000 mg-N/L, dilute with water to give 3,000 mg-N/L.
2. Dilute each sample with an equal volume of 2M Na_2CO_3; the pH should be about 10.5.
3. With the stainless-steel rod removed from both of the silicone tubing terminuses of the PTFE tubing, add 200 μL of sample with the air-displacement pipette; the plastic tip fits snugly into the silicone tubing.
4. <u>Immediately</u> connect the two terminuses with the stainless steel rod and submerge the PTFE loop in the room-temperature water bath by suspending the U-shaped rod from the two glass bars.

C. Dialysis
   1. When all of the samples have been loaded into the water bath, lift the NOGD units from the bath by grasping the glass rods. Transfer the units to the 1N sulfuric acid bath (30°C).
   2. Immediately start a timer, and ensure that the magnetic stirrer in the dialysate solution is engaged.
   3. At 15 minutes, remove the NOGD units from the acid bath, and resubmerge the loops in the room-temperature water bath.

D. Analysis: NVON
   1. Expulsion of sample.
      a. blot the PTFE tubing dry, and remove the stainless steel rod from both ends of each membrane.

      NOTE: The two ends of the PTFE tubing must be held together at the same height so that the sample does not drain.

      b. carefully drain the tubing by placing one end of the PTFE loop into a vial; expel any residuum by forcing air through the tubing with a Pasteur pipette.
   2. Analyze each dialyzed sample for total nitrogen by combustion/chemiluminescence or by Kjeldahl analysis (see Chapter V protocols). The result is nonvolatile organic nitrogen (NVON).
   3. The removal of ammonia can be verified by the phenate colorimetric ammonia method using the more sensitive standard curve obtained at 635 nm (see Chapter IV protocol).

E. Maintenance
   1. Other than the maintenance specified in Chapter V for the chemiluminescent nitrogen analyzer, more frequent maintenance of the combustion tube is required because of the high concentrations of sodium that are injected (from the sodium carbonate buffer). The injector end of the combustion tube tends to clog. This is remedied by removing the septum and breaking up the deposit with a 0.66-mm o.d. stainless steel needle. This may be necessary every four injections.

Protocol prepared by: C.G. Daughton, R.H. Sakaji, and G.J. Harris

Chapter III

CARBON ANALYSIS: UV-PEROXYDISULFATE OR HIGH-TEMPERATURE OXIDATION COUPLED WITH COULOMETRIC TITRATION

G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton

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ABSTRACT

Wastewaters from the production of synfuels, in particular oil shale retort waters, present several major problems to various instrument configurations designed for carbon analysis. A carbon analyzer was fabricated from commercially available oxidation and detection units. Carbon oxidation occurred in an ultraviolet (UV) photochemical reactor using acid peroxydisulfate as a source of oxidant; quantitation of the evolved carbon dioxide was accomplished with an automatic coulometric titrator. This new design eliminated the problems of (i) instrument downtime caused by fouling of high-temperature combustion catalysts and corrosion of furnace combustion tubes, (ii) limited linear dynamic range and upper detection limit (viz., infrared detection), and (iii) frequent detector calibration (viz., infrared and flame ionization detection).

The UV-persulfate/coulometric titration carbon analyzer was compared statistically with a high-temperature combustion system that is suitable for use with an ASTM method of carbon analysis. The basis of the comparison was: (i) the accuracy and precision of recovery of total dissolved carbon (TDC) and dissolved organic carbon (DOC) for individual nitrogen heterocycles, which were of primary interest because of their preponderance in oil shale process waters and their reported resistance to certain oxidation methods, and (ii) the precision of TDC and DOC determinations for nine oil shale process wastewaters. Several qualitative considerations are discussed for both analyzers, including ease of operation, instrument downtime, and maintenance costs.

INTRODUCTION

Oil shale process wastewaters are complex aqueous mixtures of dissolved and suspended organic and inorganic compounds. These wastes present numerous problems when attempts are made to quantify the "total" amount of solutes present or the degree of contaminant removal effected by a waste treatment process. Methods that are specific for given compounds or for entire chemical classes contribute information relevant only to a small portion of the solutes present in complex wastes; these methods can also be inaccurate because of positive and negative interferences by the sample matrix. Complex solutions and heterogeneous wastes often necessitate the measurement of "bulk" or colligative properties that are shared by as many solutes in the matrix as possible.

Bulk properties that are conducive to analytical measurements include total dissolved solids, electrical conductivity, and the oxidative states of the solutes. A method that is commonly but incorrectly employed to estimate the total concentration of organic solutes is biochemical oxygen demand (BOD). Although BOD is partially a function of the quantity of carbon and its average oxidative state, it is merely an estimator of the material that can be oxidized by acclimated, aerobic bacteria. The overall oxidative state of solutes in a solution is more closely reflected by chemical oxygen demand (COD), which also is often misused as an estimator of organic carbon. Neither of these methods can distinguish organic from certain inorganic compounds (ammonia and thiosulfate will yield BOD and COD values, respectively), and both are unable to detect compounds that are refractory to the particular means of oxidation (see Chapter VI).

Methods that determine specific elemental concentrations (e.g., C, N, S, or P) can give more direct information. One of the most widely employed

element-specific methods is carbon analysis. Inorganic and organic carbon species can be quantitated separately or together. Further qualitative information can be obtained by determining other parameters, such as COD (Chapter VI), and relating them to carbon concentration, or by fractionating solutes into chemical classes (Chapter I) prior to carbon analysis. For example, by relating the COD of an organic waste to the organic carbon concentration (i.e., "specific COD", see: Daughton, Jones, and Sakaji 1981), the overall oxidative state of organic solutes can be estimated.

Retort waters often contain large concentrations of organic and inorganic carbon. A large portion of the organic carbon is refractory to extensive mineralization by biooxidation. The inorganic carbon is partially responsible for the extreme buffering capacity and high alkalinity of these waters, which makes pH adjustment economically infeasible as a step in waste treatment. Of the numerous classes of organic solutes present in oil shale wastewaters, nitrogen heterocycles and nitriles are among the most difficult to oxidize biologically or chemically (e.g., by BOD and COD determinations) (APHA 1981; Naik et al. 1972) and have proved resistant to certain methods of oxidation used for organic carbon measurements (Armstrong, Williams, and Strickland 1966; Gershey et al. 1979). These compounds also appear to be the major factor limiting the success of biotreatment of these waters (Jones, Sakaji, and Daughton 1982).

CARBON ANALYSIS

Classes of Carbon

There are eight major classes of carbon that can be determined by carbon analysis. These classes are defined by organic and inorganic carbonaceous content and by whether suspended matter (e.g., particulates and colloids) is included (Fig. 1). Total carbon (TC) includes all forms of carbon in an aqueous sample; this in turn comprises total organic carbon (TOC) and total inorganic carbon (TIC). The term inorganic carbon in this report is synonymous with oxides of carbon, mineral carbon, and carbonate carbon; the predominant species in retort waters are carbonate and bicarbonate salts. If the particulate and colloidal materials are removed from liquid samples (e.g., via centrifugation or filtration), the carbon that remains is called total dissolved carbon (TDC). Total dissolved carbon comprises both dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC); usually "dissolved" is arbitrarily defined as material that passes through a membrane filter of specified pore diameter (e.g., 0.20- to 0.45-μm).

An operational definition of "dissolved" or "soluble" is exceedingly complex. Filtration is generally assumed to separate the particulates from the dissolved species, but problems attendant with this approach are numerous. Filtration methods other than molecular weight ultrafilters can allow the passage of colloidal material into the filtrate, while at the other extreme, filtration can actually remove dissolved compounds (Fox 1980) by various mechanisms. For oil shale process wastewaters, several variables influence the eventual separation of filtrate from retentate. The type of filter is the most important feature. "Depth" (e.g., glass fiber) versus "screen" (e.g., membrane) characteristics distinguish the two major groups of filters. Membrane filters include mixed cellulose esters and nylon (which themselves have depth-filter characteristics) and polycarbonate. The screen type filters are affected by the

loading of particles on their surfaces. During filtration, as the pores become partially blocked by particulates, the nominal pore size is reduced, thereby promoting the retention of particles that would normally not be retained (Laxen and Chandler 1982); this problem can be partially solved by the use of a tangential-flow filtration apparatus. The chemical sorption or precipitation of solutes by electrostatic (Zierdt 1979) or chemical interactions of the solution with the membrane surface also can effect removal of dissolved solutes. In addition, the partitioning of solutes into the immobilized, retained particulate phase has been hypothesized (Daughton, Jones, and Sakaji 1981). The composition of the filtrate can also be influenced by the type of filtration device. Vacuum filtration will remove portions of dissolved gases such as CO_2 and volatile organic species; pressure filtration is recommended in these instances. Most of these aspects of membrane filtration are thoroughly discussed by Brock (1983).

Contamination of the filtrate by the filter is a final consideration for samples with low solute concentrations. Water extractable materials (e.g., wetting agents), humectants, and particulate debris, all of which remain after the manufacture of membranes, can significantly contaminate the filtrate (Cooney 1980). From our experience, polycarbonate membranes offer the best compromise of features for the filtration of oil shale wastewaters; they are hydrophilic and yet have fewer extractables and smaller dispersion in nominal pore diameter than other filters, and they permit collection of larger volumes of filtrate from oil shale process waters.

There are two major rationales for distinguishing between dissolved and total carbon. First, dissolved carbon is the major form of carbon available to microorganisms in waste treatment. Second, error during subsampling of liquids that contain large quantities of both particulate and colloidal forms of carbon is minimized when the particulates are removed. For these reasons, this study was restricted to the determination of dissolved species of organic and inorganic carbon. The quantitation of carbon in particulate materials entails another major study.

Organic Carbon Quantitation: Direct Versus Indirect

The quantitation of carbon generally requires two steps: (1) the liberation of each organic or inorganic carbon atom as an identical C_1 molecule that is not influenced by the covalent bonding in the parent compounds and (2) the detection and quantitation of these carbon units. The first step usually involves conversion of covalently bound carbon to a single gaseous species (i.e., CO_2 or CH_4) by chemically or thermally mediated oxidation or reduction. The quantitation of organic carbon can be accomplished by either the indirect or direct method (Fox et al. 1980). The indirect method involves the separate determination of TDC and DIC; DOC is then calculated by difference. The direct method requires the removal of inorganic carbon prior to the determination of the remaining dissolved carbon; TDC then becomes equivalent to DOC. Removal of DIC can be accomplished by: (i) precipitation with barium hydroxide or (ii) acidification followed by either purging with an inert gas or boiling (Van Hall, Barth, and Stenger 1965). For the latter method, acidification converts inorganic carbon to carbonic acid, which subsequently hydrolyzes to H_2O and CO_2. Purging is the most widely accepted approach to direct DOC analysis (APHA 1981; ASTM 1977).

The direct method using acidification can result in the precipitation of organic compounds such as higher-molecular-weight fatty acids with the concomitant occlusion or partitioning of organic solutes by these precipitates; subsequent purging of the CO_2 can volatilize lower-molecular-weight organic solutes, especially carboxylic acids (Fox, Farrier, and Poulson 1978). The indirect method requires the least sample manipulation, but lengthens the sample-throughput time because two analyses are required for each sample DOC determination.

Quantitation of inorganic carbon can be accomplished at a low temperature (60°C) by conversion of mineral carbon species to CO_2 via acidification. Oil shale wastewater inorganic carbon is almost exclusively carbonate and bicarbonate. The unambiguous determination of inorganic carbon is dependent on the specific conversion of only mineral carbon species to CO_2 and the resistance of all organic compounds to both oxidation and detection.

Commercial Instrumentation

The conversion of carbonaceous species to CO_2 is generally accomplished by high-temperature combustion, chemical oxidation, ultraviolet (UV) oxidation, or UV-enhanced chemical oxidation. The evolved CO_2 can be detected in batch or continuous mode. Detection methods can be physical, chemical, or electrical and span the range of low-sensitivity gravimetric methods to high-specificity infrared spectroscopy (IR). The advantages and disadvantages of each conversion and detection method are described below.

Methods of oxidation. One of four oxidation methods is usually employed.

(1) High-temperature combustion (950°C). Both organic and inorganic carbon compounds can be oxidized at high temperatures to yield CO_2. Oxidation generally occurs within a ceramic, stainless steel, or quartz combustion tube that is heated in a temperature-controlled furnace. Combustion tubes are packed with an oxidation catalyst, which also serves to lengthen the sample residence time. A wide variety of sample introduction methods exist. These include syringe injection of aqueous samples and "boat" (ladle) introduction for solid or heterogeneous mixtures. Combustion products are swept from the tube to the detector by a carrier gas, usually oxygen or nitrogen; oxygen sufficient for carbon oxidation originates from the combustion catalysts and water.

One problem associated with the combustion method is the production of large quantities of water vapor and other gases, which can result in excessive pressures in the combustion tube. This procedure is therefore restricted to sample volumes less than several hundred microliters. In addition, samples with high salt concentrations, especially alkaline metals, may cause the rapid degradation of the catalyst and attack the tube material itself; this is a major problem with quartz. High-temperature combustion characteristically contributes high background values as a result of trace carbon contamination of the catalyst and carrier gas. These systems therefore lack precision and accuracy for carbon concentrations below 2 mg/L (Takahashi 1979), and they have been found unsuitable for the analysis of natural waters containing less than 15 mg-C/L (Baker et al. 1974) . High-temperature combustion, however, does provide the most complete oxidation (Collins and Williams 1977; Gershey et al. 1979), which can be accomplished in a relatively short analysis time (3 to 5 minutes; Takahashi 1979); this is an advantage not shared by alternative oxidation procedures.

(2) Chemical oxidation (peroxydisulfate; chromic acid in H_2SO_4). Wet chemical methods can be adapted for the oxidation of organic compounds (for TDC or DOC) or for the analysis of DIC. These methods typically have lower system background values than high-temperature combustion units, and therefore, they are capable of lower limits of detection. They are not, however, applicable to all waters; certain organic compounds are resistant to chemical oxidation (Menzel and Vaccaro 1964). Other classes of organic compounds may require significantly longer contact times with the oxidant, thereby increasing the analysis time beyond practical limits (Van Hall, Safranko, and Stenger 1963). The persulfate oxidation procedure described by Menzel and Vaccaro (1964) requires less time than other chemical oxidation methods, but yields incomplete recoveries of polycyclic aromatic and long-chain hydrocarbons. Persulfate oxidation, in general, gives significantly lower recoveries (by 10%) than either combustion or photooxidation methods (Gershey et al. 1979; Williams 1969). In addition, the presence of a high concentration of chloride ion in a sample can consume oxidant, contributing either a positive or negative interference, depending on the detection method.

(3) Ultraviolet oxidation. Ultraviolet energy alone is incapable of mineralizing inorganic carbon oxides for the determination of total or inorganic carbon, or of oxidizing particulates for determination of TOC. Therefore, this technique is applicable only to dissolved organic carbon analyses. For this method to be effective, samples must be irradiated at wavelengths less than 210 nm for long periods (0.5 to 3.0 hours); this is a significant disadvantage when large numbers of samples must be analyzed. Although UV oxidation compares favorably with combustion for the recovery of organic carbon from natural waters (Gershey et al. 1979; Goulden and Brooksbank 1975) it is incapable of complete mineralization of many of the nitrogen- and sulfur-containing organic compounds (Armstrong et al. 1966; Gershey et al. 1979) that typify oil shale process waters. The effects of extensive UV irradiation of oil shale retort waters have been reviewed by Jones et al. (May 1982).

The energy output of the UV lamp is critical for this method of oxidation. The output will decrease during the life of the lamp, and variability in output also exists between individual UV lamps (Collins and Williams 1977). Ultraviolet oxidation methods are compatible with detectors designed for quantitating low concentrations of carbon (e.g., 1 to 25 mg/L). Baker et al. (1974) report UV oxidation to be as efficient as chemical oxidation for the determination of organic carbon in freshwater. Gershey et al. (1979) found, in fact, that the recovery of DOC from seawater is higher with UV photooxidation than with chemical oxidation.

(4) UV-enhanced chemical oxidation. This method is applicable to TDC and DOC analyses, but not to direct DIC analyses. Most methods coupling UV and wet chemical oxidation incorporate the Technicon Auto-Analyzer system; the sample is introduced into the oxidant stream, usually potassium persulfate (Collins and Williams 1977; Goulden and Brooksbank 1975), and is pumped through a silica coil surrounding a UV lamp. Residence time in the coil varies from 8 to 45 minutes. This method results in higher precision than obtained by high-temperature combustion (Goulden and Brooksbank 1975). Leachable organic material from the pump tubing, however, can contribute a significant background signal; preconditioning of the pump tubing is often required for at least 24 hours to minimize this problem.

Another design for UV-enhanced chemical oxidation uses a UV lamp submerged in a reactor vessel containing the chemical oxidant (Wölfel and Sontheimer 1974). The sample is introduced directly to the solution, and the evolved CO_2 is swept by the carrier gas to the detector. A commercial system is available that incorporates a UV lamp submerged in an acidic potassium peroxydisulfate solution and nondispersive IR detection of CO_2 (model #DC-80, Dohrmann Division, Xertex, Inc., Santa Clara, CA). The manufacturer reports an analysis time of 3 to 4 minutes, and complete recoveries of several nitrogen heterocycles (e.g., pyridine, proline, and nicotinic acid) each at approximately 100-mg/L concentration (Takahashi, Martin, and Harper 1981).

Methods of detection. Evolved CO_2 can be quantitated by several physical methods, including manometric, gravimetric, and volumetric determinations. These are limited to batch analysis, are extremely time consuming, suffer from low sensitivity and high lower-detection limits, cannot be automated, and are subject to interferences from co-produced gases. Gravimetric determinations, for example, depend on the absorption of CO_2 on soda-asbestos, soda lime, or into an alkaline solution (Blom and Edelhausen 1955). The increase in weight as a result of gas absorption is measured, and the carbon content is interpolated from the recovery of standard solutions analyzed in parallel.

Thermal conductivity, electrical conductivity, and flame ionization detection (FID) are three methods for directly or indirectly detecting CO_2 in a gas stream on a continuous basis. These methods vary from moderate sensitivity with a narrow linear range (thermal conductivity) to high sensitivity with a wide linear dynamic range (FID). Thermal conductivity detectors determine the change in conductivity within a heated cavity as a result of changes in the gas composition. This method of detection is nonspecific and subject to interferences from co-produced gases. In addition, the sample throughput is limited (25 min/sample). Thermal conductivity is only moderately sensitive compared with other methods of detection (Willard, Merritt, and Dean 1974). In comparison, flame ionization detection is extremely sensitive. The introduced gases burn in a hydrogen flame, and a proportion of the molecules acquire sufficient energy to ionize (Jeffery and Kipping 1972). This ionization gives the flame an electrical conductivity that can be detected and amplified (Littlewood 1962). Since an FID detector responds only to oxidizable carbon atoms, CO_2 from oxidized or combusted organic material must be reduced to CH_4 over a nickel catalyst prior to detection (Willard et al. 1974). Any hydrocarbon gas that survives the combustion/oxidation step would also be quantified. The precision of an FID detector for organic carbon determinations will depend on the efficiency of conversion of CO_2 to CH_4. Flame ionization detectors are reported to have wide linear dynamic ranges (Willard et al. 1974), but require frequent calibration during daily usage.

Non-dispersive IR detection has the advantage of being highly specific for CO_2 with excellent sensitivity. The CO_2 content of the carrier gas is compared with a nonabsorbing reference gas (Delahay 1962), and the difference in absorbance at 2380 cm^{-1} (4.2 μm) is quantified (Beckman 1980). The range of the detector depends on the cell path length and detector configuration. Manufacturers claim a range for aqueous samples of 0 to 2000 mg-C/L; the standard curve from an IR detector over this range, however, is notoriously nonlinear. The precision of the instrument relies on a constant gas flow rate. Carbon dioxide is quantified by peak height interpolated from a standard curve.

This type of detector requires frequent calibration and, for high precision, the samples must fall within the linear portion of the standard curve.

Detection of CO_2 by coulometry, as in any titrimetric technique, requires the addition of titrant until a predetermined endpoint has been attained. For coulometry, the titrant (i.e., electrons) is generated electrolytically, and the quantity required for the stoichiometric indirect titration of the CO_2 is equivalent to the number of coulombs generated (Ewing 1981). Stoichiometric titration obviates the need for frequent calibration because the electron itself becomes a primary standard (Willard et al. 1974).

We have found this detection method to possess an excellent linear dynamic range (at least three orders of magnitude) and, with appropriate gas scrubbers, to be accurate and precise for the quantitation of CO_2. For oil shale wastewaters, we have found that coulometric detection is the method of choice.

COMPARISON STUDY

This section briefly discusses the advantages and disadvantages of various instrumental techniques for determining organic and inorganic carbon in highly contaminated waters such as those from oil shale retorting; a more general review of carbon analysis procedures is reported by Golterman et al. (1978) and Kübler (1977). Of the four oxidation/combustion methods, only high-temperature combustion and UV-enhanced persulfate oxidation were suitable for the routine determination of organic carbon in oil shale process waters. The alternative methods, chemical and UV oxidation, were not applicable to these waters because of reported incomplete oxidation of certain organic compounds and lengthy analysis times.

Nearly all commercial instruments for carbon analysis employ one of two designs: (1) high-temperature combustion coupled with either coulometric titrimetry (e.g., Coulometrics, Inc.) or IR detection (e.g., Ionics; O.I. Corp.; Beckman) or (2) low-temperature oxidation coupled with IR detection (e.g., Dohrmann; Astro; O.I. Corp.; Ionics). While both high-temperature combustion and IR detection are applicable to the analysis of oil shale process waters, significant problems were encountered with each. High-temperature combustion units were subject to frequent and unpredictable downtimes because of damaged combustion tubes and fouled catalysts. Infrared detectors have exhibited substantial drift, requiring frequent standard curve determinations; even then, relative standard deviations for the direct DOC method approach 10% (Wallace et al. 1982).

The analyzer used in this study, which is suitable for use with an ASTM carbon analysis method, was obtained from Coulometrics, Inc. (Model #5020; Wheat Ridge, CO). This system couples high-temperature combustion (quartz combustion tube) with an automatic coulometric titrator. The newly configured analyzer, subject of this comparison study, combines a commercially available photochemical reactor (Dohrmann Division, Xertex, Inc., Santa Clara, CA) with the Coulometrics automatic coulometric titrator. The major anticipated advantages of this new approach were reduced maintenance and downtime, lower capital and maintenance costs, and ability to be automated.

High-Temperature Combustion

The Coulometrics high-temperature combustion system (Fig. 2) oxidizes both organic and inorganic carbonaceous compounds. Samples are introduced to a quartz combustion tube by direct injection with a 200-µL syringe (i.e., Hamilton CR-700 "constant rate" carbon analyzer syringe). This syringe can be set for any volume up to 200 µL, and the contents are forcibly expelled by a spring-driven piston through a blunt needle to ensure reproducible emplacement of the sample within the heated portion of the combustion tube. The syringe and injection port form a gas-tight Luer-slip union. The combustion tube, successively packed with a WO_3-coated quartz wool plug, barium chromate catalyst, and a sintered plug of reduced silver for removal of HI and HBr, is heated to 950°C in a digitally controlled furnace. Oxygen (99.6% purity) is used as a carrier gas and as an additional oxidant source. The oxygen is pretreated by passage through a heated (950°C) "precombustion" tube packed with barium chromate; contaminative combustion products (e.g., acidic gases) and CO_2 are removed by a gas scrubber containing 45% potassium hydroxide before the oxygen passes into the injection port. The scrubbed oxygen stream sweeps the volatilized injected sample through the combustion tube. Combustion of the liquid sample converts organic and inorganic carbon to CO_2 and produces acidic gases (e.g., SO_x and NO_x) and steam. Much of the water vapor condenses and is collected in an ambient-temperature burette trap. The gaseous phase then passes through a drying tube (magnesium perchlorate) followed by a scrubber packed with acid potassium dichromate/manganese dioxide for removal of contaminative acidic gases. The gas stream, now theoretically containing only CO_2 and O_2, then enters the coulometric titration cell where the CO_2 is absorbed and quantified.

High-temperature combustion of organic compounds provides the most complete oxidation within a short time period and is thus well suited to the analysis of DOC in retort waters. These wastewaters characteristically contain large numbers of nitrogen and oxygen heterocycles that may be resistant to wet chemical or UV oxidation. Problems have been encountered, however, with combustion tube deterioration and sample introduction methods. The high salt content of retort water causes rapid deterioration of the combustion catalyst and the alkaline metals attack the quartz combustion tube. This results in frequent downtime for replacement and conditioning of new combustion tubes; these tubes can rarely be reused because of stress fractures that develop during cooling. The life of the combustion tube and packing material can be prolonged with the use of tungsten trioxide at the influent end of the combustion tube packing; this aids in the rapid oxidation of carbonates and prevents the formation of sodium carbonates, which are more thermally stable (ASTM D4129-82). In addition, the sample introduction method is somewhat unsatisfactory. The constant-rate syringe lacks precision and accuracy for measuring repetitive sample volumes; this necessitates volume corrections for each data point. Sample analysis time is increased because the syringe must remain in the injection port throughout the analysis period; this prevents preparation of the subsequent sample for injection. Furthermore, the restricted internal diameter of the needle severely limits the utility of this approach for particulate sampling.

Low-Temperature UV-Persulfate Oxidation

To circumvent the disadvantages associated with high-temperature combustion, the alternative approach of low-temperature oxidation was evaluated.

The high-temperature system with syringe injection was replaced with a modified Dohrmann UV-persulfate reactor for sample oxidation and a low-pressure injection loop for sample introduction (Fig. 3).

The design of the Dohrmann photochemical reactor eliminates many of the disadvantages of conventional UV-persulfate reactors. Direct immersion of a low-pressure mercury vapor lamp in the persulfate solution (85 mL) eliminates the need for a UV lamp surrounded by a silica coil, a common configuration for other photochemical reactors (Goulden and Brookshank 1975; Collins and Williams 1977); this significantly reduces the sample residence time for complete oxidation. In addition, attenuation of the UV output by the lamp's quartz envelope, dead air space, and coil wall is minimized. Therefore, more UV energy is available, and the time required for complete sample oxidation is minimized. A carrier gas/sparging system (O_2, N_2, He, Ar, or purified air) provides complete mixing of the reactor contents. The system described in this report uses O_2 (99.6%).

The photoreactor unit (Fig. 3) was assembled from parts that were purchased from Dohrmann (Xertex Inc., Santa Clara, CA). The following major parts were required: reactor body assembly (#512-090), reactor cap assembly (#512-091), UV lamp (#512-092), Teflon sleeve for tapered joint of reactor cap (#050-409), and transformer for UV lamp (#010-454). A power supply for the UV lamp was fabricated from the Dohrmann transformer, using readily available electrical supplies which included an aluminum instrument housing, instrument fan, ready light and on-off toggle switch, and an electrical outlet for auxiliary power supply to other equipment. In-line fuses were installed for the transformer and auxiliary electrical outlet. A Teflon gas-delivery line was connected to the fritted-glass impinger in the reactor bottom with a silicone reducing connector; the effluent port of the reactor cap was connected to the burette water trap with Teflon tubing and silicone connectors. Unused reactor ports were sealed with silicone plugs (#577-803). It is very important to ensure that the contact of all silicone rubber connectors and plugs with the carrier gas stream is minimized. Teflon lines and glass and metal fittings should be abutted to or slipped inside one another. This is necessitated by the high permeability of silicone rubber to carbon dioxide (Brookes 1969; also see Chapter II). An alternative connector material is shrink-fit Teflon tubing.

A low-pressure six-port injection valve (e.g., model #50-20, Rheodyne, Berkeley, CA) incorporates a calibrated 200-μL Teflon sample loop, which minimizes error in sample volume measurement and maximizes reproducibility. The system is designed so that the samples and reagents only contact Teflon, glass, and stainless steel. The sample is loaded into the 200-μL loop with a Glenco (#19925, Houston, TX) 1.0-mL gas-tight syringe (rotary valve in "load" position) via a Valco zero-volume fill-port assembly (#VISF-1; Houston, TX); excess sample is expelled through the waste line. Five to ten loop-volumes are loaded to ensure complete flushing of the previous contents from the loop (Rheodyne, Inc. 1979). When the valve is switched to the "inject" position, peroxydisulfate solution sweeps through the loop and carries the sample to the reactor.

The sample enters the bottom of the reactor through a sidearm (Fig. 3). The sample fluid and persulfate solution immediately enter a region of high turbulence created by oxygen that is introduced to the bottom of the reactor through a fritted-glass impinger; this oxygen "carrier gas" has been passed through aqueous potassium hydroxide to remove acidic contaminants, mainly CO_2.

A portion of the reactor fluid is withdrawn for recycle from a sidearm at the mid-portion of the reactor; this fluid is combined at a tee fitting with the flow of fresh persulfate reagent from a reservoir and recycled through the injection valve and back into the reactor via the lower sidearm. A glass loop connects the top and bottom of the reactor contents. Reactor waste fluid is drawn off from the top horizontal section of the glass loop. The upward flow of the impinged oxygen in the reactor chamber creates a downward flow of reactor fluid through the loop, because the liquid in the sidearm is more dense than that in the reactor since it lacks gas bubbles; this ensures that any nonoxidized sample does not become isolated from the main reactor and also promotes further mixing. By ensuring that the pumping rate for the waste reactor fluid is identical to the influent rate for fresh persulfate, the volume within the reactor is maintained at a constant level; this is accomplished by removing the reactor fluid from a fixed point above the surface level of the reactor fluid and by setting the combined wastage rate (i.e., fluid and headspace) higher than the influent rate. The maximum rate of combined wastage is limited, however, by the amount of reactor headspace that is removed; since the carrier gas flow rate is about 200 mL/min, a combined wastage rate of up to 2 mL/min would result in loss of nearly 1% of the CO_2 that may evolve from sample oxidation.

The influent, waste, and recycle lines were plumbed through a four-channel peristaltic pump (model 375-A; Sage Instruments Division, Orion Research Inc., Cambridge, MA). Organic contaminants were found to leach from both silicone and Tygon pump tubes; this resulted in high background carbon counts (10 mg-C/L-min; milligrams of carbon per liter-minute), most likely caused by plasticizers and unreacted oligomers. Overnight preconditioning of tubing in a hypochlorite solution could only temporarily (e.g., for several days) reduce the background (3.3 mg-C/L-min); Collins and Williams (1977) reported the need for tubing preconditioning and observed a decreased background during operation because of a reduction in leachable materials. To avoid these problems, the influent and recirculation tubes were replaced with Viton tubing (a copolymer of vinylidene fluoride and hexafluoropropylene; Cole-Parmer Instrument Co., Chicago, IL), which gave a constant, acceptable background carbon concentration (2.0 to 3.2 mg-C/L-min) without preconditioning. The disadvantages of Viton tubing are its higher cost and reduced elasticity, which necessitates more frequent replacement (lifetime = 50 to 80 hours of operation). The recirculation pump tube (0.063-inch i.d.) was manifolded to yield the desired flow rate of 3.0 mL/min, then recombined after the pump and joined via a stainless-steel tee with the influent persulfate line (0.031-inch i.d.) (0.6 mL/min) to yield a 3.6-mL/min flow rate through the injection valve into the reactor; the flow rate for the waste line (silicone pump tube, 1.0 mm i.d.) was 0.6 mL/min, balancing the flow of fresh reagent into the reactor.

Sample material entering the reactor is exposed to the individual and combined effects of persulfate- and UV-oxidation. Ultraviolet radiation enhances the disproportionation of persulfate into sulfate free radicals and hydroxyl radicals, two powerful oxidants (House 1962; Takahashi et al. 1981):

$$S_2O_8^{-2} + h\nu = 2SO_4^{\bullet-}$$

$$H_2O + h\nu = H^{\bullet} + {}^{\bullet}OH$$

$$SO_4^{\bullet-} + H_2O = SO_4^{-2} + {}^{\bullet}OH + H^+$$

Ultraviolet energy also can cause excitation of organic compounds, facilitating their oxidation to CO_2 by sulfate and hydroxyl radicals:

$$R + h\nu = R^*$$

$$R^* + SO_4^{-\bullet} + H_2O = nCO_2 + \ldots$$

$$R^* + {}^\bullet OH + H_2O = nCO_2 + \ldots$$

The oxidation of retort water organic solutes by hydroxyl radical has been discussed by Jones et al. (May 1982).

High chloride ion concentration in a sample can interfere with the mineralization of organic analytes by competing for oxidant (House 1962).

$$SO_4^{-\bullet} + Cl^- = SO_4^{-2} + Cl\bullet$$

If this interference were a problem, it could possibly be minimized by complexing the excess chloride ions with mercuric ion (Takahashi et al. 1981).

The gaseous oxidation products from the sample are swept by the oxygen carrier gas to the effluent line connected to the reactor cap. The CO_2 liberated to the headspace of the reactor is swept through two magnesium perchlorate drying tubes, an acid potassium dichromate/manganese dioxide scrubber, and into the coulometer titration cell.

Inorganic Carbon Determination

The Coulometrics carbonate-carbon apparatus (model #5030) uses the acidification/purge technique (Fig. 4). The sample is injected into the reaction tube with a 200-μL gas-tight syringe fitted with a septum-piercing needle (e.g., Unimetrics TP 4250S with repetitive volume adjustment). A repipette (e.g., 5-mL Dispensette, Brinkmann Instruments Co., Westbury, NY), connected to the top of the reactor tube with Teflon tubing and Teflon union, is used to dispense 2.0 mL of 2N perchloric acid. Ambient air, scrubbed through an aqueous solution of potassium hydroxide, sweeps the acid and sample down to the bottom of the reactor tube where the mixture is maintained at 60°C. The CO_2 that evolves from the carbon oxides is swept through a silver sulfate/H_2O_2 scrubber for removal of interfering acidic gases (e.g., SO_x and NO_x) and into the coulometer. Constant exposure to perchloric acid fumes necessitates frequent replacement of the neoprene septum; in addition, the sample injection method is somewhat awkward. Modifications to the system were made to circumvent these problems: a low-pressure injection valve (described previously) with a 200-μL sample loop was placed in-line between the perchloric acid reservoir and the reactor tube. Complete flushing of the sample from the loop and delivery line requires 5.0 mL of acid per sample. The acidification/purge method of inorganic carbon determination is only accurate if organic compounds are not oxidized by the acid treatment.

Coulometric Titrimetry

An automatic CO_2 coulometric titrator was obtained from Coulometrics, Inc. (model #5010). The titration cell consists of a 200-mL Berzelius tall-form Pyrex beaker and a rubber stopper that holds the cathode, influent gas line, and anode cell. For absorption/titration of evolved CO_2, the coulometer cell is

filled with approximately 75 mL of a proprietary monoethanolamine solution (Coulometrics, Inc.) that contains thymolphthalein blue as an indicator (pK_a = 9.4-10.0); the solution changes from blue to colorless upon acidification. The anode cell is a glass tube with a fritted-glass end and contains potassium iodide pellets, a proprietary anode solution (Coulometrics, Inc.), and a silver electrode, which is connected to the coulometer circuitry. The anode solution is most likely a saturated potassium iodide solution that acts as a salt bridge. Use of potassium chloride in place of potassium iodide would result in the precipitation of silver chloride within the anode cell. The platinum wire cathode surrounds the outside of the fritted-end of the anode cell. The major components of the coulometer are a colorimeter for detection of the titration endpoint and anticipator circuitry, which switches the titration current from high (100 milliamps) to low (5 milliamps) and from low to off as the colorimetric endpoint (i.e., transmittance value of 30% at 612 nm) is approached (Huffman 1977). The rate of current generation for titration is determined by comparing the colorimeter output with preset voltages to determine the distance from the endpoint. A logic block receives the comparator signal and sets the current source at the determined rate (Huffman 1977). The current passing through the cell is converted to a digital readout that can be manipulated to display carbon concentration as milligrams per liter.

Carbon dioxide in the gas stream is quantitatively absorbed by monoethanolamine (MEA), forming hydroxyethylcarbamic acid (RNHCOOH; Fig. 5). Dissociation of the acid yields one hydrogen ion per molecule of CO_2 absorbed. The transient carbamate is hydrolyzed by water, producing bicarbonate and regenerating MEA. The equilibrium reactions (Danckwerts and McNeil 1967; Danckwerts and Sharma 1966) occurring in the bulk solution are:

$$RNH_2 + CO_2 = RNHCOO^- + H^+$$

$$RNHCOO^- + H_2O = HCO_3^- + RNH_2$$

where R is the 2-hydroxyethyl moiety of both MEA and carbamic acid.

Absorption of CO_2 with the concomitant production of hydrogen ion decreases the pH of the coulometer solution; the hydrogen ion protonates the thymolphthalein blue indicator, yielding the colorless form (Fig. 5). The increased transmittance of the solution is detected by the photometer, which initiates the generation of electrons at the silver anode. Two possible fates for the electrons have been postulated. Hydrogen ions, produced stoichiometrically with CO_2 absorption, could be reduced by electrons leaving the platinum cathode, yielding hydrogen gas. Alternatively, the electrons leaving the platinum cathode could cause the hydrolysis of water, producing hydroxide ion and hydrogen gas. The hydroxide ion would then reduce the hydrogen ion (produced from CO_2 absorption), regenerating water. As the CO_2 concentration decreases during titration, the increase in pH causes dissociation of the indicator to the colored form. When all of the CO_2 has been titrated, the photodetector determines that the endpoint has been reached. The generation of current is then suspended, and the integrated measurement of the number of coulombs used is converted to display mg-C/L. Although the dried carrier gas continually evaporates liquid from the coulometer solution, thereby decreasing the transmittance possibly beyond the endpoint, small quantities of CO_2 serve to continuously readjust the transmittance.

The major advantage of coulometric titration is that titrant is generated stoichiometrically with 100% efficiency. The linear dynamic range and upper limit of the coulometer exceed those of detection by nondispersive IR spectroscopy, flame ionization, and thermal conductivity. This often eliminates the need for dilution of samples. The coulometer calibration, performed electronically, is extremely stable and obviates the need for frequent empirical calibration with standards, as is required for other detectors. Coulometric titrimetry for detection of CO_2 seems particularly well suited for analysis of carbon in oil shale process waters because of the wide range of concentrations of inorganic and organic carbon. An occasional problem of sample over-titration, however, has been observed; this problem appears to be related to the rate at which CO_2 enters the coulometer cell and the response lag-time for the high-to-low titration trip-point.

METHODS AND MATERIALS

The two carbon analyzers were evaluated and compared for the quantitation of TDC and direct and indirect DOC in nine oil shale process wastewaters. The accuracy and precision of recovery of carbon from 17 pure reference compounds in standard solutions were also determined. Of the organic solutes present in oil shale process wastewaters, nitrogen heterocycles were of primary interest because they are purported to be responsible for much of the difficulty in waste treatment processes (Jones, Sakaji, and Daughton 1982) and also because they resist many oxidation schemes used for analysis. A series of water-soluble, methyl-substituted pyridines, reported to occur in synfuel wastewaters (Raphaelian and Harrison 1981; Torpy, Raphaelian, and Luthy 1981) was selected for recovery studies. Acetonitrile and cyanuric acid were selected because they are resistant to complete and rapid oxidation by photochemical methods (Takahashi, Martin, and Harper 1981; Dohrmann-Envirotech 1981). Several other water-soluble aromatic and nitrogen-heterocyclic organic compounds also were included in this study because of their possible resistance to complete mineralization. A compound known to be quantitatively mineralized by less rigorous oxidative methods, potassium acid phthalate, was quantitated at several concentrations to determine the linear response of each instrument.

The nitrogen heterocycle standards (Noah Chemical, Farmingdale, NY; Jewel Nero Consulting, Sun Valley, CA) and potassium acid phthalate were analytical reagent grade. The acetonitrile was HPLC grade. A solution of each compound was prepared with acidified, CO_2-free ASTM Type I water. The CO_2-free water was prepared by boiling ASTM Type I water for one hour; before cooling, the boiling vessel was connected to a series of three drying tubes containing calcium chloride, Ascarite, and soda lime. The cooled water was acidified to pH 3. This minimized the uptake of atmospheric CO_2 and precluded the need to purge the standards prior to analysis for DOC. The possibility of loss of carbon from volatilization was minimized, and the TOC and DOC of these standards were therefore equivalent. The mass of compound added to a Class A 50-mL volumetric flask was determined with a semi-micro analytical balance (Mettler model HL52). The theoretical carbon concentration for each standard solution was calculated. Standard solutions and diluted samples were stored at 4°C in 25-mL glass scintillation vials with Teflon-lined screw caps. Ten single-operator replicate injections of CO_2-free water (blanks) were analyzed on each instrument to determine the background during a 5-minute analysis time. Ten single-operator replicates of each standard were then analyzed for TDC concentration (in this instance synonymous with DOC) on each carbon analyzer.

Samples of nine oil shale process wastewaters (Appendix I) were pressure filtered (0.4-μm pore-diameter polycarbonate membranes; Bio-Rad Laboratories, Richmond, CA) and diluted to yield concentrations of approximately 500 mg-C/L for TDC and DOC analyses. These samples were stored in a manner identical to the standards. Samples for direct DOC analysis were acidified with concentrated sulfuric acid (100 μL acid per 10.00 mL of sample) and purged for 10 minutes with high-purity helium (120 mL/min). This represents only a 1% dilution error, and the final DOC values were not corrected. It is important to note that the procedural order (i.e., filtration, dilution, and acidification) and the rate of acidification may affect the dissolved carbon concentration of a sample. The appropriate blank value for each system was determined by the method previously described. Ten single-operator replicates of each process water sample were analyzed on each system for both TDC and direct DOC.

For the determination of direct DOC, the time-dependency and precision of the purging operation were also determined. High purity helium (99.995%) was delivered through Teflon tubing to a purge station consisting of a six-place aluminum manifold; the flow rate through each outlet was 120 mL/min. The time-course removal of volatile carbon (presumably DIC) was followed for a wastewater that was composed of equal volumes of nine process waters (see Appendix I); this composite water was chosen to moderate possible idiosyncrasies of the individual waters. Duplicate samples of the composite water (filtered, diluted, and acidified) were purged for each of nine time intervals up to 15 minutes and then analyzed for TDC (i.e., what normally would be DOC if purging of DIC were complete) with three replicate determinations for each duplicate; the duplicates were purged at separate times using the same manifold station to eliminate variability from differences in flow between manifolds. The imprecision of purging was determined by purging replicate samples at five stations for 10 minutes each; five carbon determinations were completed for each replicate, and the study was repeated so that each purge station had a duplicate sample.

Each analyzer was interfaced with a programmable printing calculator (Hewlett-Packard, model HP 97S) that monitored the coulometer output (using the program in Chapter III, Appendix B) at 15-second intervals. Values for the system blank and sample dilution were stored in the calculator memory. The DOC value recorded after the 5-minute analysis time was subjected to a stability test prior to print-out. This test compared the final value with the value that was recorded 15 seconds earlier. If the difference in values was greater than 1%, the data-acquisition loop was re-entered and a subsequent value obtained and tested for stability. When the stability test was satisfied, the final value (minus the system blank and multiplied by the sample dilution factor) was automatically printed.

Samples of each process water also were analyzed for DIC concentration (10 replicates). The analysis time for DIC determinations was 3 minutes, and the data were manipulated as described above. The mean DIC values were subtracted from the respective mean TDC values for each retort water; this yielded an indirect DOC value for comparison with the direct DOC determination. All statistical analyses were based on the appropriate sections in Sokal and Rohlf (1969) and Rohlf and Sokal (1969).

Detailed operating protocols for all instruments used in the comparison study are appended.

RESULTS AND DISCUSSION

Pure Compounds: Recovery and Reproducibility Comparison Study

The theoretical concentrations and the observed recoveries of pure compounds in solutions using the high-temperature combustion unit are presented in Table I. Complete recoveries were obtained for all compounds except pyridine (95%) and acetonitrile (97%). The degree and position of alkyl substitution for the N-heterocycles did not affect the recoveries. The relative standard deviation (rsd) values were less than 1% for most compounds, and they did not exceed 3% for any compound. Recovery of the potassium acid phthalate standards deviated slightly from linearity at the lowest concentration (100 mg-C/L); a similar deviation was also noted for the UV-persulfate system. With increasing concentrations of acid phthalate standards, the imprecision of recovery decreased.

Ultraviolet-enhanced peroxydisulfate oxidation resulted in complete recoveries for the majority of pure compounds tested; acetonitrile and cyanuric acid, however, were resistant to oxidation (Table II) as reported by others (Takahashi, Martin, and Harper 1981; Dohrmann-Envirotech 1981). The recovery of pyridine (95%) was identical to that from the high-temperature system; this may indicate that the pyridine contained impurities that reduced its overall carbon concentration. The oxidation of acetonitrile was incomplete after the five-minute analysis period; higher recoveries (i.e., 100%) were obtained by increasing the analysis time to 10 minutes. Cyanuric acid, an s-triazine, was completely resistant to UV-persulfate oxidation, regardless of the analysis time. Similar findings were reported for cyanuric acid and melamine by the manufacturer of the UV reactor (Dohrmann-Envirotech). It is not known whether other triazines present a similar problem, but these compounds have not been reported in oil shale process waters. With the exception of cyanuric acid, the rsd values for sample recoveries were less than 2% for most samples and did not exceed 4% for any sample. It must be emphasized that UV-persulfate oxidation should not be used for these classes of refractory compounds without further study.

The close agreement between analyzers for the recovery of all the pure compounds, except cyanuric acid, is illustrated in Figure 6. The pattern of small range deviations above and below 100% recovery was similar for both analyzers; this was probably the result of impurities in the stock reference compounds and errors in sample preparation. The ranges of percent recoveries suggested that the high-temperature analyzer was slightly more precise.

To determine if the observed differences in sample recoveries were significant, a two-way analysis of variance (anova) was conducted. The calculated F-value (F_s) for the variability between analyzers was 1.23, which was less than the critical F-value (F_α) of 4.41 at $\alpha = 0.05$. Therefore, there was no significant difference ($P>0.05$) between carbon analyzers for the recovery of carbon from solutions of pure compounds.

Because of the anticipated problems with the ability of the UV-persulfate oxidation system to completely oxidize acetonitrile and cyanuric acid, the mean recoveries of these compounds were tested by a priori comparisons between analyzers. There was no significant difference ($P>0.05$) between analyzers for the recovery of acetonitrile: $F_s(<1)<F_{.05}(4.41)$. There was a highly

significant difference ($P<0.001$) between analyzers for the recovery of cyanuric acid, despite the nonsignificant overall anova: $F_s(19.24)>F_{.001}(15.4)$.

Process Wastewaters: TDC and DOC Reproducibility Comparison Study

Results from the time-course purge study are presented in Figure 7. The rsd values for the three replicate determinations of each duplicate were less than 1.0, except for one sample. A one-way anova conducted on these data indicated a highly significant variance component ($P<<0.001$) for purge time: $F_s(46.5)>>F_{.001}(8.96)$. Results of the Student-Newman-Keuls step-wise multiple comparison test indicated that there was a significant difference ($P<0.05$) between means for purge times of 0.0, 0.5, and 1.0 minutes; there was no significant difference between purge times of 1.0 and 15 minutes ($P>0.05$). Since oil shale wastewaters differ greatly in DIC concentrations, they may require more or less purging than the composite water used in this study. The results for this composite water showed that 10 minutes was a more than sufficient purge time for DIC removal from the nine waters used in this study. This study, however, did not address the question of whether volatile organic compounds also are lost during purging.

An alternative to purging for CO_2 removal may be the use of nonosmotic dissolved-gas dialysis (see Chapter II). Acidification of the sample would allow the CO_2 to permeate through the tubular microporous PTFE membrane, while most organic solutes may be selectively retained.

Results of the purge precision study (Table III) indicated that the variability between purge stations was small. A one-way anova conducted on these data showed no significant difference ($P>0.05$) among stations: $F_s(3.91)<F_{.05}(5.19)$.

The values obtained from each carbon analyzer for TDC, DIC, and direct and indirect DOC concentrations in nine oil shale process wastewaters are presented in Table IV. There was close agreement between the two analyzers (each labeled "A" or "B" in Table IV) for TDC and DOC determinations for each water. Since high-temperature combustion techniques are generally assumed to give complete recovery of carbon, even though there is no definitive means of proving the completeness of mineralization (Gershey et al. 1979), it therefore can be concluded from these results that UV-enhanced persulfate oxidation of oil shale process waters yielded complete oxidation of dissolved organic material. If compounds resistant to UV-persulfate oxidation were present in these retort wastewaters, their concentrations were too low to significantly affect the overall recovery of carbon.

The rsd values for the recovery of TDC and DOC by either analyzer were less than 3% and generally less than 2%. To determine if a significant difference existed between carbon analyzers for the recovery of TDC, a two-way anova was conducted on square-root transformed data. There was no significant difference ($P>0.05$) between analyzers for TDC recovery: $F_s(4.88)<F_{.05}(5.32)$. There was a significant interaction effect between analyzers and wastewaters $F_s(3.99)>F_{.05}(1.94)$, but the results of Tukey's test indicated that an insignificant portion was due to nonadditive effects, $F_s(0.21)<F_{.05}(5.59)$, and therefore did not violate the assumptions of the statistical model. The additive interaction between treatments (i.e., between wastewaters and

analyzers) obviously resulted from the wide range in TDC values between wastewaters.

A two-way anova was conducted on square-root transformed DOC data with similar results. There was no significant difference ($P>>0.05$) between carbon analyzers for the quantitation of DOC: $F_s(0.98)<F_{.05}(5.32)$. The interaction term was significant but additive. For each carbon analyzer, the direct and indirect DOC data for the nine process waters (Table IV) were compared by a two-way anova on log-transformed values. There was no significant difference ($P>>0.05$) between direct DOC and indirect DOC measurements for either high-temperature combustion or UV-persulfate oxidation: $F_s(<1)<F_{.05}$ (5.59) for both anova's. The purging of samples for direct DOC analysis therefore did not appear to remove measurable quantities of volatile organic carbon compounds nor did the acidification step result in noticeable loss of organic species by precipitation. These observations are in agreement with indirect versus direct carbon determinations for oil shale wastewaters reported by Fox et al. (1980); it should be noted, however, that extensive volatilization may have already occurred in all of these waters because they had been stored for long periods. The imprecision of the indirect DOC method was greater than that of the direct method, as shown by its larger rsd values (Table IV). Since indirect DOC is the difference between TDC and DIC, its associated rsd contains the propagated error from both the TDC and DIC analyses. The standard deviation for indirect DOC was calculated as:

$$s_{TDC-DIC} = (s^2_{TDC} + s^2_{DIC})^{1/2}$$

where s^2 is the variance for the TDC and DIC determinations.

The discrepancies between some of the paired direct and indirect DOC values possibly resulted from problems with determining DIC values which were required for calculation of indirect DOC. For S-55, Omega-9, 150-Ton, and Oxy-6 gas condensate (Table IV), TDC values were 14%, 26%, 31%, and 40% lower, respectively, than values from earlier analyses. There was agreement, however, for DOC values between data sets from different days, indicating that the TDC discrepancies resulted from variability in DIC concentrations. Although the rsd values for DIC were less than 2% (Table IV), several of the process waters exhibited TDC values that were lower than values obtained in previous analyses. The following are offered as possible origins of this problem: (i) Samples containing DIC greater than 1000 mg-C/L must be diluted prior to determination of DIC. Sodium carbonate standards of 1000 mg-C/L routinely gave 95% recovery, whereas standards diluted from this same stock gave 100% recovery. It is unknown whether this was a problem with inadequate acidification/purging or with inefficient absorption of large dosages of CO_2 by the coulometer solution. Inefficient absortion was not a problem, however, when an equivalent amount of CO_2 was generated by the high-temperature or UV-persulfate units, where the CO_2 is presumably released over a longer time interval. (ii) Certain samples (e.g., Oxy-6 gas condensate, S-55, Omega-9, and 150-Ton) yielded significantly lower TDC values when diluted and stored (4°C) for more than one week. It is not known whether storage of these diluted samples under headspace would result in uptake or loss of CO_2, but the former would seem more likely for these alkaline waters. (iii) Certain samples (e.g., Paraho) would not yield stable DIC values on particular days. This problem seemed to be related to gross interference by other gases that evolved during acidification/purging.

The number of problems encountered with the DIC determinations on oil shale wastewaters is surprising, and this method requires further validation. For this reason, we recommend that DOC be determined directly. An alternative route to DIC quantitation that deserves investigation is the use of the photochemical reactor with the UV-lamp turned off. This would preclude the need for the Coulometrics DIC unit, although it may be necessary to replace the acidic persulfate reagent with a nonoxidizing acid (e.g., dilute perchloric or sulfuric acid).

The statistical analyses of data from the comparison study indicated that no significant difference existed between the two carbon analyzers for the precision and accuracy of DOC recoveries from solutions of pure compounds or for the quantitation of TDC and DOC in retort wastewaters. Since the UV-persulfate system gave incomplete recoveries for 2 of the 17 pure compounds analyzed, use of this oxidation procedure for the analysis of waters other than those reported should be preceded by a similar validation study. The routine determination of direct DOC should always be validated by indirect DOC measurements.

Incomplete recovery of cyanuric acid may indicate an inability to completely oxidize other symmetrical triazines containing electron-donating substituents (e.g., melamine). Recalcitrant compounds could probably be more effectively oxidized by using ozone/oxygen for the carrier gas through the photooxidation unit. This would promote formation of hydroxyl radical (Jones et al. May 1982). Two problems, however, would need to be overcome: (i) the difficulty in purging the resultant carbon dioxide from an alkaline reaction medium and (ii) the difficulty in selectively removing residual ozone from the carrier gas stream prior to its entry to the coulometer.

Some important qualitative differences did exist in the performance and operation of the analyzers. The syringe injection method and the downtime from exhausted packing material and deteriorated combustion tubes severely hampered the routine use of the high-temperature unit. Following the DOC analyses of the pure compounds and retort wastewaters in the study reported here (approximately 320 sample injections), replacement of the combustion tube was necessary. Symptoms of the malfunctioning tube were an increased system blank and incomplete recoveries of acid phthalate standards. The calibration of the constant rate syringe was easily disturbed during use and required frequent checking. When calibrated according to the manufacturer's instructions, the actual volume delivered was never within several microliters of 200 μL; this necessitated different volume-correction terms for all the reported data.

The design of the UV-persulfate oxidation/coulometric titration carbon analyzer circumvented these problems. There was a minimum of downtime; maintenance was limited to replacement of worn pump tubing and replenishment of persulfate reagent. One of the few disadvantages was that the 200-μL sample loop required flushing with at least 10 loop-volumes of sample to eliminate the dilution effect of the persulfate reagent which had flushed the previous sample from the loop. If large sample volumes (e.g., 5 mL) are not available, a septum injection system (Dohrmann P/N 880-034), used in conjunction with a gas-tight syringe, could easily be installed for sample introduction. The loop injector has the main advantages of ease of use, increased precision, and reduction of intersample preparation time; it also can be easily automated.

A cost comparison of the UV-persulfate system and the Coulometrics high-temperature total-carbon analyzer showed that the UV-persulfate system ($8,500) was slightly less expensive than the Coulometrics analyzer ($9,200). The parts for the photochemical system include: UV-lamp ($212), reactor body and cap ($366), transformer ($147), injection value ($90), 4-channel peristaltic pump ($975), syringe ($28), miscellaneous electrical parts and plumbing ($408), and coulometric titrator ($6300). The photochemical system was significantly less expensive with regard to downtime, supplies, and maintenance costs. Routine annual supplies and maintenance costs include potassium persulfate ($70), Viton pump tubes ($200), and UV lamp ($212; assuming at most one per year) compared with combustion tubes ($2,000; assuming about one per month), precombustion tubes ($250), and associated energy expenses for the high-temperature system; combustion tube and catalyst lifetimes are a function of the concentration and number of process water sample injections.

Based on recoveries of the potassium acid phthalate standards (Tables I and II), it appeared that the operation of both analyzers was best at higher carbon concentrations (>500 mg/L); this affords an advantage to either system for the analysis of oil shale process waters. We have concluded, however, that the UV-persulfate oxidation/coulometric titration carbon analyzer provided improved performance over the high-temperature combustion/coulometric titration system for analysis of oil shale wastewaters on the basis of ease of operation, downtime, and maintenance costs, while maintaining accuracy and precision of sample recovery.

A major question that has not been addressed relates to the analysis of total carbon (TC). This involves both dissolved and suspended forms; the latter includes particulate and colloidal forms. The major problem with determining TC (TOC plus TIC) is representative subsampling. Particulate sampling is biased when a syringe is used for high-temperature combustion, but the combustion process itself should not be a problem. Particulate analysis with the UV-persulfate oxidation system may not be possible, because the particulates will float to the surface of the reactor fluid or sink to the bottom. Two possible approaches to the subsampling problem are (i) collection of suspended materials on quartz filters followed by introduction to the combustion furnace tube with a ladle or boat assembly and (ii) homogenization of the sample by ultrasonication followed by syringe subsampling for either combustion unit.

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Table I. Recovery Study: High-Temperature Combustion Carbon Analyzer

	DOC Concentration (mg/L)			
Compound	Theoretical (T)	Observed[a] (O)	(O/T) X 100	rsd[a]
potassium acid phthalate	100.0	104.3	104.3	1.39
potassium acid phthalate	500.0	506.5	101.3	0.54
potassium acid phthalate	1000.0	1002.6	100.3	0.50
phenol	606.8	606.0	99.9	0.61
acetonitrile	479.0	465.6	97.2	2.79
3,5-dimethylpyrazole	501.5	502.3	100.2	0.45
pyridine	476.3	454.1	95.3	0.64
2-methylpyridine	435.2	434.1	99.7	0.81
4-methylpyridine	455.3	453.5	99.6	0.69
2,4-dimethylpyridine	437.5	435.7	99.6	0.66
2,6-dimethylpyridine	431.6	430.4	99.7	1.09
2,4,6-trimethylpyridine	420.7	424.3	100.8	0.73
2,3,6-trimethylpyridine	416.7	430.4	103.3	0.58
2-ethylpyridine	443.4	441.7	99.6	0.92
3-ethylpyridine	447.8	449.3	100.3	0.69
3-ethyl-4-methylpyridine	472.1	503.8	106.7	0.21
2-*n*-propylpyridine	435.4	441.1	101.3	0.55
2-methylpyrazine	502.5	503.6	100.2	1.77
cyanuric acid	252.2	253.5	100.5	1.05

[a] n=10 for each standard solution.

Table II. Recovery Study: UV-Persulfate Carbon Analyzer

Compound	DOC Concentration (mg/L) Theoretical (T)	Observed[a] (O)	(O/T) X 100	rsd[a]
potassium acid phthalate	100.0	102.8	102.8	3.72
potassium acid phthalate	500.0	503.3	100.7	0.70
potassium acid phthalate	1000.0	999.4	99.9	0.44
phenol	606.8	602.9	99.4	0.90
acetonitrile	479.0	426.3	89.1	3.44
3,5-dimethylpyrazole	501.5	502.5	100.2	0.70
pyridine	476.3	454.5	95.4	0.65
2-methylpyridine	435.2	438.9	100.8	1.01
4-methylpyridine	455.3	447.5	98.3	1.29
2,4-dimethylpyridine	437.5	434.2	99.3	1.16
2,6-dimethylpyridine	431.6	429.9	99.6	0.88
2,4,6-trimethylpyridine	420.7	421.7	100.2	0.66
2,3,6-trimethylpyridine	416.7	430.6	103.3	0.80
2-ethylpyridine	443.4	436.3	98.4	1.66
3-ethylpyridine	447.8	453.1	101.2	1.38
3-ethyl-4-methylpyridine	472.1	495.7	104.9	0.71
2-n-propylpyridine	435.4	435.2	100.0	0.87
2-methylpyrazine	502.5	500.0	99.5	0.68
cyanuric acid	252.2	5.4	2.2	123

[a] n=10 for each standard solution.

Table III. Precision of the Purge Operation for Determining Direct Organic Carbon in a Composite Sample of Nine Oil Shale Process Waters

Purge Station	DOC[a] (mg/L)	rsd
1	6244	0.70
	6291	0.50
2	6369	0.69
	6361	0.54
3	6364	0.51
	6328	0.35
4	6369	0.32
	6394	0.19
5	6310	0.45
	6385	0.16

[a] each duplicate is the mean of 5 single-operator replicates.

Table IV. Comparison of Carbon Analyzers: Direct/Indirect Organic Carbon (mg/L)[a] in Oil Shale Wastewaters

Wastewater	DOC (direct)	rsd	DOC (indirect) (TDC-DIC)	rsd	TDC	rsd	DIC	rsd
Paraho							209.8	1.3
A (high-temperature)	41809	1.4	43205	0.55	43415	0.55		
B (UV-persulfate)	**42066**	**1.1**	**42470**	**0.66**	**42680**	**0.66**		
150-Ton							1932	1.8
A	3147	0.58	2925	1.4	4857	0.44		
B	**3259**	**0.46**	**3128**	**1.4**	**5060**	**0.53**		
Oxy-6 retort water							984.9	1.0
A	2829	0.80	2832	1.6	3817	1.2		
B	**2942**	**0.40**	**2967**	**0.79**	**3952**	**0.54**		
Geokinetics							1994	0.67
A	1627	1.1	1680	3.0	3674	1.3		
B	**1656**	**0.55**	**1688**	**1.3**	**3682**	**0.45**		
TOSCO HSP							824.8	0.54
A	2651	0.23	2545	0.81	3370	0.59		
B	**2726**	**0.61**	**2661**	**1.3**	**3486**	**0.97**		
Oxy-6 gas condensate							2213	0.38
A	651.7	2.6	522.0	3.7	2735	0.63		
B	**641.0**	**0.51**	**653.0**	**2.4**	**2866**	**0.47**		
S-55							339.5	1.6
A	2213	0.40	2256	2.1	2595	1.8		
B	**2285**	**0.34**	**2294**	**0.63**	**2633**	**0.51**		
Omega-9							1387	1.3
A	694.7	0.34	732.0	6.5	2119	2.1		
B	**718.4**	**0.44**	**787.0**	**2.5**	**2174**	**0.29**		
Rio Blanco sour							364.3	1.4
A	206.3	1.4	191.3	2.7	555.6	0.25		
B	**207.0**	**1.3**	**183.8**	**3.0**	**548.1**	**0.47**		

[a] mean of 10 single-operator replicates.

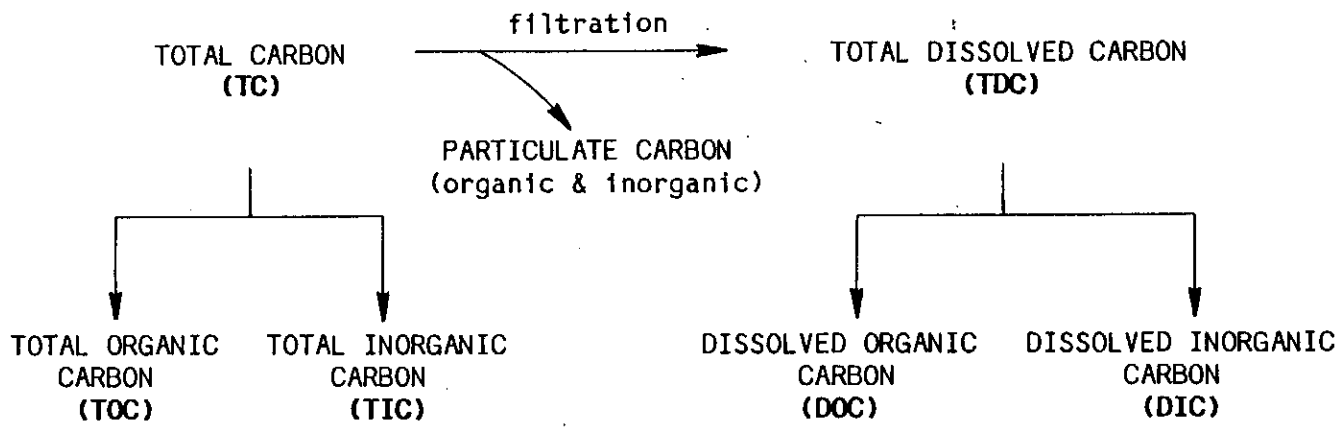

Figure 1. Terminology for carbon classifications used in carbon analysis.

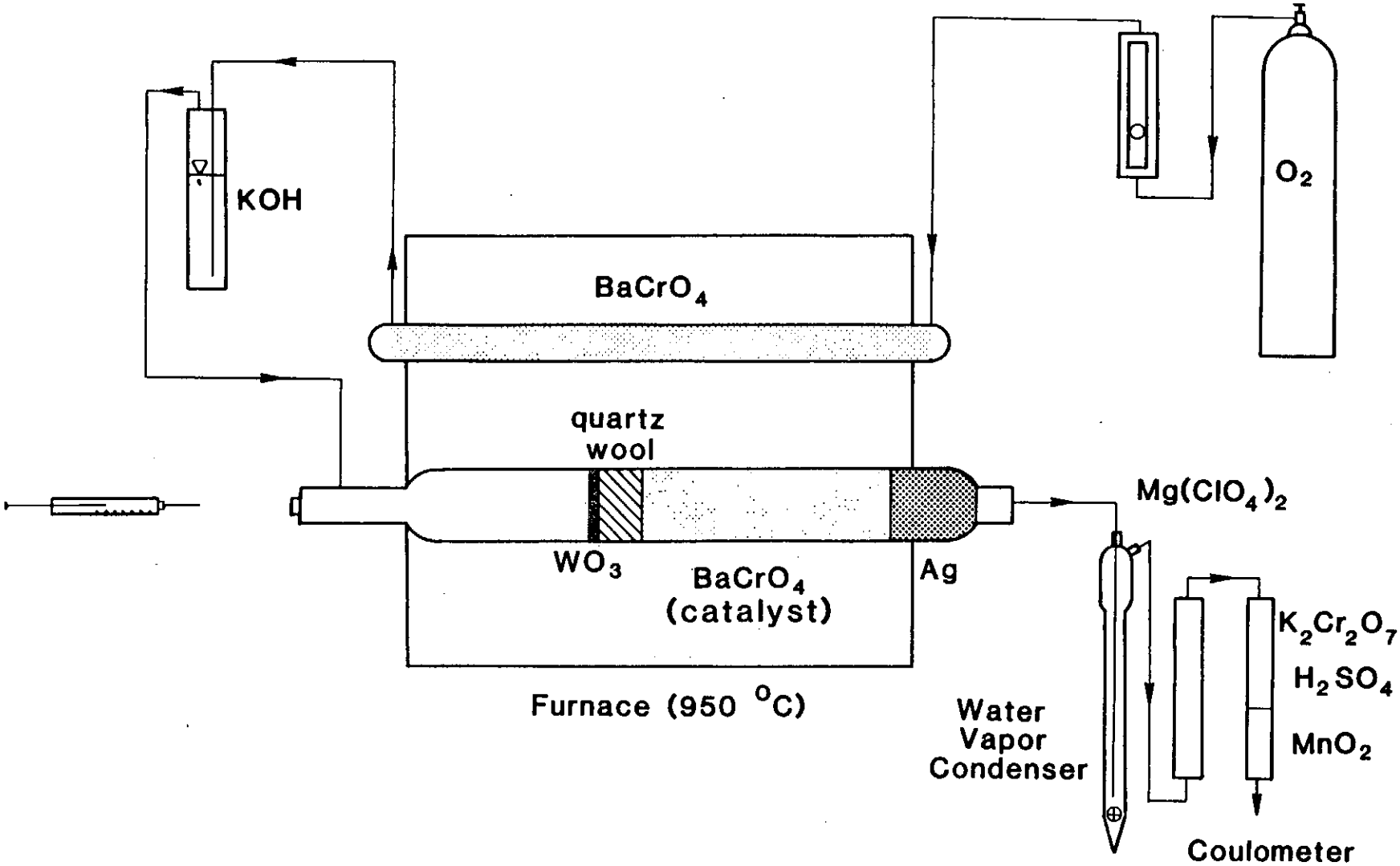

Figure 2. Schematic of high-temperature combustion apparatus (XBL 832-8325).

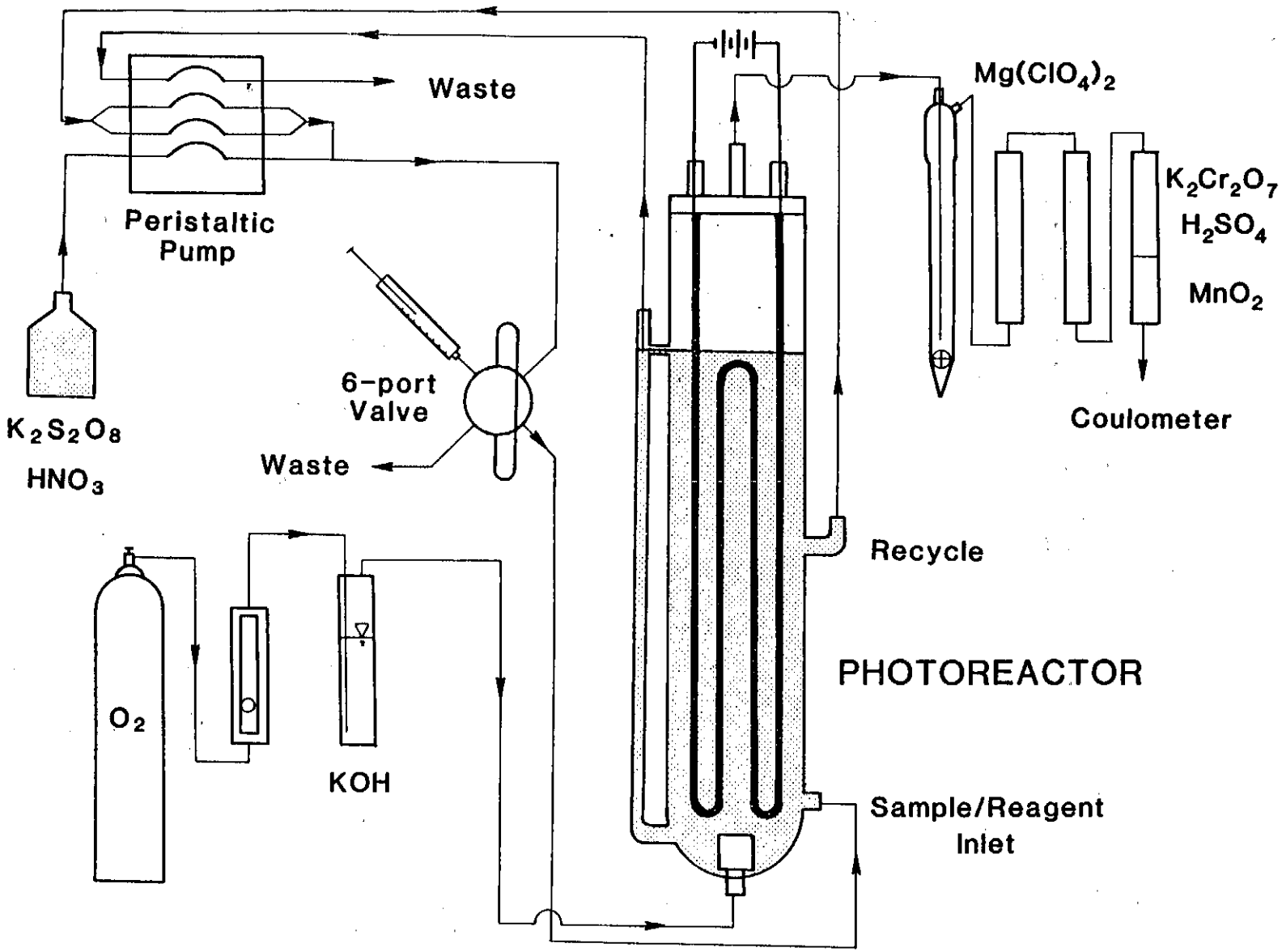

Figure 3. Schematic of UV-peroxydisulfate low-temperature oxidation apparatus (XBL 832-8326).

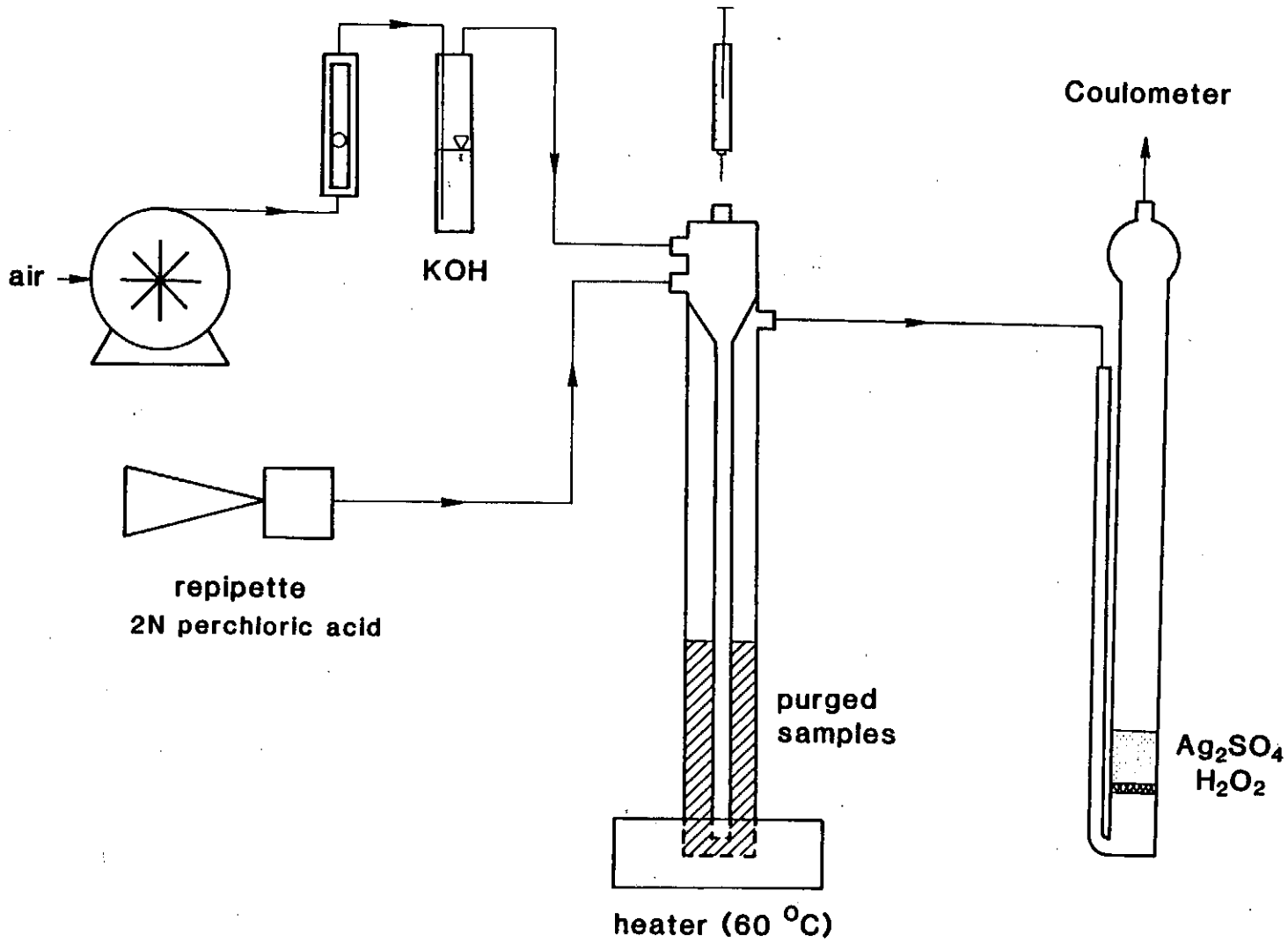

Figure 4. Schematic of acidification/purge apparatus for inorganic carbon (XBL 832-8327).

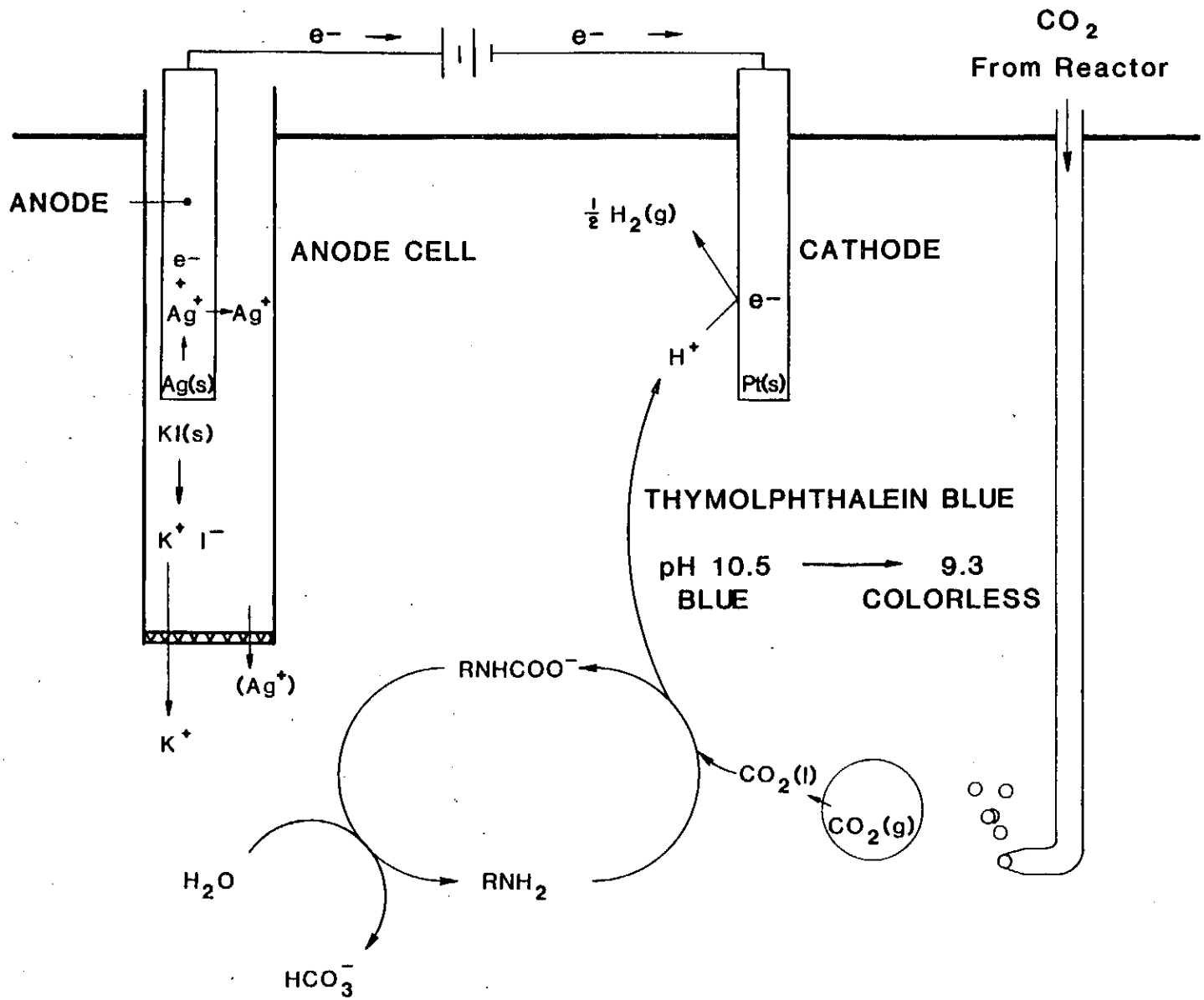

Figure 5. Coulometric titration of carbon dioxide: a proposed scheme (XBL 832-8329).

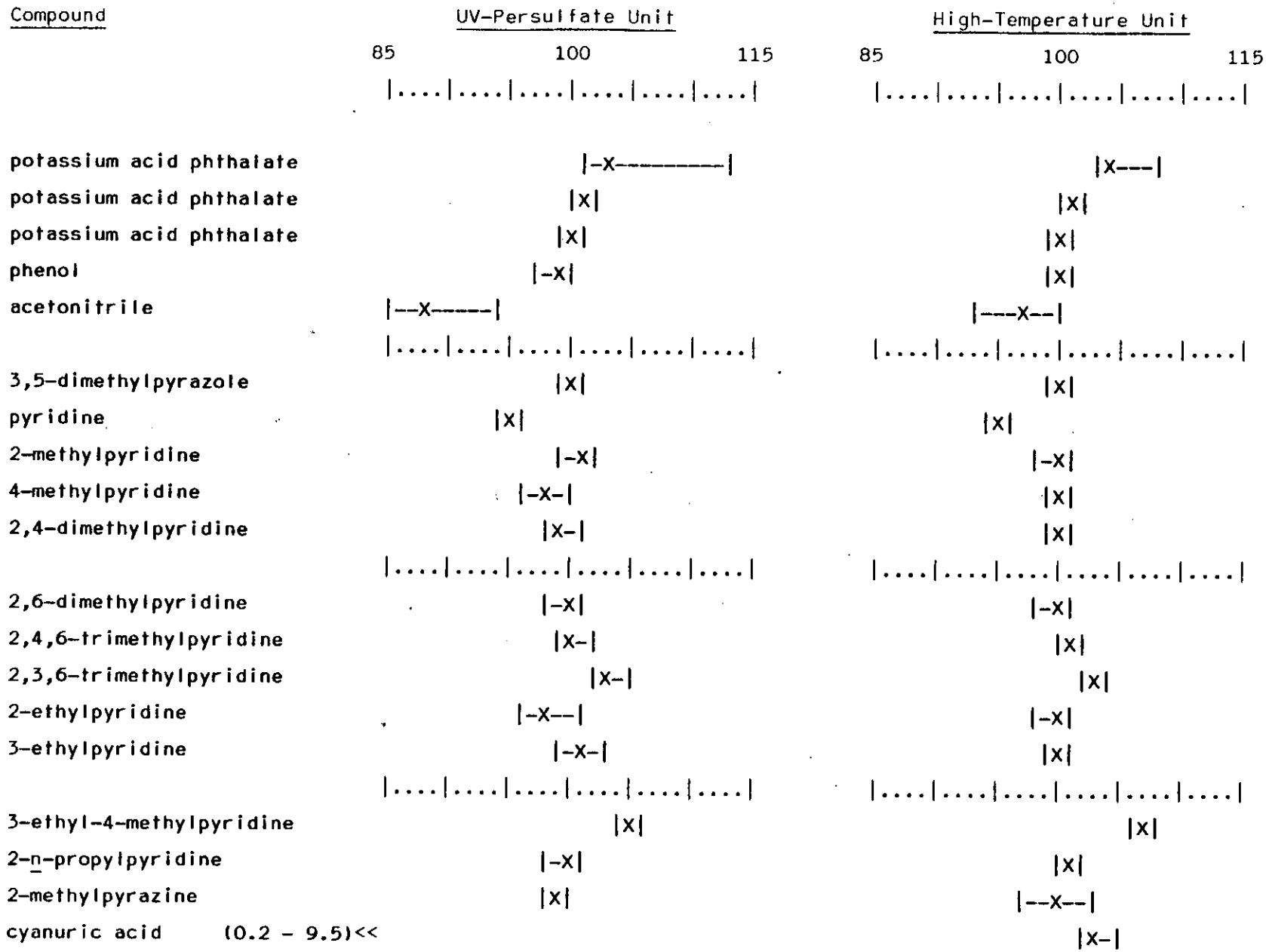

Figure 6. Comparison of percent recovery means (X) and ranges (|---|) for standard solutions; concentrations of compounds are identical to those presented in Tables I and II.

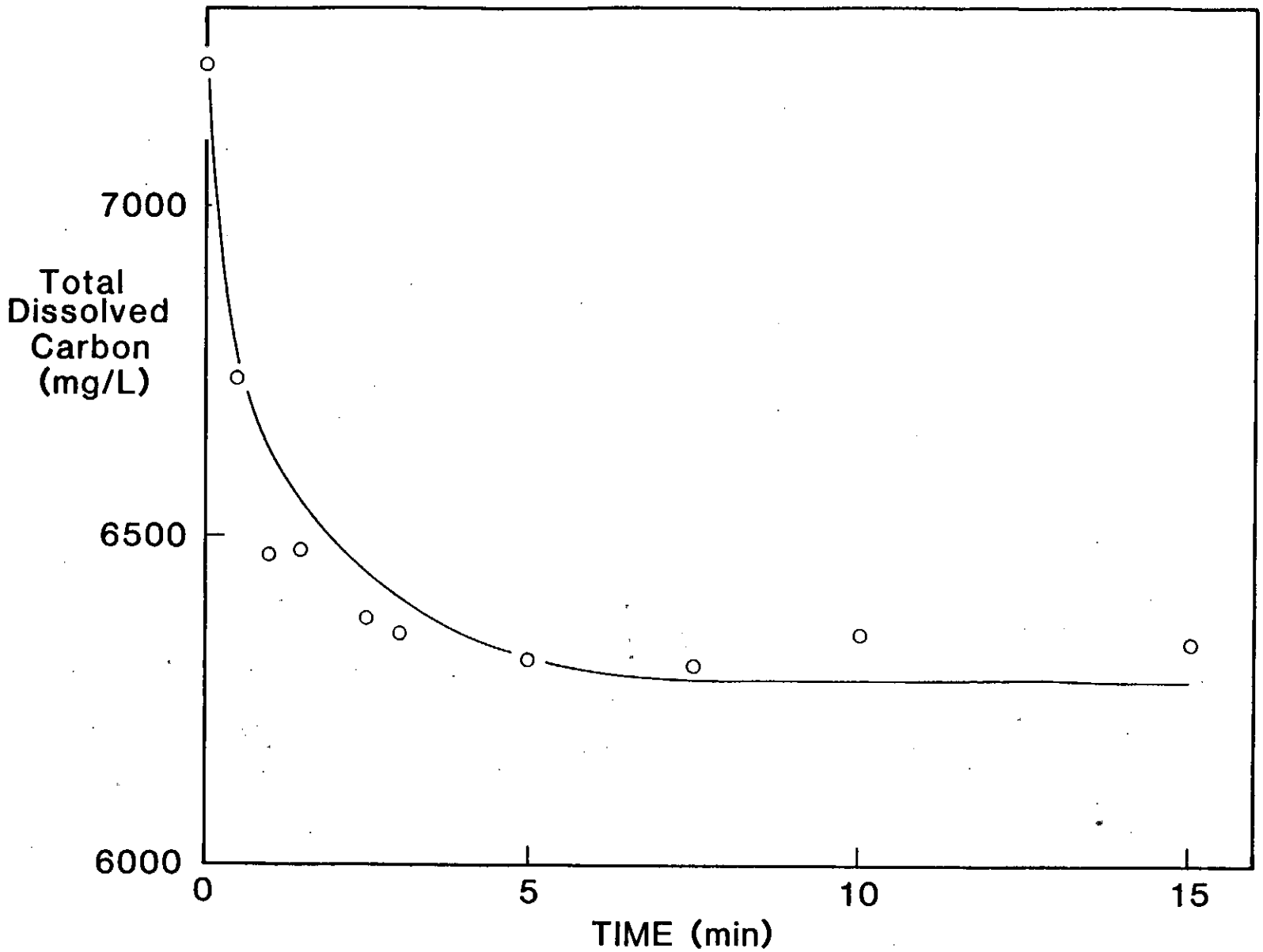

Figure 7. Time-course purge study for direct organic carbon determination (XBL 832-8328).

PROTOCOL: Dissolved Organic Carbon
(UV-Enhanced Persulfate Oxidation/Coulometric Titration)

I. Start-Up

NOTE: All reagents are made from Analytical Reagent grade chemicals unless otherwise specified. When used as a reagent, "water" refers to ASTM Type I quality. All glassware is washed in 35% nitric acid and thoroughly rinsed with ASTM Type I water. The dipotassium salt of peroxydisulfuric acid is referred to as "persulfate".

A. Prepare the persulfate oxidation solution.
   1. Weigh 20 g of persulfate, and quantitatively transfer to 300 mL of water in a 1000-mL volumetric flask.
   2. Add 1.0 mL of concentrated nitric acid, and bring to volume with water.

B. Increase the oxygen flow rate to 190 cm^3/min.
   1. Ensure that the oxygen delivery pressure is 15 psig.
   2. Ensure that at least 500 psig of oxygen is in the cylinder.
   3. If foaming occurs in the KOH scrubber, add a small amount of water to the KOH; if foaming persists, replace the contents with approximately 12 mL of fresh 45% KOH solution.

C. Repack the magnesium perchlorate scrubbers. These scrubbers are positioned directly after the burette water-trap.

NOTE: Using the old, wetted packing could result in formation of a plug, increasing the back-pressure.
   1. Wash out the old packing, rinse the scrubber tube with ASTM Type I water, then air- or oven-dry the tube.
   2. Repack and reconnect the tube.

D. Check the acid dichromate/manganese dioxide scrubber for exhaustion. This scrubber is positioned after the magnesium perchlorate scrubber.
   1. The acid dichromate packing will change from yellow-orange (oxidized) to green-orange (reduced) as it becomes exhausted. This color change will be seen as a front progressing in the direction of gas flow. When almost all of the acid dichromate has changed color, the entire scrubber must be repacked.
   2. When the manganese dioxide packing is exhausted, it will change from black to dark brown; the entire scrubber must then be repacked.

E. Position the recirculation (0.063" I.D.) and reagent delivery (0.031" I.D.) Viton pump tubes and the silicone waste-line pump tube (1.0-mm I.D.) in the peristaltic pump (Sage Instruments, model 375A), and close the platen lid.
   1. The Viton pump tubes require the 11-lb pressure plates (gray); the silicone pump tube requires the 2.12-lb pressure plate (tan).

F. Fill the UV reactor with the persulfate solution and secure the reactor cap with silicone rubber bands. Connect the persulfate reservoir using the Omnifit Teflon fittings, and connect the waste line to an appropriate receptacle.

G. Turn on the peristaltic pump.
   1. The pump setting should be preset to deliver approximately 0.6 mL/min of fresh persulfate solution to the reactor. The contents of the reactor should recycle through the valve at a rate of 3.0 mL/min. Combined flow of fresh and recycled reagent will be 3.6 mL/min. Fluid from the reactor is pumped to waste at a

rate of 0.6 mL/min.; the total waste flow rate, which includes headspace, is higher.

NOTE: Check for leaks at all tubing connections during initial pumping; a misaligned sample injection valve rotor will increase back-pressure and cause leaking.

H. Assemble the coulometer cell.
   1. Fill the coulometer cell with 75 mL of coulometer solution.
   2. Add the stir bar.
   3. Position the rubber stopper on the coulometer cell such that the anode, cathode, and gas line face the back wall of the cell (that portion of the beaker containing the volume graduations).
   4. Add 3 pellets of potassium iodide to the anode compartment.
   5. Add anode solution to the anode compartment. The anode solution level should be slightly higher than that of the coulometer cell solution.
   6. Place the silver anode in the anode compartment. Ensure that the tip of the silver anode is wetted by the anode solution.
   7. Place the assembled coulometer cell in the cell holder of the instrument; the volume graduations should face the rear of the instrument.
   8. Plug the anode (red) and cathode (black) wires into the coulometer.

NOTE: Do NOT turn on the electrolysis current.

   9. Connect the coulometer cell gas line to the one-way valve in-line from the nitrogen oxide scrubber. Check that the stopcock on the burette water trap is open (the gas flow must be diverted from the coulometer cell during adjustment of cell transmittance per step I.K.).

I. Connect the HP 97S to the coulometer interface cable.
   1. Turn on the HP 97S.
   2. With the calculator in the RUN mode, load the "background" program as per the user instructions (Chapter III, Appendix A).

J. Turn on the coulometer main power supply. Allow a warm-up period of several minutes.

K. Adjust the coulometer cell transmittance.
   1. Carefully rotate the coulometer cell until a maximum transmittance is obtained.
   2. Adjust the transmittance to 100% using the "100% adjust" knob.
   3. Close the stopcock on the burette water trap.
   4. Check that the gas flow into the coulometer cell does not deflect the 100% transmittance setting. If a deflection occurs, reposition the gas line to eliminate this interference. Open the burette stopcock and repeat steps 1-4.

NOTE: The gas line must be submerged in the coulometer solution.

L. Turn on the electrolysis current.

M. Start the UV-lamp and initiate the background program.

CAUTION: Although the ultraviolet radiation emitted by the lamp is greatly attenuated by the Pyrex glass of the reactor and by safety glasses, the intensity of the transmitted light can cause eye strain. The glare can be minimized by enclosing the reactor in 6-inch O.D. Plexiglass tubing that has been coated with reflective plastic film (e.g., solar control film). This still allows for visual inspection of the reactor during operation.

1. When the coulometer has stabilized, the background (counts per minute) should be approximately 2.0 to 3.2 mg/L (when range plug is set for mg/L readout). Stabilization should be complete within 0.5 to 2.0 hours.
2. If high background counts persist (greater than 4 mg/L per minute) for more than two hours, replace the Viton pump tubing, and repeat step M. and M.1.

NOTE: Should the high background persist after installing new Viton tubing, check for the following (in decreasing order of priority): exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; contamination of persulfate solution; coulometer performance.

II. Sample Preparation -- Total Dissolved Carbon (TDC)
- A. Filter all samples through 0.4-μm pore-diameter polycarbonate membrane filters.
- B. Dilute sample filtrates with water to yield TDC concentrations between 200 and 2000 mg/L.
- C. Prepare a TDC sample blank; a 10-mL aliquot of water should be processed with the TDC samples.
- D. Refrigerate samples until analysis time.

III. Sample Preparation -- Dissolved Organic Carbon (DOC)
- A. Filter all samples through a 0.4-μm pore size polycarbonate membrane filter.
- B. Dilute sample filtrates with water to yield DOC concentrations between 200 and 2000 mg/L.
- C. Prepare a DOC sample blank; a 10-mL aliquot of water should be processed with the DOC samples.
- D. Acidify the blank and DOC samples with 0.10 mL concentrated sulfuric acid per 10 mL of sample. A positive displacement pipette or a repipette should be used.

NOTE: Samples should have pH values of 2 after acidification.

- E. Refrigerate samples until analysis time.

IV. Purging of DOC Samples
- A. Open the helium cylinder main valve, and set the delivery pressure for the purge manifold to 10 psig. The needle valve on the helium flow meter should be preset to deliver 775 cm^3/min at 10 psig.
- B. Rinse the glass capillaries by submersion in concentrated HCl, and wipe dry.
- C. Submerge each capillary in a sample, and purge for 10 minutes.
- D. Remove the capillaries, wipe dry, and repeat steps IV.B.-C. for all samples.
- E. After all the samples have been purged, repeat step IV.B., and store the capillaries in a clean, dry vial.
- F. Turn off the helium cylinder valve.

NOTE: Purging should be conducted in an enclosed compartment to prevent deposition of acidic aerosols on nearby equipment.

V. Preparation of Standards
- A. Prepare a stock solution of potassium acid phthalate (DOC = 2000.0 mg/L as C).

1. Weigh 2127.2 mg of dried potassium acid phthalate and quantitatively transfer to a 500-mL volumetric flask.
2. Bring to volume with water.
3. Acidify the standards as instructed in III.D.

B. Prepare working standards of 100, 1000, and 2000 mg/L.

NOTE: Use air- or positive-displacement pipettes. Additional standard concentrations should be made if the sample DOC concentration is expected to be outside of this range.

1. 100 mg/L : pipette 0.5 mL of stock solution and 9.5 mL of water into a scintillation (DOC) vial.
2. 1000 mg/L : pipette 5.0 mL of stock solution and 5.0 mL of water into a DOC vial.
3. 2000 mg/L : pipette 10.0 mL of stock solution into a DOC vial.

C. Prepare a stock solution of phenol for recovery determinations (DOC = 2371.7 mg/L as C).

1. Weigh 155.0 mg of phenol, and quantitatively transfer to a 50-mL volumetric flask.
2. Bring to volume with water.
3. Dilute this stock solution 1:5 with water, and acidify as instructed in III.D. (DOC = 474.3 mg/L as C).

D. Prepare a stock solution of pyridine for recovery determinations (DOC = 1000 mg/L as C).

1. Dispense approximately 65 μL of pyridine into a tared 50-mL volumetric flask and record the exact mass.
2. Bring to volume with water.
3. Acidify as instructed in III.D.4.
4. Calculate the theoretical DOC of this solution:
   DOC (mg/L) = (15.18) x (mg pyridine added).
5. Dilute this stock solution 1:5 with water (DOC = 200 mg/L as C).

VI. Sample Analysis

A. Load the "Water Analysis" program into the HP 97S.

1. Turn off the coulometer main power.
2. With the HP 97S calculator in RUN mode, run the program card through the HP 97S card reader.
3. Initiate the program as per the user instructions (Chapter III, Appendix B).
4. Turn on the coulometer main power.

B. Sample injection

1. Rinse the 1.0-mL gas-tight HPLC syringe (Glenco) and Teflon sample loop with one milliliter of sample; this is required to exponentially dilute the persulfate reagent from the loop. Ensure that the injector waste line is connected to a waste receptacle.
2. Fill the syringe with a minimum of 0.6 mL of sample.
3. Insert the syringe needle into the injection valve port.
4. Position the rotary sample injection valve to the LOAD position.
5. Load the sample into the 200-μL injection loop. Leave the syringe in place to prevent both the introduction of air to the sample loop and drainage of sample from the loop.
6. Switch the valve to the INJECT position and initiate the sample program on the HP 97S as per the user instructions (Chapter III, Appendix B).

7. The syringe can now be removed from the injection port. The valve, however, must remain in the INJECT position during sample analysis.
8. The suggested analysis time for each sample is 5 minutes.
9. Check the burette water trap. This trap should be emptied between analyses so that the gas line does not become submerged in the condensate.
10. For replicates, repeat VI.B.1.-9.

C. Repeat VI.B. for each sample.

D. Samples should be analyzed in the following order:
1. Sample blank; the mean DOC value from the blanks must be subtracted from the DOC value of each standard and sample. This calculation is performed automatically by the HP 97S "Water Analysis" program.
2. Acid phthalate standards.

NOTE: If standard recoveries deviate more than 5% from the theoretical values, check for the following (in decreasing order of priority): accuracy of standard stock solution and standard dilutions; exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; condition of persulfate reagent; condition of UV lamp; coulometer performance.

3. Recovery standards (phenol and pyridine).
4. Samples.

NOTE: If a large number of samples is to be analyzed, the series of standards should be analyzed at intervals throughout the course of analyses.

5. Upon completion of the DOC analyses, the series of standards and blanks should be reanalyzed.

VII. Shutdown

A. Turn off the HP 97S.
B. Turn off the main power supply to the UV lamp.
C. Drain the contents of the reactor vessel and discard.
D. Disconnect the persulfate reservoir and refrigerate.
E. Turn off the peristaltic pump after the lines have been pumped dry.
1. Disengage the platen, and remove all tubing from the pump.
F. Turn off the oxygen cylinder main valve.
G. Open the stopcock on the burette water trap, and raise the sparger in the KOH scrubber above the liquid level.
H. Disassemble the coulometer cell.

NOTE: Use only ASTM Type I water for all rinsing.

1. Turn off the electrolysis current.
2. Turn off the main power.
3. Unplug the anode and cathode wires from the coulometer.
4. Disconnect the gas line at the one-way valve between the coulometer cell and the nitrogen oxide scrubber.
5. Remove the coulometer cell from the coulometer.
6. Remove the silver anode, rinse with water, and air-dry on a clean surface.
7. Remove the rubber stopper from the coulometer cell, and rinse the anode cell with acetone. Ensure that no potassium iodide deposits remain in the anode cell. Using a vacuum source and the perforated serum stopper, draw a small volume of acetone through the fritted-glass end of the anode cell.

8. Rinse the exteriors of the anode, cathode, and gas line with water, and air-dry on a clean surface.
9. Rinse the coulometer cell and stir bar several times with water, and air-dry on a clean surface.

VIII. Data Reduction

A. Calculate the mean value for each set of replicates:

1. $\bar{x} = \Sigma(x_i - b)/n$

   where x_i = each carbon value in a set of replicates
   b = the mean value of all DOC blank analyses
   n = the number of replicates per sample.

B. The HP 97S "Water Analysis" program automatically calculates [$(x_i - b)$ X (dilution factor)] for each datum point. Therefore, the mean for a set of replicates equals the sum of data outputs divided by the number of replicates (n); a separate program for calculating $\bar{x}$ and the rsd is in Chapter III, Appendix B.

C. Determine whether suspected outliers should be discarded.

1. Suspected outliers should be subjected to statistical analysis before being discarded (1).
2. If an outlying value is known to be the result of a mechanical or operator error, it may be rejected without statistical verification.

IX. Maintenance

A. Record the appropriate information in the C-Analyzer Log Book, including:

1. Date and duration of usage.
2. Number of injections (retort water samples and total) and sample dilutions.
3. Symptoms of malfunctioning.
4. Repairs.
5. Initial all entries.

X. References

(1) Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1977; 1110 pp.

Protocol prepared by: G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton

PROTOCOL: Dissolved Organic Carbon
(High-Temperature Combustion/Coulometric Titration)

I. Start-Up

NOTE: All reagents are made from Analytical Reagent grade chemicals unless otherwise specified. When used as a reagent, "water" refers to ASTM Type I quality. All glassware is washed in 35% nitric acid and thoroughly rinsed with ASTM Type I water.

A. Turn the combustion furnace temperature control knob to 950°C. Allow 0.5 to 1 hour for the furnace to reach temperature.

B. Increase the oxygen flow rate to 100 cm^3/min.
   1. Ensure that the oxygen delivery pressure is 15 psig.
   2. Ensure that at least 500 psig of oxygen is in the cylinder.
   3. If foaming occurs in the KOH scrubber, add a small amount of water to the KOH; if foaming persists, replace the contents with approximately 12 mL of fresh 45% KOH solution.

C. Assemble coulometer cell, adjust transmittance, and connect HP 97S calculator.
   1. Follow I.C.-I.D. and I.H.-I.K. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

D. Turn on the electrolysis current and initiate the background program.
   1. Allow several minutes for the titration of endogenous CO_2 in the coulometer solution.
   2. Following this initial titration, check the coulometer stability. A stable background count of 1.0 to 1.2 mg/L per minute should be obtained when the range plug is set to display mg/L. Stabilization may take as long as 30 minutes.

II. Sample Preparation -- Total Dissolved Carbon (TDC)

A. Filter all samples through a 0.4-µm pore-diameter polycarbonate membrane filter.

B. Dilute sample filtrates with water to yield TDC concentrations between 50 and 200 mg/L; higher concentrations can be analyzed, but will accelerate deterioration of the combustion tube.

C. Prepare a TDC sample blank; a 10-mL aliquot of water should be processed with the TDC samples.

D. Refrigerate samples until analysis time.

III. Sample Preparation -- Dissolved Organic Carbon (DOC)

A. Filter all samples through a 0.4-µm pore-diameter polycarbonate membrane filter.

B. Dilute sample filtrates with water to yield DOC concentrations between 50 and 200 mg/L; higher concentrations can be analyzed, but will accelerate deterioration of the combustion tube.

C. Prepare a DOC sample blank; a 10-mL aliquot of water should be processed with the DOC samples.

D. Acidify the blank and DOC samples with 0.10 mL concentrated sulfuric acid per 10 mL of sample. A positive displacement pipette or a repipette should be used.
   NOTE: Samples should have pH values of 2 after acidification.

E. Refrigerate samples until analysis time.

IV. Purging Of DOC Samples

A. Follow IV. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)"

V. Preparation of Standards

A. Prepare a stock solution of potassium acid phthalate (DOC = 1000.00 mg/L as C).

1. Weigh 1063.7 mg of dried potassium acid phthalate, and quantitatively transfer to a 500-mL volumetric flask.
2. Bring to volume with water.
3. Acidify the standards as instructed in section III.D.

B. Prepare working standards of 50, 100, and 200 mg/L.

NOTE: Use air- or positive-displacement pipettes. Additional standard concentrations should be made if the sample DOC concentration is expected to be outside of this range.

1. 50 mg/L : pipette 0.5 mL of stock solution and 9.50 mL of water into a scintillation (DOC) vial.
2. 100 mg/L : pipette 1.0 mL of stock solution and 9.0 mL of water into a DOC vial.
3. 200 mg/L : pipette 2.0 mL of stock solution and 8.0 mL of water into a DOC vial.

C. Prepare a stock solution of phenol for recovery determinations (DOC = 2371.7 mg/L as C).

1. Weigh 155.0 mg of phenol, and quantitatively transfer to a 50-mL volumetric flask.
2. Bring to volume with water.
3. Dilute this stock solution 1:10 with water, and acidify as instructed in III.D. (DOC = 237.17 mg/L as C).

D. Prepare a stock solution of pyridine for recovery determinations (DOC = 1000 mg/L as C).

1. Dispense approximately 65 μL of pyridine into a tared 50-mL volumetric flask and record the exact mass.
2. Bring to volume with water.
3. Acidify as instructed in III.D.
4. Calculate the theoretical DOC of this solution: DOC (mg/L) = (15.18) x (mg pyridine added).
5. Dilute this stock solution 1:5 with water (DOC = 200 mg/L as C).

VI. Sample Analysis

A. Load the "Water Analysis" program into the HP 97S.

1. Turn off the coulometer main power.
2. With the HP 97S in the RUN mode, run the program card through the HP 97S card reader.
3. Initiate the program as per the user instructions (Chapter III, Appendix B).
4. Turn on the coulometer main power.

B. If the Hamilton constant rate carbon analyzer syringe is used, check that it is set and locked at 200 μL. Recheck frequently during sample analysis.

NOTE: A sample injection volume of 200 μL is recommended; excessive injection volumes can damage the quartz tube and are unsafe because of the rapid expansion of gases.

C. Rinse the syringe 10 times with the sample to be analyzed.
   1. Insert the syringe needle into the sample injection port; ensure that the Luer fittings are seated.
   2. Inject the sample and simultaneously initiate the sample program as per the user instructions (Chapter III, Appendix B).
   3. The syringe must remain in the injection port throughout the analysis; improper flushing by the oxygen and loss of CO_2 will occur if the injection port/syringe Luer union does not remain sealed.
   4. The suggested analysis time for each sample is 3 minutes.
   5. For replicates, rinse the syringe twice with the sample to be analyzed and repeat VI.C.1.-4.

D. Repeat VI.C. for each sample.
   1. Check the burette water trap. This trap should be emptied between analyses so that the gas line does not become submerged in the condensate. Collect the condensate in an acid-washed vial for later validation of complete combustion (i.e., absence of DOC).

E. Samples should be analyzed in the following order:
   1. Sample blank; the mean DOC value from the blanks must be subtracted from the DOC value of each standard and sample. This calculation is performed automatically by the HP 97S "Water Analysis" program.
   2. Acid phthalate standards.

   NOTE: If standard recoveries deviate more than 5% from the theoretical values, check for the following (in decreasing order of priority): accuracy of standard stock solution and standard dilutions; exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; condition of combustion tube; coulometer performance.

   3. Recovery standards (phenol and pyridine).
   4. Samples.

   NOTE: If a large number of samples is to be analyzed, the series of standards should be analyzed at intervals throughout the course of analyses.

   5. Upon completion of the DOC analyses, the series of standards and blanks should be reanalyzed.
   6. The collected condensate sample should also be analyzed for DOC concentration. A DOC value greater than the background count indicates carry-over of uncombusted carbon.

VII. Shutdown
   A. Turn off the HP 97S.
   B. Remove the syringe and replace the end-plug over the sample injection port.
   C. Reduce the furnace temperature to approximately 750°C.
   D. Reduce the oxygen flow rate to 40 cm^3/min.
   E. Follow VII.G.-H. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

VIII. Data Reduction
   A. Follow VIII. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

IX. Maintenance

A. Follow IX. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

Protocol prepared by: G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton

PROTOCOL: Inorganic Carbon
(Acidification-Purge/Coulometric Titration)

I. Start-Up

NOTE: All reagents are made from Analytical Reagent grade chemicals unless otherwise specified. When used as a reagent, "water" refers to ASTM Type I quality. All glassware is washed in 35% nitric acid and thoroughly rinsed with ASTM Type I water.

A. Turn on the main power supply for the inorganic carbon apparatus. This controls both the air pump and the heating element.
B. The temperature control knob should be set at 60 (60°C).
C. Increase the air flow rate to 100 cm^3/min.
   1. If foaming occurs in the KOH scrubber, add a small amount of water to the KOH; if foaming persists, replace the contents with approximately 12 mL of fresh 45% KOH solution.
D. Refill the aqueous Ag_2SO_4 scrubber.
   1. The contents can be removed with a 9-inch Pasteur pipette.
   2. Refill with 3 mL of saturated Ag_2SO_4 solution containing 3% H_2O_2 (vol/vol).
E. Refill the perchloric acid ($HClO_4$) reservoir/dispenser with 2N $HClO_4$ if necessary (each analysis requires 2 mL of acid).

CAUTION: Take appropriate precautions when handling concentrated $HClO_4$. (read pertinent sections in references 1-3).

   1. To prepare a 2N solution, place 100 mL of water in a 250-mL volumetric flask, followed by 43.0 mL of $HClO_4$ (70%).
   2. Mix and bring to volume with water.
F. Check the neoprene slip-on septum. Replace the septum if signs of oxidation (i.e., from $HClO_4$) are evident (e.g., dryness, cracking).
G. Check the silicone reducing connectors on the air- and acid-delivery lines. Replace these fittings if they show signs of deterioration.
H. Assemble coulometer cell and connect HP 97S calculator.
   1. Follow I.H.-J. (except I.H.9.) of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".
I. Adjust the coulometer cell transmittance.
   1. Carefully rotate the coulometer cell until a maximum transmittance is obtained.
   2. Adjust the transmittance to 100% using the "100% adjust" knob.
   3. Connect the coulometer cell gas line to the one-way valve in-line from the Ag_2SO_4 scrubber.
   4. Check that the gas flow into the coulometer cell does not deflect the 100% transmittance setting. If a deflection occurs, reposition the gas line to eliminate this interference; disconnect the gas line and repeat steps 1-4.

   NOTE: The gas line must be submerged in the coulometer solution.
J. Turn on the electrolysis current and initiate the background program.
   1. Allow several minutes for the titration of endogenous CO_2 in the coulometer solution.
   2. Following this initial titration, check the coulometer stability. A stable background count of 1.0 to 1.2 mg/L per minute should be obtained when the range plug is set to display mg/L. Stabilization may take as long as 30 minutes.

II. Sample Preparation -- Dissolved Inorganic Carbon (DIC)
   A. Filter all samples through 0.4-μm pore-diameter polycarbonate membrane filters.
   B. Dilute sample filtrates with water to yield DIC concentrations between 100 and 500 mg/L.
   C. Prepare a DIC sample blank; a 10-mL aliquot of water should be processed with the DIC samples.
   D. Refrigerate samples until analysis time.

III. Preparation of Standards
   A. Prepare a stock solution of sodium carbonate (DIC = 1000.0 mg/L).
      1. Weigh 4414.5 mg of dried sodium carbonate, and quantitatively transfer to a 500-mL volumetric flask.
      2. Bring to volume with water.
   B. Prepare working standards of 100, 250, and 500 mg/L.
   NOTE: Use air- or positive-displacement pipettes. Additional standard concentrations should be made if the sample DIC concentration is expected to be outside of this range.
      1. 100 mg/L : pipette 1.0 mL of stock solution and 9.0 mL of water into a scintillation (DOC) vial.
      2. 250 mg/L : pipette 2.5 mL of stock solution and 7.5 mL of water into a DOC vial.
      3. 500 mg/L : pipette 5.0 mL of stock solution and 5.0 mL of water into a DOC vial.

IV. Sample Analysis
   A. Load the "Water Analysis" program into the HP 97S.
      1. Turn off the coulometer main power.
      2. With the HP 97S in the RUN mode, run the program card through the HP 97S card reader.
      3. Initiate the program as per the user instructions (Chapter III, Appendix B).
      4. Turn on the coulometer main power.
   B. Sample injection
      1. If a gas-tight syringe (e.g., Unimetrics) is used, check that the constant-volume adaptor is set for 200 μL. Recheck frequently during sample analysis.
      2. Rinse the syringe 10 times with the sample to be analyzed.
      3. Insert the syringe needle through the septum injection port.
      4. Inject the sample, and withdraw the syringe.
      5. Depress the plunger on the perchloric acid reservoir, and initiate the program loop as per the user instructions (Chapter III, Appendix B). The repipette should be set to deliver 2.0 mL of acid.
      6. The suggested analysis time for each sample is 3 minutes.
      7. For replicates, rinse the syringe twice with the sample to be analyzed and repeat IV.B.3.-6.

      NOTE: If analyses are conducted without the HP 97S, a stopwatch should be used to measure the analysis time. The coulometer should be reset at the start of the analysis time.
   C. Empty contents of reactor tube before the level reaches the ground-glass connection.
      1. Reconnect and wait for pulse of introduced atmospheric CO_2 to be titrated.

D. Repeat IV.B. for each sample.
E. Samples should be analyzed in the following order:
   1. Sample blank; the mean DIC value from the blanks must be subtracted from the DIC value of each standard and sample. This calculation is performed automatically by the HP 97S "Water Analysis" program.
   2. Sodium carbonate standards.

   NOTE: If standard recoveries deviate more than 5% from the theoretical values, check for the following (in decreasing order of priority): accuracy of standard stock solution and standard dilutions; exhaustion of scrubbers; clogging of anode-cell glass frit; silver deposition on platinum wire cathode; contamination of reactor tube; coulometer performance.

   3. Samples.

   NOTE: If a large number of samples is to be analyzed, the series of standards should be analyzed at intervals throughout the course of the DIC analyses.

   4. Upon completion of the DIC analyses, the series of standards and blanks should be reanalyzed.

V. Shutdown
   A. Turn off the HP 97S.
   B. Turn off the inorganic carbon apparatus.
      1. Remove the reactor tube, and rinse thoroughly with water.
   C. Disassemble the coulometer cell.
      1. Follow VII.H. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

VI. Data Reduction
   A. Follow VIII. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

VII. Maintenance
   A. Follow IX. of protocol for "Dissolved Organic Carbon (UV-Enhanced Persulfate Oxidation/Coulometric Titration)".

VIII. References

(1) Lefevre, M.J.
First Aid Manual for Chemical Accidents; Dowden, Hutchinson and Ross, Inc.: Stroudsburg, PA, 1980; 218 pp.

(2) Manufacturing Chemists Association
Guide for Safety in the Chemical Laboratory; Van Nostrand Reinhold Company: New York, NY, 1972; 505 pp.

(3) National Research Council
Prudent Practices for Handling Hazardous Chemicals in Laboratories; National Academy Press: Washington, D.C., 1981; 291 pp.

Protocol prepared by: G.W. Langlois, B.M. Jones, R.H. Sakaji, and C.G. Daughton

C-ANALYZER: BACKGROUND COUNTS PROGRAM

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position, coulometer turned off.			
2	PRESS"E": display will go to 0.0		E	
3	PRESS "R/S": display will go to 0.		R/S	
4	ENTER NUMBER OF DATA INPUTS FROM COULOMETER (e.g., 20 inputs at 15 sec intervals = 5 min analysis time). PRESS "R/S".	"n"	R/S	
5	TURN COULOMETER ON.			
6	PRESS "R/S": prints number of inputs, spaces 2 lines, begins data acquisition loop.		R/S	"n"
7	When data input is completed (i.e., after the "n"th data input) the system background for the selected analysis time is printed; the data acquisition loop is automatically reentered.			Background value
8	PRESS "R/S" to terminate the program.		R/S	
9	To change the value for number of data inputs turn off the coulometer, PRESS "f" "e" and repeat instructions beginning at step 4.		f e	

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	001 *LBLE	21 15	
	002 CF3	16 22 03	
	003 R/S	51	
	004 SF3	16 21 03	
	005 *LBLe	21 16 15	
	006 SF3	16 21 03	
	007 DSP0	-63 00	
	008 0	00	
	009 R/S	51	
010	010 PRTX	-14	
	011 SPC	16-11	
	012 SPC	16-11	
	013 1	01	
	014 +	-55	
	015 STOC	35 13	
	016 R/S	51	
	017 *LBLD	21 14	
	018 DSP0	-63 00	
	019 0	00	
020	020 RCLC	36 13	
	021 STOI	35 46	
	022 DSP1	-63 01	
	023 SF0	16 21 00	
	024 PSE	16 51	
	025 *LBLB	21 12	
	026 CF3	16 22 03	
	027 CF0	16 22 00	
	028 R/S	51	
	029 *LBLF	21 11	
030	030 1	01	
	031 0	00	
	032 ÷	-24	
	033 DSZI	16 25 46	
	034 GTOB	22 12	
	035 CF3	16 22 03	
	036 PSE	16 51	
	037 SF3	16 21 03	
	038 PRTX	-14	
	039 GTOD	22 14	
040	040 R/S	51	
050			
060			
070			
080			
090			
100			

LABELS

A DATA INPUT LOOP	B DATA LOOP	C	D INITIATE DATA LOOP	E INITIATE PROGRAM
a	b	c	d	e MANUAL DATA INPUTS
0	1	2	3	4
5	6	7	8	9

REGISTERS

0	1	2	3	4	5	6	7	8	9
		S2	S3	S4	S5	S6	S7	S8	S9

B	C # DATA INPUTS	D	E	I COUNTER FOR DATA INPUTS	

1 WATER ANALYSIS PROGRAM: TDC, DOC, and DIC 2

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position, coulometer turned off.			
2	PRESS "E": display will go to 0.0		E	
3	PRESS "R/S": display will go to 0.000		R/S	
4	ENTER STABILITY FACTOR (e.g., 0.990).	Value		
5	PRESS "R/S": prints stability factor, display goes to 0.		R/S	Value
6	ENTER BLANK VALUE	Value		
7	PRESS "R/S": prints blank value, display goes to 0.		R/S	Value
8	ENTER NUMBER OF DATA INPUTS FROM COULOMETER (e.g., 20 inputs at 15 sec intervals = 5 min analysis time).	"n"		
9	PRESS "R/S": prints number of inputs, spaces 3 lines, displays 0.		R/S	"n"
10	TURN COULOMETER ON.			
11	ENTER SAMPLE NUMBER (integer only)	Value		
12	PRESS "R/S": prints sample number, displays 0.0		R/S	Value
13	ENTER DILUTION FACTOR (e.g., "10" for a 1:10 dilution).	Value		
	NOTE: THE FOLLOWING STEP INITIATES THE DATA ACQUISITION LOOP; THE LOADED SAMPLE MUST BE INJECTED AT THIS STEP.			
14	INJECT SAMPLE, PRESS "R/S": prints dilution factor, starts analysis time.		R/S	Value

WATER ANALYSIS PROGRAM (continued)

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS		OUTPUT DATA/UNITS
15	When data input is completed (i.e., after the				Final Value minus blank
	"n"th data input) the final value minus the				multiplied by the
	blank is printed; this value is multiplied by				dilution factor
	the dilution factor and printed.				
16	To analyze the next sample repeat steps 11-14.				
17	To change the STABILITY FACTOR, BLANK, or		f	e	
	NUMBER OF DATA INPUTS, turn off the coulometer,				
	PRESS "f" "e", and repeat instructions				
	beginning at step 4.				
18	To calculate the mean and rsd for a set of		f	a	$\bar{x}$
	replicates, PRESS "f" "a"; the mean, rsd, and				rsd
	number of replicates will be printed. If one				n
	or more replicates are outliers (see protocol				
	for Dissolved Organic Carbon, UV-enhanced				
	persulfate oxidation/coulometric titration,				
	section VIII.C.), they can be removed from the				
	data set as follows: enter the value and				
	PRESS "f" "Σ-". Proceed with step 18 when				
	outliers have been removed.				
	NOTE: If step 18 is omitted, the storage				
	registers used for $\bar{x}$ and rsd calculations				
	will continue to accumulate sums among sets of				
	replicates. This will invalidate subsequent				
	$\bar{x}$ and rsd determinations.				

CARBON ANALYSIS

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLE	21 15	
002	CF3	16 22 03	
003	R/S	51	
004	SF3	16 21 03	
005	*LBLe	21 16 15	
006	SF3	16 21 03	
007	DSP3	-63 03	
008	0	00	
009	R/S	51	
010	PRTX	-14	
011	STOA	35 11	
012	DSP1	-63 01	
013	0	00	
014	R/S	51	
015	PRTX	-14	
016	STOB	35 12	
017	DSP0	-63 00	
018	0	00	
019	R/S	51	
020	PRTX	-14	
021	SPC	16-11	
022	SPC	16-11	
023	SPC	16-11	
024	1	01	
025	+	-55	
026	STOC	35 13	
027	RCLB	36 12	
028	X⇄Y	-41	
029	÷	-24	
030	STO2	35 02	
031	*LBLD	21 14	
032	DSP4	-63 04	
033	0	00	
034	R/S	51	
035	PRTX	-14	
036	DSP1	-63 01	
037	0	00	
038	R/S	51	
039	X=0?	16-43	
040	1	01	
041	PRTX	-14	
042	STO1	35 01	
043	RCLC	36 13	
044	STOI	35 46	
045	STO3	35 03	
046	DSP1	-63 01	
047	SF0	16 21 00	
048	PSE	16 51	
049	*LBLB	21 12	
050	STOE	35 15	
051	CF3	16 22 03	
052	CF0	16 22 00	
053	R/S	51	
054	*LBLA	21 11	
055	1	01	
056	0	00	
057	÷	-24	
058	DSZI	16 25 46	
059	GTOB	22 12	
060	CF3	16 22 03	
061	PSE	16 51	
062	SF3	16 21 03	
063	STOD	35 14	
064	RCLA	36 11	
065	×	-35	
066	RCLE	36 15	
067	-	-45	
068	RCL2	36 02	
069	-	-45	
070	X>0?	16-44	
071	GTOC	22 13	
072	DSP1	-63 01	
073	RCLD	36 14	
074	ENT↑	-21	
075	RCL2	36 02	
076	ENT↑	-21	
077	RCL3	36 03	
078	×	-35	
079	-	-45	
080	PRTX	-14	
081	RCL1	36 01	
082	×	-35	
083	PRTX	-14	
084	Σ+	56	
085	SPC	16-11	
086	GTOD	22 14	
087	*LBLC	21 13	
088	ISZI	16 26 46	
089	RCL3	36 03	
090	1	01	
091	+	-55	
092	STO3	35 03	
093	RCLD	36 14	
094	GTOB	22 12	
095	R/S	51	
096	R/S	51	
097	*LBLa	21 16 11	
098	SPC	16-11	
099	DSP2	-63 02	
100	x̄	16 53	
101	PRTX	-14	
102	STO4	35 04	
103	S	16 54	
104	ENT↑	-21	
105	RCL4	36 04	
106	÷	-24	
107	1	01	
108	0	00	
109	0	00	
110	×	-35	
111	PRTX	-14	
112	0	00	

REGISTERS

0	1 Dilution Factor	2 Blank Increment	3 # Data Inputs	4 x̄	5	6	7	8	9
S0	S1	S2	S3	S4 x̄, rsd	S5 x̄, rsd	S6 x̄, rsd	S7 x̄, rsd	S8 x̄, rsd	S9 x̄, rsd

A Stability Factor	B Blank Value	C # Data Inputs	D Final Data Input Value	E Previous Data Input Value	I Counter for Data Inputs

Program Listing

STEP	KEY ENTRY	KEY CODE	COMMENTS
113	STO4	35 04	
114	P⇄S	16-51	
115	RCLS	36 09	
116	PRTX	-14	
117	0	00	
118	ENT↑	-21	
119	STO4	35 04	
120	STO5	35 05	
121	STO6	35 06	
122	STO7	35 07	
123	STO8	35 08	
124	STO9	35 09	
125	P⇄S	16-51	
126	SPC	16-11	
127	SPC	16-11	
128	GTOD	22 14	
129	R/S	51	
130			
140			
150			
160			
170			
180			
190			
200			
210			
220			

LABELS					FLAGS	SET STATUS		
A Data Input Loop	B Data Loop	C Reset for Add. Data	D Initiate Data Loop	E Initiate Program	0	FLAGS (ON OFF)	TRIG	DISP
a $\bar{x}$, rsd	b	c	d	e Manual Data Input	1	0 ☐ ☐	DEG ☐	FIX ☐
		2	3	4	2	1 ☐ ☐	GRAD ☐	SCI ☐
		7	8	9	3	2 ☐ ☐	RAD ☐	ENG ☐
						3 ☐ ☐		n_____

Chapter IV

AMMONIA: COLORIMETRIC AND TITRIMETRIC QUANTITATION

C.G. Daughton, J. Cantor, B.M. Jones, and R.H. Sakaji

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INTRODUCTION

Ammonia gas is soluble in water in a hydrated form, which equilibrates with its protonated form, ammonium ion, as dictated by its pK_a (9.3 at 25 °C). Ammonia and ammonium ion are present in wastewaters from oil shale retorting at concentrations generally greater than several thousand milligrams per liter. The origin of these high concentrations in oil shale process waters has not been elucidated; evidence exists that both organic and mineral assemblages in the raw shale are contributors. Methods for the quantitation of ammoniac species in synfuel wastewaters are necessary for research, treatment monitoring, and establishment of regulatory standards. The method of choice should be sufficiently simple to find applicability in a routine wet-chemistry laboratory. Published methods for determining ammonia in water, however, may be applicable only to the particular sample matrix for which they were developed or tested, and even conventional "standard methods" should not be used for oil shale wastewaters without validation, because these methods often require modification.

In this report, ammonia will be used as a colligative term for both of the ammoniac species. Only three approaches are available to a routine wet-chemistry laboratory for quantitating these species as total ammonia: (1) colorimetry, (2) titrimetry, and (3) direct ammonia-selective electrode; other, less routine methods, include ion chromatography, gas-liquid chromatography, combustion/chemiluminescence, diffusion/UV absorbance, and highly specific enzymatic assays; three of these methods (ammonia electrode, ion chromatography, and gas diffusion) have been evaluated for retort waters by Wallace et al. (October 1982). Methods that employ the routine approaches are numerous, and most of them give excellent results for particular sample matrices.

These three routine methods were extensively evaluated for oil shale wastewaters. Methods for colorimetry and acidimetric titrimetry proved comparable for "accuracy". Although both methods were very reproducible, the titrimetric method, when automated, was superior for precision. Gas-sensing electrodes from two manufacturers proved to be extremely unreliable even though they could possibly provide the fastest and easiest means of quantitation. Ammonia-sensing electrodes generate unstable response curves in oil shale retort waters, probably because their membranes become easily fouled and because surfactants (e.g., fatty acids) increase the membrane permeability to other interfering solutes. One electrode gave extremely stable readings in standard solutions, exhibited severe drift when immersed in oil shale wastewater samples, and subsequently failed to produce stable readings when re-immersed in standard solutions. Changes in the slope of the response curve made frequent recalibration prohibitively time consuming. Erratic response of ammonia electrodes has also been reported by Wallace et al. (1982). Ammonia-sensing electrodes also respond to organic amines (Lopez and Rechnitz 1982). We strongly recommend that ammonia electrodes not be used for these waters, although they may be useful for range-finding to facilitate the subsequent application of other methods. For these reasons, a study was performed for the statistical comparison of only two methods, colorimetry and automated acidimetric titrimetry, on nine oil shale wastewaters.

THEORY

Distillation/Titrimetry

Ammonia quantitation by titrimetry requires the quantitative distillation of the ammonia from an aqueous sample to eliminate interference of other titratable species (e.g., carbonate alkalinity) and color. This is a straightforward method that makes use of the pK_a of the ammonium-ammonia equilibrium. If a sample is maintained at a sufficiently high pH and temperature, the equilibrium is shifted toward free hydrated ammonia, allowing quantitative distillation of the hydrated ammonia as ammonia gas until the remaining ammonium ion has completely dissociated. If the adjusted pH is too high, however, hydrolysis of cyanates or organically bound nitrogen (e.g., urea) may occur and contribute a positive interference; samples are therefore buffered at about pH 9.5 during distillation to retard hydrolysis in the distilland. Volatile alkaline compounds such as amines, ketones, alcohols, and aldehydes also can distill and interfere. The latter three can possibly be eliminated by predistilling at a low pH, discarding the distillate, and then distilling at the high pH.

The distilled ammonia gas is captured in a weakly acidic receiving solution (e.g., boric acid), and the pH of the receiving solution is thereby increased. The two routine methods for quantitating the captured ammonia are colorimetry and acidimetric titrimetry. The former is more involved and is not the preferred finish following distillation. Acidimetric titrimetry simply involves the titration of the receiving solution with a standardized strong acid (e.g., sulfuric acid) until the pH is lowered to equal that of the original receiving solution. The titration endpoint can be most easily detected by measurement with a pH electrode (ASTM 1980) or by color-matching when a pH indicator dye is used.

Endpoint detection by pH electrode. If nondistilled blanks, distilled blanks, and distilled samples are diluted to equal volumes, then the equivalents of standardized acid titrant required for lowering the pH of the distillates to match the value of the nondistilled blanks is identical to the equivalents of ammonia present in the distillates. The endpoint is on the steep portion of the titration curve when boric acid is used to capture the ammonia, and it therefore is easily detected. A complicating factor, however, is the importance of ensuring that the volumes of all samples are also equal after titration. If the final volumes are not equal, then the pH values are no longer comparable. This problem is best avoided by ensuring that the quantity of ammonia in each distillation flask is about the same and that the volumes of the diluted distillates are large when compared with the titrant volumes. The use of a pH electrode to follow the titration is most easily accomplished with an automatic titrator; spurious problems have been encountered, however, with electrode stability in boric acid.

Endpoint detection by pH indicator dye. There are no pH indicators that give sharp endpoints when the equivalency point (approximately pH 4.8) is reached during titration of ammonia in boric acid. Methyl red is the most widely used indicator, and it exhibits little salt error (Stover and Sandin 1931). Protonation of the benzoic azo-nitrogen of methyl red yields the red form in the acid region of the pH range of 4.2 to 6.2; at the basic end of this range, the nonprotonated form is yellow. The titration of distillates that

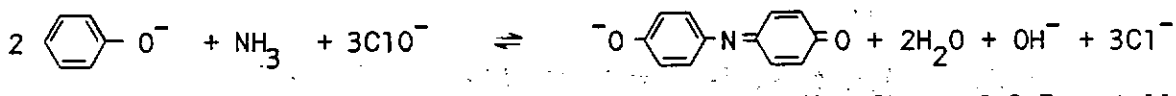
contain ammonia therefore proceeds from the higher end of the range (yellow) to the lower end (red). The intermediate colors of the two forms are gradations of pink, and the endpoint can only be determined by carefully comparing the distillate color with that of a nondistilled blank (Meeker and Wagner 1933). Visual detection of the endpoint can be ameliorated by addition of a blue "enhancer" dye such as methylene blue (Johnson and Green 1930) whose color does not change with pH. The color that results when methylene blue is combined with methyl red is green in basic solutions and violet in acidic solutions. Regardless of whether such an enhancer dye is used, experience and patience are required for accurate and reproducible color-matching titration. This is an inferior endpoint detection method except when the detection is performed by determining the absorbance of the solution and matching it to that of the blank; this is best done by automated colorimetry. The problem of unequal sample volumes during titration, which was noted for endpoint detection by pH electrode, is also applicable to detection using an indicator dye. Furthermore, uniformity of ionic strength and pH of the distillates is important because of their effects on salt error and color intensity of indicator dyes (Stover and Sandin 1931).

Colorimetry

Ammonia quantitation by direct colorimetry (i.e., without distillation) involves the formation of an ammonia reaction-product whose chromophore has a molar absorptivity sufficiently high to alleviate interferences by endogenous chromophores. Routine colorimetric methods include Nesslerization, pyridine/pyrazole, oxidation to nitrite followed by nitrite quantitation, and various adaptations of the indophenol-blue procedure, which is also called the "phenate", "phenolate", "alkaline phenol-hypochlorite", or "Berthelot reaction" methods.

The Nessler reagent can only be used for relatively clean waters and involves the use of mercury. The pyridine/pyrazole method is highly specific for ammonium ion, but it is cumbersome. All of the colorimetric methods usually require distillation of the ammonia if the sample contains interferences. Distillation can be avoided by oxidation of the sample to yield nitrite. The nitrite can then be quantitated by diazotization, which is highly sensitive. The oxidation step, however, is very time consuming. The phenate method, in contrast, is simple to perform, and it was the only rapid colorimetric method that was applicable to the highly complex sample matrix of oil shale wastewater. The phenate method, as described by Weatherburn (1967), was evaluated for direct quantitation of ammonia in several of these waters, and it was statistically compared with the distillation/titrimetric method.

Phenate method. The phenate method is based on the Berthelot color reaction catalyzed by nitroprusside (Scheiner 1976):



Ammonia is converted to monochloramine between pH values of 9.7 and 11.5. Losses to dichloramine, nitrite, and other species occur above pH 11.5. Monochloramine then reacts with phenol in the presence of chlorine to form indophenol blue via intermediates (Hampson 1977) such as quinonechloramine (Ngo et al. 1982).

Interferences to phenate method. Hampson (1977) states that nitrogen from nitroprusside can enter the indophenol blue molecule and cause high blank absorbance values. High blank values were not found to be a problem in the 10- to 1000-mg/L NH_3-N range, but if blank values are found to be too high for determination of low concentrations of ammonia, other catalysts (e.g., ferrocyanide or manganese [II] ion) should be considered. We have found that high background (blank) values will also result from screw caps that have been washed in acid; subsequent contact with water will continually leach a yellow substance (possibly a substituted phenol) from the plastic. It is extremely important that all glassware be scrupulously clean; all soaps should be avoided.

Although Weatherburn (1967) does not discuss interferences with the phenol-hypochlorite reaction, other authors have investigated the subject. Aliphatic amines and nitrite suppress color development significantly when present in excess of the quantity of ammonia in the sample (Hampson 1977). Ngo et al. (1982) tested the effect of 72 compounds (10mM) on the recovery of ammonia (0.5mM). All primary and secondary aliphatic amines strongly inhibit the development of indophenol, possibly by nucleophilic addition to the quinonechloramine intermediate at the ortho position, resulting in steric hindrance; sulfide and thiols also inhibit indophenol formation. Zadarojny, Saxton, and Finger (1973) describe the effects of amino acids, urea, and 24 inorganic electrolytes on the analysis of a 0.2-mg/L NH_3-N solution. Apparent recoveries of 109% to 214% resulted from additions of 100 mg/L of KCN, KSCN, Na_2S, NaF, $CuSO_4$, $CoCl_2$, and NiCl; recoveries of 38% to 93% were reported for additions of $NaNO_2$, $Na_2S_2O_2$, $HgCl_2$, and $AlCl_3$ at 100 mg/L, and of L-cystine, L-methionine, and L-phenylalanine at 0.2 mg/L amino acid-N. Interference was not evident below 5 mg/L of electrolyte.

Gravitz and Gleye (1975) report the formation of an interfering substance having an absorbance maximum at 640 nm when the standards are exposed to sunlight. Standards so affected fail to follow Beer's law with respect to ammonia concentration, and absorbance values for blanks are high. The interfering substance has been tentatively identified as indophenol, formed with nitrogen from the nitrosyl group of the nitroprusside molecule. In our laboratory, the test has been performed routinely under fluorescent lighting, and these problems have not been encountered. If desired, light may be excluded from the reaction mixture by wrapping the test tubes in aluminum foil. This procedure may be helpful for samples with low ammonia concentrations. Use of a vacuum-operated flow-through spectrophotometer cuvette minimizes light exposure during sample transfer. Verdouw, van Echteld, and Dekkers (1978) recommend potassium ferrocyanide as the catalyst for better color stability in light and dark.

It must be emphasized that observations on one variation of the phenate method may not be applicable to another. Presumably, certain interferences (including color) are minimized in Weatherburn's version because of the 500-fold dilution of the sample by the reagents. In our laboratory, this method has yielded results for ammonia concentrations in oil shale process waters statistically comparable to those obtained by distillation/titrimetry, and no color interference has been observed. Distillable bases, especially aliphatic amines, will cause interference for both methods.

PROTOCOL SUMMARY

Detailed laboratory protocols for determining ammonia by the colorimetric phenate and distillation/titrimetric methods are appended. The importance of representative subsampling, especially with regard to loss of ammonia by degassing, cannot be overemphasized. Degassing is especially a problem with oil shale wastewaters because of the high concentrations of ammonia coupled with high pH. Normally, it is best to acidify wastewater samples to pH values below 2.0 prior to storage. With oil shale wastewaters, however, the high alkalinity would necessitate addition of large amounts of acid which then causes other problems such as foaming and precipitation (see: Chapter III, Carbon Analysis).

Acidimetric Titration Method

Samples are diluted to give ammonia-N masses for a subsample in the range of 0.1 to 5 mg. The appropriate volume (generally 5 to 300 mL) is added to an 800-mL Kjeldahl distillation flask that contains an alkaline borate buffer solution; the amounts of ammonia in each flask should be as equal as possible. Upon the addition of sufficient sodium hydroxide to maintain the pH of the solution at about 9.5, the flask is immediately connected to a distillation/condensation apparatus. Immediate connection is important to avoid loss of ammonia to the headspace; losses can be further minimized by carefully adding the dense caustic down the inside of the flask so that it underlays the sample solution. The flask contents (about 300 mL) are thoroughly mixed after connection to a spray-trap and condenser, the contents are heated to a boil, and the first 100 mL of distillate is collected by bubbling it through 50 mL of a 4% boric acid solution. The quantity of boric acid in the receiving solution is important for good precision; it is most conveniently measured with a tilting dispenser, although highest precision is obtained with a volumetric pipette or repipette. Mixed indicator/dye (methyl red indicator and methylene blue dye) is added to the boric acid receiving solution if an indicator endpoint is followed, but it is omitted if the endpoint is determined with a pH electrode. This acidic solution ensures quantitative capture of the ammonia gas as nonvolatile ammonium ion, with a concomitant stoichiometric rise in pH. Although over 90% of the ammonia is distilled in the first couple of minutes, the distillation is continued until over 100 mL of distillate is collected.

The distillates are diluted to equivalent final volumes with water and titrated with standardized sulfuric acid. When mixed indicator/dye is used in the receiving solution, the distillates that contain sufficient ammonia are initially bluish-green. During titration, the color proceeds through a transition point of dirty gray followed by gradations of more intense shades of violet. Storage of the boric acid solution in glass containers should be avoided since alkali from the glass has been suggested as a cause of slowly rising pH, making the boric acid unusable (Meeker and Wagner 1933). It is extremely important that the spray-traps and condenser tubes be steamed clean before and after each series of distillations; otherwise, deposits tend to accumulate in the condenser tubes.

Detection is not very accurate or precise when performed manually. Automation can be easily accomplished, however, with an automatic titrator interfaced with either a pH electrode or colorimeter. The distilled samples are then titrated to either the pH of the nondistilled blanks or to the absorbance of the nondistilled blanks (at about 545 nm) if indicator dye is used.

Colorimetric Phenate Method

Samples are diluted to give ammonia-N concentrations in the ranges of 10 to 100 mg/L or 100 to 1000 mg/L. If the samples have been acidified, the pH values must be raised and made consistent. Five milliliters of phenol/nitroprusside reagent (5 g phenol and 25 mg nitroferricyanide dihydrate diluted to 500 mL with water) is added per tube to 20- X 150-mm Pyrex culture tubes with Teflon-lined screw caps. Samples or standards (prepared from ammonium sulfate) are withdrawn with 20-µL glass capillaries which are calibrated "to-contain" (end-to-end). The entire capillary is carefully added to the reagent mixture, and the tube is sealed and shaken vigorously. Immediately after adding 5 mL of alkaline hypochlorite solution (2.5 g sodium hydroxide and 4.2 mL of 5% sodium hypochlorite diluted to 500 mL), the tubes are resealed, shaken vigorously, and immersed in a 37°C bath for 20 minutes. The tubes are cooled to room temperature and the samples' absorbance values are read versus a reagent blank at 635 nm or 520 nm for low or high concentration ranges, respectively, using a spectrophotometer equipped with a 1-cm path length micro flow-through cell.

The use of glass capillaries (e.g., Drummond Microcaps) can be simplified by not using the dispenser that is usually supplied by the manufacturer. Standards or samples should be prepared in small wide-mouthed containers, such as scintillation vials. The capillary should be grasped near its top with forceps, placed in the container, and leaned against the rim. When the container is tilted, the capillary becomes filled as it approaches the horizontal. Adequate headspace must be left in the container to avoid spilling the sample, but it is imperative to work quickly because degassing of ammonia could be extensive in samples of high pH such as oil shale wastewaters. The capillary is removed from the container with forceps and kept horizontal. This ensures that the capillary remains filled from end to end. Excess liquid on the outer surface of the capillary should be removed by gently scraping its entire length against the rim of the container. The capillary should not be tilted from the horizontal until this step is completed. Tilting the capillary will result in the formation at its lower end of a drop of liquid that will be lost during the scraping step. Relative standard deviation values (rsd's) of less than 1.5% can be easily achieved using this method.

The use of capillaries can be avoided by combining the sampling step with the alkaline/hypochlorite addition step with the aid of an automatic dilutor-pipettor (e.g., Brinkmann Instruments, Inc., Digital Dilutor 9200). The sample is drawn into the Teflon tip on the pipetting mode and and then expelled on the dilution mode with the alkaline/hypochlorite reagent into the phenol/nitroprusside reagent. This method is highly accurate, precise, and rapid.

The colorimetric method has been evaluated in our laboratory using only Drummond Microcaps. It is not known whether the use of other brands of capillary pipettes having different dimensions would necessitate variations in operator technique to achieve good reproducibility.

A spectrophotometer cell with a 1-cm path length has been used in the protocol reported here. A longer path length may be required for determination of lower ammonia concentrations. The lower detection limit for the phenate method as adapted for oil shale process wastewaters is about 10 mg/L. This limit could be reduced only by increasing the sample size, which would in turn

increase the background. For the determination of ammonia concentrations less than 10 mg/L, other methods are available (see: Jenkins 1977), including several other variations of the phenate method.

RESULTS AND DISCUSSION: Comparison of Colorimetry and Titrimetry

Ammonia concentrations in nine oil shale wastewaters were determined by the phenate colorimetric method for homologous unfiltered and filtered (0.4-μm pore-diameter polycarbonate membranes, Bio-Rad Laboratories, Richmond, CA) samples (Table I). The phenate ammonia values for unfiltered waters were no more than 5% higher than the values for filtered waters, with the exception of TOSCO HSP process water, and Oxy-6 and 150-Ton retort waters. Since only slight differences were observed between filtered and unfiltered samples, the time-consuming pressure filtration step was eliminated; particulates apparently did not interfere with the absorbance of the samples nor did they release interfering compounds.

Ammonia concentrations determined by newly automated versions of distillation/titrimetry (using the basic method of APHA [1976]) and colorimetry (using the method of Weatherburn 1967) for nine oil shale process wastewaters are presented in Table II. Also presented are results from a "composite" water, which comprised equal volumes of each of the nine waters. Each sample was diluted to give an ammonia-N concentration of about 500 mg/L to minimize differences between waters for subsequent statistical analysis, and five replicate subsamples were analyzed by each method.

The average ammonia-N values for the nine waters were 6536 and 6286 mg/L for the titrimetric and colorimetric methods, respectively, compared with 6564 and 6225 mg/L for the Composite water (Table II); both values for the Composite water were within 1% of the respective averages. For all but one of the waters (Oxy-6 gas condensate), the titrimetric values were 1% to 7% higher than the respective colorimetric values (Table II). To determine if a significant difference existed between the two methods for the determination of ammonia in this series of oil shale wastewaters, a two-way analysis of variance (anova) was conducted on reciprocal-transformed replicate data ($n = 5$). For the variability between methods, the calculated F-value was less than 1.0, which was less than the critical F-value of 3.92 at $\alpha = 0.05$. Therefore, there was no significant difference ($P>0.05$) between the colorimetric and titrimetric methods for determining ammonia in these wastewaters.

The precision for both methods was excellent (Table II). The rsd values for titrimetry ranged from 0.08% to 1.3%; those for colorimetry ranged from 0.7% to 1.4%, with the exception of the Composite water (4.0%). The seemingly better precision of the titrimetric method was probably a result of automation of the titrimetric step.

The question of accuracy is more difficult to address. Although the two methods employed entirely different approaches and achieved comparable ammonia concentrations for each of the waters, they are subject to similar interferences (e.g., distillable amines). Accuracy was partly addressed by running standard additions recovery series on three of the waters: Paraho, Composite, and Oxy-6 gas condensate. Each water was diluted to yield a concentration of about 500 mg/L ammonia-N, and then an equal portion of one of each of four ammonium sulfate standards was added to duplicate samples: 100, 300, 600, and 800 mg/L.

The least-squares linear regression lines of total ammonia-N recovered versus ammonia-N added to each spiked sample yielded the data shown in Table III. In general, the recoveries of ammonia spikes from these three waters were greater than 100% for the titrimetric method and lower than 100% for the colorimetric method. If these recoveries were used to correct the apparent concentrations in Table II, the difference between the two methods would be even less. An EPA quality control nutrient sample that contained 1.52 mg/L NH_3-N was also analyzed; since this concentration was below the detection limit for the colorimetric method, the sample size was increased from 0.020 to 1.00 mL. Recoveries were 102.0% (rsd = 3.2%) and 104.6% (rsd = 1.1%) for the titrimetric and colorimetric methods, respectively.

Mean concentrations determined on different days for a sample type were found to vary because subsamples were obtained from different lots and because of degassing during storage; this was the cause of the discrepancies between the two sets of colorimetric data in Tables I and II. Degassing is probably a major cause of problems in interlaboratory comparisons. Interlaboratory comparison results reported by Fox, Farrier, and Poulson (1978) for Omega-9 retort water gave NH_3-N concentrations that ranged from 2650 to 3457 mg/L for different methods. Wallace (1982) reported values from different methods of 3300 to 3600 mg/L for Omega-9. The values reported here for Omega-9 were 3551 to 3690 mg/L. Interlaboratory comparison values for other waters are not available.

The alkalinity of these waters apparently does not interfere with the colorimetric method. Alkalinity concentrations greater than 500 mg/L, however, do interfere with the standard phenate method, in which the sample is diluted very little by the reagents (APHA 1976, p 466). A major advantage of the phenate method is that of sample throughput. In our laboratory, an analyst can perform over 90 determinations per day (excluding sample preparation) versus 20 for the titrimetric method. This number can be processed in three batches of 30 samples each. The rate of color development at ambient temperature is sufficiently great that when more than about 10 samples are being prepared, considerable color-development for the samples prepared earliest will have occurred by the time the entire batch is ready to be immersed in the warm water bath. Studies to determine whether this affects final absorbance values have been inconclusive, but as a precaution, premature development may be prevented by maintaining the reaction mixtures at 0°C during preparation. Cooling may also increase the time allowable for sample preparation, as reagent stability limits the number of samples that may be prepared in one batch. Tubes need not be cooled until the sample is added to the phenol/nitroprusside reagent and the tubes shaken; this will minimize condensation of atmospheric moisture in the test tubes, which could measurably dilute the samples.

The success of the colorimetric method when applied directly to retort waters results from its low inherent minimum detection limit (i.e., 10 ppb); this allows for extensive dilution of the sample by the reagents, which effectively eliminates interference by endogenous chromophores. The disadvantage of any of the colorimetric methods is that several range-finding dilutions may be required to bring the absorbance of an unknown sample within the standard-curve range. Precision and accuracy are sacrificed when dilutions are performed, but more significantly, the large, exposed surface-to-volume ratio of samples contained in measuring devices (e.g., pipettes, syringes) exacerbates the loss of ammonia by degassing; this problem is magnified with the necessity of measuring small volumes and by the transient headspace pressure

reduction during sample withdrawal. We have found that the most satisfactory solution to this problem is the use of to-contain glass capillaries for accurate volume measurement while sampling or the use of a dilutor-pipettor for simultaneous sample measurement and reagent delivery. If filtration of samples is required, vacuum filtration should never be used, as extensive degassing will occur. Range-finding is also required for the titrimetric method; the appropriate normality of the titrant must be determined for each unknown, or the unknown must be appropriately diluted before distillation. This problem can be alleviated by using an autotitrator that is capable of delivering microliter-volumes of titrant. Automation of the colorimetric method would be possible by segmented-flow or flow-injection analysis.

Several problems were encountered with the distillation/condensation apparatus that was used in this study. The spray traps had been connected to the condenser tubes with silicone-rubber tubing and fitted with silicone rubber stoppers for the Kjeldahl flasks. The extreme permeability of silicone rubber to gases (Brookes 1969; also see Chapter II) necessitates that all connections be made with neoprene rubber and Tygon or Teflon tubing. Another, more serious problem was the inability to recover more than 89% of the ammonia from numerous replicates of standard solutions, and rsd values always exceeded 8%. The cause of this incomplete recovery was finally traced to the scavenging of ammonia by the block-tin condenser tubes. Whether these losses were caused by absorption into the pitted inner surfaces of the tin or by formation of insoluble complexes with volatile compounds from wastewater samples that had precipitated onto the tin from previous use is not clear. The problem was solved by replacing the condenser tubes with heavy-walled Pyrex tubing.

The use of tubular microporous polytetrafluoroethene membranes for the separation of ammonia from aqueous matrices is described in Chapter II. This nonosmotic dissolved-gas dialysis technique could be used for the direct determination of ammonia in the dialysate. Such an approach has been used by others (see Chapter II) and should be evaluated for oil shale process waters. Once the ammonia is separated from the organic nitrogen, it can be rapidly determined as total nitrogen by combustion/chemiluminescence (see: Chapter V).

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Table I. Comparison of Colorimetric Ammonia Determinations on Filtered and Unfiltered Oil Shale Wastewaters[1]

	Filtered		Unfiltered		
Process Water	mean	rsd (%)	mean	rsd (%)	(% diff)[2]
Paraho	23 750	0.7	24 655	0.7	3.7
150-Ton	11 180	1.1	10 516	1.1	-6.3
Oxy-6 gas condensate	6 933	1.0	6 994	0.7	0.9
S-55	4 005	2.0	4 188	1.5	4.4
Omega-9	3 551	4.9	3 638	1.4	2.4
TOSCO HSP	2 346	6.9	2 097[3]	1.5	-11.9
Geokinetics	1 505	1.8	1 589	0.6	5.3
Oxy-6 retort water	1 136	0.9	1 117	0.9	-1.7
Rio Blanco sour water	1 032	1.1	1 061	1.1	2.7

1 mg/L NH_3-N; n=10 for each sample

2 (colorimetric unfiltered mean) - (colorimetric filtered mean) divided by (colorimetric unfiltered mean)

3 second operator determination

Table II. Comparison of Colorimetric and Titrimetric Methods for Determining Ammonia in Unfiltered Oil Shale Wastewaters[1]

	Titrimetric		Colorimetric	
Process Water	mean	rsd (%)	mean	rsd (%)
Paraho	26 385	1.3	24 689	1.0
150-Ton	10 838	1.3	10 662	0.7
Oxy-6 gas condensate	7 157	0.1	7 202	0.7
Composite	6 564	0.5	6 225	4.0
S-55	4 079	0.08	4 047	1.4
Omega-9	3 690	0.4	3 583	1.1
TOSCO HSP	2 401	0.1	2 292	1.2
Geokinetics	1 991	1.0	1 905	0.7
Oxy-6 retort water	1 161	0.5	1 127	0.9
Rio Blanco sour water	1 118	0.3	1 065	0.9
average[2]	6 536		6 286	

[1] mg/L NH_3-N; n=5 for each sample

[2] mean for nine waters, excluding Composite

Table III. Standard Additions Results: Ammonium Sulfate in Oil Shale Process Waters[1]

	Colorimetric					Titrimetric				
Process Water	m	b	r^2	zero spike[2]	percent recovery[3]	m	b	r^2	zero spike[2]	percent recovery[3]
Paraho	0.967	484	1.000	485	96.9	1.065	543	1.000	537	105.3
Composite	0.987	490	1.000	504	101.5	1.037	534	0.998	532	103.3
Oxy-6 gas condensate	0.964	478	1.000	1484	97.6	1.040	518	0.999	514	103.2

1 ammonia-N spike levels of 100, 300, 600, and 800 mg/L (n = 2)
2 ammonia concentration of diluted sample (i.e., without spike)
3 (zero spike) divided by b/m X 100

m = regression coefficient (slope)
b = y-intercept
r^2 = coefficient of determination

PROTOCOL: PHENATE/COLORIMETRY

I. Apparatus

A. Glassware (one each per sample)

1. 20- X 150-mm or 16- X 150-mm culture tube with Teflon-lined screw cap; Parafilm "M" may be used instead of screw caps. The culture tubes should be soaked in 35% nitric acid for one day prior to use. They should then be rinsed thoroughly in ASTM Type I water and dried in a convection oven. The screw caps should never be soap washed or contacted with acid; they should be thoroughly rinsed with ASTM Type I water and air dried. Do not use screw-capped tubes with chipped rims, as they will leak when shaken.
2. Drummond Microcap 20-μL capillary (Drummond Scientific Co., Broomall, PA); substitutes <u>must</u> be calibrated "to contain".

B. Repetitive dispensing device (e.g., Brinkmann Instruments Digital Dilutor 9200) or automatic pipette (e.g., 5-mL Gilson Pipetman, Rainin Instrument Co., Emeryville, CA; or 10-mL Brinkmann macro-Transferpettor).

C. Water bath with heater (Haake D-1, Haake Inc., Saddle Brook, NJ).

D. Forceps, preferably flexible.

II. Reagents

NOTE: All reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.

A. Phenol/Nitroprusside Solution: dissolve 5 g phenol and 25 mg sodium nitroferricyanide (nitroprusside) dihydrate [$Na_2Fe(CN)_5NO \cdot 2H_2O$] in water in a 500-mL volumetric flask, and bring to volume with water. Store in amber or foil-covered bottle (4°C). Discard after one month.

B. Alkaline Hypochlorite Solution: dissolve 2.5 g sodium hydroxide in water in a 500-mL volumetric flask, add 4.2 mL of 5% sodium hypochlorite solution (J.T. Baker or equivalent), and bring to volume with water. Store in amber or foil-covered bottle at 4°C. Discard after one month or when high blank values or change in slope of calibration curve are observed.

C. Ammonia-Nitrogen Standard Stock Solution (1000 mg/L as N): dissolve 0.4717 g ammonium sulfate in water and dilute to 100 mL, or use Hellige standard solution R-2434 (1 mL = 1 mg N) (Hellige, Inc., Garden City, NY) or equivalent. Store at 4 °C.

III. Protocol

A. Dilute sample concentration to within a 10- to 100-mg/L or 1000- to 1000-mg/L NH_3-N range.

B. Prepare working standards.

1. For the 10- to 100-mg/L and 100- to 1000-mg/L ranges, add the following quantities of the Standard (II.C) and water to a suitable container (e.g., scintillation vials):

working standard (mg-N/L)	standard (mL)	water (mL)
blank	0.00	10.00
10.0	0.100	9.90
20.0	0.200	9.80
50.0	0.500	9.50
100	1.000	9.00
200	2.000	8.00
500	5.000	5.00
1000	10.00	0.00

C. Place tubes for reagent blank, standards and samples in test tube rack. Samples and standards should be prepared and developed together.

D. Adjust water-bath temperature to 37°C.

E. Add 5 mL phenol/nitroprusside solution to each tube; an automatic pipette or repipette is recommended for convenience.

F. Fill capillary (held in forceps) with sample or standard and drop into tube containing phenol/nitroprusside solution without contacting rim of tube.

NOTE: See "Protocol Summary: Colorimetric Phenate Method" for discussion on filling capillaries.

1. seal tube **immediately** and shake vigorously about 10 times. (No more than 1/2 hour should be allowed to elapse before immersion of all samples in the water bath; the number of samples that can be prepared in this interval will depend on the operator's speed). Repeat for all tubes.

NOTE: Breakage of the capillary during shaking is inconsequential.

G. Immediately after adding 5 mL alkaline hypochlorite solution to each tube, replace cap and shake vigorously.

NOTE: Vortex mixing is inadequate to flush the capillaries.

H. Immerse test tube rack in water bath for 20 minutes.

I. Cool to approximately room temperature with cold tap water and promptly read absorbance of each sample against a water blank (to allow detection of high reagent blanks) at 635 nm (for 10- to 100-mg/L NH_3-N range) or at 520 nm (for 100- to 1000-mg/L range).

NOTE: In our laboratory, absorbance of blanks is usually less than 0.015 AU at 520 nm.

IV. Waste Disposal

A. Ensure that the contents of the assay tubes are separated from the capillaries before disposal.

V. Data Reduction

A. Subtract absorbance of the blank from that of each standard or sample. Calculate least-squares regression equation for standards. Over the ranges of 10 to 100 mg/L and 100 to 1000 mg/L NH_3-N, response should be sufficiently linear to allow use of the regression equation for determining sample concentrations (routinely r^2 = 0.999+). Between 1000 and 3000 mg/L, the curve is usable, though non-linear, and curve fitting methods are recommended. Multiply result by appropriate dilution factor.

Example:

	A_{520}	blank subtracted
Blank	0.008	----
100 mg NH_3-N/L	0.064	0.056
200	0.122	0.114
500	0.316	0.308
1000	0.628	0.620

NH_3-N (mg/L) = 1589(A_{520}) + 14 (r^2 = 0.999+)

sample absorbance	blank subtracted	NH_3-N (mg/L) calculated from regression equation
0.472	0.464	751
0.464	0.456	739
0.469	0.461	747

Protocol prepared by: J. Cantor, B.M. Jones, R.H. Sakaji, and C.G. Daughton

PROTOCOL: DISTILLATION/TITRIMETRY

I. Apparatus
   A. Glassware (one each per sample)
      1. 500-mL Kjeldahl flask (discard any Kjeldahl flask with star-shaped cracks)
      2. 250-mL Erlenmeyer flask (or sampling cup for autotitrator); the masses of these containers should be within several grams of each other
   B. 50-mL tilting dispenser
   C. Volumetric flasks (250-mL, 1-L; Class A)
   D. 100-mL graduated cylinder (TD)
   E. Magnetic stirring bar (one each per sample for manual titration)
   F. Top-loading electronic balance (e.g., Mettler PC 2000)
   G. pH meter with pH probes (e.g., Corning model 135); for detection of pH endpoint during manual titration
   H. Titration equipment
      1. Detection option 1 (automatic titration): Sybron/Brinkmann (Westbury, NY) Autotitrator (Metrohm model 655 Dosimat, E 526 titrator, 643 control unit/624 auto sampler), and appropriate electronic burette (e.g., 10- or 20-mL).
         a. pH endpoint: combination pH electrode (Metrohm EA 157), or
         b. indicator endpoint: submersible colorimeter probe with 1-cm path length, 545-nm filter, and colorimeter (Brinkmann PC 800).
      2. Detection option 2 (manual titration): 50-mL precision-bore burette, or Metrohm model 655 Dosimat with push-button controller.
   I. Kjeldahl distillation apparatus (e.g., 12-unit Labconco combination digestion/distillation); it is highly recommended that Pyrex condenser tubes with Teflon ferrules and fittings be used rather than the standard block-tin condenser tubes.
   J. Zetex insulated gloves

   IMPORTANT: The Kjeldahl distillation unit must <u>never</u> be plumbed with silicone rubber stoppers or tubing because of the permeability of this rubber to ammonia gas; neoprene Kjeldahl stoppers are highly recommended. It is also very important to ensure that extraneous ammonia vapors are not present in the lab.

   CAUTION: Safety face-shields or glasses with side-shields should be worn while performing this assay.

II. Reagents

   NOTE: All reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.
   A. Borate Buffer Solution: add 88 mL of 0.1N NaOH (prepared from Hellige standard R-1226C) to approximately 500 mL of 0.025M sodium tetraborate solution (5.0 g $Na_2B_4O_7$ or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ per L) in a 1-L volumetric flask, and bring to volume with water; store in polyethylene or polypropylene.
   B. Mixed-Indicator Solution (for titration to indicator endpoint): dissolve 300 mg of methyl red (acid) indicator and 200 mg of methylene blue in 250 mL of 95% ethanol. Fresh indicator should be prepared each month.

C. Boric Acid Receiving Solution:
   1. pH endpoint: dissolve 40 g of H_3BO_3 in water in a 1-L volumetric flask and bring to volume with water; store in polyethylene or polypropylene.
   2. Indicator endpoint: indicator/boric acid receiving solution. Add 10 mL of indicator (II.B.) before bringing boric acid receiving solution (II.C.1.) to volume.

D. 0.02N H_2SO_4 Titrant: either use commercially prepared H_2SO_4 concentrate (e.g., Hellige R-1241C), or prepare a 0.10N H_2SO_4 stock solution (e.g., from Hellige R-1238C), and dilute 200 mL (quantitatively transferred from 200-mL volumetric flask) of this solution to 1 L with water.

E. Phenolphthalein Indicator: dissolve 0.5 g phenolphthalein in 50 mL of 95% ethanol, add 50 mL of water, and mix.

F. Sodium Hydroxide Solution (6N): use commercially prepared solution (e.g. 50% NaOH, J.T. Baker Chemical Co., Phillipsburg, NJ), or carefully dissolve 240 g NaOH in 800 mL of water (1-L volumetric flask), and bring to volume; store in screw-capped polypropylene bottle.

III. Protocol

A. Preparation of apparatus and glassware
   1. Steam apparatus clean; prior to each series of analyses, place 300 to 400 mL water and 2 or 3 boiling chips or glass beads in each Kjeldahl flask and attach to distillation apparatus. With cooling water off, distill approximately 150 mL; collect distillate in any convenient container and discard.
   2. All glassware should be soap-washed followed by acid washing in 35% nitric acid. Rinse with ASTM Type III water.

B. Receiving flasks: add 50.0 mL of boric acid solution (tilting dispenser) into each 250-mL Erlenmeyer flask (or preweighed sampling cup) and place on Kjeldahl rack so that the distillation effluent tube-tips are completely submerged in the solution; use boric acid solution for pH endpoint or indicator/boric acid solution for indicator endpoint.

C. Samples: place boiling chips or glass beads and appropriate volume of sample in Kjeldahl flask. Sample volume may be adjusted to optimize titrant volume based on expected sample concentration; a 5-mL sample containing 1000 mg/L ammonia-N should require 17.86 mL of 0.02N H_2SO_4 titrant; the mass of ammonia-nitrogen added to the sample flask should not exceed 5 mg, nor should it be lower than 0.1 mg. The quantities of ammonia in each flask should be equalized as best as possible.
   1. Add water to bring the total volume to approximately 300 mL; add 2 drops of phenolphthalein and 10 mL of borate buffer solution (automatic pipette recommended for convenience, but the volume is not critical).
   2. Randomize flasks by consulting the "Ten Thousand Random Digit" Table; (Table "O" in Statistical Tables, F.J. Rohlf and R.R. Sokal, W.H. Freeman and Co.: San Francisco, CA; 1969, pp 152-156).
      a. randomly select a group of five digits from the table.
      b. pick any two adjacent digits within the group of five.

c. for two-digit random numbers, begin with the pair selected, move horizontally, and record the next two consecutive digits; continue this process in a consistent manner until two-digit numbers are generated for each flask.
d. record the random numbers and the corresponding sample number.
e. relabel each flask with its assigned random number.

3. Slowly add several drops of NaOH solution down the inner side of each Kjeldahl flask so that it layers beneath the aqueous sample, and immediately connect to distillation/condenser apparatus. Place flasks on distillation unit in ascending order of assigned random numbers. Ensure that the stoppers are firmly seated. Swirl the flask contents to mix.

D. Standards: use Hellige 1000-mg/L ammonia-N standard R-2434 or equivalent (e.g., ammonium sulfate) and prepare as in III.C.

E. Blank (distillation): omit sample (substitute water) and prepare the flask contents as in III.C.

F. Blanks (nondistilled): prepare three as in III.B. using the Boric Acid Receiving Solution prepared in II.C.; these will be used to determine either the pH endpoint (IV.C.2.i.) or the colorimetric indicator endpoint (IV.B.2.i.). Allow these blanks to sit under the same conditions as the receiving flasks (i.e., exposed to ambient air), but do not place under the distillation tubes.

G. Distillation

1. Turn on condenser cooling water and set heating mantles to maximum temperature. After samples begin to boil, reduce heat as necessary to control boiling and foaming.
2. Collect approximately 100 mL of distillate. Though considerable variation in the volume collected is allowed, the distillate volumes must be equalized before titration. Distillation must not progress too long because the distilland can become concentrated and viscous; this can lead to explosive bumping over into the condenser tubes.

NOTE: Ensure that the distilland retains a pinkish hue during distillation.

3. Upon completion of distillation, remove the effluent tubes from the receiving-container solutions, and rest the tubes on the flask rims to prevent sample drawback during cooling. Turn off heat and continue collection of distillate until distillation ceases.
4. Place each receiving vessel on the balance, and add water until the contents of each is within one gram in weight (e.g., 200 g); alternatively, dilute all samples to equivalent volumes (e.g., 200 mL).

H. Manual Titration (alternatively, see IV: Automatic Titration)

1. Endpoint detection by pH indicator
   a. add stirring bar to each flask
   b. if the indicator/boric acid receiving solution (II.C.2.) was not used, add 0.2 mL of mixed-indicator solution (II.B.) to each 200-mL distillate and nondistilled blank.
   c. titrate with 0.02N H_2SO_4 titrant until the color matches that of the nondistilled blanks. Samples containing sufficient ammonia will begin bright green and progress through darker shades of blue-green until the grayish transition point is reached; the sample will then gradually develop more intense

hues of violet. The blank should require no more than one drop of titrant.

NOTE: The endpoint is not sharp.

2. Endpoint detection by pH electrode

NOTE: Do not use indicator in the receiving container solutions.

a. add stirring bar to each flask.
b. determine the average pH of the three nondistilled blanks prepared in III.F.; this value is approximately 4.8 if the samples have been diluted to 200 mL.
c. titrate samples with 0.02N H_2SO_4 titrant until the pH of the blanks is reached.

IV. Automatic Titration (instructions for Sybron/Brinkmann Metrohm Autotitrator)

A. Setup

1. Fill rinse-water reservoir with water and plug solenoid into outlet "J" on the back of the 643 control unit.
2. Install burette containing 0.02N H_2SO_4.
3. Connect HP 97S calculator to external power supply and connect Amphenol connector to 643 control unit.
4. Switch 643 control unit to "manual".
5. Turn on the following units in sequence: titrator E 526, Dosimat 655, control unit 643, and calculator.

B. Indicator Endpoint Calibration (alternatively, see IV.C.: pH Enpoint Calibration)

1. E 526 titrator:
   a. plug shorting strap into the reference and indicator electrode inputs
   b. set: thumbwheels to 0000, calibration knob to ''calibr", selector knob to +mV
   c. turn "U" knob until analog meter is nulled

   NOTE: It is important that the setting of the "U" knob not be touched once it is set.

   d. disconnect shorting strap
2. PC 800 Colorimeter:
   a. connect colorimeter leads; black to reference electrode input and red to indicator electrode input.
   b. install 545-nm filter and turn colorimeter power switch to %T; allow at least 5 minutes for warm-up.

   WARNING: NEVER turn on the colorimeter without a filter in the filter block.

   c. ensure that the colorimeter probe tip is the appropriate path length.
   d. adjust colorimeter to 100% T with coarse/fine knobs.
   e. thoroughly rinse colorimeter probe.
   f. disconnect mixer from 643 control unit and connect to 655 Dosimat; turn mixer on and adjust by turning the knob on top of the mixer counterclockwise. Avoid vortex formation to prevent air bubbles from becoming entrapped in the colorimeter probe tip.
   g. use sample cup that contains the most ammonia (i.e., brightest green) to set 100% T on colorimeter.
   h. determine transmittance (545 nm) of three nondistilled blanks from III.F.

i. average these transmittance values, and set titrator thumbwheels to match (e.g., for an average transmittance value of 40.2%, the thumbwheels are set to read 0402); this setting will be about 0400-0440.
j. set calibration knob to first -mV setting.

3. Proceed to IV.D.

C. pH Endpoint Calibration

NOTE: Do not use indicator in the receiving boric acid solutions.

1. E 526 Titrator:
   a. set selector knob to "pH" and calibration knob to "calibr".
   b. connect pH electrode lead to indicator electrode inlet jack of E 526 titrator.
2. pH Calibration:
   a. open filler port on pH electrode.
   b. disconnect mixer from 643 control unit and connect to 655 Dosimat; turn mixer on and adjust by turning the knob on top of the mixer counterclockwise.
   c. set thumbwheels to 0700, raise sample cup containing pH 7.00 buffer, turn mixer on, and null analog meter with "U" knob.
   d. lower sample cup, stop mixer, rotate table, and rinse electrode.
   e. set thumbwheels to 0401 and raise sample cup containing pH 4.01 buffer.
   f. null meter using the slope correction knob (located with the temperature knob on the far right of the control panel; slope correction is the inner knob).
   g. set calibration knob to "pH 0" and turn mixer on.
   h. lower sample cup, turn off mixer, and rinse electrode.
   i. set thumbwheels to match the average pH of the three nondistilled blanks (III.F.); this setting will be about 0482, which corresponds to a pH of 4.82.
3. Proceed to IV.D.

D. Operation

1. Reconnect the mixer to the 643 control unit and rotate mixer knob to the "on" position. Adjustment can be made once the control unit is engaged (IV.D.9.).
2. Close control panel cover.
3. Ensure that the turntable surface is clean.
4. Place samples on turntable and record their positions; the 624 control unit only holds 10 samples and the calculator program can only accommodate 10 samples before it must be restarted.
5. Load "Basic Titration Program" (Chapter IV, Appendix A) and depress "E" button to initiate.
6. Set titration rate knob on 655 Dosimat between "5" and "6" (for 10-mL burette) or "2" and "3" (for 20-mL burette).
   a. if samples contain low concentrations of ammonia, then the speed must be accordingly decreased to prevent overtitration.
7. Set 643 control unit to "auto".
8. Ensure that the titrator delivery-line diffuser is installed.
9. Press "Start" switch on 643 control unit.

NOTE: The titration sequence can be stopped between samples by switching the 643 control unit to "stop"; to restart, depress the calculator "E" button.

E. Shutdown: reverse order of sequence at IV.A.5.

V. Data Reduction

$$\text{ammonia nitrogen (mg/L)} = \frac{\text{sample titrant volume (mL)} - \text{blank titrant volume (mL)} \times \text{normality of acid (meq/mL)} \times 1000\ \text{mL/L} \times 14\ \text{mg/meq}}{\text{sample volume (mL)}}$$

Protocol prepared by: C.G. Daughton, G. Harris, B.M. Jones, and R.H. Sakaji

1 BASIC TITRATION PROGRAM 2

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS		OUTPUT DATA/UNITS
1	LOAD PROGRAM: Calculator in RUN mode, PRINT in				
	manual position.				
2	PRESS "E": Program is initiated and will wait			E	
	for input signal from autotitrator.				
	NOTE: Calculator records sample position				
	(1-10) and volume of titrant used for each of				
	10 samples.				

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLE	21 15	
002	CF0	16 22 00	
003	CF1	16 22 01	
004	0	00	
005	STOI	35 46	
006	RTN	24	
007	R/S	51	
008	*LBLA	21 11	
009	CF3	16 22 03	
010	STOA	35 11	
011	ISZI	16 26 46	
012	RCLI	36 46	
013	DSP0	-63 00	
014	PRTX	-14	
015	1	01	
016	0	00	
017	X=Y?	16-33	
018	GSBE	23 15	
019	RCLA	36 11	
020	1	01	
021	0	00	
022	÷	-24	
023	FRC	16 44	
024	1	01	
025	0	00	
026	×	-35	
027	10^x	16 33	
028	RCLA	36 11	
029	1	01	
030	0	00	
031	0	00	
032	÷	-24	
033	INT	16 34	
034	X⇄Y	-41	
035	÷	-24	
036	DSP2	-63 02	
037	PRTX	-14	
038	SFC	16-11	
039	GSB1	23 01	
040	R/S	51	
041	*LBL1	21 01	
042	RTN	24	
043	R/S	51	

STEP	KEY ENTRY	KEY CODE	COMMENTS
060			
070			
080			
090			
100			
050			

LABELS

A START DATA COLLECTION	B	C	D	E INITIATE PROGRAM
a	b	c	d	e
0	1	2	3	4
5	6	7	8	9

REGISTERS

0	1	2	3	4	5	6	7	8
S0	S1	S2	S3	S4	S5	S6	S7	S8

S9

A DATA INPUT	B	C	D	E	I COUNTER FOR # SAMPLES

Chapter V

NITROGEN: KJELDAHL AND COMBUSTION/CHEMILUMINESCENCE

B.M. Jones, G.J. Harris, and C.G. Daughton

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ABSTRACT

The quantitation of total nitrogen in a heterogeneous mixture of organic and inorganic nitrogenous compounds requires the conversion of each species to a common detectable unit (e.g., N_2 or NH_3). This usually involves either wet-chemical digestion or combustion followed by catalytic conversion of the combustion products to the detectable species. Detection methods can be physical, chemical, or electrical. For aqueous samples, the quantitation of total nitrogen has generally been done by the laborious wet-chemical Kjeldahl method. The sample is digested in H_2SO_4 to release bound nitrogen as NH_3, which, together with endogenous NH_3, is separated from the sample by distillation and quantified by either acidimetric titrimetry or colorimetry. Within the last decade, a new approach for determining total nitrogen has been developed that relies on combustion followed by chemiluminescent detection of an electronically excited, oxidized nitrogen species.

Combustion/chemiluminescence (C/CL) is an extremely rapid and reliable method for <u>total</u> nitrogen analysis, but a rapid method still does not exist for quantifying <u>organic</u> nitrogen. Determination of organic nitrogen in biological and agricultural samples has been traditionally accomplished by modifications of the Kjeldahl method. Although C/CL is not limited by many of the drawbacks of the Kjeldahl method, the same time-consuming separation methods used in Kjeldahl analysis would be required for the determination of organic nitrogen by C/CL. These generally involve a predistillation step to remove endogenous ammonia so that the remaining nitrogen is composed solely of organic nitrogen.

We have developed two techniques for rapidly removing inorganic nitrogen from an aqueous sample so the remaining organic nitrogen can be quantified as total nitrogen (TN) by C/CL. (1) Adaptation of a simple and rapid chromatographic method (reverse-phase fractionation; RPF) to separate polar from nonpolar nitrogen. The nonpolar fraction can then be analyzed for TN. (2) Use of nonosmotic, dissolved-gas dialysis with microporous tubular polytetrafluoroethene membranes to separate volatile from nonvolatile nitrogen. The dialysis process yields an ammonia-free sample that can also be directly analyzed for TN. The TN values obtained using these separation techniques are <u>direct</u> measures of "nonpolar organic nitrogen" (NPON) and "nonvolatile organic nitrogen" (NVON), respectively. Both measures also serve as direct <u>estimates</u> of organic nitrogen.

The Kjeldahl and C/CL methods were evaluated for the quantitation of: (i) nitrogen in various classes of N-containing reference solutions, (ii) total nitrogen in 10 oil shale wastewaters, and (iii) organic nitrogen in 10 oil shale wastewaters. The C/CL method gave quantitative recovery of nitrogen from each of 52 organic nitrogen compounds tested except pyrazoles and an azoxy compound; enhanced recovery was found for nitrate and nitrite salts. In contrast, only 3 of 17 compounds tested yielded greater than 90% recovery of their theoretical nitrogen contents using the Kjeldahl method. No statistically significant difference ($P>0.10$) was found between the two methods for quantifying total nitrogen in oil shale wastewaters. Both the NPON and NVON proved to be rapid estimators of organic Kjeldahl nitrogen (OKN) for oil shale wastewaters; they were, on the average, 67% and 114% of the OKN values, respectively; the relative standard deviations for these three methods were generally less than 4%.

INTRODUCTION

The heterogeneous organic polymers that compose oil shale kerogen can be thermally decomposed into petroleum-like crude oil by pyrolytic retorting processes. In addition to oil, retorting also generates a large stream of wastewater that extracts many of the nitrogenous organic compounds from the cogenerated shale oil. The majority of the dark amber/brown color and noxious odor associated with these process waters can be attributed to nitrogenous heterocycles and aromatic amines, which account for a large portion of the organic nitrogen; many of these compounds have mutagenic potential (Santodonato and Howard 1981). Furthermore, the resistance of these classes of nitrogen compounds to microbial degradation has been postulated to account for the failure of biological treatment schemes to upgrade these waters (Jones et al. 1981). Subsequent research has shown that most of the biorefractory organic carbon is associated with nitrogen (Healy et al. 1983; Torpy, Luthy, and Raphaelian 1982).

The importance of organic nitrogen in aqueous synfuel effluents has been underscored by the U.S. Environmental Protection Agency. To establish a data base and for baseline monitoring, it has been recommended that nitrogenous organic compounds be monitored in any stream unique to a particular synthetic fuel industry (Henschel and Stemmle 1983), even though these compounds are not currently priority pollutants. Although individual compounds can be determined by gas-liquid chromatography with nitrogen-specific detection, a rapid and colligative measurement for total nitrogen would be preferred for the frequent assessment of waste treatment processes designed to remove nitrogenous organic compounds from aqueous waste streams.

NITROGEN ANALYSIS

Among the many methods available for determination of nitrogen, three have been used extensively in different disciplines, and a relatively new method, based on chemiluminescent detection, is gaining acceptance. The quantification of total and organic nitrogen in biological and agricultural samples has been traditionally accomplished using the time-consuming, wet-chemical Kjeldahl method. The major failings of this method include the loss of volatile species such as aliphatic amines (during predistillation) and the incomplete recovery of nitrogen from many nitrogenous heterocycles (during digestion). In contrast to the Kjeldahl method, the Merz modification of the Dumas method has been characterized as the most rigorous method for total nitrogen analysis and is preferred by organic chemists (Fleck 1974). For the Dumas methods, a sample is generally mixed with a metal catalyst (e.g., copper[II] oxide) and combusted at 850 to 900 °C. Oxides of nitrogen evolve and are reduced by contact with metallic copper to molecular nitrogen, which is quantified by gas-volume measurements using a nitrometer. Excellent recoveries from nitrogenous heterocycles have been reported (Pella and Colombo 1973); this method is not well suited, however, for aqueous samples. Oil chemists have used catalytic hydrogenation, first described by ter Meulen in 1924 (Fleck 1974), to transform nitrogen compounds to ammonia. Automated microcoulometric titration of ammonia is generally used as the detection step. The combustion/chemiluminescent (C/CL) method relies on the combustion of a sample in an oxygen-rich atmosphere followed by chemiluminescent detection of electronically excited nitrogen dioxide. The method is suitable for both aqueous and non-aqueous liquid samples, and commercial instrumentation can be adapted for solid samples.

The undiscerning application of an unvalidated analytical method to a complex waste stream, such as oil shale process water, may yield uninterpretable data. For example, it has been reported that the organic Kjeldahl nitrogen (OKN) concentration of an ammonia-stripped, ozonated oil shale retort water (Oxy-6) exceeds the OKN of the raw water. This could occur only if the oxidative treatment altered refractory nitrogenous organic compounds so that the nitrogen became amenable to Kjeldahl digestion (Torpy, Luthy, and Raphaelian 1982). To validate a method for use with oil shale wastewaters, one must investigate the recovery of nitrogen from representative members of the major classes of nitrogenous compounds present in oil shale wastewaters, ensure the absence of matrix effects, and establish the precision.

Total and Organic Kjeldahl Nitrogen

The development of the Kjeldahl method for quantifying organic nitrogen was prompted by the need of the Danish brewing industry to detect changes in grain protein during fermentation (Bradstreet 1965). The method described in Johann Kjeldahl's original paper in 1883 (cited in Bradstreet 1965) was immediately embraced because of its relative simplicity (compared with existing methods), reliability, and adaptability to various applications. The method was long considered one of the most significant advances in analytical chemistry (Kirk 1950). Kjeldahl was aware, however, of the limitations of the method and recognized that it was not applicable to samples containing many of the numerous forms of nitrogen.

Total Kjeldahl nitrogen (TKN) is determined by digesting a sample in boiling sulfuric acid. The carbonaceous portion of organic compounds is oxidized, and the organic nitrogen is released as ammonia. The ammonia is separated from the digestate by distillation and quantified by acidimetric titrimetry or colorimetry. The procedure for OKN is identical to the TKN method except that endogenous ammonia is eliminated by distillation prior to sample digestion. Countless modifications have been made to the original method, most of which are variants of two aspects: (1) addition of salts to the sulfuric acid to increase the severity of the digestion by elevating the boiling point and (2) addition of metal catalysts to increase the oxidation rate of many organic materials and promote oxidation of some that normally resist digestion.

Salt additions. In 1889, Gunning first proposed (Bradstreet 1965) the addition of potassium sulfate to raise the boiling point of the digestion mixture. Other salts have been proposed (e.g., sodium sulfate and phosphates), but most official methods (APHA 1981; AOAC 1975; ASTM 1980; U.S. EPA 1979) stipulate the use of potassium sulfate because the alternative salts are not as soluble in sulfuric acid (Fleck 1974). The optimum salt-to-acid ratio has been determined as 0.5 to 1.0 g of potassium sulfate for each 1.0 mL of sulfuric acid. This yields digestion temperatures of 360 to 385 °C (Bradstreet 1965) as opposed to the boiling point of sulfuric acid, which is about 290°C. The salt-to-acid ratio in the official methods is generally 0.67, although the version of the ASTM method that uses premeasured reagent packets (Kel-Pak; now available as KELMATE; EM Science, Gibbstown, NJ) has a salt-to-acid ratio of 0.75. Reasons for adhering to this ratio range are threefold.

(1) Below this salt-to-acid ratio, the digestion temperature will not be high enough to yield full recovery of nitrogen from the pyridine nucleus. Digestion at 370°C for one hour is reported to be required to obtain quantitative recovery of nitrogen from refractory heterocycles (Lake et al. 1951).

(2) Exceeding this ratio or using less-soluble salts can cause the digestate to solidify upon cooling; this solidification has been associated with irreproducible losses of nitrogen (Bradstreet 1965; Fleck 1974). In addition, when the solubility of the salt is exceeded, bumping and foaming occur during digestion, resulting in loss of a portion of the digestate and therefore to nonquantitative results (Bradstreet 1965).

(3) An excessive ratio yields digestion temperatures that cause loss of nitrogen through the thermal decomposition of ammonium hydrogen sulfate to volatile nitrogenous products (Ingram 1962; Lake et al. 1951; White and Long 1951). For this reason, it has been recommended that digestion temperatures not exceed 400°C (Bradstreet 1965; Lake et al. 1951). In an attempt to increase the digestion temperature without incurring the loss of nitrogen, White and Long (1951) developed a microprocedure for Kjeldahl digestion using sealed Carius tubes and a digestion temperature of 470°C for 15 minutes. The method apparently yields excellent recovery for refractory heterocyclic nitrogen compounds, but it has never gained wide acceptance, because of the cumbersome procedure.

Catalysts. In 1885, Wilfarth noted that copper salts appreciably reduce the time required for complete recovery of nitrogen by the Kjeldahl procedure (Bradstreet 1965). Mercury was also investigated, but it was discounted at the time as a usable catalyst, because it formed a mercuroammonium sulfate complex that remains intact during distillation. It was later discovered, however, that the addition of thiosulfate decomposes the complex and precipitates mercury as HgS, and the addition of alkali allows the ammonia to be distilled. This reaction has been summarized by Clark in 1943 (cited in Bremner 1965):

$$Hg\begin{matrix}NH_3\\NH_3\end{matrix}SO_4 + Na_2S_2O_3 + H_2O \longrightarrow HgS + Na_2SO_4 + (NH_4)_2SO_4$$

A systematic study of the catalytic potential of 39 metals by Osborn and Wilkie in 1935 (Bradstreet 1965; Glowa 1974) revealed that 10 of these resulted in a shorter digestion period: mercury, selenium, tellurium, titanium, molybdenum, iron, copper, vanadium, tungsten, and silver (listed in order of decreasing catalytic ability). In 1961, Baker examined 21 metals as catalysts for the micro-Kjeldahl procedure and found mercury to be the most effective (Bradstreet 1965). Concern over the disposal of the spent digestate when mercury is used as a catalyst has prompted investigation of the minimal amount required. It was reported by Rexroad in 1973 (Fleck 1974) that the optimal amount of mercury required is about one sixth of the amount commonly recommended in official methods (160 mg vs 1000 mg). In addition to reducing the amount of mercury used, an efficacious method for precipitation and collection has been described (Dillon, Caldwell, and Gehrt 1972).

Current research has re-examined some of the early work on catalysts. Of the suggested substitutes for mercury, selenium is the most controversial. Selenium is superior to mercury for enhancing the speed of clearing of the digestate and has the added advantage of allowing direct distillation of ammonia without pretreatment. Many accounts (e.g., Fleck 1974; Patel and Sreenivasan 1948) indicate, however, that prolonged digestion after clearing and excessive concentrations of selenium cause the irreproducible loss of nitrogen from the digest. From a thorough study of the catalytic potential of selenium, Patel and Sreenivasan (1948) conclude that the digestion time after clearing must be standardized for each type of nitrogenous organic compound; therefore selenium was not recommended for general Kjeldahl applications. In contrast, Bremner (1965) maintains that by strict adherence to his procedure selenium is a safe and effective catalyst for soil samples. Combining selenium with mercury (one part to five parts) minimizes the undesirable aspects of selenium while maintaining the increased rate of clearing of the digestate (Ingram 1962). Copper and its salts are effective for recovering the nitrogen from natural products, but there is some question as to its effectiveness for refractory nitrogenous compounds (Fleck 1974). Zirconium dioxide has been proposed as a substitute for mercuric oxide (Glowa 1974); it is readily soluble in sulfuric acid, less toxic, and inexpensive (5% of the cost of mercuric oxide). For samples of feedstock, foods, and fertilizers, the alternative catalysts give equivalent recoveries of nitrogen compared with mercuric oxide. Many of the catalysts, however, have not been demonstrated to yield full recovery of nitrogen from refractory nitrogenous compounds.

Duration of digestion. Prolonged heating is reported to cause the components of the Kjeldahl digestate to condense, forming potassium hydrogen sulfate. At the point of complete conversion of the digestate to potassium hydrogen sulfate, quantitative loss of ammonia occurs (Ingram 1962; Kirk 1950). An additional hazard associated with a long digestion period is the volatilization of sulfuric acid with the concomitant rise in temperature because of the increased salt-to-acid ratio (Bremner 1965). If the temperature increases sufficiently, thermal decomposition of ammonium hydrogen sulfate will result in the loss of nitrogen (Ingram 1962). Some analysts recommend adding a second dose of acid at the midpoint of the digestion period to compensate for volatilization losses (Kirk 1950). In addition to the loss of sulfuric acid by a prolonged digestion period, the oxidation of organic matter in the sample consumes acid in a manner similar to the chemical oxygen demand assay (Chapter VI). Therefore, acid is lost in direct proportion to the chemical oxygen demand of the sample. This will cause a rise in the salt-to-acid ratio and a concomitant rise in temperature (Randtke 1983). The acid requirement of the Kjeldahl digestion has been extensively studied by Bradstreet (1957).

Chemistry of digestion. Despite numerous modifications that have been made to the Kjeldahl method, little has been published on the chemistry of the Kjeldahl digestion (Bradstreet 1957). Kirk (1950) notes that the majority of the hundreds of articles published on the Kjeldahl method are merely anecdotal accounts of empirical studies on the effects of catalyst addition, oxidizing and reducing agents, and other conditions of digestion. Accurate information is lacking on the chemistry associated with the breakdown of organic compounds during the Kjeldahl digestion process. He notes that two major, seemingly contradictory, features are common to all Kjeldahl digestions.

(1) Sulfuric acid is a very weak oxidizing agent; it would not be expected to oxidize organic materials rapidly. Addition of catalysts and salts are designed to aid the digestion of organic compounds, and many researchers have proposed the use of oxidizing agents to accelerate digestion of structurally stable compounds. The addition of oxidizing agents has been the subject of debate, however, because the requisite conditions for reduction of nitrogen must coexist with oxidizing conditions for the decomposition of organic molecules. A shift from this narrow oxidation-reduction range by the addition of oxidants risks the oxidation of the resultant ammonia to molecular nitrogen. Bradstreet (1965) includes an extensive discussion on the merits of different oxidizing agents and their proper application.

(2) The conditions existing in the Kjeldahl digestion favor reduction. This is indicated by the fact that ammonia is not oxidized to molecular nitrogen and that oxidized forms of nitrogen may be partly or wholly reduced during digestion. The identity of the reducing agent remains unclear. Kirk (1950) maintains that reducing conditions are generated directly by easily oxidized organic carbon. An alternative explanation is that sulfur dioxide, generated from the reduction of sulfuric acid during the oxidation of carbonaceous compounds to carbon dioxide, is the reductant for a portion of the more oxidized nitrogen (Bradstreet 1957, 1965).

In contrast to the assertion that a portion of the oxidized nitrogenous species is recovered with the trivalent organic nitrogen, it is stated in Standard Methods (APHA 1981) that nitrogen of the trinegative valence state is the only form of organic nitrogen recovered by the Kjeldahl procedure. This appears to be a simplistic view, especially because research has shown that some oxidized nitrogen is indeed reduced to ammonia. Furthermore, the concept of "oxidation state" may often have little to do with chemical reactions (Stumm and Morgan 1970) and should not be relied on to define the classes of compounds that may be susceptible to the digestion conditions.

The reducing conditions of the Kjeldahl digestion are not sufficient to recover nitrogen in N-N or N-O linkages (Mancy and Weber 1971). A prereduction step using an electron source such as salicylic acid can reduce the nitrogen of hydrazine and of azo (diazane and diazene), nitro, and nitroso compounds (Bradstreet 1965). The combined reducing and hydrolyzing capacity of hydriodic acid and red phosphorus can aid in the recovery of nitrogen from heterocyclic rings (e.g., pyridine); this method is also successful when applied to nitro, nitroso, and azo compounds. Sucrose and other labile organic compounds are excellent sources of reducing power for nitro, nitroso, azo, and some polyaromatic heterocyclic nitrogen compounds (Bradstreet 1965); Bradstreet includes an extensive review of the different reducing agents and necessary pretreatments.

The disadvantage of the many oxidation and reduction pretreatments is that, although they may be adequate to recover refractory nitrogen from pure solutions, when applied to a complex matrix such as oil shale wastewaters, they may be ineffective or many different pretreatments may be required to fully recover the nitrogen. In addition, these various pretreatments are extremely time consuming and do not lend themselves to routine analysis.

Applicability to shale oil. The applicability of Kjeldahl analysis to oil shale process waters has not been previously investigated. During the early 1950's, however, the recovery of nitrogen from shale oils by standard methods was researched. Ball and Van Meter (1951) compiled the results from 17 laboratories comparing the nitrogen recoveries from shale oil samples and two fractions using micro-, semimicro-, and macro-Dumas and Kjeldahl methods. The macro-Kjeldahl method was found to yield more reproducible results, but the Kjeldahl-nitrogen values tended to be lower than the Dumas results. This was attributed to incomplete conversion of nitrogenous heterocycles to ammonia. In a two-laboratory comparison study, the form of the mercuric catalyst (mercury/selenium oxychloride vs mercuric oxide/copper sulfate) had little effect on the recovery of nitrogen from shale oil; the digestion temperature was the critical factor (Lake et al. 1951). For full recovery of nitrogen from shale oils, the digestion temperature range was restricted to 370 to 405 °C with one hour of digestion. In a later round-robin study (Lake 1952), 20 laboratories participated in a comparison of the Kjeldahl, Dumas, and ter Meulen (catalytic hydrogenation) methods for the determination of nitrogen in petroleum and shale oil. For the seven laboratories submitting Kjeldahl results, mercury was found to be the preferred catalyst and, for maximum recovery of nitrogen, the digestion temperature had to exceed 370°C. These laboratories were able to achieve complete recovery of nitrogenous heterocycles (pyridine, quinoline, and isoquinoline) by careful application of the macro-Kjeldahl method. Nitrogen results from shale oil naptha samples were also in close agreement among the seven laboratories. The micro-Kjeldahl values were inconsistent; lack of adequate temperature control was cited as being responsible for the erratic results. It was suggested that a constant-temperature bath or block digestor would yield more consistent, controllable temperatures.

Semiautomated methods. Using a Technicon AutoAnalyzer II and a Technicon BD-40 block digestor to carefully maintain digestion temperatures between 360 and 370 °C, Jirka et al. (1976) developed and validated an ultra-micro semiautomated method for combined total phosphorus and TKN in wastewaters. The method uses the same reagents as the macro-Kjeldahl method (APHA 1981), but only requires 10 mL of sample and substantially reduces the volume of waste (to 1/25) that must be treated before disposal. The recoveries of nitrogen from environmental samples and from those spiked with nicotinic acid were excellent, and there was no significant difference in recoveries between the standard and ultra-micro methods. Bowman and Delfino (1982) modified the original ultra-micro method by doubling the sample size to increase the sensitivity, increasing the digestion time at 370°C from 30 to 75 minutes, and by using two block digestors to increase the digestion capacity from 80 to 160 samples per 8-hour day. Compared with the macro method, both the precision and accuracy were improved. The recovery of nitrogen was generally higher using the micro method; this was attributed to more complete recovery of nitrogen from refractory compounds because of the more rigorous digestion conditions (Randtke 1983). These ultra-micro and micro methods require open-tube digestion for evaporation of water from the sample; it is unclear if solutes in the sample digestate become concentrated as a result of evaporation or consumption of sulfuric acid, thereby artificially increasing the nitrogen concentration. We have not evaluated these methods for use with oil shale wastewaters.

Semiautomated macro-Kjeldahl systems are available from Brinkmann Instruments, Inc. (Westbury, NY). They are more compact than the traditional digestion/distillation Kjeldahl rack, but are extremely expensive and do not

offer the advantages of increased sample-throughput and decreased waste volume of the micro methods.

Nitrogen Determination by Combustion/Chemiluminescence (C/CL)

A chemiluminescence analyzer is basically a transducer that transforms a flow of molecules into a flow of detectable photons (Mehrabzadeh, O'Brien, and Hard 1983). The chemiluminescence from the reaction of ozone (O_3) with nitric oxide (NO) was first exploited as an analytical tool for the measurement of atmospheric air pollution and to directly monitor automobile emissions. Fine, Lieb, and Rufeh (1975) developed the thermal energy analyzer (TEA) for the trace analysis of both volatile and non-volatile N-nitroso compounds in foods, beverages, and complex biological mixtures. The sample is first pyrolyzed (thermally decomposed at 300°C in the absence of oxygen). The N-NO bond is thermally labile, and the nitrosyl radical ($NO^{\bullet}$) is released and reacted with O_3 yielding either NO_2 or electronically excited nitrogen dioxide (NO_2^*). This metastable species decays by one of three possible paths:

$$NO_2^* \longrightarrow NO_2 + h\nu$$
$$NO_2^* + M \longrightarrow NO_2 + M$$
$$NO_2^* + M + M \longrightarrow NO_2 + M + M$$

"M" refers to species that participate in multi-body collisions, competing reactions during relaxation of NO_2^*. At ambient temperatures, only about 8% of the NO is excited to NO_2^* by O_3.

The emitted quanta are amplified by a photomultiplier tube (PMT). Fine and coworkers noted that the desired series of reactions is extremely inefficient for the production of NO_2^*. The geometry of the pyrolysis unit, the relative proportion of NO excited to NO_2^*, and the 2- and 3-body reactions contribute to this inefficiency; multi-body collisions are a function of pressure, and the detector reaction-chamber geometry must ensure the appropriate residence time for decay of the NO_2^*.

Antek nitrogen analyzer. In 1976, Antek Instruments, Inc. (Houston, TX) introduced a nitrogen analyzer that coupled the microcombustion techniques developed for determination of sulfur by microcoulometry with detection based on principles similar to those of TEA. The major fundamental difference between the Antek nitrogen analyzer and the TEA is that the Antek analyzer uses a much higher furnace temperature and an oxidizing atmosphere in order to detect total nitrogen. Although Antek terms the furnace a "pyrolysis" unit, the nitrogen-containing analytes undergo oxidation to yield NO; competing reactions during combustion include formation of N_2O and NO_2. We call this method of total nitrogen analysis, which couples combustion with chemiluminescence, C/CL analysis. A schematic of the reactions and equipment involved is shown in Figure 1.

For determination of nitrogen in a liquid sample, 5 μL is injected (10-μL septum-piercing syringe, Precision Sampling, Baton Rouge, LA) through a Teflon-lined silicone septum at a constant rate of 1.2 μL/sec using a syringe pump (model #735, Antek Instruments, Inc.). This sample is delivered through an orifice/burner tip into a quartz combustion tube packed with quartz chips; the chips increase the turbulence in the combustion zone, mitigate the effects of flash vaporization of aqueous samples, and slow the attack of the tube by

alkaline metals (a major problem with retort waters). A ceramic sleeve insert can also be used to alleviate the corrosive effects of alkaline metals. The actual sample injection rate is critical; it must also be constant (Drushel 1977). If the sample is delivered too slowly, the concentration in the gas phase will be too low for accurate detection. If the rate is too fast, either incomplete combustion of the sample (because of residence time or burner-tip temperature depression) or incomplete conversion to NO can result.

Oxygen is delivered to the combustion tube at two points: as carrier gas (100 cm^3/min) and as combustion gas (275 cm^3/min). The flow rate of O_2 and the furnace temperature determine the relative proportions of NO, N_2O, and NO_2 that are produced from the combusted sample (Drushel 1977). We experienced a great deal of difficulty determining the optimum operating conditions (carrier gas flow rates, ozone generator oxygen flow rate, and syringe injection speed) to maximize response, while maintaining a wide linear dynamic range. The response from chemiluminescence analyzers is notoriously difficult to optimize by an empirical approach, because there can be up to five independent instrumental parameters (Mehrabzadeh et al. 1983). A more theoretical approach to system optimization has been advocated (Mehrabzadeh et al. 1983), but is difficult to apply in a laboratory situation. Drushel (1977) details an optimization process for a combustive chemiluminescence system and includes an extensive discussion on the effects of changing each parameter.

The combustion tube is maintained at 1100°C by a furnace ("pyroreactor" model #771, Antek Instruments, Inc.) to ensure complete oxidation of aqueous samples; with organic solvents, exothermic reactions allow a lower furnace temperature to suffice. The gas stream leaving the combustion tube, which consists of NO, CO_2, H_2O, and other oxidized species (e.g., SO_x, ClO_2), passes through a membrane dryer (for removal of H_2O) and then enters the detector (model #720, Antek Instruments, Inc.), where NO reacts with O_3 and is partially oxidized to NO_2^* (Schuchardt 1980). When the NO_2^* decays to its ground state, it emits radiant energy in the range of 600 to 3000 nm with a maximum intensity near 1200 nm (Clifford and McGaughey 1982). The intensity of the radiant energy is directly proportional to the mass flow rate of NO through the reaction chamber. The emitted photons are detected by a PMT that is sensitive to long wavelength light and is protected from low- to mid-range visible and ultraviolet light by appropriate filters (Ward, Owens, and Rennie 1980). A 650- to 900-nm bandpass (high-range visible to near infrared) eliminates chemiluminescent interference by unsaturated hydrocarbons, chlorine, and sulfur, which also react with O_3, but emit light of shorter wavelengths. The collection of the light quanta is only about 80% efficient, and the PMT only amplifies a small percentage of this available signal, because the majority of the NO_2^* emission is in the far infrared and is not detected by PMTs (Stedman 1981). The amplified binary output is coded into decimal (BCD) and is received by a Hewlett-Packard (HP-97S) programmable printing desk-top calculator; the BCD interface cable was fabricated by our project, and a pin-to-pin diagram is shown in Appendix B (Chapter V). The calculator is programmed to determine nitrogen values from integrator counts using a least-squares regression curve generated from ammonium sulfate standards (counts vs mass of NH_3-N) that are analyzed on the same day as the samples.

Comparison of C/CL with TKN recoveries. A method for total nitrogen that is rapid, reproducible, requires extremely small sample volumes (5 μL), and can be automated has numerous advantages compared with the wet-chemical Kjeldahl

method. The question of accuracy, however, is difficult to address. How do results from C/CL compare with those from Kjeldahl analyses for determination of nitrogen? Snodgrass (1981) reports that the sum of TKN and NO_3-N from fertilizer processing wastewater equals the total nitrogen yielded by C/CL analysis. Similarly, Clifford and McGaughey (1982) report an excellent agreement between instrumental and wet-chemical results for wastewater samples. For aqueous biological and clinical samples (rat urine and human urine and feces), Ward et al. (1980) find no significant difference between the C/CL method and a Kjeldahl method that uses a mixed $CuSO_4/SeO_2$ catalyst. For the distillate fraction of shale oil, Drushel (1977) notes that Antek C/CL results were approximately 10% higher than Kjeldahl results. He attributes this to incomplete recovery by the wet-chemical method for some of the refractory nitrogenous compounds in shale oil rather than to a fundamental problem with the C/CL method of analysis.

In addition to its advantages over the time-consuming Kjeldahl method, C/CL is reported to have significant advantages over other instrumental methods for total nitrogen. Compared with the Dumas method (used by the Carlo Erba ANA 1400), the C/CL method is reported to be superior for concentrations below 1000 mg-N/L (Schuchardt 1980). Nitrogen in an aqueous sample can be readily determined by C/CL, whereas water may interfere with the Dumas method. The advantages of the C/CL method compared with catalytic hydrogenation followed by microcoulometric detection have been detailed by Drushel (1977). The major advantages the C/CL system offers are reduction in time of setup, equilibration, and calibration, elimination of microcoulometer cell maintenance, and complete combustion of high-boiling residual fuels (incomplete vaporization and coking are serious problems with catalytic hydrogenation). Additionally, the C/CL system is amenable to automation and requires less professional supervision for routine application. The total nitrogen results from the two instrumental methods (C/CL vs catalytic hydrogenation/microcoulometric detection) were identical for a distillate fraction of shale oil (Drushel 1977).

<u>Organic nitrogen by C/CL</u>. A major disadvantage of the Antek nitrogen analyzer is its inability to directly determine organic nitrogen. To circumvent this shortcoming, we have adapted two methods that can remove the inorganic nitrogen from the organic nitrogen, thereby allowing for the <u>direct</u> estimation of organic nitrogen as total nitrogen (TN) by C/CL.

Reverse-phase fractionation (RPF) (Chapter I) was evaluated as a method to rapidly separate most of the organic nitrogen from the large amount of ammonia in oil shale process waters. Disposable cartridges containing C_{18}-bonded silica are activated with methanol. The wastewater sample is applied to the cartridge, which is then rinsed with a small volume of water. Polar compounds are not retained; they pass through with the aqueous effluent (hydrophilic fraction; HpF). Nonpolar compounds are retained; they can be eluted with methanol (lipophilic fraction; LpF). The HpF contains ammonia, nitrate and nitrite salts, and polar organic-nitrogen compounds (e.g., hydroxylated and oxygenated heterocycles and nitriles). Alkylated pyridines, quinolines, and other nitrogenous heterocycles and aromatic amines reside in the methanolic LpF. Since a small portion of organic nitrogen passes through the cartridge and is collected with the HpF and since some azaarenes are irreversibly retained by the stationary phase, LpF-TN would be expected to be an underestimate of organic Kjeldahl nitrogen. LpF-TN is a direct measure, however, of nonpolar organic nitrogen (NPON). With RPF, approximately 40 samples can be fractionated and

analyzed in triplicate in eight hours; each replicate determination requires only approximately 90 seconds. In contrast, five hours are required for analysis of three samples (in triplicate) using the Kjeldahl method with a 12-place digestion/distillation unit and automated titration.

Microporous tubular polytetrafluoroethene (PTFE) membranes can be used to effect a rapid separation of dissolved ammonia from aqueous samples. This process, called nonosmotic dissolved-gas dialysis (NOGD), is described in Chapter II. The result of this dialysis process, when used for oil shale process waters, is an ammonia-free sample that contains nonvolatile organic nitrogen (NVON) that can be directly analyzed for TN by C/CL. Nitrogen oxide ions and cyanates do not diffuse through the membrane, and therefore contribute to the estimation of organic nitrogen. Volatile monoalkylamines can permeate the membrane, however, and would not be accounted for in the estimate of organic nitrogen. Solution pH determines the fraction of compounds removed by this process. Dialyzed-TN is a direct measure of nonvolatile organic nitrogen. With NOGD, approximately 30 samples can be degassed and analyzed in eight hours.

The five methods discussed in this chapter, for the direct determination of total and organic nitrogen, are summarized in Table I. The advantages and disadvantages of direct and indirect determinations of organic nitrogen are listed in Table II.

Other Commercial Instrumentation for Nitrogen Analysis

The quantitation of total nitrogen in a heterogeneous mixture of organic and inorganic nitrogen compounds requires the conversion of each species to a common detectable unit (e.g., N_2 or NH_3). For non-Kjeldahl analyses, this usually involves a combustion step followed by the catalytic conversion of the combustion products to the detectable species. Detection methods can be physical, chemical, or electrical. Nitrogen may be the only element of interest, or it may be one of a suite of elements (e.g., carbon, hydrogen, oxygen, nitrogen, and sulfur). The advantages and disadvantages of each approach used in commercial instrumentation will be described for several methods.

Dumas. In the classical Dumas method, the sample is mixed with copper(II) oxide and introduced through a funnel to an automatic burner where it is heated. The resulting pyrolysis products are further oxidized by passing them over heated copper(II) oxide in a stream of CO_2. The oxygen and oxides of nitrogen are then reduced by contact with metallic copper in a reduction tube (Brewer 1977; Ingram 1962). Carbon dioxide is absorbed from the effluent gas stream in a potassium hydroxide scrubber, and the amount of nitrogen in the sample is determined by the measurement of the remaining gas volume (presumably 100% N_2) in a nitrometer. Atmospheric nitrogen is an obvious interference with this detection method, and precautions are required at all steps of the procedure to minimize the introduction of air. The entire procedure requires a high degree of skill and experience; attempts to achieve complete sample oxidation have prompted the substitution of different catalysts and oxygen donors in an effort to improve the recovery of nitrogen. These variations include a micro-Dumas method that uses a copper(II) oxide-platinum catalyst and a combustion temperature of 850 to 900 °C. Combustion at 750°C in the Coleman analyzer is reported to be inadequate for the recovery of heterocyclic nitrogen (Fleck 1974); others (Sternglanz and Kollig 1962) report better recovery of refractory

compounds by use of vanadium pentoxide in a platinum sleeve within the quartz combustion tube at 900°C. The platinum serves as a catalyst, and the molten vanadium pentoxide fuses with the sample and acts as a combustion aid.

Automated Dumas. The Merz modification serves to partially automate the Dumas method; a fully automated instrument, Micro-Rapid-N, has been developed by W.C. Heraeus (Hanau, Germany). The sample is weighed into either an aluminum boat or glass capillary and placed in a three-way stopcock for introduction to a furnace. Air is removed by purging with CO_2 or O_2. The combustion tube has different temperature regions; the upper part is maintained at 1000 to 1050 °C and the lower part at 850 to 900 °C. The lower part of the tube is filled with copper(II) oxide and has a concentric baffle to provide a longer path length for pyrolysis products. The instrument is fully described by Brewer (1977).

Multi-element analyzers. Multi-element (CHN or CHNO) analyzers made by Carlo Erba, Perkin Elmer, Fisher, and Technicon are similar in principle to the Dumas approach for the determination of nitrogen. These analyzers use a stream of helium that serves as the carrier gas and also purges any introduced air. Oxygen is introduced simultaneously with the preweighed sample (in quartz, aluminum, tin, or silver boats or in capillary tubes) into the 900 to 1000 °C combustion tube. Combustion converts carbon to CO_2 and hydrogen to H_2O; nitrogen is released as a mixture of molecular nitrogen and nitrogen oxides. After the sample has been oxidized, helium sweeps the H_2O, CO_2, and NO_x to a reduction tube (700 to 750 °C) packed with metallic copper or nickel oxide; any remaining oxygen is removed and the oxides of nitrogen are reduced to molecular nitrogen (Cottrell and Cottrell 1977; Pella and Colombo 1973; Willard, Merritt, and Dean 1974).

The combustion/reduction gases are separated either by gas chromatography (GC) or by means of specific absorbents. Thermal conductivity is the detection method for either technique (Willard et al. 1974). Separation of the gases by GC first requires the reaction of H_2O vapor with calcium carbide to yield acetylene; the sample gases are then frozen and isolated in an injection loop. The injection loop is heated, and a stream of dry helium carries the gases as a plug into the chromatographic column. The emerging analytes are quantified by integrating the areas of the peaks that result from changes in the thermal conductivity of the gas stream (Howarth 1977; Willard et al. 1974). In contrast, the other general method separates the gases by specific absorbents (magnesium perchlorate for H_2O and either LiOH, Na_2O, or Ascarite for CO_2). The thermal conductivity is determined after each absorbent trap and at the influent to determine the contribution from each sample gas (Colombo and Giazzi 1982; Willard et al. 1974).

The methods based on the Dumas method are cumbersome, because each sample must be individually weighed and sealed in a sample boat. This can be difficult for liquid samples and has led to the use of glass or silver capillary tubes for liquid introduction (Howarth 1977; Pella and Colombo 1973). Furthermore, the expansion of water contributes to inadequate combustion (Huffman 1982). The multi-element analyzers are complex and require frequent adjustment and maintenance. Only 40 CHN analyses or 23 CHNO analyses can be performed per day (Fleck 1974). The method is not well suited for samples containing less than 5 000 mg-N/L (Lake 1952). There have been many favorable reports, however, regarding the performance of these types of analyzers for the analysis of

atmospheric aerosols and for use in the petroleum industry. Excellent recoveries have been reported for nitrogenous heterocycles such as nicotinic acid, 8-hydroxyquinoline, pyridine, 4-aminoantipyrine, antipyrine, and aminopyrine (Huffman 1982; Pella and Colombo 1973). Others have reported low results, however, for biologically important nitrogenous heterocycles such as pyrimidines and purines (Ingram 1962).

For shale oil, a comparative study compiled by Ball and Van Meter (1951) reveals that Dumas results are generally higher than the Kjeldahl values. As mentioned above, this was in part a result of low Kjeldahl recoveries, but was also a problem of enhanced recoveries by the Dumas method. If nitrous oxide, carbon monoxide, or cracking products (such as methane) enter the nitrometer, the apparent nitrogen recovery will be artificially high. In addition, if the absorption of carbon dioxide is incomplete, carbon dioxide will also be quantified as nitrogen. In a round-robin study (Lake 1952), the results were extremely erratic (rsd = 11%) from six laboratories, each of which performed their individual modifications of the Dumas method. It was concluded that the method was extremely sensitive to individual technique and that an experienced technician could achieve precise results. Furthermore, it was concluded that the Dumas method was the only one of the three methods tested (Dumas, Kjeldahl, and ter Meulen) that was insensitive to compound type and that the Dumas method should be used to confirm the accuracy of the results from the less vigorous methods.

Catalytic hydrogenation/microcoulometric titration. As opposed to the combustion/reduction treatment of nitrogen-containing compounds to yield N_2, the microcoulometric method relies on quantitative conversion of nitrogen compounds to NH_3 by catalytic hydrogenation over a nickel-on-magnesium oxide catalyst at 440°C (Fleck 1974; Martin 1966). The NH_3 is then stoichiometrically titrated with electronically generated hydrogen ions (Killer 1977). The area under the peak gives a direct measurement of titrant generated. The original method has been modified for the determination of total nitrogen in aqueous samples (Oita 1968), and a modification to the sample entry port allows the sample to bypass the catalysts so that endogenous NH_3 can be determined (Albert et al. 1969). The major advantage of the catalytic hydrogenation/coulometric (ter Meulen) method compared with the Dumas or Kjeldahl methods is the low detection limit (Martin 1966). The microcoulometric method was primarily designed for the determination of total nitrogen in the range of 1 to 200 mg-N/L in samples that are completely volatilized at 550°C (Killer 1977). The method has been reported to be capable of being used for concentrations below 1 mg-N/L as well as up to 5 000 mg-N/L. The method yields full recovery for most nitrogenous compounds, including such heterocycles as pyridine, quinoline, pyrrole, indole, and carbazole (Killer 1977; Martin 1966). Triazines gave less than 100% recovery in one study, because two of the three nitrogen atoms in the triazine linkage are converted to molecular nitrogen, which cannot be converted to ammonia (Martin 1966); azo compounds were not quantitatively recovered in another study (Killer 1977). Since any strong acid reaching the titration cell will be titrated, it is recommended that the method not be used for samples containing more than 20% halogen or 5% sulfur (Killer 1977). The method is fairly time consuming; only four samples per hour can be analyzed. In addition, there is the risk of explosion if the hydrogen gas required for catalytic hydrogenation is improperly handled.

The catalytic hydrogenation (ter Meulen) method is reported to be applicable to petroleum and shale oils. Four reporting laboratories in a round-robin study (Lake 1952) found catalytic hydrogenation applicable to a greater variety of nitrogenous compounds than the Kjeldahl method. In the early 1950's, however, when this round-robin study was completed, the catalytic hydrogenation method was unfamiliar to most analysts and could not be recommended for general use. Recovery of nitrogen from shale-oil naptha by catalytic hydrogenation/microcoulometric titration was equivalent to Kjeldahl recovery (Martin 1966).

The microcoulometer developed by Martin and Flannery can also be used as a nitrogen-selective detector for GC (Martin 1966). Electrical conductivity (following the Coulson or Hall approach) can also be coupled to GC for the determination of nitrogenous compounds. Organic nitrogen compounds are first separated by GC and converted to ammonia in a high-temperature pyrolyzer using hydrogen as a reactor gas, nickel wire catalyst, and strontium hydroxide post-pyrolysis scrubber. The effluent gas from the furnace is combined with deionized liquid and the conductivity is monitored using an AC bridge circuit. The conductivity is continuously measured and any change corresponds to the presence of ammonia. In other modes of pyrolysis, the unit can be used to detect chlorine or sulfur (Tracor Instruments 1976).

Other Approaches

Spectrophotometric detection of NH_3 and oxides of nitrogen that are liberated by ultraviolet irradiation affords a 100-fold decrease in detection limit (2 to 5 μg/L) compared with the micro-Kjeldahl method. This allows the quantitation of two separate organic nitrogen fractions based on the amounts of NH_3 and oxides of nitrogen released (Manny, Miller, and Wetzel 1971). Irradiation for 1.5 to 3.0 h is recommended for complete recovery of nitrogen compounds from aqueous samples; it is also recommended that the samples should be fully oxygenated and buffered to pH 4.0. Incomplete recovery of urea, however, has been reported (Henriksen 1970 cited in D'Elia, Steudler, and Corwin 1977). An automated continuous-flow method in which the sample is subjected to sequential acidic and basic UV irradiation and then analyzed for nitrate-nitrite content has been reported to yield excellent results for total soluble nitrogen. This method yields incomplete recoveries from hydrazines and ethylenediamine-tetraacetic acid following two hours of irradiation (Afghan, Goulden, and Ryan 1971).

Ultraviolet oxidation of nitrogen-containing compounds followed by a heterogeneous reduction of the irradiation products to ammonia and detection by ion selective electrode offers an alternative procedure for the determination of organic nitrogen (Lowry and Mancy 1978). Quantitative recoveries of many organic nitrogen compounds common to natural waters are reported following 17 minutes of exposure to UV irradiation; carbazones, hydrazines, and a pyrazolin-5-one, however, yielded only a portion of their nitrogen content (Lowry and Mancy 1978).

Peroxydisulfate digestion to nitrate followed by detection by ion specific electrode or reduction of nitrate to nitrite with spectrophotometric detection has been adopted as a standard method in Sweden, Denmark, Finland, and Norway for the determination of total nitrogen in natural waters (Nydahl 1978). Alkaline peroxydisulfate digestion at 100 to 120 °C yields NO_3 as the sole

nitrogen product (Solórzano and Sharp 1980). Careful attention to buffer strength, pH, reaction vessels, and dilution factors is required to attain accurate and precise results with natural waters. In a comparison study between the total persulfate nitrogen and TKN methods, the persulfate method was found to be more precise for standard solutions and samples; the accuracies of nitrogen recoveries for the two methods were equivalent (Smart, Reid, and Jones 1981). It has been reported, however, that 5-membered nitrogenous heterocycles or N=N double bonds are not oxidized by this method (Nydahl 1978). Among the major advantages the peroxydisulfate method offers are convenience (the procedure can be completed aboard ship), cost (in one study the chemical costs for a parallel set of Kjeldahl analyses were eight times that of peroxydisulfate analyses), and detection of all N-containing compounds (i.e., nitrate and nitrite are included by TPN, whereas they are not detected by TKN).

METHODS AND MATERIALS

An Antek nitrogen analyzer (model #703C, Houston, TX) was used for the determination of nitrogen by C/CL. The instrument and principles of operation are discussed in the section "Nitrogen Determination by Combustion/Chemiluminescence: Antek Nitrogen Analyzer". The nitrogen analyzer was interfaced with an Hewlett-Packard (HP-97S) calculator, which registered the integrated detector output (if stable) 50 seconds after sample injection. Values (slope and y-intercept) for the ammonium sulfate standard curve (20 to 100 mg-N/L) and sample dilution factor were stored in the calculator memory; the nitrogen value for a sample was interpolated from the standard curve. All statistical analyses were based on the appropriate sections in Rohlf and Sokal (1969) and Sokal and Rohlf (1969).

The ASTM recommended procedure (ASTM 1980), using either the digestion solution or Kel-Pak alternative, was followed for all Kjeldahl analyses. Detailed operating protocols for all nitrogen methods used are appended. Carbon concentration of 3,5-dimethylpyrazole was determined by the UV-persulfate method detailed in Chapter III.

To investigate the effects of solvents on C/CL recovery of nitrogen, standard curves between 20 and 100 mg-N/L were produced for 2,4,6-trimethylpyridine in either nanograde toluene or ASTM Type I water, ammonium sulfate in water, and 9-methylcarbazole in toluene. These solutions were prepared from 1 000 mg-N/L stock solutions by dilution in Class A 10-mL volumetric flasks.

The nitrogen compounds used in the pure compound and comparison studies were of the highest grade commercially available (manufacturers listed in Table III). Each of 52 compounds was placed in a Class A volumetric flask (25- to 100-mL) and weighed using a semi-micro analytical balance (model HL 52, Mettler Instrument Corp.). The amount of each compound was chosen to yield standard solutions of approximately 80 mg-N/L. The solutions were made to volume using either ASTM Type I water or appropriate solvent (4,4'-azoxyanisole, 1,5-dimethyltetrazole, 2,5-dimethyl-1,3,4-thiadiazole, indazole, m-nitrophenol, o-nitrophenol, p-nitrophenol, 6-nitroquinoline, and quinoline in HPLC grade methanol; carbazole, indole, isoquinoline, and 9-methylcarbazole in nanograde toluene). For the TKN analyses of 4-amino-2,3-dimethyl-2-phenyl-3-pyrazolin-5-one, 3,5-dimethylpyrazole, pyrazole, cyanuric acid, and nicotinic acid, 1.5-mL samples were drawn from 1 000 mg-N/L stock solutions and added directly to each Kjeldahl flask. Stock solutions were stored at 4°C in 25-mL glass scintillation

vials with Teflon-lined screw caps; diluted samples were also stored at 4°C in either 25-mL glass scintillation vials with Teflon-lined screw caps or in 100-mL reagent bottles with Teflon-lined screw caps, depending on required volume. Three single-operator replicates of the 52 compounds were analyzed by C/CL. Duplicate samples of 17 compounds (listed in Table IV) were analyzed for nitrogen content by the Kjeldahl method, and their TKN values were compared with the results from C/CL.

To determine if the oil shale process waters exerted a matrix effect, a standard additions study was designed to compare the TKN values with the TN values from C/CL. A composite sample of unfiltered oil shale process waters (equal volumes of nine process waters listed in Table V; the origins of these waters are listed in Appendix I) was diluted 1:200 so that the nitrogen concentration was approximately 35 mg/L. Nicotinic acid (3-pyridinecarboxylic acid), a nonhygroscopic compound that is reported to be one of the more difficult compounds to recover by Kjeldahl digestion (Bowman and Delfino 1982), was added to samples of the diluted composite water so that the nicotinic acid concentrations were 15, 35, and 55 mg-N/L. The final TN concentrations of these spiked samples were 50, 70, and 90 mg-N/L.

To compare TN values for different retort waters and to determine method imprecision, samples of nine oil shale process wastewaters and a composite sample (Table V) were filtered (0.4-μm pore-diameter polycarbonate membranes, Bio-Rad Laboratories, Richmond, CA) under pressure and diluted to yield concentrations of between 30 and 75 mg-N/L. These samples were stored in a manner identical to the standards. Ten single-operator replicates of each process water sample were analyzed for nitrogen by C/CL, and three single-operator replicates were analyzed for total nitrogen by the Kjeldahl method.

The separation of lipophilic organic solutes from ammonia in the parent process water was accomplished by reverse-phase fractionation (Chapter I). The standard fractionation procedure was modified slightly; all of the aqueous effluent (sample and rinse) was collected with the hydrophilic fraction (HpF), and the LpF was eluted with methanol followed by tetrahydrofuran. Nine oil shale wastewaters and a composite water were filtered and fractionated. The total nitrogen content of both the LpF and HpF was determined by C/CL in triplicate for each of three replicate sample fractionations. To determine the effectiveness of RPF in separating organic nitrogen from inorganic nitrogen species, the NPON value of each process water was compared with the results from triplicate determinations of OKN for each sample.

The effectiveness of microporous tubular PTFE membranes for the separation of ammonia from the NVON in oil shale wastewaters was evaluated by determining TN on the dialyzed sample and comparing these results with OKN and NPON. Seven oil shale process waters and a composite water were filtered and diluted with 2M Na_2CO_3 buffer solution to yield ammonia concentrations of approximately 1500 mg-N/L. The dilution of two oil shale wastewaters deviated from this scheme; Oxy-6 gas condensate and Rio Blanco sour water were diluted so that their ammonia concentrations were approximately 2700 and 800 mg-N/L, respectively; these dilutions ensured that the nitrogen concentrations of these two dialyzed samples would be above 10 mg/L. Triplicate samples of all the waters were dialyzed according the procedure given in the protocol of Chapter II. The dialyzed fraction of each replicate was diluted (when necessary) to

yield a nitrogen concentration between 10 and 100 mg/L, and analyzed for TN by C/CL.

RESULTS AND DISCUSSION

Solvent Effects

Water has been reported to depress detection of nitrogen with C/CL by lowering the burner-tip temperature, quenching the chemiluminescence, and contributing to 2- and 3-body reactions. An 1100°C furnace mitigates the effect of burner-tip temperature depression by aqueous samples. The Antek 703C nitrogen analyzer has a membrane dryer that eliminates water from the gas stream and thereby minimizes quenching. The slopes of standard curves between 20 and 100 mg-N/L (attenuation = 20) for 2,4,6-trimethylpyridine in either nanograde toluene or ASTM Type I water were nearly identical: 6.07×10^6 and 6.14×10^6 counts/mg-N, respectively (Fig. 2). Two other standard curves obtained at the same time (ammonium sulfate in water and 9-methylcarbazole in toluene) also had slopes that were virtually indistinguishable from those of 2,4,6-trimethylpyridine (6.08×10^6 and 6.07×10^6 counts/mg-N, respectively) (Fig. 2). Compounds dissolved in methanol (quinoline and 6-nitroquinoline) did not exhibit an enhanced response when compared with an ammonium sulfate standard in water (Fig. 3). These results indicated that water probably does not interfere with the combustion of the sample or with the detection of nitrogen by chemiluminescence and that either toluene or methanol can be used interchangeably with water, as required by the solubility of the analyte.

Pure Compounds: Recovery Study

To ensure that the C/CL system would be applicable to detection of nitrogen in oil shale process waters, the recovery of nitrogen from compounds reported to be resistant to Kjeldahl digestion (pyridines and quinolines) and from compounds reported to be prevalent in process waters (alkyl-substituted pyridines) was determined. The majority of the 52 compounds tested yielded 90% to 110% of their theoretical nitrogen contents. Preliminary studies indicated that 3,5-dimethylpyrazole was either refractory to oxidation or its ozone reaction products were not detected; in contrast, carbon analysis gave 103% of theoretical recovery. Recovery of nitrogen from standard solutions (Fig. 3) supported the hypothesis that compounds containing the pyrazole nucleus give low recoveries, probably because of resistance to quantitative liberation of the nitrogens as NO. The important aspect of this chemical structure is a diaza N-N bond coupled with only one N=C double bond in a 5-membered ring that is able to tautomerize. Less than 15% of the nitrogen was recovered from pyrazole, whereas 35% of the nitrogen was recoverable from benzopyrazole (indazole). The 4-membered unsaturated structure coupled with the 5-membered pyrazole nucleus either slightly destabilized the compound or prevented tautomerization. Half of the nitrogen in tetrazole, a 5-membered ring consisting of four nitrogen atoms and one carbon, was recovered by C/CL; the resistance to oxidation may be restricted only to the pyrazole moiety of the structure.

In contrast, complete recovery of nitrogen was obtained from pyridazine (6-membered ring with a resonant N-N bond), imidazole (5-membered ring with two nitrogen atoms, but without an N-N bond), 2,5-dimethyl-1,3,4-thiadiazole (5-membered ring with an N-N bond, two N=C double bonds, and a sulfur instead of the carbon atom in the 4-position of pyrazole; this substitution appears to

prevent tautomerization), and 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (4-aminoantipyrine; 5-membered ring with an N-N bond but without an N=C double bond). These results are summarized in Figure 4. Curiously, only 78% recovery was observed from 2,3-dimethyl-1-(4-methylphenyl)-3-pyrazolin-5-one, which has a structure similar to 4-aminoantipyrine (4-amino-2,3-dimethyl-2-phenyl-3-pyrazolin-5-one).

Another compound that yielded incomplete recovery of nitrogen was 4,4'-azoxyanisole. Azo compounds have been reported to be somewhat resistant to C/CL nitrogen analysis presumably because of the liberation of the N=N moiety as molecular nitrogen. Inorganic nitrate and nitrite salts yielded enhanced responses; 114% and 122% nitrogen were recovered, respectively. This is attributed to relatively higher efficiencies of conversion to NO because of the higher oxidative state of nitrogen oxide salts (Clifford and McGaughey 1982). This may be a serious drawback for the application of C/CL methods to agricultural wastewaters or biological samples. Organic nitrogen oxides are not generally found in shale oils (Lake et al. 1951), and early accounts of high nitrate concentrations in oil shale wastewaters have been discounted by subsequent research using a method less prone to interferences (i.e., Devardo's alloy method) (Fox 1980). Since inorganic nitrogen oxides and pyrazoles are present at extremely low concentrations in oil shale process wastewaters, the application of C/CL to the determination of nitrogen in oil shale wastewaters seems justified.

In contrast, only 3 of 17 compounds tested yielded greater than 90% recovery of their theoretical nitrogen contents using the TKN method (Table IV). Despite the reported resistance of nicotinic acid to Kjeldahl digestion, 99% of the theoretical nitrogen was recovered from this compound. Similarly, pyridazine and cyanuric acid yielded more than 90% of their theoretical nitrogen values. Compounds containing the pyrazole nucleus and tetrazole, however, yielded only 15% to 79% of their theoretical nitrogen values. Imidazole and 2,5-dimethyl-1,3,4-thiadiazole yielded only 5% to 25% of their theoretical nitrogen. These results are not surprising, because the N-N linkage in pyrazolones and similar compounds has been reported as extraordinarily resistant to Kjeldahl digestion (Lake 1952; Lennox and Flanagan 1982; Steyermark et al. 1958; White and Long 1951). None of the three nitrophenols yielded its theoretical nitrogen content. Approximately half of the nitrogen was recovered from m-nitrophenol and p-nitrophenol; o-nitrophenol was completely resistant to Kjeldahl analysis. The purity of this compound could not be guaranteed by the manufacturer, but, since full recovery of nitrogen was obtained by C/CL, it would indicate that the compound was in fact resistant to digestion. These results are not in agreement with earlier work on nitro aromatic compounds; Margosches and coworkers, in 1919 to 1923 (cited in Bradstreet 1965), tried to develop a correlation between the recovery of nitrogen and the position of substituent groups in mononitro compounds. He found that only ortho substituents could be determined without modification of the standard Kjeldahl digestion procedure (0.5 salt-to acid ratio; 10 g potassium sulfate to 20 mL of sulfuric acid). By the addition of 1 g of salicylic acid, a reducing agent, m-nitro groups could be recovered, but p-nitro groups remained resistant. The reasons for the discrepancy between our results and Margosches's conclusions are unknown. The Kjeldahl method of nitrogen analysis should be used with caution for aqueous waste streams, such as oil shale wastewaters, that may contain some of these refractory classes of compounds.

Matrix Effects: Standard Additions

The addition of nicotinic acid to retort water to give various known concentrations was used to detect matrix effects (e.g., enhanced or depressed responses) (Fig. 5). The recovery of nicotinic acid spikes from diluted composite samples ranged from 98% to 103% for C/CL and from 102% to 104% for the Kjeldahl method. The extrapolated x-intercept values were within 5% of the respective zero-spike values indicating that matrix effects were at worst minimal. For the diluted samples, the extrapolated x-intercept for the C/CL method was 36.52 mg-N/L, and the zero-spike value was 38.55 mg-N/L. The extrapolated x-intercept for the Kjeldahl method was 35.51 mg-N/L, and the zero-spike value was 34.27 mg-N/L. The coefficient of determination (r^2) values for both methods exceeded 0.9990 (TN = 0.9992; TKN = 0.9998).

Total Nitrogen: Comparison of C/CL and Kjeldahl Analysis for Retort Waters

The values for TN by C/CL (n = 10) were compared with those for TKN (n = 3) for nine oil shale process waters and a composite water; each water was diluted to yield concentrations between 30 and 75 mg-N/L. The results from this study are presented in Table V. The values obtained by the two methods agreed remarkably well. The difference in recovery of nitrogen by the two methods ranged from -5.5% to +5.6%. The relative standard deviation (rsd) values for TN were less than 3.5%, and those for TKN were less than 2.5%. The average TN and TKN values for the nine waters agreed with the TN and TKN values for the composite water (6740 and 6915 mg/L vs 6712 and 7108 mg/L, respectively); this verifies the internal consistency of the values.

To determine if a significant difference existed between the two methods, a two-way analysis of variance (anova) was conducted using the first three TN determination values obtained for each sample and the triplicate TKN results. There was no significant difference ($P>0.10$) between the two nitrogen methods, $F_s<F_{.10}$ ($2.30<2.84$), although there was a significant interaction effect between methods and wastewaters, $F_s>F_{.005}$ ($3.90>3.22$). The results of Tukey's test for nonadditivity indicated that an insignificant portion ($P>0.10$) of the interaction was nonadditive; therefore the assumptions of the anova were not violated. This interaction effect was most likely a result of the wide range of nitrogen concentrations among the waters.

Organic Nitrogen: Comparison of C/CL and Kjeldahl Analysis for Retort Waters

The quantitation of organically bound nitrogen is traditionally accomplished by a combination of wet-chemical methods (e.g., ammonia predistillation followed by Kjeldahl digestion for organic Kjeldahl nitrogen; or total Kjeldahl digestion and a separate ammonia analysis). The TKN and OKN values for the nine oil shale wastewaters and a composite sample are given in Table VI. The percentage of TKN accounted for by OKN was highest for Paraho (14.5% of TKN). The average organic nitrogen content of the nine waters was 7.7% of TKN, and that of the composite sample was 9.2% of TKN. Oxy-6 gas condensate (GC), a condensate of process off-gases collected simultaneously with Oxy-6 retort water (RW), had one of the lowest concentrations of OKN (21 mg-N/L). The average OKN value for the nine waters agreed with the OKN value for the composite water (671 vs 652 mg/L, respectively).

The direct determination of organic nitrogen by C/CL has not been reported. Currently, "organic" nitrogen can only be determined by C/CL indirectly, by analysis for total nitrogen and then subtracting ammonia-nitrogen as determined by a separate method (e.g., colorimetry, distillation/titrimetry). Since this approach compounds the errors of two distinctly different analytical methods and since organic nitrogen concentrations in oil shale wastewaters can be orders of magnitude less than ammonia concentrations, a sample pretreatment method that would effect a physical separation of inorganic from organic nitrogen would be extraordinarily valuable. This would allow the direct analysis of the organic nitrogen fraction for total nitrogen.

We have developed two novel approaches to separate ammonia from the sample matrix by adapting the methods discussed in Chapters I and II. A modification of the RPF procedure (Chapter I) and the dialysis method (Chapter II) were evaluated for their abilities to separate the ammonia in oil shale process wastewaters from the organic nitrogen. Previous studies (Sakaji, unpublished data) indicated that ammonia removal by purging with inert gas (N_2) was extremely inefficient for retort waters; this purging process was extraordinarily time consuming and ineffective even at high temperature and pH.

RPF-TN. To test the effectiveness of the RPF method, we analyzed unfractionated filtrates and the two RPF fractions (HpF and LpF) of nine oil shale process waters and a composite water for TN using the prescribed C/CL procedure. These results were compared with those from the respective wet-chemical method (OKN with LpF-TN, and titrimetric ammonia-N with HpF-TN). The results of these analyses are presented in Figure 6.

For all but two of the process waters analyzed (Paraho and Oxy-6 gas condensate), the NPON concentrations, as measured by LpF-TN, ranged from 46% to 150% of the respective OKN concentrations; the average NPON concentration (excluding Paraho) was 67% of the average OKN concentration (excluding Paraho) (Table VII). The average NPON value for the nine waters, however, did not agree particularly well with the NPON value for the composite water (202 vs 311 mg/L, respectively).

The nitrogen content of the LpF was an underestimator of the OKN, and the difference between these two values was strictly dependent on the relative polarity of the nitrogen-bearing organic solutes. From 8% to 24% of the organic nitrogen was not partitioned by the stationary phase, but was collected with the ammonia in the hydrophilic fraction and quantified as HpF-TN. For all but two of the wastewaters, the TN determined on the unfractionated sample exceeded the sum of the LpF-TN and HpF-TN as well as the sum of NH_3-N and OKN. This indicated in the first instance that a portion ("residual-N" in Figure 6) of the organic nitrogen was irreversibly retained by the C_{18} stationary phase. In the second instance, it indicated that a portion of the nitrogen was unrecovered by the Kjeldahl procedure; some of the OKN was either steam distilled or hydrolyzed to ammonia prior to OKN digestion, or a portion of the solutes was refractory to Kjeldahl digestion. Even though organic nitrogen may be incompletely recovered by the RPF method, LpF-TN is a direct measure of nonpolar organic nitrogen and has been successfully applied to yield valuable information about the fate of organic nitrogen solutes during biotreatment (Healy et al. 1983).

Dialyzed-TN. Treatment of oil shale wastewaters by NOGD yielded an ammonia-free (NH_3-N < 10 mg/L) dialyzed sample that was analyzed for TN. The

resulting NVON concentrations ranged from 89% to 154% of the respective OKN concentrations; the average NVON concentration was 114% of the average OKN (Table VII). The average NVON value for the nine waters agreed with the NVON value for the composite water (768 vs 766 mg/L, respectively).

Inorganic nitrogen oxides and cyanates would not be expected to degas from the sample, and therefore would be sampled with the NVON and contribute a positive interference to this method of determining organic nitrogen. As mentioned above, however, oil shale wastewaters contain negligible amounts of NO_x. Therefore, the consistently higher results from NOGD may indicate that this method has successfully recovered a more complete spectrum of nitrogen compounds than either OKN or NPON, and is a closer estimate of the true organic nitrogen concentration.

The precision of the OKN, NPON, and NVON values was excellent. Almost all of the rsd values were less than 4% (Table VII). The only rsd value that exceeded 6% was the NVON value for Oxy-6 gas condensate (17.9%); this value was probably high because this wastewater was composed exclusively of volatile compounds, which did not diffuse at a constant rate through the microporous tubing.

Although the nitrogen values obtained by the RPF and dialysis methods were not equivalent to OKN in these oil shale process waters, these methods of solute separation followed by analysis with C/CL for TN may be the most rapid methods available for estimating organic nitrogen.

Cost Comparison

A cost comparison of the macro-Kjeldahl apparatus and the Antek 703C nitrogen analyzer showed that the capital expense of the Kjeldahl apparatus and flasks was approximately half that of the Antek nitrogen analyzer and syringe drive ($7,800 vs $14,800). The yearly costs of expendable parts were approximately equal for the two approaches. Assuming two full-rack Kjeldahl digestions per day, 100 days per year, the acid, base and digestion reagents would cost approximately $1600. For the nitrogen analyzer, replacement combustion tubes, syringes, scrubbers, septa, and high-purity oxygen for 100 days of operation would be approximately $1950 per year; the cost of determining NPON would be substantially higher because of the expense of the chromatographic cartridge (approximately $100 per 100 cartridges), and would depend on the number of analyses and reusability of the cartridge. Similarly, the cost of NVON analyses would depend on the reusability of the PTFE tubing. None of these estimates includes the electrical demand of the units. Operator's time for the C/CL methods (per sample) is considerably less than for the Kjeldahl method.

CONCLUSIONS

Wastewaters from the recovery of shale oil are highly contaminated; organic nitrogen compounds (i.e., nitrogenous heterocycles and aromatic amines) have been postulated as responsible for a large portion of the biorefractory solutes. Total Kjeldahl nitrogen and organic Kjeldahl nitrogen, the standard methods for quantifying nitrogen in agricultural and biological wastewaters, are extremely time-consuming procedures, and nitrogenous heterocycles are notoriously resistant to the Kjeldahl digestion step. Total nitrogen (TN) as determined by combustion at 1100°C followed by excitation of the by-products with ozone to an

an electronically excited species (NO_2^*) and chemiluminescent detection, was demonstrated to recover a wide range of nitrogenous heterocycles. There was no statistically significant difference between TKN and TN for nine oil shale wastewaters.

Two novel techniques (reverse-phase fractionation and nonosmotic dialysis) for the separation of ammonia from the sample matrix were evaluated for their ability to broaden the scope of C/CL analysis. Total nitrogen values for either the RPF nonpolar fraction or the dialyzed portion of oil shale wastewaters revealed that these methods of solute separation followed by analysis with C/CL may be the most rapid methods available for directly estimating organic nitrogen; these methods yield nitrogen values comparable with those for organic Kjeldahl nitrogen.

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Table I. Five Methods for Direct Determination of Total and Organic Nitrogen

measurement designation	pretreatment	discarded fraction	organic-N abstraction technique	quantitation method	value yielded
TKN	——	——	acid digestion	distillation/ titrimetry	total-N (Kjeldahl)
TN	——	——	high-temperature combustion	chemiluminescence	total-N (C/CL)
OKN	predistillation	distillate (NH_3, C_1-C_n amines)	(distilland) acid digestion	distillation/ titrimetry	organic-N (underestimate)
LpF-TN (NPON)	reverse-phase fractionation	HpF (NH_3, polar organic-N, nitrites, nitrates, cyanates)	(LpF) high-temperature combustion	chemiluminescence	organic-N (underestimate)
dialyzed-TN (NVON)	nonosmotic dissolved-gas dialysis	permeate/dialysate (NH_3, volatile organic-N, amines)	(retentate) high-temperature combustion	chemiluminescence	organic-N (overestimate)

Table II. Direct and Indirect Methods of Estimating Organic Nitrogen: Advantages and Disadvantages

Method	Advantages	Disadvantages
-indirect-		
(TN)-(NH_3)	-if no nitrogen oxide salts present, this method could yield correct value	-requires two methods of analysis -compounded error can lead to over- or under-estimation, especially if the NH_3 value is large
(TN)-(HpF-TN)	-one method of analysis -rapid; requires one fractionation and two determinations	-HpF-TN contains some polar organic-N (e.g., oxygenated heterocycles); therefore this method underestimates organic-N -compounded error
(TKN)-(NH_3)	-saves time compared with OKN, since separate, but simultaneous analyses can be conducted	-time consuming -compounded error -organic-N compounds can volatilize or hydrolyze during pretreatment; therefore this method is an underestimator of organic-N
-direct-		
OKN	-standard method of analysis	-extremely time consuming -organic-N compounds can volatilize or hydrolyze during pretreatment -certain compounds resist digestion -this method is an underestimator of organic-N
LpF-TN (NPON)	-only one determination required (on a fractionated sample) -extremely rapid (90 s per sample after fractionation)	-some solutes irreversibly retained on stationary phase, and some organic-N not retained -this method underestimates organic-N, but is a direct measure of nonpolar organic-N
dialyzed-TN (NVON)	-only one determination required (on degassed sample) -extremely rapid (90 s per sample after dialysis)	-nitrate, nitrite, and cyanate salts contribute to TN values; this method therefore overestimates organic-N, but is a direct measure of nonvolatile N

Table III. Sources and Purities of Nitrogen Heterocycles

Alfa Products; Danvers, MA
piperidine (98%)

Aldrich; Milwaukee, WI
4,4'-azoxyanisole (NA[2])
2,3-dimethyl-1-(4-methylphenyl)-3-pyrazolin-5-one (99+%)
3,5-dimethylpyrazole-1-carboxamide (NA)
1,5-dimethyltetrazole (97+%)
2,5-dimethyl-1,3,4-thiadiazole (99%)
2-hydroxy-6-methylpyridine (97%)
2-hydroxypyridine (97%)
3-hydroxypyridine (97%)
imidazole (99%)
indazole (98%)
6-nitroquinoline (98%)
piperazine (99%)
pyrazole (98%)
pyridazine (97%)
pyrazine (99+%)

J.T. Baker; Phillipsburg, NJ
acetonitrile (HPLC grade)
diethanolamine (99.9%)
potassium nitrate (AR grade)

Burdick & Jackson; Muskegon MI
N-N-dimethylformamide (HPLC grade)

Calbiochem-Behring; La Jolla, CA
nicotinic acid (NA)

Carnegie-Mellon University; Pittsburg, PA
9-methylcarbazole (NA)

Chem Service; West Chester, PA
benzimidazole (NA)
carbazole (99+%)
cyanuric acid (NA)
3,5-dimethylpyrazole (99%)
2,4-dimethylpyridine (NA)
2,6-dimethylpyridine (NA)

Chem Service; West Chester, PA (cont.)
indole (99+%)
isoquinoline (NA)
2-methylpyrazine (99%)
2-methylpyridine (NA)
3-methylpyridine (NA)
4-methylpyridine (99%)
pyridine (99%)
quinoline (NA)
2,4,6-trimethylpyridine (99%)

Eastman Organic Chemicals; Rochester, NY
m-nitrophenol (NA)
o-nitrophenol (NA)
p-nitrophenol (98%)

Fisher; Fair Lawn, NJ
glycine (NA)

Fluka AG; Buchs, Switzerland
5-ethyl-2-methylpyridine (NA)

Hellige; Garden City, NJ
nitrite salt solution (50 mg-N/L)
urea (NA)

Mallinckrodt; St. Louis, MO
4-amino-2,3-dimethyl-2-phenyl-3-pyrazolin-5-one (NA)

Matheson Coleman & Bell; East Rutherford, NJ
ethylenedinitrilotetra-acetic acid (NA)

NOAH Chemical; Farmingdale, NY
3-ethyl-4-methylpyridine (NA)
4-ethyl-3-methylpyridine (NA)
2-ethylpyridine (NA)
3-ethylpyridine (NA)
4-ethylpyridine (NA)
2-n-propylpyridine (NA)
2,3,6-trimethylpyridine (NA)

[1] purities given in parentheses
[2] not available

Table IV. Percent Recoveries of Nitrogen from 17 Compounds Using the Total Kjeldahl Nitrogen (TKN) Method

Compound	(% theoretical)
pyridine	81
nicotinic acid	99
piperidine	87
pyridazine	94
4-amino-2,3-dimethyl-2-phenyl-3-pyrazolin-5-one	53
2,3-dimethyl-1-(4-methylphenyl)-3-pyrazolin-5-one	79
pyrazole	15
3,5-dimethylpyrazole	17
indazole	46
1,5-dimethyltetrazole	46
2,5-dimethyl-1,3,4-thiadiazole	5
imidazole	25
cyanuric acid	102
m-nitrophenol	57
o-nitrophenol	< 1
p-nitrophenol	51
4,4'-azoxyanisole	72

Table V. Determination of Total Nitrogen in Oil Shale Process Waters: Combustion/Chemiluminescence (TN) versus Kjeldahl (TKN)

Process Water	TN	rsd	TKN	rsd	(% diff)[1]
Paraho	28 805	3.5	29 661	1.9	2.9
150-Ton	10 084	1.3	10 453	1.2	3.5
Oxy-6 GC	6 886	3.5	6 985	1.9	1.4
Composite	6 712	2.3	7 108	1.3	5.6
S-55	4 196	2.1	4 379	2.3	4.2
Omega-9	3 574	1.9	3 698	1.3	3.4
TOSCO HSP	2 826	2.6	2 809	1.7	-0.6
Geokinetics	1 844	2.1	1 826	0.5	-1.0
Oxy-6 RW	1 313	1.8	1 349	0.9	2.7
Rio Blanco sour	1 133	3.4	1 074	1.5	-5.5
average[2]	6 740		6 915		

[1] [(TKN - TN)/(TKN)] X 100
[2] mean for nine waters, excluding Composite

Table VI. Total Kjeldahl Nitrogen (TKN) and Organic Kjeldahl Nitrogen (OKN) for Nine Oil Shale Process Waters and a Composite Water

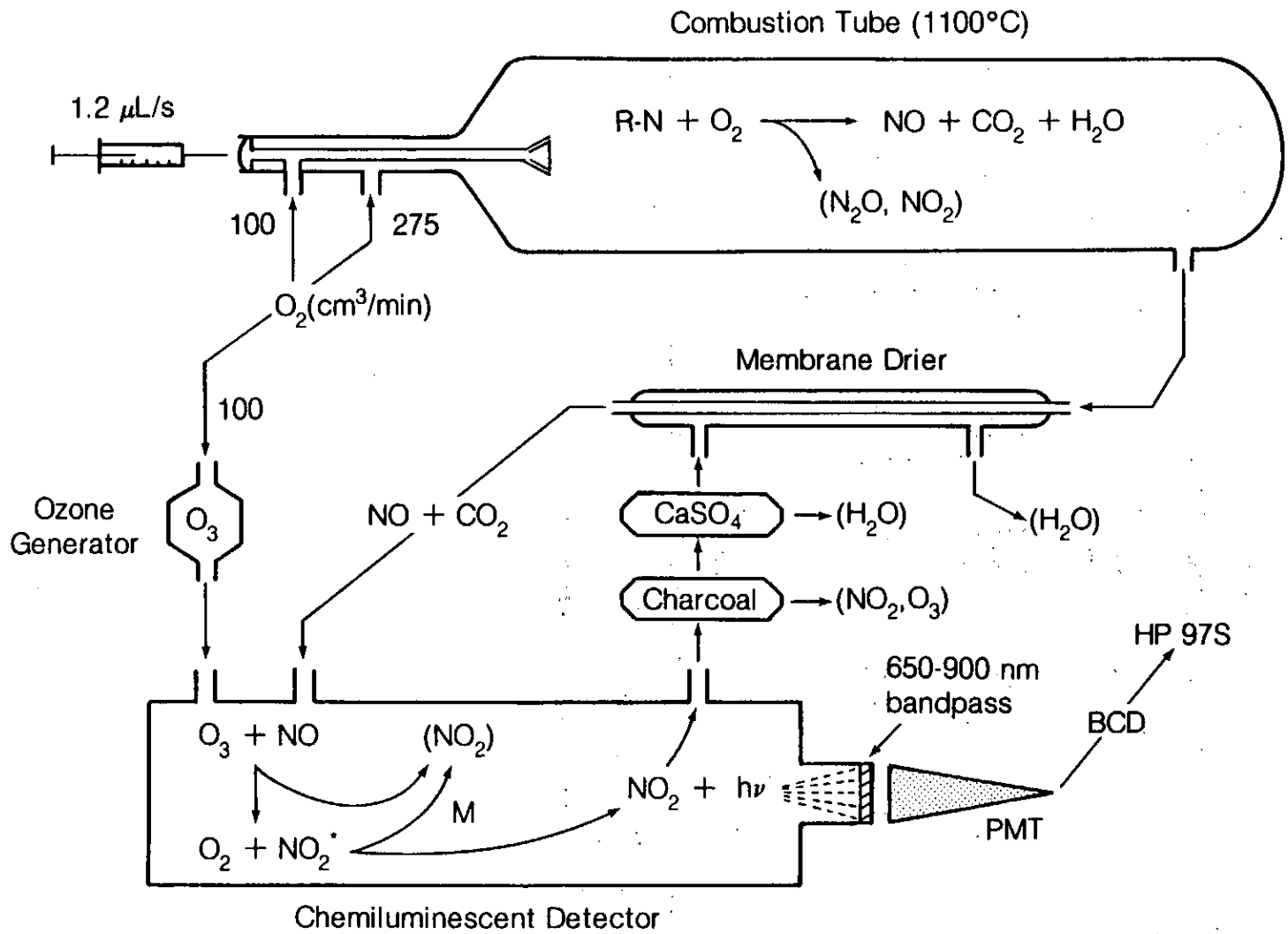
Process Water	TKN	rsd (%)	OKN	rsd (%)	% OKN[1]
Paraho	29 661	1.9	4 299	1.8	14.5
150-Ton	10 453	1.2	541	3.4	5.2
Oxy-6 GC	6 985	1.9	21	1.4	0.3
Composite	7 108	1.3	652	1.0	9.2
S-55	4 379	2.3	372	0.9	8.5
Omega-9	3 698	1.3	112	0.9	3.0
TOSCO HSP	2 809	1.7	305	1.2	10.9
Geokinetics	1 826	0.5	194	3.4	10.6
Oxy-6 RW	1 349	0.9	183	3.0	13.6
Rio Blanco sour water	1 074	1.5	14	4.8	1.3
average[2]	6 915		671		

1 [(OKN)/(TKN)] X 100
2 mean for nine waters, excluding Composite

Table VII. Organic Kjeldahl Nitrogen (OKN), Nonpolar Organic Nitrogen (NPON), and Nonvolatile Organic Nitrogen (NVON) Values for Nine Oil Shale Process Waters

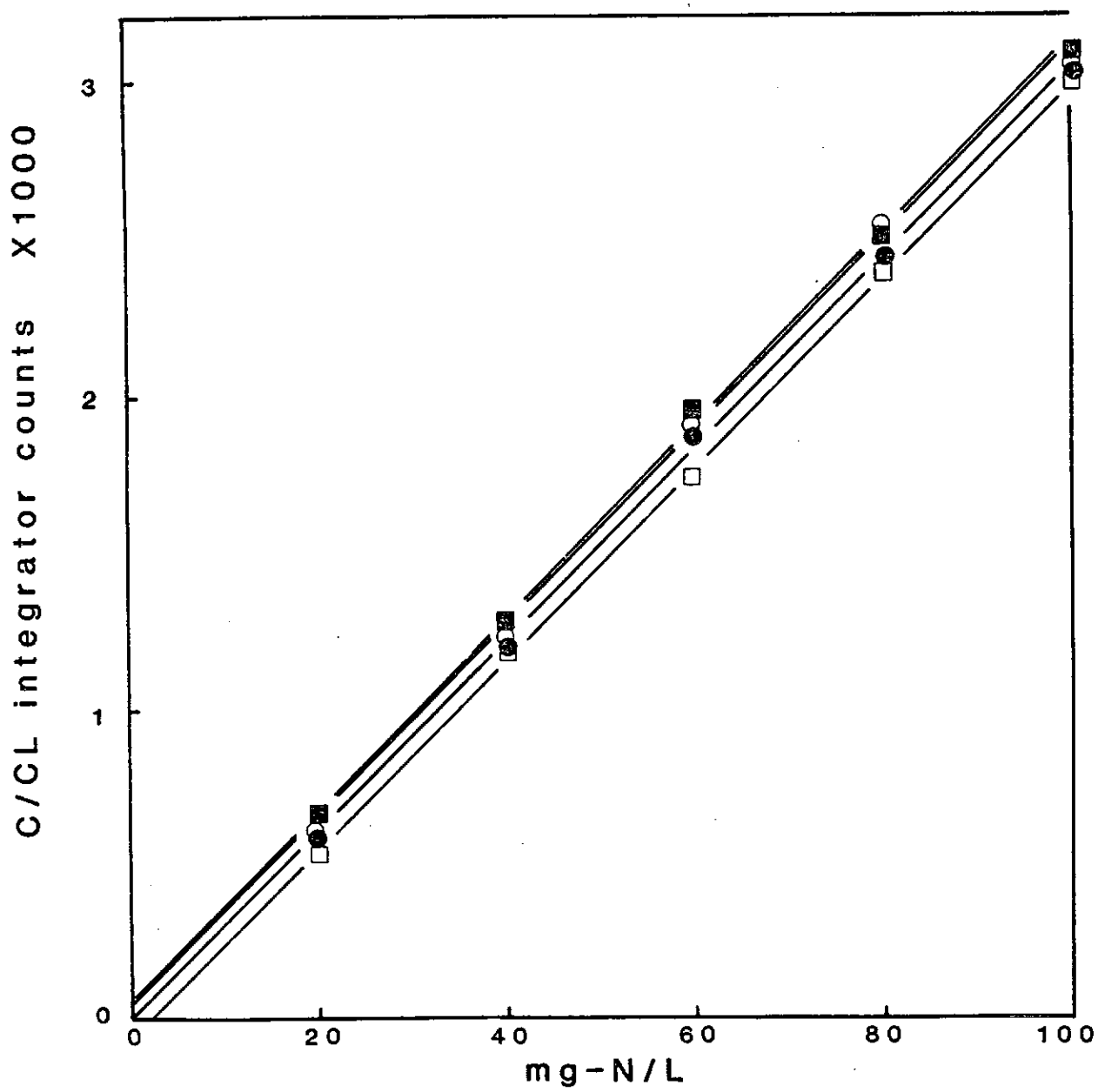
Process Water	OKN	rsd (%)	NPON	rsd (%)	NVON	rsd (%)
Paraho	4299	1.8	645	5.7	4839	1.1
150-Ton	541	3.4	295	2.7	587	3.7
Oxy-6 GC	21	1.4	79	1.2	30	17.9
Composite	652	1.0	311	1.6	766	1.2
S-55	372	0.9	172	0.5	414	1.5
Omega-9	112	0.9	81	2.0	100	2.9
TOSCO HSP	305	1.2	236	1.9	470	1.9
Geokinetics	194	3.4	133	3.2	242	4.1
Oxy-6 RW	183	3.0	159	1.7	218	0.5
Rio Blanco sour	14	4.8	21	2.9	17	2.3
average[1]	671		na[2]		768	
average[3]	218		147		na	

[1] mean for nine waters, excluding Composite
[2] not applicable
[3] mean for eight waters, excluding Composite and Paraho



XBL 8312-6910

Figure 1. Reaction schematic of a chemiluminescence total-nitrogen analyzer (Antek model #703C).



XBL 843-993A

Figure 2. C/CL standard curves for three compounds in toluene and water:
■ ammonium sulfate in water (y = 30.37x + 75.09)
● 9-methylcarbazole in toluene (y = 30.38x - 1.90)
□ 2,4,6-trimethylpyridine in toluene (y = 30.37x - 53.65)
○ 2,4,6-trimethylpyridine in water (y = 30.70x + 40.50).

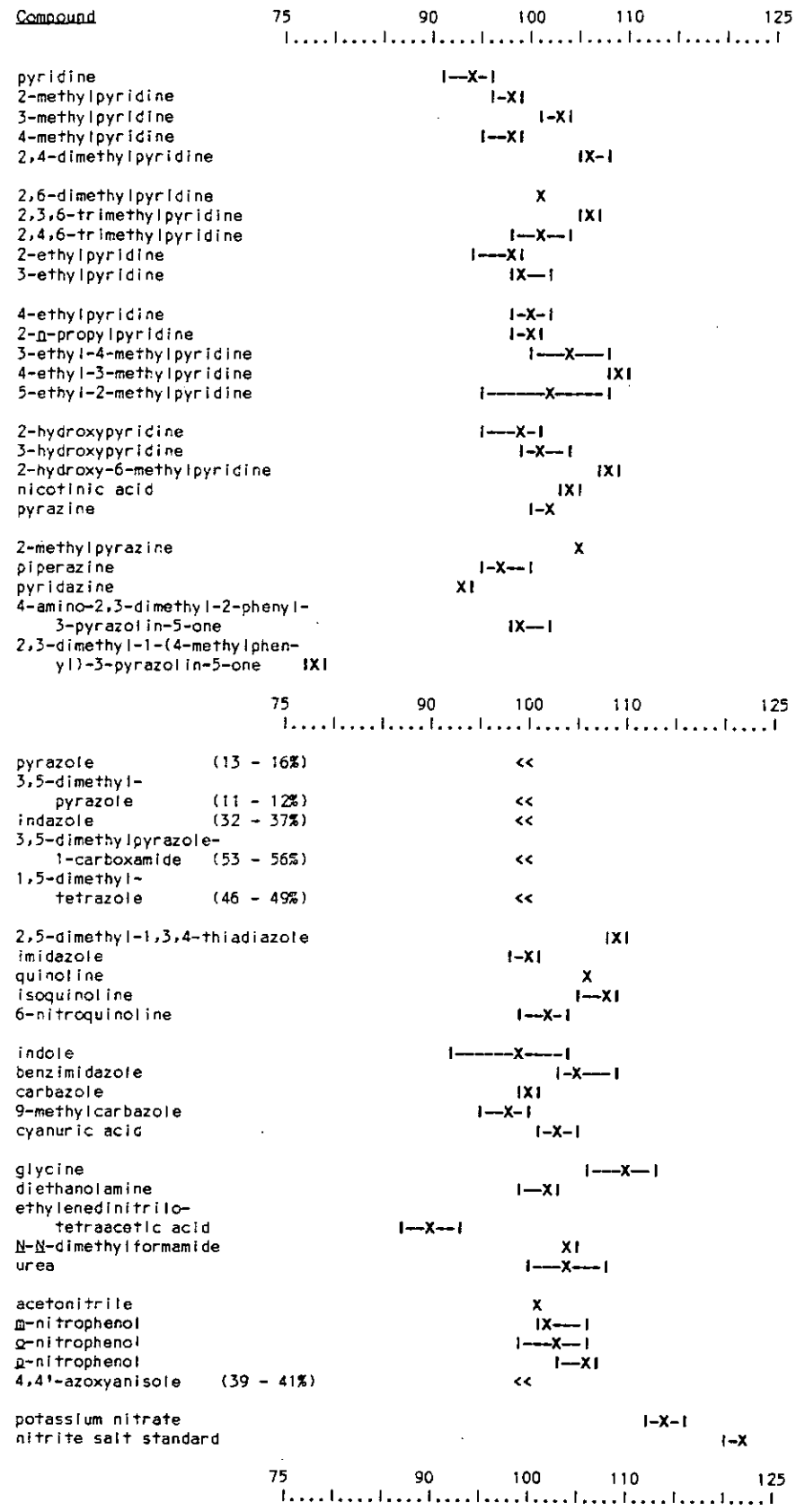
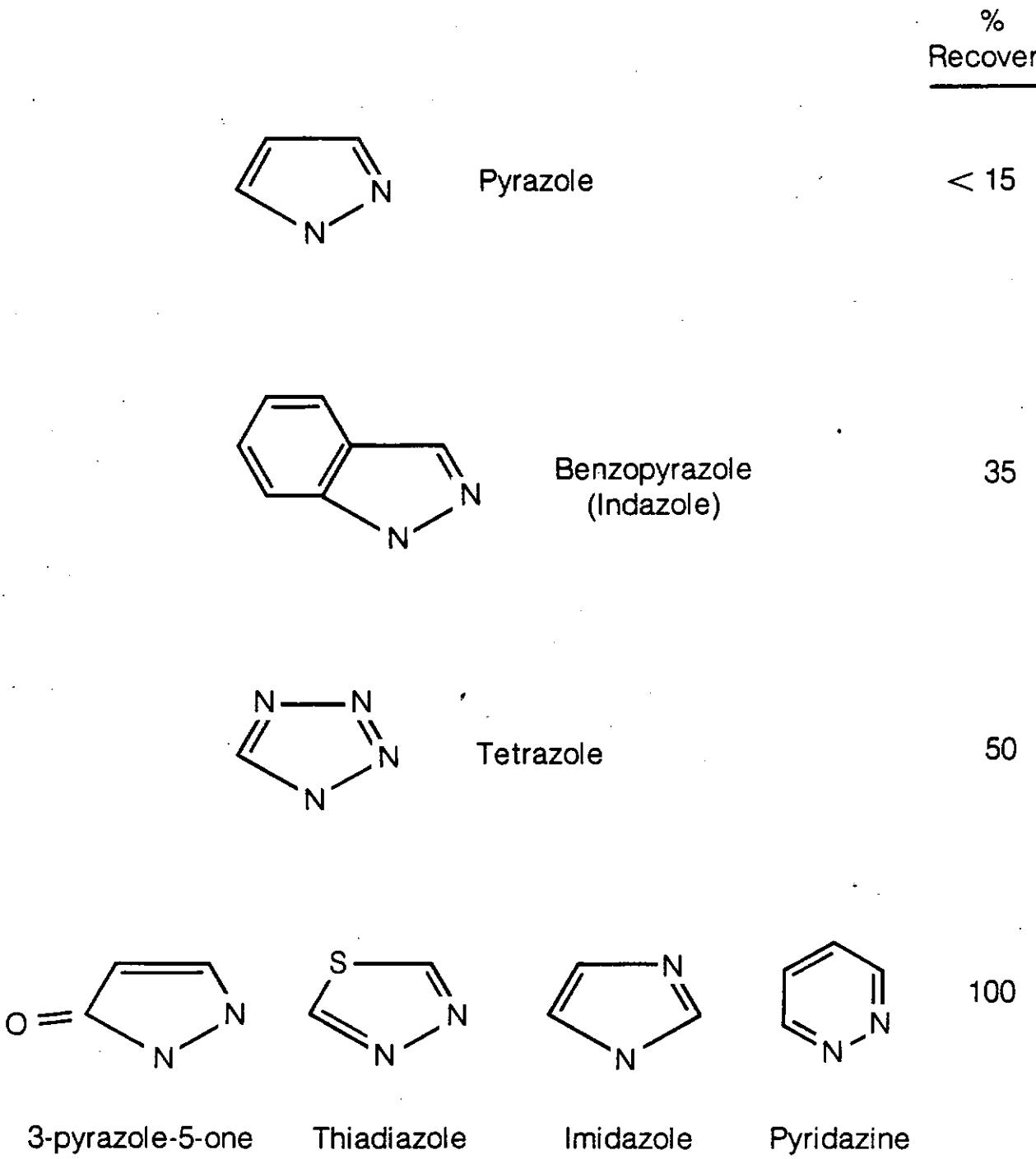


Figure 3. Percent recovery of nitrogen from 52 standard solutions by C/CL nitrogen analysis using an ammonium sulfate standard curve (mean = X) (range = I——I) (XBL 8310-12246).



XBL 8312-6909

Figure 4. Effect of Structure on Recovery of Nitrogen by C/CL.

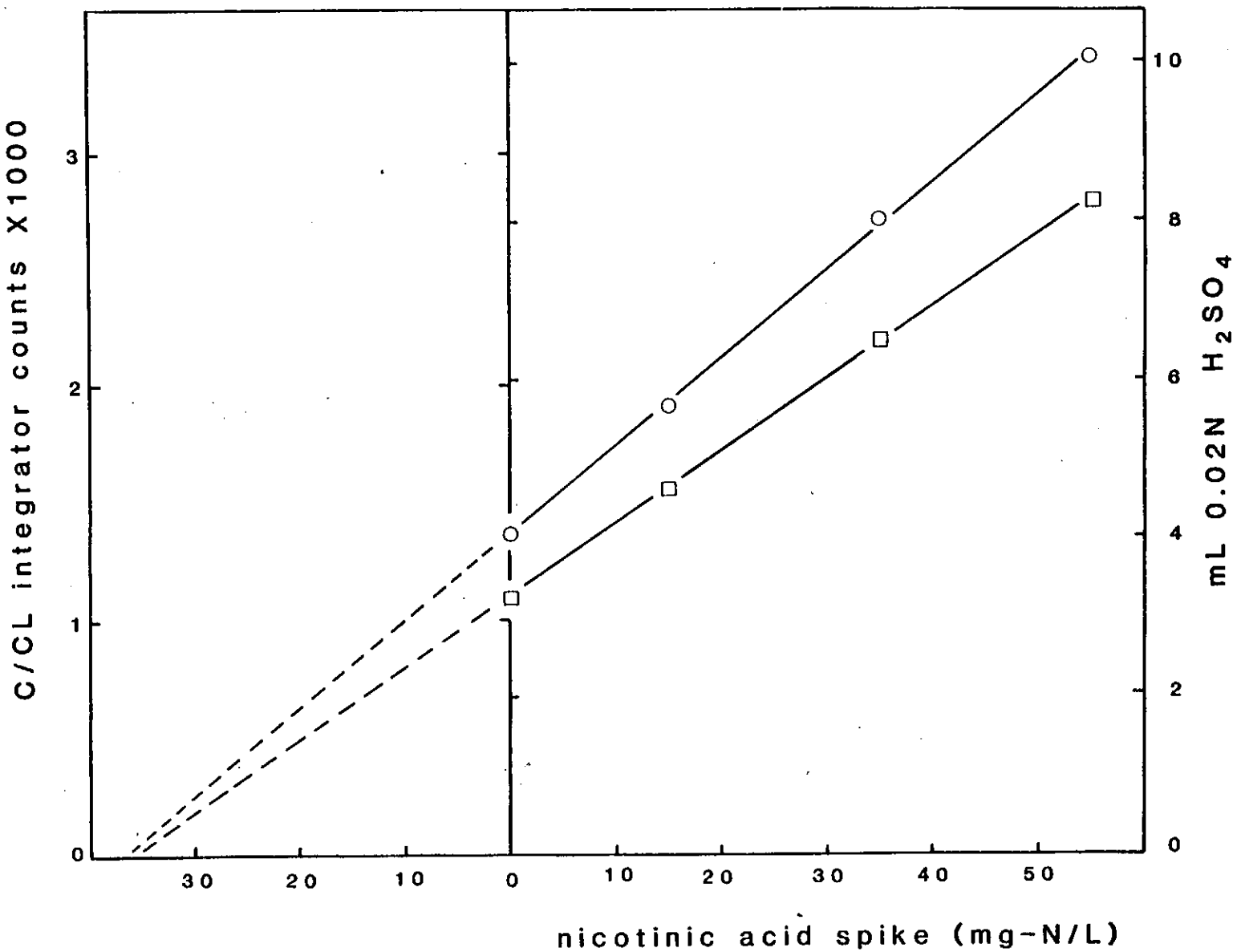


Figure 5. C/CL and Kjeldahl standard-addition curves for nicotinic acid in a composite oil shale wastewater. (○) C/CL: ($y = 37.46x + 1368$; x-intercept = 36.52 mg-N/L; $r^2 = 0.9992$); (□) Kjeldahl: ($y = 0.0917x + 3.254$; x-intercept = 35.49 mg-N/L; $r^2 = 0.9998$) (XBL 843-992A).

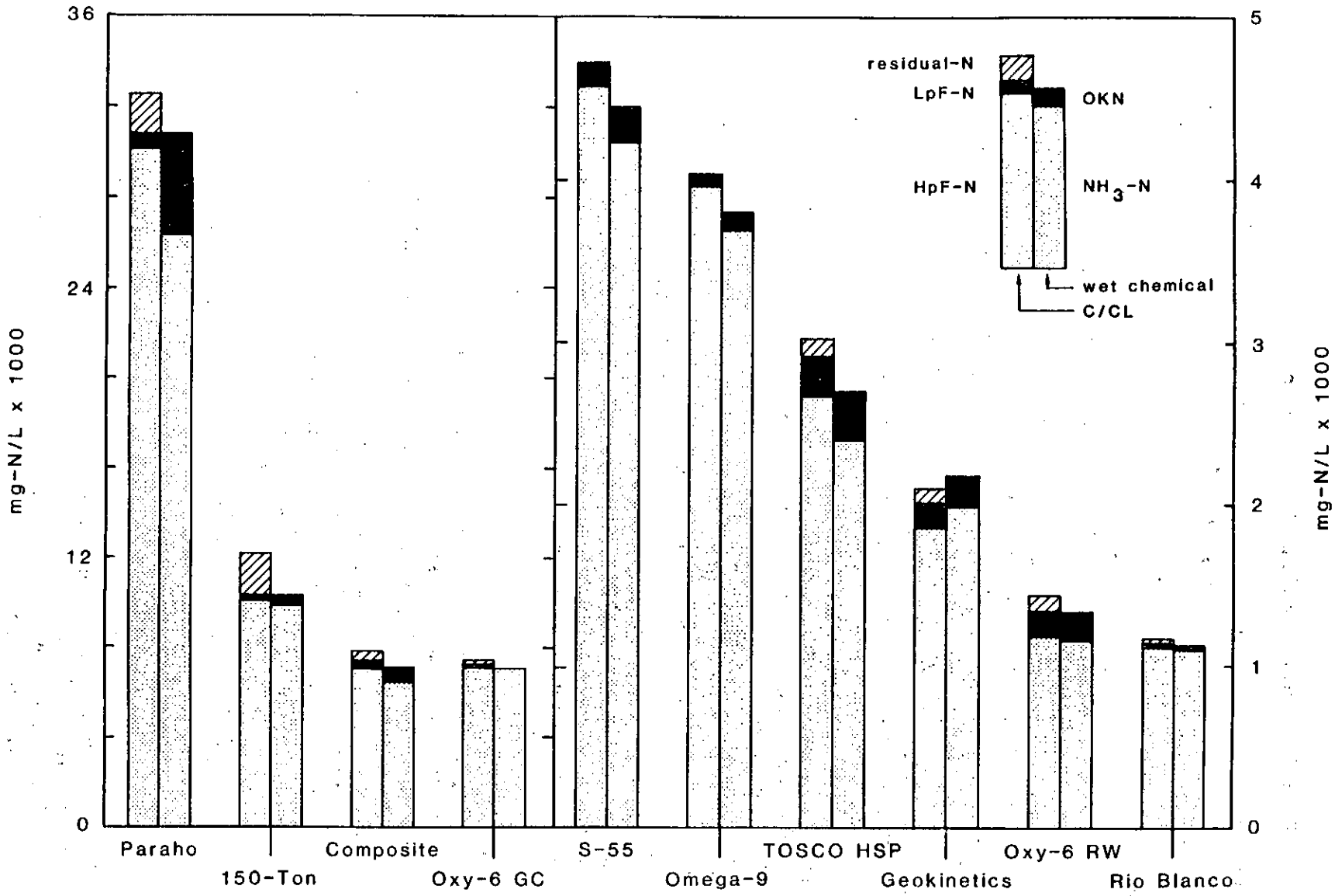


Figure 6. Nitrogen values for oil shale process waters obtained by C/CL and Kjeldahl. For each pair of bars, the top of the left member is total nitrogen (TN) as determined by C/CL and the top of the right member is total Kjeldahl nitrogen (TKN) as determined by wet-chemical analysis. The C/CL nitrogen values for the reverse-phase fractions, HpF and LpF, are estimators of NH_3-N and organic Kjeldahl nitrogen (OKN), respectively. The cross-hatched areas are "residual-N", that portion of the TN not accounted for by the sum of the two fractions; for S-55, the total nitrogen value was 111 mg-N/L less than the sum (XBL 8310-12247).

PROTOCOL: TOTAL NITROGEN (Combustion/Chemiluminescence)

(for the Antek model 703C nitrogen analyzer, which includes 771 Pyroreactor, 720 Chemiluminescence Detector, and 735 Syringe Drive; Antek Instruments, Inc., Houston, TX)

I. Start Up

A. Insert a new Teflon-backed septum in the combustion tube entry port. Ensure that the Teflon side faces into the combustion tube and that it is properly seated.

B. Perform any routine maintenance (IX.B.-D.).

C. Depress the 1100°C button on the pyroreactor to bring the furnace and combustion tube to operating temperature. Allow 30 to 60 minutes for the furnace temperature to stabilize.

D. Adjust the oxygen flow rotameters so that the settings listed below bisect the floats (ensure that the oxygen delivery pressure is 20 psig and that the tank pressure is at least 400 psig).

1. pyro-oxygen: 2.0 (275 cm^3/min).
2. inlet-oxygen: 3.7 (100 cm^3/min).
3. ozone-oxygen: 4.0 (100 cm^3/min).

E. Zero the signal-level analog meter display.

1. Set manual/automatic button to MAN position.
2. Set SENSITIVITY button to HIGH position.
3. Depress ATTENUATION "1" button.
4. Turn ZERO-adjust knob to null the signal-level analog meter.
5. Depress RESET button. It must be held in for at least two seconds; the digital display will then read zero.
6. Depress ATTENUATION "20" button; the signal-level analog meter needle should not shift from zero.
7. Set manual/automatic button to AUTO position.
8. Repeat I.E.5.

II. Sample Preparation

NOTE: When used as a reagent, "water" refers to ASTM Type I quality.

A. Total nitrogen for aqueous samples

1. Filter all samples through a 0.4-µm pore-diameter polycarbonate membrane filter.
2. Dilute sample filtrates with appropriate solvent to yield nitrogen concentrations between 20 and 100 mg-N/L.

B. HpF nitrogen (estimator of inorganic nitrogen)

1. Fractionate 5 mL of sample (as described in Chapter I, part III.A.1.-3. of protocol) with the following modifications:
   a. collect <u>all</u> of the aqueous effluent in a 10-mL Class A volumetric flask; do NOT discard any of the initial effluent.
   b. rinse cartridge with 1 mL of water; pool with the aqueous effluent.
   c. dilute a subsample as in II.A.2.

C. Nonpolar organic nitrogen (estimator of organic Kjeldahl nitrogen)

1. Elute the rinsed cartridge (from II.B.1.b.) with approximately 4 mL of methanol followed by 1 mL of THF. Collect eluent in a 5-mL Class A volumetric flask; bring to volume with methanol.
2. Dilute a subsample with methanol as in II.A.2.
3. Save cartridge for reuse.

D. Nonvolatile organic nitrogen (estimator of organic Kjeldahl nitrogen)
   1. Follow procedure outlined in the protocol of Chapter II.
E. Store samples in either 20-mL scintillation vials with Teflon-lined screw caps (II.A.-C.) or in 600-µL conical-bore vials with Teflon-lined screw caps (II.D.)at 4°C until time of analysis.
   IMPORTANT: Do not acidify samples prior to analysis; this causes the buildup of deposits that clog the syringe needle bore after the sample vaporizes from the needle while it is in the combustion tube.

III. Preparation of Nitrogen Standards
NOTE: Use Analytical Reagent grade chemicals only; glassware should be washed in 35% nitric acid.
A. Prepare an aqueous stock solution of ammonium sulfate $(NH_4)_2SO_4$ (100 mg-N/L).
   1. Weigh 235.85 mg of oven-dried (105°C) ammonium sulfate and quantitatively transfer to a 500-mL volumetric flask; bring to volume with water.
B. For the following working standards, add the indicated volume of stock solution to a 10-mL Class A volumetric flask and bring to volume with water.
   NOTE: Use calibrated positive-displacement pipettes.
   1. 20 mg-N/L: 2.0 mL.
   2. 40 mg-N/L: 4.0 mL.
   3. 60 mg-N/L: 6.0 mL.
   4. 80 mg-N/L: 8.0 mL.
   5. 100 mg-N/L: use a portion of the stock solution neat.
C. Store standards at 4°C; prepare new standards monthly.

IV. Analysis of Standards
NOTE: Follow VI. instead of IV.A.-C. if automated injection is used.
A. Load the "Standard-Curve Program" (Chapter V, Appendix A) into the HP-97S.
   1. Connect the nitrogen analyzer to the HP-97S with the interface strap. A pin-to-pin diagram for the BCD-output wiring from the nitrogen detector to the amphenol connector of the HP-97S is included in Chapter V, Appendix B.
   2. Turn on the calculator.
   3. With the HP-97S in RUN mode, run the program card through the HP-97S card reader.
      a. ensure that the LED display reads zero before loading program.
      b. if an error message is displayed, push "CL X" and reload program.
   4. Initiate the program as per the user instructions (Chapter V, Appendix A).
      IMPORTANT: Maximize the distance between the nitrogen detector and the calculator and program packet to avoid magnetic alteration of the program cards. The card reader will not function properly in the presence of the magnetic field produced by an instrument such as the ozone generator used in the detector.
B. Set the syringe drive to allow for full depression of the syringe plunger (10-µL syringe with extended handle, Precision Sampling, Baton Rouge, LA) at 1.2 µL/sec (setting 900).
   NOTE: After the correct position has been established, do not move syringe drive. The syringe should always be positioned in the same

place in the clamp to ensure reproducible sample injection.

1. Height adjustment.
   a. adjust height of syringe drive by turning leg screws so that, when the syringe is under the rocker-arm clamp, the needle can pierce the center of the septum.
2. Adjust the forward limit.
   a. set the syringe drive to manual and turn power on.
   b. turn the forward-limit adjust-knob fully clockwise; this sets the limit to the extreme forward position. Press the FWD button.
   c. align the syringe drive with the combustion tube sampling port. Press the REV button to move the drive plate to the rear of the syringe drive. Place the syringe under the rocker-arm clamp of syringe drive, and fully insert the needle through the septum; approximately 7.5 cm of syringe barrel should extend from behind the rocker-arm.
   d. with a marking pen, mark on the syringe drive housing the position at which the syringe plunger is fully depressed. Remove the syringe.
   e. set the forward limit of the drive plate by turning the limit adjust knob counterclockwise. Press the FWD button to ensure that the drive plate stops at the marked point. Repeat if necessary.
   f. press the REV button. Position the syringe to determine that the forward limit point is set correctly; the drive plate should stop just when the syringe plunger is fully depressed. **NOTE:** Ensure that the syringe drive does not push the syringe barrel into the septum; this would damage the combustion tube.
   g. if the forward limit is set incorrectly, remove syringe and repeat steps e.-f.
3. Adjust the rear limit.
   a. turn the rear-limit adjust knob fully clockwise; this sets the limit to the extreme rear position. Press the REV button.
   b. place the syringe under the rocker-arm clamp and pull the plunger back so that the plunger tip is approximately at the 7-μL mark. With a marking pen, mark the syringe drive housing approximately 2.5 cm behind the extended syringe plunger. Remove the syringe.
   c. set the rear limit of the drive plate by turning the limit adjust knob counterclockwise until the drive plate stops at the marked point.
   d. press the REV button, place the syringe (with plunger extended) under the rocker-arm clamp; press the FWD button. If the rear limit has been set correctly, the drive plate will stop approximately 2.5 cm behind the plunger.
   e. if the rear limit point is set incorrectly, remove syringe and repeat steps c.-d.
4. Check syringe drive limits.
   a. press REV to position the drive plate to the rear.
   b. place syringe (with plunger extended) under the rocker-arm clamp.
   c. set syringe drive to auto.
   d. press the FWD button.
   NOTE: Syringe drive plate should move forward, fully depress

syringe plunger, and return to the rear set-point.

5. Allow syringe drive to warm up for 10 minutes.

C. Injection

IMPORTANT: This portion of the protocol must be followed <u>exactly</u> for reproducible results.

1. Rinse the syringe 10 times with the solvent in which the sample is dissolved.
2. Slowly draw solvent into the syringe (ensure the absence of air bubbles) and then fully depress the plunger so that the solvent remains only in the needle bore. Tap needle against the rim of a beaker to remove any excess solvent from outside of needle.
3. Withdraw the plunger until the lower meniscus of the solvent plug is at the 0.2-μL mark (this places a plug of air in the syringe after the solvent plug).
4. <u>Without moving the plunger</u>, immerse the needle in the analyte solution and slowly draw up the solution so that the lower meniscus of the solvent plug is at the 5.2-μL mark. Withdraw the needle from solution and tap against the rim of a beaker to remove any excess sample from outside of needle. Do not remove excess sample by wiping.
5. Pull the sample into the syringe barrel, and determine the volume contained between the upper and lower menisci of the sample plug; this volume should be 5.0 μL. Always pull the lower sample-plug meniscus back to the 1.0-μL mark. If the volume differs from 5.0 μL, repeat steps IV.C.1.-5.

   IMPORTANT: It is essential that exactly the same volume be injected for each determination, since detector counts are proportional to the mass of nitrogen injected, not to the concentration.
6. Place the syringe under the rocker-arm clamp of the syringe drive, and insert the needle through the septum. Position the syringe so that it is perpendicular to the septum and press the FWD button.
7. When the drive first touches the syringe plunger, press the R/S button on the HP-97S to initiate the timing loop for data collection.

D. Blanks

1. Make several injections of water or appropriate solvent to ensure zero response. Up to 10 injections may be required to rinse system and to obtain zero counts for background.
2. If a nonzero blank value persists, rinse syringe with methanol and repeat IV.D.1.

E. Inject each standard in triplicate (from low to high concentration).

F. Determine the slope and intercept of the standard curve (counts vs mass).

1. If the slope deviates significantly from previous results, check flow rates and septum; slopes have ranged from 6.0×10^6 to 7.8×10^6 counts/mg-N.
2. Significant deviations in slope indicate that the combustion tube may need replacement. The Antek operation/service manual, reference (1) section 4.1.4., has detailed replacement instructions.

V. Sample Analysis

NOTE: Follow VI instead of V.B. if automated injection is desired.

A. Load the "Data-Reduction Program" (Chapter V, Appendix C) into the HP-97S.
   1. with the HP-97S in RUN mode, run the program card through the HP-97S card reader.
      a. if error message is displayed, push "CL X" and reload program.
   2. Initiate the program as per the user instructions (Chapter V, Appendix C).
      a. if error message is displayed, push "CL X" and continue as outlined.
   3. Enter the slope (m) and y-intercept (b) from IV.F. (see Chapter V, Appendix C).

B. Sample injection: follow the syringe protocol (steps IV.C.1.-5.)
   1. After the syringe has been positioned (IV.C.6.), allow the furnace to combust all volatile nitrogen residue in the syringe needle (this is called the <u>needle blank</u>), and then depress the reset button.
   2. Press the FWD button on the syringe drive. When the drive first touches the syringe plunger, press the R/S button on the HP-97S to initiate the timing loop for data collection.
   3. When all of the nitrogen has been oxidized and detected, the detector signal (counts) stabilizes. The needle can be withdrawn from the septum before the detector has stabilized.
   4. Analyze each sample in triplicate. Follow the syringe protocol (IV.C.1.-5.), and repeat steps V.B.1.-3. for each sample.

VI. Operation of Autosampler (Precision Sampling IDP Controller 311-0200 with GC 311-H Autosampler)

A. Installation of GC 311-H Autosampler
   1. Height adjustment
      NOTE: The pyroreactor (Antek #771) must be raised 5 cm to accommodate the GC 311-H injector.
   2. Alignment: follow "Mounting Instrument and Alignment" procedure as outlined in the GC 311-H operator's manual.
      NOTE: Proper vertical and horizontal alignment of the GC 311-H allows needle to pierce center of septum at entry port of the quartz combustion tube.

B. Set up
   1. Ensure that pneumatic lines are properly connected (see operator's manual).
   2. Ensure that the supplies of air and helium are filtered.
      a. regulator pressure for operating gas (air) is 70 psig
      b. regulator pressure for pressurizing gas (He) is 20 psig
      c. secondary regulator pressure for He is 5 psi.

C. Sample volume adjustment

NOTE : The autosampler is supplied with a factory-installed 10 μL syringe. If replacement is necessary, refer to GC 311-H operator's manual.
   1. Turn on power to autosampler.
   2. Loosen locking nut on volume adjustment screw by turning counterclockwise. Screw is located directly under syringe.
   3. Adjust screw by turning it counterclockwise to decrease or clockwise to increase volume sampled by syringe. Volume required

for nitrogen analysis is 5 μL.

4. Flip toggle switch on rear of controller to displace plunger; determine volume delivered.
5. Repeat step VI.C.3. until syringe samples exactly 5 μL.
6. Ensure locking nut is tight.

D. Injection speed adjustment
1. Turn screw located on top of mineral oil reservoir fully counterclockwise.
2. Flip toggle switch and determine injection speed.
3. Adjust screw if necessary until injection speed is 1.2 μL/sec.

E. Standard and sample analysis
1. Completely fill each 1.5-mL autosampler vial with standard or sample solution, and cap (sample concentrations should be between 10 and 100 mg-N/L).
NOTE: Ensure cap is tightened securely to prevent gas leakage during vial pressurizing.
2. Place vials for standards and samples in autosampler carousel, and record the corresponding numbers. Place an empty vial in the "last-sample ring" between the standard vials and samples. The series of numbers on the lower level are offset by eight; an arrow points to the sample number being analyzed.
3. Turn carousel until sampling vial pointer is on number 42 (carousel will advance one space to position 1 when autosampler program is initiated).
4. Program autosampler (mode = program); refer to Antek's addendum, "IDP Controller # 311-0200," (section E: Controls and Display).
a. cycle: 2 min
b. aux 1: 0
c. aux 2: 0
d. dwell: 5 sec
e. flush: 7 sec
5. Change mode to auto.
6. Load the "Autosampler Standard-Curve Program" (Chapter V, Appendix D) into the HP-97S.
7. Initiate program as per user instructions.
8. Determine r^2, slope (m) and y-intercept (b) of standard curve.
9. Load the "Autosampler Data-Reduction Program" (Chapter V, Appendix E) into the HP-97S.
10. Inititate autosampler program as per users instructions.

F. Maintenance and repair (GC 311-H)
1. refer to operator's manual, chapter 7-7.

VII. Shutdown
A. Turn off the HP-97S, and disconnect the amphenol connector from nitrogen detector.
B. Press the STBY button on the pyroreactor; this lowers the temperature of the furnace to 650°C, thereby preventing the development of stress fractures in the quartz furnace tube.
IMPORTANT: It is imperative that the furnace not be turned off. Cooling a combustion tube that has been etched by the alkaline metals in retort water will craze the tube.
C. Reset the rotameters for the standby oxygen flow rates to the pyroreactor.
1. pyro-oxygen: 0.0.

2. inlet-oxygen: 0.0.
3. ozone-oxygen: 1.0 (20 cm^3/min); continual flow is required to prevent condensation of water in the ozone generator.

D. Rinse the syringe with methanol, water, and followed again by methanol.

VIII. Data Reduction

A. The "Data Reduction" program for the HP-97S automatically determines mg-N/L by interpolation of the detector signal (counts) from the standard curve; the program then multiplies this value by the dilution factor for each sample. The "Label E" function in the program will calculate the mean for a set of replicates.

B. Determine whether outliers should be discarded.
   1. Outliers should be subjected to statistical analysis before being discarded (2).
   2. If an outlying value is known to be the result of a mechanical or operational error, it may be rejected without statistical verification.

IX. Maintenance and Replacement Supplies

A. Record the appropriate information in the N-Analyzer Log Book:
   1. Date and duration of usage.
   2. Number of injections (retort water samples and total) and sample dilutions.
   3. Symptoms of malfunctioning.
   4. Repairs or replacements.
   5. Initial all entries.

B. Combustion tube replacement:
   1. Option 1: Fill new quartz combustion tube (#7061) with quartz chips (#7129). The quartz chips increase the turbulence in the combustion zone and extend the effective tube life by minimizing the attack of the quartz tube by alkaline metals.
   2. Option 2: A quartz combustion tube with a ceramic sleeve insert is available (#7141). Filling this modified tube with quartz chips extends the effective life.

C. On a weekly basis, clean old grease from the 18/9 ball-joint fittings on combustion tube; regrease with high-temperature, silicone vacuum grease.

D. On a monthly basis:
   1. Clean air filters on nitrogen detector and pyroreactor. Remove filters and wash in a solution of warm water and mild soap. Rinse thoroughly and blow dry with compressed air.
   2. Clear deposits in combustion tube by reaming orifice with a long piece of wire.

E. When the indicating Drierite in the activated carbon/Drierite scrubber or in the oxygen delivery line scrubber turns <u>pink</u> (or at least every six months), empty and recharge with fresh material.
   DANGER: If the indicating Drierite in the activated carbon/Drierite scrubber turns <u>black</u>, this is an indication that ozone has penetrated the activated carbon portion of the scrubber. Prior to this condition, the activated carbon will begin to turn whitish; this is an indication of peroxide formation. Exercise extreme CAUTION when replacing the scrubber. DO NOT attempt to repack the scrubber without proper safety equipment. The scrubber should be replaced (refill kits

#7128 and #7132) as soon as oxidation of the activated carbon is first observed. The scrubber should contain approximately 80% charcoal and 20% Drierite.

F. Ensure that the maximum ambient room temperature does not exceed 26°C.
G. Maintain a supply of 1-amp replacement fuses (SLO-BLO).
H. Oxygen should be dry and of at least 99.6% purity. If the oxygen is wet, an in-line oxygen drier is required to ensure a dry source of oxygen to the ozone generator.

X. Troubleshooting

A. See Antek operation/service manual (1) Appendix B.1. for extensive troubleshooting guide.
B. Diagnostic LEDs:
   1. Electronics - power supply on Main PC board out of specifications.
   2. Gas Flow - reduction in gas flow through detector (below 300 cm^3/min) or capacity of membrane dryer exceeded.
   3. Cooler - malfunction in thermoelectric circuit.
   4. Ozone - electrical problem with ozone generator.

XI. References

1. Antek Instruments, Inc. Operation/Service Manual: Model 703C Chemiluminescent Nitrogen System: Houston, TX.
2. ASTM "Standard Test Method for Total Kjeldahl Nitrogen in Water," in 1980 Annual Book of ASTM Standards, Part 31, Water; American Society for Testing and Materials: Philadelphia, PA, 1980, 1404 pp.

Protocol prepared by: B.M. Jones, G.J. Harris, and C.G. Daughton.

PROTOCOL: ORGANIC KJELDAHL NITROGEN

I. Apparatus
   A. Glassware
      1. 800-mL Kjeldahl flask (one each per sample; discard any Kjeldahl flasks with star-shaped cracks)
      2. Receiving flasks:
         a. 250-mL Erlenmeyer flask (two each per sample), or
         b. 250-mL Erlenmeyer flask (one each per sample) and 250-mL sampling cup for autotitrator (one each per sample; the masses of these containers should be within several grams of each other)
   B. 50-mL tilting dispenser
   C. Volumetric flasks (2-L, 1-L, 500-mL, 200-mL; Class A)
   D. Volumetric pipettes (10-mL; size depends on sample volume)
   E. Graduated cylinders (25-mL, 100-mL; TD)
   F. Graduated beaker (1-L)
   G. Stirring rod (glass)
   H. Kjeldahl stoppers (one each per sample; these stoppers are Neoprene, longer than regular stoppers, and specially tapered)
   I. Magnetic stirring bar (one each per sample for manual titration)
   J. Top-loading electronic balance (e.g., Mettler PC 2000)
   K. pH meter with pH probes (e.g., Corning model 135); for detection of pH endpoint during manual titration
   L. Titration equipment
      1. Detection option 1 (automatic titration): Sybron/Brinkmann (Westbury, NY) Autotitrator (Metrohm model 655 Dosimat, E 526 titrator, 643 control unit/624 auto sampler), and appropriate electronic burette (e.g., 10- or 20-mL).
         a. pH endpoint: combination pH electrode (Metrohm EA 157), or
         b. indicator endpoint: submersible colorimeter probe with 1-cm path length, 545-nm filter, and colorimeter (Brinkmann PC 800).
      2. Detection option 2 (manual titration): 50-mL precision bore burette, or Metrohm model 655 Dosimat with push-button controller.
   M. Kjeldahl digestion/distillation apparatus (e.g., 12-unit Labconco combination digestion/distillation); it is highly recommended that Pyrex condenser tubes with Teflon ferrules and fittings be used rather than the standard block-tin condenser tubes.
      IMPORTANT: The Kjeldahl distillation unit must <u>never</u> be plumbed with silicone rubber stoppers or tubing because of the permeability of this rubber to ammonia gas. It is also very important to ensure that extraneous ammonia vapors are not present in the lab.
   N. Zetex insulated gloves
      CAUTION: Safety face-shields or glasses with side-shields should be worn while performing the digestion/distillation.

II. Reagents
   NOTE: All reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.
   A. Borate Buffer Solution: add 88 mL of 0.1N NaOH (prepared from Hellige standard R-1226C) to approximately 500 mL of 0.025M sodium tetraborate solution (5.0 g $Na_2B_4O_7$ or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ per L) in a 1-L volumetric flask, and bring to volume with water; store in

polyethylene or polypropylene.

B. Digestion Solutions:
   1. Digestion option 1: use commercially prepared digestion packets (e.g., KELMATE N #500, EM Science, Gibbstown, NJ).
   2. Digestion option 2:
      a. mercuric sulfate solution: dissolve 8 g of red mercuric oxide (HgO) in a mixture of 10 mL of sulfuric acid (H_2SO_4; sp gr 1.84) and 40 mL of water in a 100-mL volumetric flask and bring to volume with water.
      b. digestion solution: to a 2-L volumetric flask containing a mixture of 1300 mL of water, add 400 mL of concentrated H_2SO_4 (add acid to water and mix); while the acid solution is still hot, dissolve 267 g of K_2SO_4. While stirring, add 50 mL of mercuric sulfate solution (II.B.2.a.) and bring to volume with water.

C. Mixed-Indicator Solution (for titration to indicator endpoint): dissolve 300 mg of methyl red (acid) indicator and 200 mg of methylene blue in 250 mL of 95% ethanol. Fresh indicator should be prepared each month.

D. Boric Acid Receiving Solution:
   1. pH endpoint: dissolve 40 g of H_3BO_3 in water in a 1-L volumetric flask and bring to volume with water; store in polyethylene or polypropylene.
   2. Indicator endpoint: indicator/boric acid receiving solution. Add 10 mL of indicator (II.C.) before bringing boric acid receiving solution (II.D.1.) to volume.

E. 0.02N H_2SO_4 Titrant: either use commercially prepared H_2SO_4 concentrate (e.g., Hellige R-1241C), or prepare a 0.10N H_2SO_4 stock solution (e.g., from Hellige R-1238C), and dilute 200 mL (quantitatively transferred from 200-mL volumetric flask) of this solution to 1 L with water.

F. Phenolphthalein Indicator: dissolve 0.5 g phenolphthalein in 50 mL of 95% ethanol, add 50 mL of water, and mix.

G. Sodium Hydroxide Solution (6N): use commercially prepared solution (e.g. 50% NaOH, J.T. Baker Chemical Co., Phillipsburg, NJ), or carefully dissolve 240 g NaOH in 800 mL of water (1-L volumetric flask), and bring to volume; store in screw-capped polypropylene bottle.

H. Sodium Hydroxide-Sodium Thiosulfate Solution: carefully dissolve 500 g of NaOH and 25 g of $Na_2S_2O_3 \cdot 5H_2O$ in water (approximately 800 mL) contained in a 1-L graduated beaker and mix using stirring rod; cover and allow to cool. Quantitatively transfer to 1-L volumetric flask and dilute to volume. Store in screw-capped polyproplene reagent bottle.

III. Protocol

NOTE: This protocol is for the determination of organic Kjeldahl nitrogen. Predistillation is required to remove ammonia from the organically bound nitrogen. If total Kjeldahl nitrogen is required, delete step III.F.

A. Preparation of glassware
   1. All glassware should be soap-washed followed by washing in 35% nitric acid. Rinse with ASTM Type III water.
   2. Add two glass beads to each Kjeldahl flask.

CAUTION: The samples will bump resulting in loss of sample if the glass beads are omitted.

B. Preparation of samples

1. Place appropriate volume of sample in Kjeldahl flask. The sample size can be determined from the anticipated organic nitrogen concentration and optimal titrant volume. For example, a 25-mL sample with organic nitrogen concentration of 80 mg-N/L should require 7.38 mL of 0.02N H_2SO_4 titrant; the mass of organic nitrogen added to the sample flask should not exceed 12.5 mg, nor should it be lower than 1.25 mg. The quantities of organic nitrogen in each flask should be equalized as best as possible.

OKN in sample (mg-N/L)	sample size (mL)
5- 10	250
10- 20	100
20- 50	50
50- 500	25
500-5000	2.5

C. Preparation of standards

1. Prepare a glycine standard with nitrogen concentration equivalent to the estimated organic nitrogen concentration in the samples (e.g., if a 25-mL sample volume is used, the glycine nitrogen concentration should not exceed 500 mg-N/L).

D. Preparation of blanks

1. Digested/distilled: omit sample and substitute a volume of water equivalent to the average sample volume.
2. Nondigested/nondistilled: prepare three (follow III.H.5.). Use 50 mL of boric acid receiving solution (prepared in II.D), but do not use for collection of distillate; these blanks will be used to determine the titrimetric endpoint (pH or colorimetric). Allow these blanks to sit under the same conditions as the receiving flasks (i.e., exposed to laboratory air). These blanks should be prepared at the same time as the receiving flasks or cups (III.H.5.).

E. Randomization of Kjeldahl flasks

1. Randomize flasks (samples, standards, and digestion blank) by consulting Table O in reference (1).
   a. randomly select a group of five digits from the table.
   b. pick any two adjacent digits within the group of five.
   c. for two-digit random numbers, begin with the pair selected, move horizontally, and record the next two consecutive digits; continue this process in a consistent manner until two-digit numbers are generated for each flask.
   d record the random numbers and the corresponding sample number.
   e. relabel each Kjeldahl flask with its assigned random number.
   f. ensure that receiving flasks are labeled with the appropriate random number.

F. Predistillation (for removal of ammonia)

1. Steam apparatus clean. Prior to each distillation, place 300 to 400 mL of water and two glass beads into each Kjeldahl flask and attach to distillation apparatus. With cooling water off, distill approximately 150 to 200 mL; collect distillate in any convenient container and discard.

2. Containers for collection of distillate.
   a. option 1: if ammonia determination is required, measure 50.0 mL of boric acid solution (tilting dispenser) into each 250-mL Erlenmeyer flask (or sampling cup), and place on Kjeldahl rack so that the distillation effluent tube-tips are completely submerged in the solution; use boric acid solution for pH endpoint or indicator/boric acid solution for indicator endpoint.
   b. option 2: if distillate is to be discarded, replace receiving flasks with any convenient container.
3. Add water to each Kjeldahl flask to bring the total volume to approximately 300 mL; add two drops of phenolphthalein, two glass beads and 10 mL of borate buffer solution (automatic pipette recommended for convenience, but the volume is not critical).
4. Slowly add several drops of NaOH solution down the inner side of each Kjeldahl flask so that it layers beneath the aqueous sample, and immediately connect to distillation/condenser apparatus. Place flasks on distillation unit in ascending order of assigned random numbers. Ensure that the stoppers are firmly seated. Swirl the flask contents to mix.
5. Turn on condenser cooling water and set heating mantles to maximum temperature. After samples begin to boil, reduce heat as necessary to control boiling and foaming.
6. Collection of distillate.
   a. option 1: collect approximately 100 mL of distillate in boric acid receiving solution; this portion of the distillate can be later analyzed for ammonia (Chapter IV, parts III.H. or IV.A.-E. of Distillation/Titrimetry protocol). To remove excess water from distilland, continue distillation, and collect approximately 125 mL of distillate in any convenient container. Discard distillate from secondary distillation.
   b. option 2: collect approximately 225 mL of distillate; discard.
      CAUTION: Do not over-distill; if the distilland becomes excessively concentrated it will bump, and flasks may overheat and crack.
   c. upon completion of either distillation, remove the effluent tubes from the receiving-container solutions, and rest the tubes on the flask rims to prevent sample drawback during cooling. Turn off heat and continue collection of distillate until distillation ceases.
   d. 50 to 75 mL of distilland should remain in the Kjeldahl flask following predistillation.
      NOTE: The distilland should retain a pinkish hue during distillation to ensure proper pH for removal of ammonia.

G. Digestion
1. At the conclusion of predistillation, allow the Kjeldahl flasks to cool for 30 minutes.
2. If total Kjeldahl nitrogen is required (i.e., step III.F. has been deleted), add two glass beads and appropriate volume of sample (see III.B.) to each Kjeldahl flask.
3. Addition of digestion reagents
   a. digestion option 1: to each flask, add the contents of one KELMATE N packet followed by 20 ml of concentrated H_2SO_4;

carefully swirl the flask to mix contents.

b. digestion option 2: add 100-mL of digestion solution (II.B.2.b.) to each Kjeldahl flask, and swirl to mix.

4. Place flask on digestion apparatus with the mouth of the flask in the exhaust manifold; set heating mantle to "high."
5. The samples should boil briskly; white SO_3 fumes should become evident and then disappear. The digestates should "clear," becoming pale yellow or colorless. Time to achieve clearing is about 30 minutes.
6. Continue the digestion for an additional hour. Adjust temperature if necessary to control boiling.

H. Distillation

1. Steam apparatus clean (follow III.F.1.).
2. Allow sample digestates to cool for approximately one hour; the flasks can be stoppered (after one hour of cooling) and refrigerated if the procedure cannot be completed.
3. Add water to each cooled Kjeldahl flasks to bring the total volume to approximately 300 mL; add 2 drops of phenolphthalein and 10 mL of borate buffer solution (automatic pipette recommended for convenience, but the volume is not critical).
4. Slowly add 100 mL of sodium hydroxide-sodium thiosulfate solution (II.H.) down the inner side of each Kjeldahl flask so that the alkali layers beneath the aqueous sample, and immediately connect to distillation/condenser apparatus. Place flasks on distillation unit in ascending order of assigned random numbers. Ensure that the stopper is firmly seated. Swirl the flask contents to mix.
5. Receiving flasks: measure 50.0 mL of boric acid solution (tilting dispenser) into each 250-mL Erlenmeyer flask (or sampling cup) and place on Kjeldahl rack so that the distillation effluent tube-tips are completely submerged in the solution; use boric acid solution for pH endpoint or indicator/boric acid solution for indicator endpoint.

   a. if samples had been refrigerated, the solution may solidify, making mixing difficult. After addition of water, allow samples to sit at room temperature for 20 minutes; mix again.

6. To distill, follow procedure in Chapter IV, part III.G. of Distillation/Titrimetry protocol.

I. Titration

1. Detection option 1 (automatic titration): proceed as outlined in Chapter IV, part IV.A.-E. of Distillation/Titrimetry protocol.
2. Detection option 2 (manual titration): proceed as outlined in Chapter IV, part III.H.1. or part III.H.2. of Distillation/Titrimetry protocol.

IV. Waste Disposal

A. Pour spent digestates into 5-gallon polypropylene carboy. Store carboy in a warm area. Periodically decant supernatant and discharge to sanitary sewer while running tap water (2). Contact appropriate authorities for proper disposal of mercuric sludge.

V. Data Reduction

$$\text{organic nitrogen (mg-N/L)} = \frac{\text{sample titrant volume (mL)} - \text{blank titrant volume (mL)} \times \text{normality of acid (meq/mL)} \times 1000\ \text{(mL/L)} \times 14\ \text{(mg-N/meq)}}{\text{sample volume (mL)}}$$

VI. References

1. Rohlf, F.J.; Sokal, R.R. Statistical Tables; W.H. Freeman and Co.: San Francisco, CA; 1969, pp. 152-156,
2. Dillon, P.L.; Caldwell, M.J.; Gehrt, A.J. "Recovery System for Mercury Catalyst Used in Kjeldahl Analysis," J. Assoc. Off. Anal. Chem. 1972, 55, 101-2.

Protocol prepared by: G.J. Harris, B.M. Jones, and C.G. Daughton

User Instructions

STANDARD-CURVE PROGRAM

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position.			
2	PRESS "f" "a": display will go to 0.000.		f a	
3	ENTER STABILITY FACTOR (e.g., 0.990).	Value		
4	PRESS "R/S": prints stability factor, display goes to 0.		R/S	Value
5	ENTER TIME FACTOR (e.g., 75 = 50 sec)	Value		
6	PRESS "R/S": prints time factor, spaces 3 lines, display goes to 0.		R/S	Value
7	CONNECT INTERFACE STRAP.			
8	PRESS ANTEK RESET BUTTON: Antek 720 display goes to 00.			
9	ENTER STANDARD CONCENTRATION (integer only)	Value		
10	PRESS "R/S": prints and displays standard concentration		R/S	Value
11	PRESS "FWD" ON SYRINGE DRIVE AND PRESS "R/S" SIMULTANEOUSLY: initiates timing loop.		R/S	Value
12	When timing loop is completed, the final value is evaluated for stability; if the value passes the stability test this value is printed.			
13	To analyze the next standard, repeat steps 8-11.			
14	To change the STABILITY FACTOR or TIME FACTOR, disconnect the interface strap, press "f" "a" and repeat instructions beginning at step 3.		f a	

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLa	21 16 11	
002	CLRG	16-53	
003	PSE	16-51	
004	CLRG	16-53	
005	PSE	16-51	
006	DSP3	-63 03	
007	R/S	51	
008	STO3	35 03	
009	PRTX	-14	
010	DSP0	-63 00	
011	CLX	-51	
012	R/S	51	
013	STO4	35 04	
014	STO5	35 05	
015	PRTX	-14	
016	CLX	-51	
017	SPC	16-11	
018	SPC	16-11	
019	*LBLb	21 16 12	
020	R/S	51	
021	PRTX	-14	
022	R/S	51	
023	*LBLc	21 16 13	
024	CF3	16 22 03	
025	RCL4	36 04	
026	STO5	35 05	
027	ENTP	-21	
028	*LBLd	21 16 14	
029	RCL5	36 05	
030	1	01	
031	-	-43	
032	STO5	35 05	
033	2	02	
034	X≠Y?	16-35	
035	GTOd	22 16 14	
036	*LBLA	21 11	
037	CF3	16 22 03	
038	PSE	16 51	
039	*LBLA	21 11	
040	STO6	35 06	
041	RCL5	36 05	
042	X>0?	16-44	
043	GTOe	22 16 15	
044	RCL6	36 06	
045	RCL2	36 02	
046	X≠Y	-41	
047	÷	-24	
048	RCL3	36 03	
049	X>Y?	16-34	
050	GTOe	22 16 15	
051	RCL6	36 06	
052	PRTX	-14	
053	SPC	16-11	
054	GTOb	22 16 12	
055	*LBLe	21 16 15	
056	RCL6	36 06	
057	STO2	35 02	
058	GTOd	22 16 14	
059	R/S	51	
060			
070			
080			
090			
100			
110			

REGISTERS

0	1	2 NEXT TO LAST VALUE	3 STABILITY FACTOR	4 TIME FACTOR	5 DECREMENTED TIME FACTOR	6 LAST VALUE	7	8	9

LABELS

A DATA ACQUISITION	B	C	D	E	S6	S7	S8
a START MANUAL INPUT	b STANDARDS LABEL	c INITIATE DATA LOOP	d TIMING LOOP	e INCREMENT DATA LOOP		E	

Appendix B

Pin-to-Pin Diagram for Interface Strap between BCD Port of Antek Nitrogen Detector and Hewlett-Packard 97S

	Antek	97S			97S	Antek	
	29	GND	1	26	$\overline{LE}$		
		$\overline{F3}$	2	27	$\overline{LOAD}$		
		$\overline{F2}$	3	28	INHIBIT	30	
		$\overline{F1}$	4	29	LOAD		
		$\overline{F0}$	5	30	T/C		
		I1	6	31	J1		
		I2	7	32	J2		
		I4	8	33	J4		
		I8	9	34	J8		
		G1	10	35	H1		
	32	G2	11	36	H2		
		G4	12	37	H4		
		G8	13	38	H8		
10^1	14	E1	14	39	F1	22	10^0
	16	E2	15	40	F2	24	
	18	E4	16	41	F4	26	
	20	E8	17	42	F8	28	
10^3	3	C1	18	43	D1	6	10^2
	1	C2	19	44	D2	8	
	2	C4	20	45	D4	10	
	4	C8	21	46	D8	12	
10^5	19	A1	22	47	B1	11	10^4
	17	A2	23	48	B2	9	
	15	A4	24	49	B4	7	
	13	A8	25	50	B8	5	

User Instructions

1 DATA-REDUCTION PROGRAM 2

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position.			
2	PRESS "f" "a": display will go to 0.000.		f a	
3	ENTER STABILITY FACTOR (e.g., 0.990).	Value		
4	PRESS "R/S": prints stability factor, display goes to 0.		R/S	Value
5	ENTER TIME FACTOR (e.g., 75 = 50 sec)	Value		
6	PRESS "R/S": prints time factor display goes to 0.00.		R/S	Value
7	ENTER SLOPE OF STANDARD CURVE (m).	Value		
8	PRESS "R/S": prints slope, display goes to 0.000		R/S	Value
9	ENTER Y-INTERCEPT (b).	Value		
10	PRESS "R/S": prints y-intercept, space 3 lines, displays 0.		R/S	Value
11	CONNECT INTERFACE STRAP.			
12	PRESS ANTEK RESET BUTTON: Antek 720 display goes to 00.			
13	ENTER SAMPLE NUMBER (integer only)	Value	.	
14	PRESS "R/S": prints sample number, displays 0.		R/S	Value
15	ENTER DILUTION FACTOR (e.g., "10" for a 1:10 dilution)	Value		
16	PRESS "R/S": prints sample number, displays 0.		R/S	Value
17	PRESS "FWD" ON SYRINGE DRIVE AND PRESS "R/S" SIMULTANEOUSLY: initiates timing loop.		R/S	Value

DATA-REDUCTION PROGRAM (continued)

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
18	When timing loop is completed, the final value is evaluated for stability; if the value passes the stability test this value is printed (integrator counts). The nitrogen concentration is interpolated from the standard curve data and printed. This value is multiplied by the dilution factor and this corrected value is printed.			
19	To analyze a replicate, repeat steps 12-17.			
20	PRESS "f" "e": this calculates the $\bar{x}$ and rsd		f e	$\bar{x}$ rsd
21	To analyze the next sample, repeat steps 12-21.			

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLa	21 16 11	
002	CLR6	16-53	
003	P⇄S	16-51	
004	CLR6	16-53	
005	P⇄S	16-51	
006	DSP3	-63 03	
007	R/S	51	
008	STO3	35 03	
009	PRTX	-14	
010	DSP0	-63 00	
011	CLX	-51	
012	R/S	51	
013	STO4	35 04	
014	STO5	35 05	
015	PRTX	-14	
016	CLX	-51	
017	DSP2	-63 02	
018	R/S	51	
019	STO0	35 00	
020	PRTX	-14	
021	DSP3	-63 03	
022	CLX	-51	
023	R/S	51	
024	STO1	35 01	
025	PRTX	-14	
026	SFC	16-11	
027	SFC	16-11	
028	CLX	-51	
029	*LBLB	21 12	
030	DSP0	-63 00	
031	R/S	51	
032	PRTX	-14	
033	CLX	-51	
034	R/S	51	
035	STO7	35 07	
036	PRTX	-14	
037	CLX	-51	
038	R/S	51	
039	*LBLC	21 13	
040	CF3	16 22 03	
041	RCL4	36 04	
042	STO5	35 05	
043	ENT↑	-21	
044	*LBLD	21 14	
045	RCL5	36 05	
046	1	01	
047	-	-45	
048	STO5	35 05	
049	2	02	
050	X≠Y?	16-35	
051	GTOD	22 14	
052	*LBLA	21 11	
053	CF3	16 22 03	
054	PSE	16 51	
055	*LBLA	21 11	
056	STO6	35 06	
057	RCL5	36 05	
058	X>0?	16-44	
059	GTOE	22 15	
060	RCL6	36 06	
061	RCL2	36 02	
062	X⇄Y	-41	
063	X=0?	16-43	
064	GSBb	23 16 12	
065	÷	-24	
066	RCL3	36 03	
067	X>Y?	16-34	
068	GTOE	22 15	
069	RCL6	36 06	
070	DSP2	-63 02	
071	PRTX	-14	
072	X=0?	16-43	
073	1	01	
074	ENT↑	-21	
075	RCL1	36 01	
076	-	-45	
077	RCL0	36 00	
078	÷	-24	
079	PRTX	-14	
080	RCL7	36 07	
081	×	-35	
082	PRTX	-14	
083	Σ+	56	
084	CLX	-51	
085	SFC	16-11	
086	GTOB	22 12	
087	*LBLE	21 15	
088	RCL6	36 06	
089	STO2	35 02	
090	GTOD	22 14	
091	*LBLb	21 16 12	
092	1	01	
093	ENT↑	-21	
094	RTN	24	
095	R/S	51	
096	R/S	51	
097	*LBLe	21 16 15	
098	SFC	16-11	
099	DSP2	-63 02	
100	x̄	16 53	
101	PRTX	-14	
102	STO8	35 08	
103	S	16 54	
104	ENT↑	-21	
105	RCL8	36 08	
106	÷	-24	
107	1	01	
108	0	00	
109	0	00	
110	×	-35	
111	PRTX	-14	
112	0	00	

REGISTERS

0 SLOPE	1 Y-INTERCEP	2 NEXT TO LAST VALUE	3 STABILITY FACTOR	4 TIME FACTOR	5 DECREMENTED TIME FACTOR	6 LAST VALUE	7 DILUTION FACTOR	8 X̄	9
S0	S1	S2	S3	S4	S5	S6	S7	S8	

A	B	C	D	E

Program Listing

STEP	KEY ENTRY	KEY CODE	COMMENTS
113	STO6	35 06	
114	RCL	16-51	
115	CLR	16-53	
116	RCL	16-51	
117	DSP0	-63 00	
118	SPC	16-11	
119	SPC	16-11	
120	GTO	22 10	
121	R/S	31	

STEP	KEY ENTRY	KEY CODE	COMMENTS
170			
180			
190			
200			
210			
220			

LABELS					FLAGS	SET STATUS		
A DATA ACQUISITION	B INPUT SAMPLE DATA	C SAMPLE ANALYSIS	D TIMING LOOP	E STABILITY TEST RESET	0	FLAGS	TRIG	DISP
a START, MANUAL INPUT	b CORRECTION FOR 0 VALUES	c	d	e CALCULATE $\bar{x}$, rsd	1	ON OFF 0 ☐ ☐ 1 ☐ ☐ 2 ☐ ☐ 3 ☐ ☐	DEG ☐ GRAD ☐ RAD ☐	FIX ☐ SCI ☐ ENG ☐ n ____
		2	3	4	2			
		7	8	9	3			

User Instructions

1 AUTOSAMPLER STANDARD-CURVE PROGRAM 2

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position.			
2	PRESS "f" "a": display will go to 0.000.		f a	
3	ENTER STABILITY FACTOR (e.g., 0.990).	Value		
4	PRESS "R/S": prints stability factor, display goes to 0.		R/S	Value
5	ENTER TIME FACTOR (e.g., 75 = 50 sec).	Value		
6	PRESS "R/S": prints time factor, display goes to 0.		R/S	Value
7	ENTER NUMBER OF STANDARDS TO BE RUN.	Value		
8	PRESS "R/S": prints number of standards, spaces 3 lines, display goes to 0.		R/S	Value
9	CONNECT INTERFACE STRAP.			
10	PRESS ANTEK RESET BUTTON: Antek 720 display goes to 00.			
11	Ensure that autosampler is programmed correctly; see VI.E.4. for programming instructions.			
12	PRESS "ENTER" ON AUTOSAMPLER: autosampler will start its cycle.			
13	WHEN THE STANDARD IS INJECTED, PRESS "R/S": this initiates timing loop for data acquisition, and prints replicate identification number.		R/S	Value
14	When timing loop is completed, the final value is evaluated for stability; if the value passes the stability test this value is printed.			
15	The calculator then compares the detector display with zero. When the autosampler resets the detector (upon injection of the next replicate) this condition is satisfied and the calculator goes to the beginning of the timing loop.			
16	After three injections, the autosampler carousel advances; the calculator determines the mean and rsd value of the replicates.			

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLa	21 16 11	
002	CLRG	16-53	
003	P⇄S	16-51	
004	CLRG	16-53	
005	P⇄S	16-51	
006	DSP3	-63 03	
007	R/S	51	
008	STO3	35 03	
009	PRTX	-14	
010	DSP0	-63 00	
011	CLX	-51	
012	R/S	51	
013	STO4	35 04	
014	STO5	35 05	
015	PRTX	-14	
016	CLX	-51	
017	R/S	51	
018	STO7	35 07	
019	PRTX	-14	
020	CLX	-51	
021	SPC	16-11	
022	SPC	16-11	
023	R/S	51	
024	*LBLc	21 16 13	
025	ISZI	16 26 46	
026	RCLI	36 46	
027	PRTX	-14	
028	CF3	16 22 03	
029	RCL4	36 04	
030	STO5	35 05	
031	ENT↑	-21	
032	*LBLd	21 16 14	
033	RCL5	36 05	
034	1	01	
035	-	-45	
036	STO5	35 05	
037	2	02	
038	X≤Y?	16-35	
039	GTOd	22 16 14	
040	*LBLA	21 11	
041	CF3	16 22 03	
042	PSE	16 51	
043	*LBLA	21 11	
044	STO6	35 06	
045	RCL5	36 05	
046	X>0?	16-44	
047	GTOe	22 16 15	
048	RCL6	36 06	
049	RCL2	36 02	
050	X⇄Y	-41	
051	÷	-24	
052	RCL3	36 03	
053	X>Y?	16-34	
054	GTOe	22 16 15	
055	RCL6	36 06	
056	PRTX	-14	
057	SPC	16-11	
058	Σ+	56	
059	RCLI	36 46	
060	ENT↑	-21	
061	3	03	
062	X>Y?	16-34	
063	GTOB	22 12	
064	*LBLC	21 13	
065	SPC	16-11	
066	DSP2	-63 02	
067	x̄	16 53	
068	PRTX	-14	
069	STO8	35 08	
070	S	16 54	
071	ENT↑	-21	
072	RCL8	36 08	
073	÷	-24	
074	1	01	
075	0	00	
076	0	00	
077	×	-35	
078	PRTX	-14	
079	DSP0	-63 00	
080	0	00	
081	STO8	35 08	
082	STOI	35 46	
083	ISZI	16 26 45	
084	P⇄S	16-51	
085	STO4	35 04	
086	STO5	35 05	
087	STO6	35 06	
088	STO7	35 07	
089	STO8	35 08	
090	STO9	35 09	
091	P⇄S	16-51	
092	SPC	16-11	
093	SPC	16-11	
094	RCLI	36 45	
095	ENT↑	-21	
096	RCL7	36 07	
097	X>Y?	16-34	
098	GTOB	22 12	
099	GTOa	22 16 11	
100	*LBLe	21 16 15	
101	RCL6	36 06	
102	STO2	35 02	
103	GTOd	22 16 14	
104	*LBLB	21 12	
105	*LBLA	21 11	
106	CF3	16 22 03	
107	PSE	16 51	
108	*LBLA	21 11	
109	X=0?	16-43	
110	GTOc	22 16 13	
111	GTOB	22 12	
112	R/S	51	

REGISTERS

0 SAMPLE COUNTER	1	2 NEXT TO LAST VALUE	3 STABILITY FACTOR	4 TIME FACTOR	5 DECREMENTED TIME FACTOR	6 LAST VALUE	7 NUMBER OF STANDARDS	8	9
S0	S1	S2	S3	S4 $\bar{X}$, rsd	S5 $\bar{X}$, rsd	S6 $\bar{X}$, rsd	S7 $\bar{X}$, rsd	S8 $\bar{X}$, rsd	S9 $\bar{X}$, rsd

B	C	D	E	I REPLICATE COUNTER

LBL-17421; UCB/SEEHRL 84-2 **Program Listing** NITROGEN

STEP	KEY ENTRY	KEY CODE	COMMENTS	STEP	KEY ENTRY	KEY CODE	COMMENTS
				170			
120				180			
130				190			
140				200			
150				210			
160				220			

LABELS					FLAGS
A DATA ACQUISITION	B RESET LOOP	C X, RSD LOOP	D	E	0
a START, MANUAL INPUT	b	c INITIATE DATA LOOP	d TIMING LOOP	e INCREMENT DATA LOOP	1
0	1	2	3	4	2
5	6	7	8	9	3

SET STATUS		
FLAGS	TRIG	DISP
ON OFF	DEG	Y - 65
0 ☐ ☐	GRA	
1 ☐ ☐	RAD	
2 ☐ ☐		
3 ☐ ☐		

User Instructions

AUTOSAMPLER DATA-REDUCTION PROGRAM

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: calculator in RUN mode, PRINT in manual position.			
2	PRESS "f" "a": display will go to 0.000.		f a	
3	ENTER STABILITY FACTOR (e.g., 0.990).	Value		
4	PRESS "R/S": prints stability factor, display goes to 0.		R/S	Value
5	ENTER TIME FACTOR (e.g., 75 = 50 sec)	Value		
6	PRESS "R/S": prints time factor display goes to 0.00.		R/S	Value
7	ENTER SLOPE OF STANDARD CURVE (m).	Value		
8	PRESS "R/S": prints slope, display goes to 0.000		R/S	Value
9	ENTER Y-INTERCEPT (b).	Value		
10	PRESS "R/S": prints y-intercept, display goes to 0.		R/S	Value
11	ENTER NUMBER OF SAMPLES TO BE RUN.	Value		
12	PRESS "R/S": prints number of samples, spaces 3 lines, display goes to 1.		R/S	Value
13	CONNECT INTERFACE STRAP.			
14	PRESS ANTEK RESET BUTTON: Antek 720 display goes to 00.			
15	Ensure that autosampler is programmed correctly; see VI.E.4. for programming instructions.			
16	PRESS "ENTER" ON AUTOSAMPLER: autosampler will start its cycle.			
17	WHEN THE SAMPLE IS INJECTED, PRESS "R/S": this initiates timing loop for data acquisition, and prints sample identification number.		R/S	Value

User Instructions

AUTOSAMPLER DATA-REDUCTION PROGRAM (continued)

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
18	When timing loop is completed, the final value is evaluated for stability; if the value passes the stability test this value is printed (integrator counts). The nitrogen concentration is interpolated from the standard curve data and printed.			
19	The calculator then compares the detector display with zero. When the autosampler resets the detector (upon injection of the next replicate) this condition is satisfied and the calculator goes to the beginning of the timing loop.			
20	After three injections, the autosampler carousel advances; the calculator determines the mean and rsd value of the replicates.			

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBLa	21 16 11	
002	CLRG	16-53	
003	P⇄S	16-51	
004	CLRG	16-53	
005	P⇄S	16-51	
006	DSP3	-63 03	
007	R/S	51	
008	STO3	35 03	
009	PRTX	-14	
010	DSP0	-63 00	
011	CLX	-51	
012	R/S	51	
013	STO4	35 04	
014	STO5	35 05	
015	PRTX	-14	
016	CLX	-51	
017	DSP2	-63 02	
018	R/S	51	
019	STO0	35 00	
020	PRTX	-14	
021	DSP3	-63 03	
022	CLX	-51	
023	R/S	51	
024	STO1	35 01	
025	PRTX	-14	
026	CLX	-51	
027	DSP0	-63 00	
028	R/S	51	
029	STO9	35 09	
030	PRTX	-14	
031	6	06	
032	5	05	
033	STOB	35 12	
034	1	01	
035	STOA	35 11	
036	SPC	16-11	
037	SPC	16-11	
038	R/S	51	
039	*LBLC	21 13	
040	ISZI	16 26 46	
041	RCLA	36 11	
042	ENT↑	-21	
043	PRTX	-14	
044	CF3	16 22 03	
045	RCL4	36 04	
046	STO5	35 05	
047	ENT↑	-21	
048	*LBLD	21 14	
049	RCL5	36 05	
050	1	01	
051	-	-45	
052	STO5	35 05	
053	2	02	
054	X≥Y?	16-35	
055	GTOD	22 14	
056	*LBLA	21 11	
057	CF3	16 22 03	
058	PSE	16 51	
059	*LBLA	21 11	
060	STO6	35 06	
061	RCL5	36 05	
062	X>0?	16-44	
063	GTOE	22 15	
064	RCL6	36 06	
065	RCL2	36 02	
066	X⇄Y	-41	
067	X=0?	16-43	
068	GSBB	23 12	
069	÷	-24	
070	RCL3	36 03	
071	X>Y?	16-34	
072	GTOE	22 15	
073	RCL6	36 06	
074	DSP2	-63 02	
075	PRTX	-14	
076	X=0?	16-43	
077	1	01	
078	ENT↑	-21	
079	RCL1	36 01	
080	-	-45	
081	RCL0	36 00	
082	÷	-24	
083	PRTX	-14	
084	DSP0	-63 00	
085	SPC	16-11	
086	Σ+	56	
087	RCL6	36 06	
088	X=0?	16-43	
089	GSBc	23 16 13	
090	RCL1	36 46	
091	ENT↑	-21	
092	3	03	
093	X>Y?	16-34	
094	GTOb	22 16 12	
095	*LBLe	21 16 15	
096	SPC	16-11	
097	DSP2	-63 02	
098	x̄	16 53	
099	PRTX	-14	
100	STO8	35 08	
101	S	16 54	
102	ENT↑	-21	
103	RCL8	36 08	
104	÷	-24	
105	1	01	
106	0	00	
107	0	00	
108	×	-35	
109	PRTX	-14	
110	RCLA	36 11	
111	1	01	
112	+	-55	

REGISTERS

0 SLOPE	1 Y-INTERCEPT	2 NEXT TO LAST VALUE	3 STABILITY FACTOR	4 TIME FACTOR	5 DECREMENTED TIME FACTOR	6 LAST VALUE	7 DILUTION FACTOR	8 X	9 NUMBER OF SAMPLES
		S2	S3	S4 $\bar{X}$, rsd	S5 $\bar{X}$, rsd	S6 $\bar{X}$, rsd	S7 $\bar{X}$, rsd	S8 $\bar{X}$, rsd	S9 $\bar{X}$, rsd

A SAMPLE COUNTER	B SLEEP VALUE	C DECREMENTED SLEEP VALUE	D	E	I REPLICATE COUNTER

Program Listing

NITROGEN

STEP	KEY ENTRY	KEY CODE	COMMENTS	STEP	KEY ENTRY	KEY CODE	COMMENTS
113	STOa	35 11					
114	0	00		170			
115	STO8	35 08					
116	STO7	35 46					
117	P25	16-51					
118	STO4	35 04					
119	STO5	35 05					
120	STO6	35 06					
121	STO7	35 07					
122	STO8	35 08					
123	STO9	35 09					
124	P25	16-51		180			
125	SPC	16-11					
126	SPC	16-11					
127	DSP0	-53 00					
128	RCL4	36 11					
129	ENT↑	-21					
130	1	01					
131	-	-45					
132	RCL9	36 09					
133	X>Y?	16-34					
134	GTOa	22 16 12		190			
135	GTOa	22 16 11					
136	*LBLE	21 12					
137	1	01					
138	ENT↑	-21					
139	RTN	24					
140	*LBLE	21 15					
141	RCL6	36 06					
142	STO2	35 02					
143	GTOD	22 14					
144	*LBLa	21 16 12		200			
145	*LBLA	21 11					
146	CF3	16 22 03					
147	PSE	16 51					
148	*LBLA	21 11					
149	X=0?	16-43					
150	GTOC	22 13					
151	GTOa	22 16 12					
152	*LBLa	21 16 13					
153	RCLB	36 12					
154	STOC	35 13		210			
155	*LBLa	21 16 14					
156	RCLC	36 13					
157	1	01					
158	-	-45					
159	STOC	35 13					
160	X≠0?	16-42					
161	GTOa	22 16 14					
162	RTN	24					
163	R/S	51					
				220			

LABELS					FLAGS	SET STATUS
A DATA ACQUISITION	B CORRECTION FOR 0 VALUES	C SAMPLE ANALYSIS	D TIMING LOOP	E STABILITY TEST RESET	0	FLAGS ON OFF / TRIG / DISP
a START, MANUAL INPUT	b RESET LOOP	c SLEEP LOOP	d DECREMENTED SLEEP LOOP	e CALCULATE $\bar{X}$, rsd	1	0 ☐ ☐ DEG
0	1	2	3	4	2	1 ☐ ☐ GRAD
5	6	7	8	9	3	2 ☐ ☐ RAD 3 ☐ ☐

Chapter VI

CHEMICAL OXYGEN DEMAND (COD): COLORIMETRIC AND TITRIMETRIC QUANTITATION

B.M. Jones, R.H. Sakaji, and C.G. Daughton

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INTRODUCTION

The chemical oxygen demand (COD) test was originally used as a rapid estimator of the biochemical oxygen demand (BOD) of wastewater organic material (Moore, Kroner, and Ruchhoft 1949). Of numerous possible oxidants, four have been used for determining the oxygen demand of aqueous effluents: potassium permanganate, potassium dichromate, ceric acid, and iodic acid. Of these four, potassium dichromate in sulfuric acid is the prescribed method in the United States. Specific correlations have been developed to relate the COD of a waste to its 5-day BOD (Rhame 1947). The correlations between BOD and COD must be applied with caution because potassium dichromate does not oxidize ammonia; therefore, the COD value does not reflect the contribution of ammonia to ultimate BOD. The original, routine method for COD determination has been revised (i.e., by the addition of silver as a catalyst) to yield oxidation of certain biodegradable compounds (i.e., aliphatic hydrocarbons, straight-chain alcohols, and fatty acids) that are not fully susceptible to the chemical oxidant. In addition to saving time, the COD test was recognized as being extremely valuable for determining the oxygen demand of wastes that are toxic to microorganisms. Chemical oxygen demand is often determined in lieu of BOD and has achieved an intrinsic meaning separate from its biochemical counterpart.

THEORY

Chemical Oxygen Demand

The numerical value obtained from a COD test reflects in theory both the carbon concentration and the overall oxidative state of the oxidizable organic material. The COD of a sample is defined by the absolute amount of hexavalent chromium that is reduced during two hours of digestion by potassium dichromate in a solution of 50% sulfuric acid. Ideally, organic compounds are completely oxidized to carbon dioxide and water with the simultaneous stoichiometric reduction of the orange hexavalent dichromate ion (Cr +VI) to the green trivalent chromic ion (Cr +III). The degree of reduction is quantified either by colorimetry (i.e., by determination of the remaining dichromate ion or, alternatively, by determination of the newly produced chromic ion) or by titrimetry (i.e., redox titration of the remaining dichromate ion). This value is then related stoichiometrically to oxygen equivalents. The reduction of each mole of Cr(VI) to Cr(III) is equivalent to the consumption of 1.5 moles of O_2; the results therefore can be expressed in terms of milligrams of O_2 per liter (mg-O_2/L) even though oxygen has no direct role in the chemical oxidation process.

Soluble COD (SCOD), the COD of dissolved species, is of primary interest because of (i) the availability of dissolved organic compounds to microorganisms and (ii) the minimization of sampling error. For these reasons, our laboratory has limited itself to the determination of SCOD. An extensive discussion of the problems associated with filtration is included in Chapter III.

Oxidant. Potassium dichromate is used both as a source of Cr(VI) and as a primary standard for determining the normality of the titrant in the COD test. Potassium dichromate is preferred over other oxidants because it can be highly purified, it is not hygroscopic, and it can be dried without decomposition (Waser 1966). In aqueous solution, two forms of hexavalent chromium exist: chromate ion CrO_4^{2-} and dichromate ion $Cr_2O_7^{2-}$ (Latimer and Hildebrand 1951).

The equilibrium between the two species is represented by:

$$2\ CrO_4^{2-} + 2\ H^+ \rightleftharpoons Cr_2O_7^{2-} + H_2O \qquad K = 1.2 \times 10^{14}$$

The highly acidic sulfuric acid solution of the COD digestion reagent ensures that the predominant species is the very potent oxidant, dichromate ion (half-reaction potential = 1.33 volts). The acidic environment and elevated temperatures of the COD assay are essential for dichromate to mineralize carbonaceous material to CO_2 and H_2O (Sawyer and McCarty 1967).

Organic compounds, however, are not equally susceptible to dichromate oxidation; aliphatic hydrocarbons, straight-chain organic acids, and alcohols, for instance, are resistant to oxidation unless silver is added as an oxidation catalyst (APHA 1981). Even then, heterocyclic compounds, such as many nitrogen and oxygen heterocycles, are incompletely oxidized by the prescribed procedure (Table I) (Gibbs 1979; APHA 1981).

<u>Interferences</u>. Certain inorganic compounds consume oxidant and therefore exert a COD. Nitrites, sulfites, sulfides, and ferrous and chloride ions are oxidized by dichromate (ASTM 1981) and therefore are calculated as part of the oxygen demand. For example, each mole of thiosulfate loses eight equivalents of electrons when completely mineralized (Wong and Mercer 1979); this is equivalent to the consumption of two moles of oxygen per mole of thiosulfate.

$$S_2O_3^{2-} + 2\ O_2 + H_2O \longrightarrow 2\ SO_4^{2-} + 2\ H^+$$

Chloride represents the most serious inorganic interference to the determination of COD. Chloride ion will precipitate the silver catalyst as silver chloride; this both reduces the effectiveness of the catalyst and obscures the titrimetric endpoint (Dobbs and Williams 1963). Secondly, chloride ion is oxidized by dichromate, giving a positive interference to the assay (Moore et al. 1949). This oxidation is not stoichiometric in the presence of organic material (Dobbs and Williams 1963; Gibbs 1979); moreover, the oxidation of chloride can generate a second oxidant, gaseous chlorine, which in turn may react with organic components in a sample and thereby lower the apparent COD (Dobbs and Williams 1963). Although reduced nitrogen, such as ammonia, does not exert a COD (Foulds and Lunsford 1968), the combination of chloride ion and high concentrations of ammonia, or even organic amines or nitrogenous compounds, can lead to the continuous reduction of dichromate by a cyclic series of changes from chloride to chlorine (Dobbs and Williams 1963). Chlorine that is produced from oxidation of chloride ion by dichromate reacts with ammonia to form chloramines; if the molar ratio of chlorine to ammonia exceeds unity, chloride ion is produced (Jenkins et al. 1976) and reinitiates further reduction of dichromate. Therefore, the combination of these two inorganic constituents in a sample can lead to a tremendous positive interference.

To minimize the involved interactions of chloride ion, mercuric sulfate is added to complex the chloride, essentially protecting it from oxidation and preventing its precipitation with silver ion (APHA 1981). It has been noted that mercuric sulfate is not completely soluble in the cooled digestion mixture (Gibbs 1979; Jirka and Carter 1975; Jones et al. unpublished observations). The precipitate that develops can interfere with quantitation by clouding the titrimetric endpoint or by contributing severe noise to the spectrophotometric determination (Jirka and Carter 1975; Jones et al. unpublished observations).

Digestion. The COD digestion mixture includes sample, dichromate reagent, and acid/catalyst solution. Volatile compounds may be lost from the sample during the addition of acid/catalyst because of the heat of mixing, unless certain precautions are followed. The acid must be added carefully by allowing it to run down the inside of the flask or tube, and the contents should not be mixed until connected to a reflux condenser or capped. For two hours, the mixture is then either maintained at 150°C (micro-colorimetric COD) or refluxed (macro-titrimetric COD). With the latter method, the sample and reagents reach a rapid boil and a portion of the volatile gases are captured during refluxing. It has been postulated that this refluxing may lead to more complete mixing of the sample and therefore better digestion of solids if total COD is being determined (Messenger 1981). A major disadvantage of the macro reflux-titrimetric method is the loss of volatile compounds during refluxing. This loss can be eliminated by the use of sealed tubes, but in the absence of refluxing, unoxidized volatile compounds can still escape from the digestion mixture into the headspace, and the mixing of particulates is impeded.

Quantitation by redox titrimetry. At the conclusion of two hours of digestion, the extent of Cr(VI) reduction can be determined by either redox titration or spectrophotometry. The titrimetric procedure involves the reduction of the remaining Cr(VI) with ferrous ion, which is provided as the standardized titrant, ferrous ammonium sulfate (FAS): $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$.

$$6\ Fe^{2+} \longrightarrow 6\ Fe^{3+} + 6\ e^-$$

$$Cr_2O_7^{2-} + 14\ H^+ + 6e^- \longrightarrow 2\ Cr^{3+} + 7\ H_2O$$

The COD is calculated by subtracting the volume of FAS required for sample titration from that required for a digested blank. The difference is a measure of the amount of Cr(VI) that was reduced by the sample. This difference is multiplied by the product of (i) the normality (N) of the FAS (determined daily by titration of a nondigested blank, i.e., normalization blank), (ii) the molecular weight of oxygen, and (iii) the equivalency of 1000 mg/g. The entire product is then divided by the product of the sample volume (S) and the electron equivalents transferred per mole of oxygen.

$$COD = \frac{N \times (\text{blank titrant} - \text{sample titrant}) \times 32\ g\text{-}O_2/mol \times 1000\ mg/g}{S \times 4\ eq/mol}$$

Recognition of the endpoint is assisted by the addition of the redox indicator 1,10-phenanthroline, which forms a red undissociated cation when complexed with divalent metal ions (e.g., ferrous ion). At the completion of titration, when dichromate is no longer present to oxidize excess ferrous ion from the titrant, the ferrous ion-phenanthroline complex forms, and the color of the reaction mixture abruptly changes from green (typical of Cr +III) to red. Formation of sufficient ferrous ion-phenanthroline complex to color the solution red is actually indicative of overtitration; the true endpoint, which is gray, exists when all of the dichromate has been reduced, but before any <u>excess</u> of ferrous ion has been added. The amount of ferrous ion required to go from transition to endpoint, however, is very small.

The indicator is added to the digestates as the ferrous-chelated form of 1,10-phenanthroline (known commercially as "ferroin"). The ferroin should be added in reproducible quantities since the chelated ferrous ion will reduce

dichromate. In addition, the oxidation of one mole of phenanthroline would consume 14 moles of oxygen (0.15 mL of ferroin would have a COD of 253 mg/L); the oxygen demand of the nitrogenous heterocycle indicator itself would, however, probably be minimal because of the lack of susceptibility of this class of chemical compounds to dichromate oxidation.

The COD titration lends itself easily to automation. The molar absorptivity of the two chromium species is equivalent at the isosbestic point (535 nm) (Fig. 1 and inset); therefore, at this wavelength, any change in absorbance during the titration of Cr(VI) must result from the appearance of another chromophore. The appearance of the ferrous ion-phenanthroline complex at the endpoint immediately changes the absorbance (Fig. 2), indicating completion of titration. A colorimeter whose wavelength is set near the isosbestic point can detect this change in absorbance and can control titrant addition, if connected to an autotitrator.

<u>Quantitation by colorimetry</u>. An alternative method for quantitating COD is by direct colorimetry. The COD of an unknown can be interpolated directly from a standard curve that is constructed from the absorbance of either the amount of orange Cr(VI) remaining (440 nm) or the amount of green Cr(III) produced (600 nm) from the digestion of standards. Although the volume of each digestate must be reproducible in such a colorimetric analysis, the quantity of Cr(VI) added to each sample is not critical when Cr(III) is quantitated, whereas it is when Cr(VI) is measured.

For samples with low chemical oxygen demand (e.g., less than 250 mg/L), the disappearance of Cr(VI) should be quantitated directly. The micro-colorimetric COD method is eight times more sensitive for the detection of Cr(VI) in the 400-nm range than for the detection of Cr(III) in the 600-nm range because of differences in the molar absorptivities of the two species (see: Fig. 1). For the high-range samples (250 to 850 mg/L), the appearance of Cr(III) should be followed (i.e., A_{600}).

Two significant problems have been encountered with the colorimetric determination of COD: (i) the appearance of catalyst-induced precipitates of mercuric and silver salts (Gibbs 1979; Jirka and Carter 1975; Messenger 1981; Jones et al. unpublished observations) and (ii) "stratification" of the acid-water mixture following digestion (Messenger 1981). When a temperature gradient exists in the digestate, schlieren lines form (Daughton unpublished observations). This severely interferes with colorimetric quantitation because the varying refractive index causes substantial drift and inaccurate spectrophotometric readings (Daughton unpublished observations). We have found that extensive mixing of the tube contents following digestion and a prolonged cooling period reduces the appearance of schlieren lines and allows the analyst to sample the tube contents without aspirating the catalyst-induced precipitates.

A semi-micro titrimetric method that uses sealed tubes instead of refluxing flasks has been reported (Himebaugh and Smith 1979). We have not evaluated this method, but it reportedly affords many of the advantages of the micro-colorimetric method while permitting titrimetric quantitation.

Specific COD

Chemical oxygen demand is often misinterpreted as an estimate of the total concentration of organic solutes. Rather, in the absence of oxidizable inorganic solutes, it is a vague measure of both the quantity of organic solutes and their associated oxidative states. When SCOD is normalized to dissolved organic carbon (DOC) concentration, however, the ratio yields considerable information about the types of chemicals that are present in a heterogeneous sample matrix. The amount of COD yielded per unit of organic carbon is defined as "specific COD" (Daughton, Jones, and Sakaji 1981). The higher the average reductive state of the compounds, the higher will be the specific COD. The two extremes for this concept are represented by CHOOH and CH_4, which contain the same amount of carbon on a molar basis yet their theoretical specific COD values span the range available to organic material: 1.33 and 5.33, respectively. The specific COD values for the oil shale process waters in this study ranged from 3.05 to 5.01 (Table II).

The specific COD of a heterogeneous sample may be higher than 5.33; this would indicate that inorganic constituents of the sample matrix have contributed a "positive interference" to the COD test or that there was a severe problem with the DOC determination (e.g., incomplete recovery). An abnormally low specific COD indicates that either (i) a substantial portion of the sample was resistant to oxidation by dichromate, but was susceptible to combustion or oxidation for the DOC determination, or (ii) inorganic carbon was mistakenly included in the determination of the organic carbon.

Oil Shale Wastewaters and Theoretical COD

The determination of the COD of oil shale process waters may be confounded by the particulate turbidity and numerous chemical classes that typify these wastewaters. Process waters can contain thiosulfate, chloride, and ammonia; up to 20% of the COD of a retort water was reportedly contributed by thiosulfate (Wong and Mercer 1979). Much of the characteristic organoleptic properties of these waters are contributed by nitrogen and oxygen heterocycles, which are only partially accounted for by the COD test because of their resistance to dichromate digestion (Table I). Heterocyclic triazines are particularly resistant to wet-chemical oxidation (see: Chapter III).

For any COD analysis, the anticipated oxygen demand must be estimated before digestion so that the capacity of the oxidant is not exceeded. It is advisable to run several dilutions of a sample if the COD is unknown. Alternatively, if the DOC is known, the COD can be estimated by assuming a specific COD of about 3.6 for oil shale wastewaters. To determine the theoretical COD, the compound of interest is assumed to be mineralized completely to CO_2, H_2O, SO_4^{2-}, and other mineral species. Examples:

Potassium Hydrogen Phthalate:

$$C_8H_5KO_4 + 7.5\ O_2 + H^+ \longrightarrow 8\ CO_2 + 3\ H_2O + K^+$$

1 mole of KHP theoretically consumes 7.5 moles of O_2;
100 mg/L of KHP should exert an oxygen demand of 118 mg/L.

Glucose:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

1 mole of glucose theoretically consumes 6 moles of O_2;
100 mg/L of glucose should exert an oxygen demand of 107 mg/L.

Pyridine:

$$C_5H_5N + 6.25\ O_2 \longrightarrow 5\ CO_2 + 2.5\ H_2O + 0.5\ N_2$$

1 mole of pyridine theoretically consumes 6.25 moles of O_2;
100 mg/L of pyridine should exert an oxygen demand of 252.8 mg/L.

The calculations of theoretical COD for nitrogen heterocycles are complex because it is unclear if the hetero atom is oxidized by dichromate; the available literature is contradictory. For example, as to the fate of nitrogen in pyridine, Dobbs and Williams (1963) assume that the nitrogen is reduced to ammonia, whereas the values calculated by Moore et al. (1949) presume that the nitrogen would be released as molecular nitrogen. The actual theoretical COD is impossible to verify because pyridine is not oxidized by dichromate, and other nitrogen heterocycles (e.g., quinoline) are only partially oxidized (Table I).

PROTOCOL SUMMARY

Laboratory protocols for determining COD by reflux/titrimetry and colorimetry are appended. The importance of representative subsampling and laboratory safety cannot be overemphasized. The use of safety equipment and proper technique for dispensing reagents (i.e., mouth pipetting is absolutely unacceptable) and the proper disposal of the digestate following titration should be primary concerns to the analyst.

Macro-Titrimetric COD

Accurate and precise addition of the dichromate reagent and careful titration are critical for accurate and reproducible results. Two glass beads and 12.00 mL of dichromate reagent are added to each reflux flask. In contrast to the protocol detailed by APHA (1981), it is better to dissolve the mercuric sulfate in the dichromate reagent rather than dispensing it as a solid. This minimizes the hazard of inhaling mercurial dust and increases the precision of the mercuric sulfate addition. Twenty milliliters of an appropriate dilution of the sample is added. The COD of the 20-mL sample should be between 50 and 500 mg/L for best results. Acid/catalyst solution consists of concentrated sulfuric acid and 10 g/L silver sulfate; 28 mL is added very carefully to the sample, and the flask is immediately connected to the appropriate condenser to minimize the loss of volatile components. The addition of the acid/catalyst solution through the condensers, as recommended by APHA (1981), is very awkward and potentially dangerous; a double-necked flask would be preferred. The contents of the flask should then be very carefully mixed to prevent localized superheating that could result in violent expulsion of the flask contents (U.S. EPA 1979). Two sets of blanks are also prepared. The normalization blanks are not digested and are used to determine the normality of the titrant. The digestion blanks are heated together with the samples and are used for determining the oxygen demand of the dilution water and reagent contaminants. It is important to randomize the

positions of all the flasks on the hot plate to eliminate bias from the temperature differential across the plate surface. The samples and digestion blanks are then refluxed for two hours, and the amount of dichromate that remains in the cooled digestates is determined by titration with 0.10N FAS to the gray ferrous ion-phenanthroline endpoint.

A standard of a known concentration of potassium hydrogen phthalate should also be analyzed with each series of digestions. The complete recovery of COD from this easily digested organic compound verifies correct addition of reagents and accurate titration. In addition to standards, with each new type of sample, a potassium hydrogen phthalate-fortified sample should be analyzed to ensure that matrix effects have not influenced the oxidation process.

The macro-titrimetric COD method requires extensive bench space and substantial quantities of glassware and expensive reagents (silver and mercuric salts). It also produces large quantities of hazardous, acidic wastes that require special handling; various means have been proposed, however, for reclamation and recycle of the mercury and silver (Gould, Masingale, and Miller 1984). In addition, the open configuration of the reflux condensers allows for the escape of volatile organic compounds. This macro-titrimetric COD method does offer, however, the advantage of reduced scale in comparison with the method outlined by APHA (1981) (waste volumes are approximately 150 vs. 315 mL/assay, respectively).

Micro-Colorimetric COD

The method reported here is an adaptation of the method of Jirka and Carter (1975). It is imperative that the reagents are dispensed in a precise manner because absorbance values are a function of volume; accurate reagent volume dispensation is especially important when A_{440} is followed. The method uses the same reagents and order of addition as in the macro-titrimetric COD method.

The acid/catalyst reagent should be added quickly to a Pyrex culture tube (ensure that rims are not chipped), and the tube should be closed immediately with a Teflon-lined cap to minimize the loss of volatile organic compounds. The contents are vortexed and placed in a fluidized sand bath at 150°C for two hours. A sand bath has the advantage over a water bath in that the tops of the tubes remain cool; this encourages refluxing and minimizes the possibility of leakage of steam through the cap seals, which leads to irreproducible losses in the volumes of the digestate and thereby to inaccurate colorimetric quantitation. At the conclusion of digestion, it is essential that the tube digestates equilibrate with the ambient air temperature to prevent schlieren lines from forming. When the tubes have cooled, the sample contents are repeatedly mixed by vortexing, any precipitate is allowed to settle, and the sample COD values are quantitated colorimetrically. The importance of the cooling and mixing steps cannot be overemphasized.

The absorbance of the digestates is best determined by sampling into a spectrophotometer micro-flow-through cell. Pouring the digestates into cuvettes is both messy and hazardous, and the sampling of particulates then cannot be avoided. Flow-through cells that use vacuum for sample introduction are not well-suited for the high viscosity and density of sulfuric acid; a low-speed peristaltic pumping system therefore is recommended (e.g., Bausch & Lomb 2000).

The absorbance is then used to determine the quantity of Cr(VI) remaining (440 nm) or the quantity of Cr(III) produced (600 nm); absorbance is read against the appropriate blank (250-mg/L standard or water, respectively). For quantitation at 600 nm, the digested reagent blank should not be used in the reference cell because the slow and uneven heating of the solution at the incident-light side of the cell produces schlieren lines resulting in severe drift. Fortunately, the absorbance of the blank is close to zero with respect to water, which should be used in place of the blank in the reference cell. For quantitation at 440 nm, the reference standard should be repeatedly mixed to minimize the production of schlieren lines.

The COD values are determined by interpolation from a standard curve prepared from reference standards (e.g., potassium hydrogen phthalate) that are treated in parallel with the digested samples. The slope of the A_{600} standard curve will be positive with a y-intercept close to zero absorbance units (Fig. 3). In contrast, the slope of the A_{440} standard curve is negative, and the y-intercept should be close to the absorbance of the reagent blank (e.g., about 0.53 AU). It is imperative that the colorimetric method be validated by determining the actual COD of the standards with the reflux-titrimetric method every time new stock solutions of standards are made.

In contrast to the macro-titrimetric method, the micro-colorimetric COD method of Jirka and Carter (1975) requires a minimum of bench space and can be performed using either laboratory reagents in screw-capped tubes or prepackaged ampules (e.g., Hach Chemical, Loveland, CO). The colorimetric method consumes only 7% of the reagents used by the macro-titrimetric COD method and thereby reduces the attendant waste disposal problems (Table III). An additional benefit of the reduced scale is the minimization of sample consumption; this may be critical when assessing the progress of bench-scale batch treatment experiments that have small volumes. As mentioned earlier, the use of screw-capped tubes minimizes the escape of volatile gases, but it does not improve the recovery of COD from nitrogen heterocycles (Jirka and Carter 1975). The advantages and disadvantages of the five COD methods (standard, macro-titrimetric, semi-micro-titrimetric, and two micro-colorimetric methods) are listed in Table IV.

COMPARISON STUDY

Two methods (macro-titrimetric and micro-colorimetric) were compared for the determination of COD in oil shale process waters. The results from nine oil shale wastewaters and from a composite sample (comprising equal volumes of each water) showed that both methods were very precise; the precision of the macro-titrimetric method, however, was superior to the micro-colorimetric method (Table V). The results from a two-way analysis of variance (anova) on log-transformed data showed that there was no significant difference ($P>0.05$) between COD methods: $F_s(2.50)<F_{.05}(3.92)$. The anova data also indicated that there was no significant interaction ($P>0.05$) between methods and waters: $F_s(1.04)<F_{.05}(2.01)$. Although it has been hypothesized in the literature that sealed-tube digestion methods have improved COD recovery because of the capture of volatile compounds that would be lost during refluxing (Jirka and Carter 1975), the statistical analysis of the comparison study data did not reflect any difference between the two methods for oil shale process waters.

The accuracy of the two COD procedures was assessed for high (192.7-mg/L) and low (10.4-mg/L) EPA quality control standards. For the titrimetric method, the recoveries were within 4% of the theoretical COD and within 1% of the empirical value reported by EPA; the relative standard deviations (rsd values) for five replicates were 2.4% and 5.7% for the high and low samples, respectively. For the colorimetric method, the recoveries were within 5% of the theoretical COD and within 1% of the empirical value reported by EPA (rsd = 13.9%) for the high-range standard. The colorimetric procedure was inaccurate and imprecise for the low-range standard.

The accuracy and precision of the macro-titrimetric COD method also was determined by analyzing potassium hydrogen phthalate standards in triplicate. The COD recovery from five different digestions was within 3% of theoretical, and the precision was excellent (rsd values generally below 1%) (Table VI).

The "accuracy" of COD values is impossible to validate for a complex matrix such as oil shale process water; each wastewater is an unknown mixture of hundreds of organic compounds each of which may be oxidized by a COD method to various degrees. From one perspective, the accuracy of the method for a complex waste is merely an academic exercise, because the "true" value for colligative properties, such as COD, can be defined simply as the value actually obtained by the careful application of the prescribed method.

The incomplete recovery of a spike of an easily mineralized organic compound, such as potassium hydrogen phthalate, from a waste gives an indication of matrix effects. The composite oil shale process water was diluted 1:40, and one of four levels of potassium hydrogen phthalate spike was added to duplicate samples: 100, 200, 300, and 400 mg/L (COD). The least-squares linear regression line of total COD found versus COD added to each spiked sample yielded the data shown in Table VII. The COD value determined by standard additions using the colorimetric procedure for this composite water was 5% higher than that from the titrimetric procedure (25 835 vs. 24 886 mg/L), and the percent recoveries were equivalent.

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Table I. Reported COD Recoveries from Nitrogen and Oxygen Heterocycles

Compound	Recovery[1]	Method	Reference
Pyridine	nil	macro	APHA (1981)
	nil	macro[2]	Moore et al. (1949)
	nil	micro	Jirka and Carter (1975)
3-Methylpyridine	27	macro	Jirka and Carter (1975)
	32	micro	Jirka and Carter (1975)
Quinoline	44	macro[3]	Medalia (1951)
	86	macro	ASTM (1980)
2-Furoic Acid	86	macro	Moore et al. (1949)

1 percentage of theoretical
2 no addition of silver or mercuric salts
3 no addition of mercuric salts

Table II. Specific COD of Oil Shale Process Waters

Process Water	Specific COD[1]
Omega-9	5.01
Rio Blanco sour water	4.41
Geokinetics	4.34
S-55	4.12
Oxy-6 gas condensate	3.60
Paraho	3.60
150-Ton	3.39
TOSCO HSP	3.37
Oxy-6 retort water	3.05

[1] specific COD = macro-titrimetric COD/DOC (direct/UV-persulfate)

Table III. Comparison of Reagent Concentrations for Four COD Methods

	Reagent Volumes (mL) per Assay Mixture			
	Standard Method[1]	Macro-Titrimetric[2]	Semi-Micro-Titrimetric[3]	Micro-Colorimetric[2]
sample	50	20	5.0	2.5
dichromate reagent	25	12	3.0	1.5
acid/catalyst reagent	75	28	7.0	3.5
water	150	75	0.0	0.0
titrant (ca)	15	15	4	0.0
total waste	315	150	19	7.0
	Concentrations in the Digestate			
H_2SO_4	50%	50%	50%	50%
Ag^+	15.87 mM	14.81 mM	14.81 mM	14.81 mM
Hg^{2+}	22.47 mM	22.45 mM	22.49 mM	22.45 mM
$Cr_2O_7^{2-}$	6.95 mM	6.95 mM	6.67 mM	6.95 mM

1 APHA (1981)
2 as described in the appended protocols
3 Himebaugh and Smith (1979)

Table IV. Advantages and Disadvantages of Five COD Methods

Method	Advantages	Disadvantages
Macro-Titrimetric and Standard Method	-reflux achieved -spectrophotometer not required	-low sample throughput -large volumes of waste -extensive space and glassware requirement -expense of reagents -escape of volatile compounds
Semi-Micro-Titrimetric	-spectrophotometer not required -method applicable if precipitates and schlieren lines make colorimetric finish impossible	-larger scale than micro-colorimetric
Micro-Colorimetric (screw-capped tubes)	-minimal space and glassware -minimal expense for reagents -minimal waste disposal -high sample throughput -less analyst skill required	-standards must be validated by titrimetric method -requires spectrophotometer with micro-flow-through sipper cell -precipitate and schlieren line interfere
Micro-Colorimetric (commercial ampules)	-need only colorimeter -all advantages of screw-capped tube method without hazard associated with dispensing toxic chemicals	-need sealer and ampule breaker -expense of pre-prepared reagents

Table V. Comparison of Macro-Titrimetric and Micro-Colorimetric Methods for Determination of COD in Filtered Oil Shale Process Waters[1]

	Macro-Titrimetric		Micro-Colorimetric	
Process Water	Mean	rsd (%)	Mean	rsd (%)
Paraho	151 600	0.79	141 379	4.5
Composite	22 114	0.59	23 282	2.5
150-Ton	11 048	1.1	11 662	2.4
S-55	9 414	0.35	9 781	2.4
TOSCO HSP	9 193	0.47	9 459	2.3
Oxy-6 retort water	8 967	0.52	8 990	0.86
Geokinetics	7 191	0.81	7 578	1.5
Omega-9	3 596	1.2	3 729	1.2
Oxy-6 gas condensate	2 308	1.0	2 074	6.4
Rio Blanco sour water	912.4	0.33	924.8	0.74
average[2]	22 692		21 731	

[1] mg/L COD; n=5 for each sample
[2] average of nine waters, excluding Composite

Table VI. COD Recovery from Potassium Hydrogen Phthalate Standards

Sample Set[1]	Recovery[2]	rsd (%)[3]
a	100.55	0.17
b	98.72	0.68
c	98.81	1.01
d	97.69	0.21
e	98.46	0.48

1 each set analyzed on a different day
2 percentage of theoretical
3 n=3

Table VII. Standard Additions Results: Potassium Hydrogen Phthlate in Composite Water[1]

Titrimetric					Colorimetric				
m	b	r^2	zero spike[2]	percent recovery[3]	m	b	r^2	zero spike[2]	percent recovery[3]
0.953	297	1.000	296	95.11	0.946	306	0.996	311	96.30

1 COD spike levels of 100, 200, 300, and 400 mg/L (n=2)
2 COD of diluted sample (i.e., without spike)
3 (zero spike) divided by b/m X 100

m = regression coefficient (slope)
b = y-intercept
r = coefficient of determination

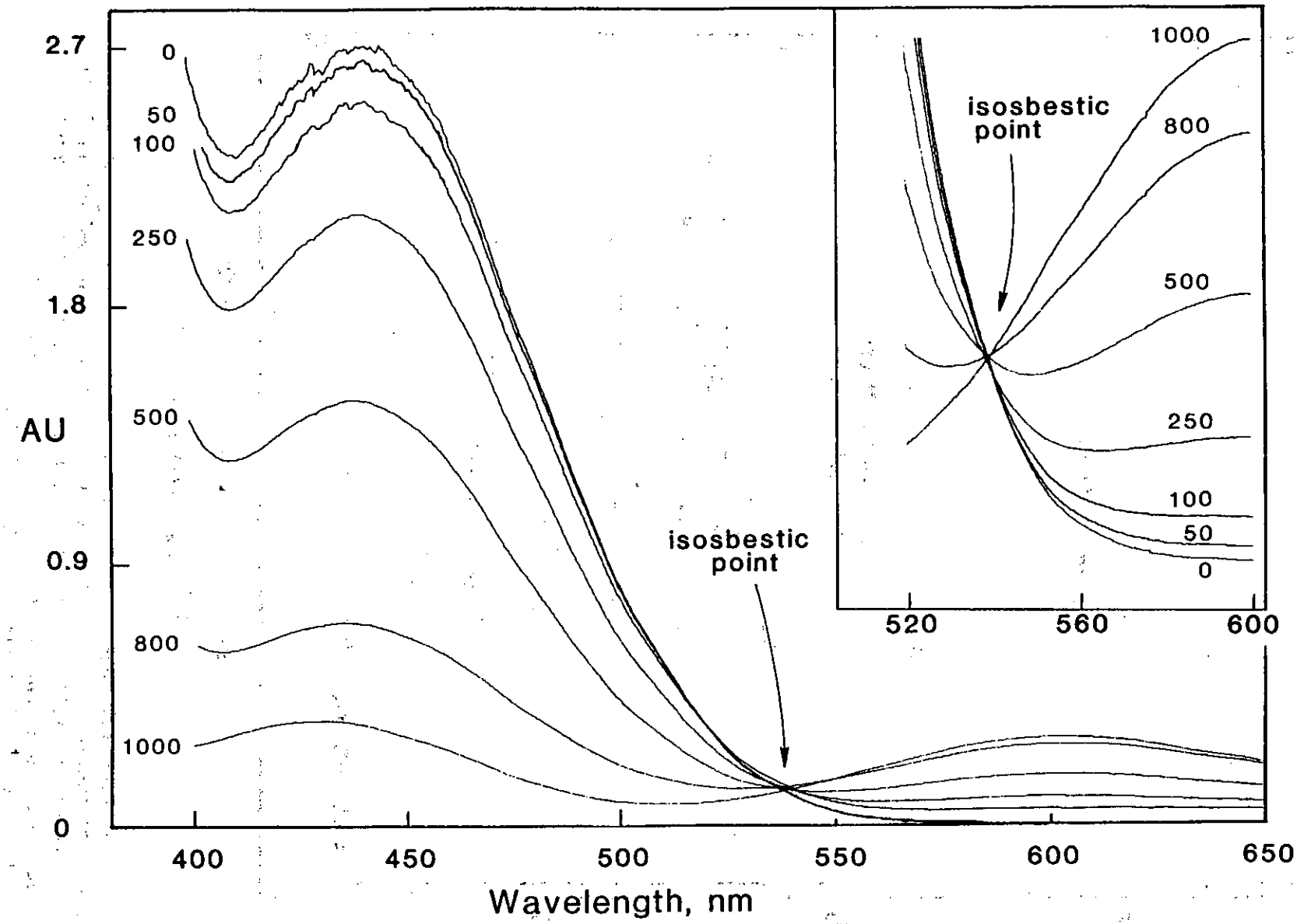

Figure 1. Absorbance scans of digested phthalate standards (mg/L COD) (inset: detail around isosbestic point at 535 nm) (XBL 832-8332).

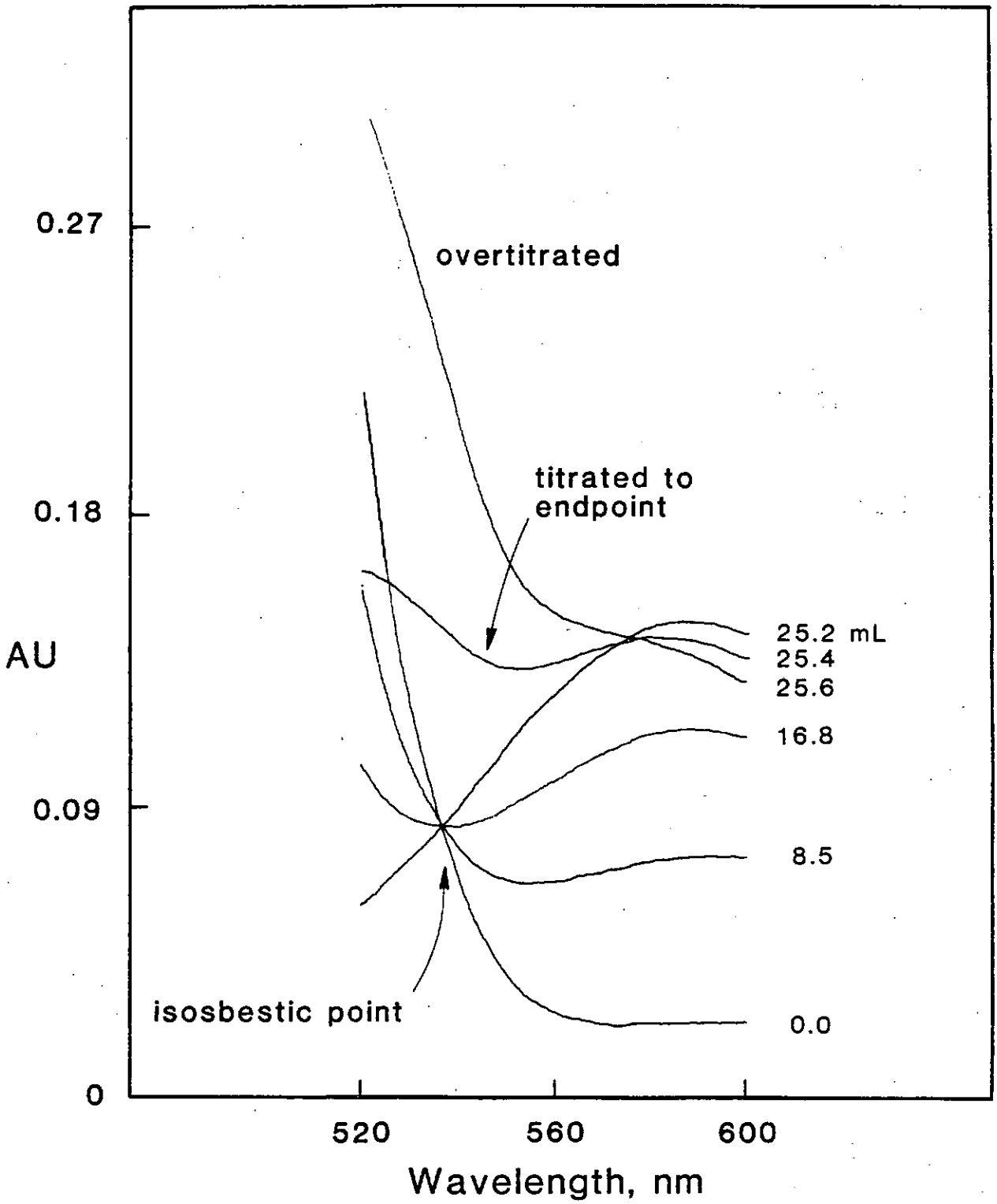

Figure 2. Absorbance changes around the isosbestic point (A_{535}) during titration of dichromate ion in the presence of ferroin indicator. Milliliters of 0.10N FAS titrant indicated at right. Scan at titration endpoint shows the shift in absorbance at the isosbestic point as a result of ferrous-ion/phenanthroline complex (XBL 832-8331).

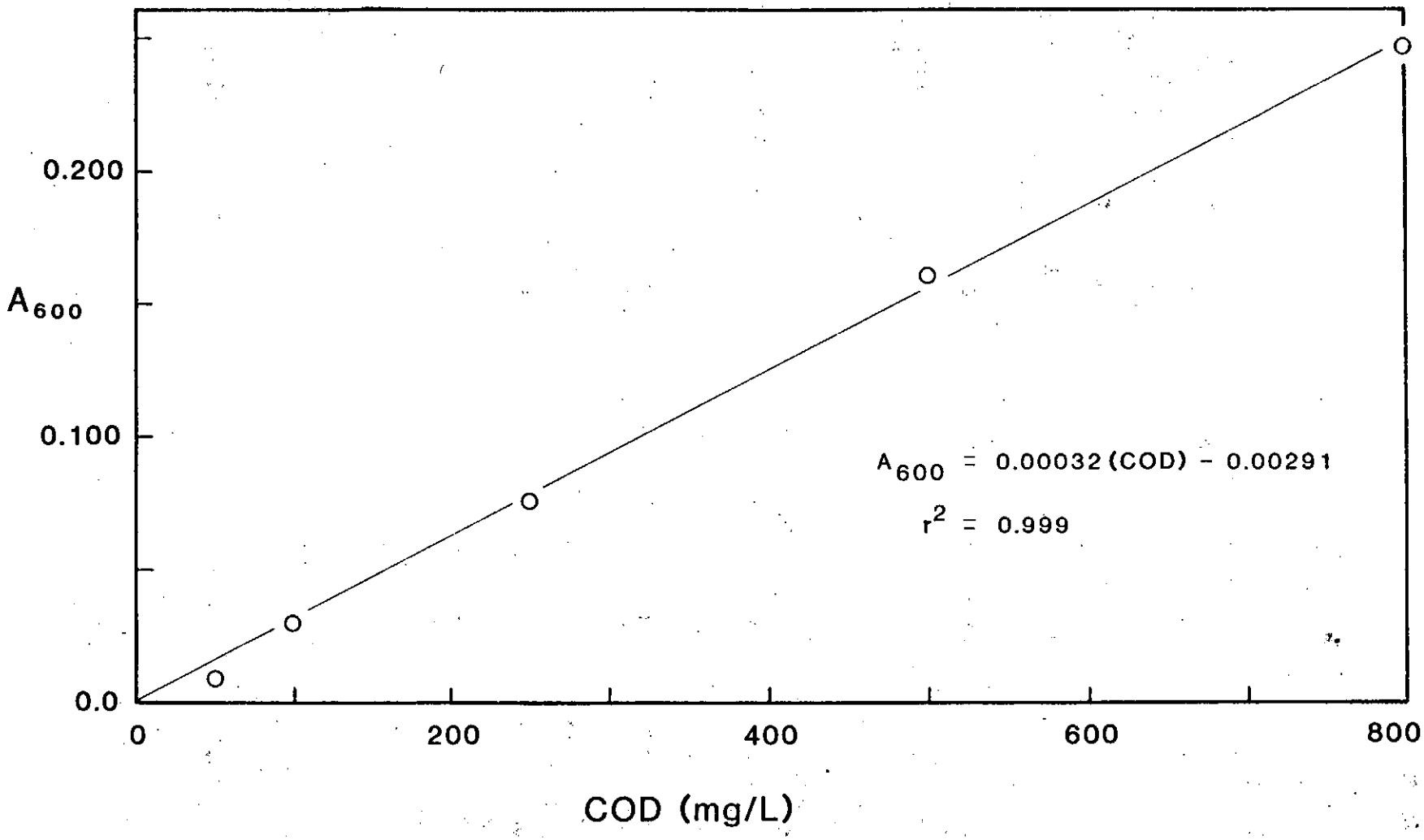

Figure 3. COD standard curve at A_{600}. Data points are averages of duplicates (XBL 846-2319).

PROTOCOL: MACRO-TITRIMETRIC COD

I. Apparatus
   A. Glassware (one unit per sample)
      1. 250-mL Erlenmeyer flask; 24/40 Ŧ outerjoint
      2. 50-mL beaker
      3. two glass beads
      4. 30-cm West condenser, 24/40 Ŧ outerjoint at top, 24/40 Ŧ inner drip joint at bottom; cover outerjoint with 50-mL beaker.
   B. Titration equipment
      1. Option 1: Automatic titrator: e.g., Sybron/Brinkmann (Westbury, NY) Autotitrator (Metrohm model 655 Dosimat, E 526 titrator, 643 control unit/624 auto sampler, and appropriate electronic burette, e.g., 10- or 20-mL).
         a. submersible colorimeter probe with 1-cm path length probe tip, 545-nm filter, and colorimeter (Brinkmann PC 800) for automated titration to indicator endpoint.
      2. Option 2: 50-mL precision-bore burette.
   C. Pipettes (calibrated): air-displacement, e.g., 5.0-mL digital adjust Gilson; positive displacement, e.g., Scientific Manufacturing Industries (SMI), Emeryville, CA, Micro/Pettors, digital adjust "F" (100 to 500 μL) and fixed volumes "H" (2.0-2.5-3.0 mL) and "K" (4.0-5.0-6.0 mL)
   D. Volumetric flasks (1-L, 500-mL; class A)
   E. Repipette (50-mL; Labindustries, Berkeley, CA)
   F. Tilting dispensers (25- and 50-mL): e.g., catalog #K759300, Kontes, Vineland, NJ
   G. Graduated cylinders (100- and 500-mL)
   H. Forceps
   I. Teflon-coated magnetic stir bars (one per Erlenmeyer flask)
   J. Magnetic stirring plate with light source
   K. Cold water bath
   L. 3600 W hot plate (model #53015, Lindberg, Watertown, WI)
   M. Polyethylene carboy (5 gal.) for disposal of waste
   N. Safety face-shield or glasses with side shields
   O. Zetex safety gloves
   P. Semi-micro analytical balance

II. Reagents
   NOTE: All reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.
   A. Potassium Hydrogen Phthalate (KHP) Standard: dissolve 850.2 mg of KHP (dried at 105°C) in 300 mL of water in a 1-L volumetric flask and bring to volume with water; solution has a COD of 1000 mg/L (i.e., 1 mL = 1 mg COD).
   B. Dichromate Reagent:
      1. Potassium dichromate ($K_2Cr_2O_7$) (dried at 105°C), 10.216 g
      2. Concentrated H_2SO_4 (sp gr = 1.84), 167 mL
      3. Mercuric sulfate, 33.3 g
      4. Add the above to 500 mL of water in a 1-L volumetric flask and bring the <u>cooled</u> solution to volume with water.
   C. Acid/Catalyst Reagent: add 22 g of Ag_2SO_4 to a 4.09-kg (9-lb) bottle of concentrated H_2SO_4; mix on stir plate with a large Teflon-coated stir bar until completely dissolved (six to eight hours).

D. Titrant:
   1. 0.25N FAS stock solution: dissolve 100 g of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (FAS) in 800 mL of water in a 1-L volumetric flask; add 20 mL concentrated H_2SO_4, and bring to volume with water.
   2. 0.10N FAS titrant solution: add 400 mL of 0.25N FAS stock solution to a 1-L volumetric flask and bring to volume with water (titrant solution should be discarded when the concentration falls below 0.08N).

E. Ferroin Indicator:
   1. 1.49 g of 1,10-phenanthroline monohydrate.
   2. 0.70 g of $FeSO_4 \cdot 7H_2O$.
   3. Dissolve the above in 100 mL of water.

   NOTE: Ferroin indicator may be purchased commercially.

III. Protocol

NOTE: The analyst should wear protective eye-wear during all steps of this procedure.

A. Glassware preparation
   1. Wash flasks and beakers in a 35% nitric acid bath, and rinse with water. Dry thoroughly.
   2. cover each flask with a 50-mL beaker until connected to the condenser.

B. Apparatus preparation
   1. Turn on hot plate at least 15 minutes prior to the beginning of the digestion.
   2. Turn on water to condensers. The water flow-rate should be sufficient to ensure that the condensers are cool to the touch.

C. Normalization blank preparation
   1. Prepare two normalization blanks for each series of digestions. These are required for determining the normality of the FAS titrant.
   2. To each flask add:
      a. 12.00 mL dichromate reagent (use "K" SMI pipette)
      b. 70.00 mL water (use 100-mL graduated cylinder)

D. Digestion blank preparation
   1. Prepare two digestion blanks for each series of digestions. These are required for assessing the contribution of the reagents to the sample COD values.
   2. To each flask add:
      a. 2 glass beads
      b. 12.00 mL dichromate reagent (use "K" SMI pipette)
      c. 20.00 mL water (use "K" SMI or 5.0-mL Gilson pipette)

E. Standard preparation
   1. Prepare at least one standard for each series of digestions.
   2. To the flask add:
      a. 2 glass beads
      b. 12.00 mL dichromate reagent (use "K" SMI pipette)
      c. 10.00 mL water (use "K" SMI or 5.0-mL Gilson pipette)
      d. 10.00 mL KHP standard solution (use "K" SMI or 5.0-mL Gilson pipette)

F. Sample preparation

   NOTE: Samples should be well-mixed and filtered (to eliminate the affect of particulates). The resulting value will be soluble COD (i.e., SCOD).

1. The reagents should be added in the order specified to minimize the interaction of chloride ion in the sample with the silver catalyst.
2. To each flask add:
   a. 2 glass beads
   b. 12.00 mL dichromate reagent (use "K" SMI pipette)
   c. 20.00 mL of sample or appropriate amount of sample and dilution water so that the total addition equals 20.00 mL (use "K" SMI or 5.0-mL Gilson pipette). The maximum COD concentration that could theoretically be determined on a 20-mL sample is 1000 mg/L; it is advisable to dilute each sample so that the COD is in the range of 500 mg/L or less. The minimum COD that can be determined accurately is 10 to 15 mg/L.

G. Fortified sample preparation
1. One fortified sample should be analyzed with each digestion.
2. To each flask add:
   a. 2 glass beads
   b. 12.00 mL dichromate reagent (use "K" SMI or 5.0-mL Gilson pipette)
   c. 20.00 mL of a combination of known volume of sample solution, known volume of KHP, and water (use "K" SMI or 5.0-mL Gilson pipette). COD of this combination should not exceed 500 mg/L; the COD of the KHP addition should be approximately 50% of the total COD.

H. Flask randomization and digestion
1. Randomize flasks by consulting the "Ten Thousand Random Digit" Table; (Table "0" in Statistical Tables, F.J. Rohlf and R.R. Sokal, W.H. Freeman and Co.: San Francisco, CA; pp 152-6, 1969).
   a. randomly select a group of five digits from the table.
   b. pick any two adjacent digits within the group of five.
   c. for two-digit random numbers, begin with the pair selected, move horizontally, and record the next two consecutive digits; continue this process in a consistent manner until two-digit numbers are generated for each flask, except for one normalization blank if autotitration is used.
   d. record the random number and the corresponding sample number.
   e. relabel each flask with its random number.
2. Add 28.00 mL acid/catalyst reagent to each flask (use 50-mL repipette)
   a. acid/catalyst reagent should be introduced by carefully touching the repipette tip to the inside of the flask neck.
3. **Immediately** connect each flask (except normalization blanks) to a condenser; then raise the flask and condenser off of the hot plate and mix the contents by swirling to avoid localized superheating, which may result in violent expulsion of the flask contents. Replace the flask on the hot plate and ensure that the bottom fully contacts the surface.
4. Place flasks on hot plate in ascending order of assigned random numbers.

CAUTION: If a flask should break on the hot plate, try to turn off the hot plate and evacuate the lab immediately. Personnel should avoid breathing the SO_3 fumes and should not re-enter the laboratory until fumes have been vented.

5. Note the time when five flasks have come to a rapid boil. This is considered the beginning of heated digestion.
6. At the end of 2 hours of heated digestion, turn off the hot plate and allow flasks to stand for at least 30 minutes. A fan will expedite cooling.
7. After 30 minutes of cooling, the flasks can be raised onto inverted 50-mL beakers while still connected to their condensers.
   a. alternatively, the flasks can be raised onto inverted 50-mL beakers immediately at the conclusion of the digestion without turning off the hot plate; this facilitates cooling and allows a second digestion to proceed with minimum delay.
8. When the flasks are cool to touch, wash residual condensate into each flask by adding 50 mL of water to the top of the condenser with a 50-mL tilting dispenser.
9. Remove flasks from the hot plate, cover with 50-mL beakers, and place in cooling bath.

I. Manual titration (also see option at: IV. Automatic Titration)
1. Fill titrant reservoir with 0.10N FAS solution; ensure that the solution is mixed.
2. Add a Teflon-coated stir bar and 150 µL of ferroin indicator with a positive displacement pipette to each flask immediately prior to manual titration.
3. Titrate to gray endpoint. A transient red color indicates approach of endpoint, while persistent red indicates overtitration. Record encoded sample number and volume of FAS that was required to titrate the sample to the endpoint.

IV. Automatic Titration (instructions for Sybron/Brinkmann Metrohm Autotitrator)

A. Setup
1. Fill rinse-water reservoir with water and plug solenoid into outlet "J" on the back of the 643 control unit.
2. Install burette containing 0.10N FAS titrant solution.
3. Connect HP 97S calculator to external power supply and connect Amphenol connector to 643 control unit.
4. Switch 643 control unit to "manual".
5. Turn on the following units in sequence: titrator E 526, Dosimat 655, control unit 643, and calculator.

B. Sample transfer
1. Quantitatively transfer the contents of the Erlenmeyer flasks to the sample cups using 25 mL of water (tilting dispenser) to rinse the flasks.
2. Transfer the encoded number of the flask to appropriate sample cup.

C. Indicator endpoint calibration
1. E 526 titrator:
   a. plug shorting strap into the reference and indicator electrode inputs
   b. set: thumbwheels to 0000, calibration knob to "calibr", selector knob to +mV
   c. turn "U" knob until analog meter is nulled

NOTE: The setting of the "U" knob must not be disturbed once it is set.

   d. disconnect shorting strap

2. PC 800 Colorimeter:
   a. connect colorimeter leads; black to reference electrode input and red to indicator electrode input.
   b. install 545-nm filter and turn colorimeter power switch on to %T mode; allow at least 5 minutes for warm-up.

   WARNING: NEVER turn on the colorimeter without a filter in the filter block.

   c. ensure that the colorimeter probe tip is the appropriate path length.
   d. adjust colorimeter to 100%T with coarse/fine knobs.
   e. thoroughly rinse colorimeter probe.
   f. disconnect mixer from 643 control unit and connect to 655 Dosimat; turn mixer on and adjust by turning the knob on top of the mixer counterclockwise. Avoid vortex formation to prevent air bubbles from becoming entrapped in the colorimeter probe tip.
   g. use normalization blank to set 100% T on colorimeter.
   h. add 150 µL of ferroin to a digestion blank and titrate manually to the gray endpoint; determine transmittance (545 nm) and record volume of titrant required.
   i. set titrator thumbwheels for a value slightly (4%) above endpoint transmittance to compensate for overtitration error by autotitrator (e.g., endpoint is generally 88%, the thumbwheels are set to read approximately 0920); this setting will be about 0900-0940.
   j. set calibration knob to first -mV setting.

D. Operation
   1. Reconnect the mixer to the 643 control unit and rotate mixer knob to the "on" position. Adjustment can be made once the control unit is engaged (IV.D.10.).
   2. Close control panel cover.
   3. Ensure that the turntable surface is clean.
   4. Place samples on turntable and record their positions; the 624 control unit only holds 10 samples and the calculator program can only accommodate 10 samples before it must be restarted.
   5. Load "COD Titration Program" (see: Appendix A), and depress "E" button to initiate.
   6. Set titration rate knob on 655 Dosimat between "5" and "6".
   7. Set 643 control unit to "auto".
   8. Ensure that the titrator delivery-line diffuser is installed.
   9. Add 150 µL of ferroin to each sample cup.
   10. Press "Start" switch on 643 control unit.

D. Shutdown: reverse order of sequence at IV.A.5.

V. Data Reduction

A. Calculate the normality of the FAS titrant:

$$N = \frac{(12.00 \text{ mL } K_2Cr_2O_7) \times (0.208 \text{ eq-}K_2Cr_2O_7/L)}{V_n}$$

$$= \frac{2.50 \text{ mL-eq/L}}{V_n}$$

where V_n = average mL of FAS required for normalization blanks
N = normality of FAS (eq/L).

B. Calculate the COD (mg/L) for each sample, standard, and fortified sample using the following equation:

$$\text{COD (mg/L)} = \frac{N \times (V_b - V_s) \times 32\ \text{g-}O_2\text{/mol} \times 1000\ \text{mg/g}}{S \times 4\ \text{eq/mol}}$$

$$\text{COD (mg/L)} = \frac{N \times (V_b - V_s) \times 8000\ \text{mg/eq}}{S}$$

where N = normality of FAS (eq/L)
V_b = mL FAS required for digestion blank
V_s = mL FAS required for sample
S = mL of sample added to flask.

V. Waste Disposal

A. Carefully decant the contents of each flask into the waste carboy. Retrieve stir bars and glass beads and rinse them in water. The silver from the catalyst reagent can be reclaimed by precipitation with NaCl.

Protocol prepared by: B.M. Jones, G.W. Langlois, R.H. Sakaji, and C.G. Daughton

PROTOCOL: MICRO-COLORIMETRIC COD

I. Apparatus
   A. Glassware (one each per sample)
      1. Culture tubes: Pyrex (16-mm O.D. X 150 mm) with Teflon-lined screw caps
   B. Pipettes (calibrated): air-displacement, e.g., 5.0-mL digital adjust Gilson; positive displacement, e.g., Scientific Manufacturing Industries (SMI), Emeryville, CA, Micro/Pettors, digital adjust "K" (2.0 to 6.0 mL) and fixed volume "H" (2.0-2.5-3.0 mL)
   C. Vortex mixer
   D. Heating unit: Tecam fluidized sandbath SBL-1 (Techne, Inc., Princeton, NJ) with overflow flange (accessory #1133).
   E. Circular stainless-steel 42-place test-tube rack for sand bath.
   F. Spectrophotometer: e.g., Bausch & Lomb Spectronic 2000 with micro flow-through cell, 1-cm path length; ensure that Viton O-rings are used. Flow-through cells must use a peristaltic pumping system rather than a vacuum system because of the density and viscosity of the sulfuric acid.
   G. Volumetric flasks (1-L, 10-mL; class A)
   H. Polyethylene carboy for disposal of waste
   I. Safety face-shield or glasses with side shields
   J. Semi-micro analytical balance

II. Reagents
   NOTE: all reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.
   A. Potassium Hydrogen Phthalate (KHP) Stock Solution: dissolve 850.2 mg of KHP (dried at 105°C) in 300 mL of water in a 1-L volumetric flask and bring to volume with water; this solution has a COD of 1000 mg/L (i.e., 1 mL = 1 mg COD).
   B. Dichromate Reagent:
      1. Potassium dichromate ($K_2Cr_2O_7$) (dried at 105°C), 10.216 g
      2. Concentrated H_2SO_4 (sp gr = 1.84), 167 mL
      3. Mercuric sulfate, 33.3 g
      4. Add the above to 500 mL of water in a 1-L volumetric flask, and bring the <u>cooled</u> solution to volume with water.
   C. Acid/Catalyst Reagent: add 22 g of Ag_2SO_4 to a 4.09-kg (9-lb) bottle of concentrated H_2SO_4; mix on stir plate with a large Teflon-coated stir bar until completely dissolved (six to eight hours).

III. Protocol
   NOTE: The analyst should wear protective eye-wear during all steps of this procedure.
   A. Apparatus preparation
      1. Turn on sandbath. Adjust temperature setting and air flow rate (maximum delivery pressure = 35 psig) to stabilize temperature at 150°C. Place a thermometer in a test tube containing silicone oil, and place test tube in the sand bath to monitor the temperature.
      2. Use only culture tubes without chipped rims (to ensure a gas-tight seal). Wash tubes in a 35% nitric acid bath, and rinse with water. Dry thoroughly. <u>Do not</u> acid-wash caps; rinse thoroughly only in water.

B. Digestion blank preparation
   1. Prepare three digestion blanks for each series of digestions.
   2. To each tube add:
      a. dichromate reagent, 1.50 mL (use 5.0-mL Gilson pipette)
      b. water, 2.50 mL (use "H" SMI pipette)
      c. acid/catalyst reagent, 3.50 mL (use "K" SMI pipette)
      CAUTION: add acid/catalyst reagents carefully; mouth of tube should be facing away from analyst when adding this reagent in the event of explosive bumping.
      d. Seal tubes tightly with Teflon-lined screw caps.
      e. Vortex
   3. Digestion blanks are heated with samples (see: III.E.).

C. Standard curve preparation
   1. For each of the following COD concentrations, add the respective volume of KHP to a 10-mL volumetric flask and bring to volume with water:

      800 mg/L: 8.00 mL
      500 mg/L: 5.00 mL
      250 mg/L: 2.50 mL
      100 mg/L: 1.00 mL
      50 mg/L: 0.50 mL

      NOTE: Use positive displacement pipettes whenever possible.
   2. Prepare duplicates of each standard concentration for each series of digestions.
   3. To each tube add:
      a. dichromate reagent, 1.50 mL (use 5.0-mL Gilson pipette)
      b. appropriate standard solution, 2.50 mL (use "H" SMI pipette)
      c. acid/catalyst reagent, 3.50 mL (use "K" SMI pipette); solution should be carefully added down the inside of the tube so that the acid forms a layer on the bottom.
      CAUTION: See III.B.2.c.
      d. Immediately seal tubes tightly with Teflon-lined screw caps.
      e. Vortex
   4. Standards are heated with samples (see: III.E.).

D. Sample preparation
   1. Samples should be well-mixed and filtered (to eliminate the affect of particulates). The resulting value will be soluble COD (i.e., SCOD).
   2. The reagents should be added in the order specified to minimize the interaction of chloride ion in the sample with the silver catalyst.
   3. To each tube add:
      a. dichromate reagent, 1.50 mL (use 5.0-mL Gilson pipette).
      b. sample (diluted or neat), 2.50 mL (use "H" SMI pipette). The maximum COD concentration that could theoretically be determined for a 2.50-mL sample is 1000 mg/L. It is advisable to dilute each sample so that the COD is in the upper portion of the standard curve (i.e., 600 to 800 mg/L for A_{600} or up to 200 mg/L for A_{440}); this increases the absorbance values for A_{600} and serves to decrease the variance of replicate readings.
      c. acid/catalyst reagent, 3.50 mL (use "K" SMI pipette).
      CAUTION: See III.B.2.c.

d. Immediately seal tubes tightly with Teflon-lined screw caps.
e. Vortex

E. Sample digestion
1. Place tubes in sandbath rack. Heat at 150°C for 2 hours.
2. Remove sample tubes, brush off sand, and cool to ambient air temperature.

F. Quantitation

IMPORTANT: Follow these instructions exactly to obtain accurate and precise results.
1. Vortex each tube repeatedly to ensure complete mixing.
2. If a precipitate forms, allow crystals to settle. Ensure that digestate remains mixed after this settling period. Schlieren lines must be totally absent from sample digestates.
3. Read the absorbance of the blanks, duplicate series of standards, and samples at 600 nm against a water reference. Avoid aspiration of the precipitate into the cell.
4. If COD values are low (i.e., 0 to 250 mg/L), read the absorbance at 440 nm against the 250-mg/L standard.

NOTE: Ensure that the standard in the reference cell remains well-mixed.

IV. Data Reduction

A. Determine the linear regression equation of the COD standard curve by least-squares analysis. Calculate the coefficient of determination (r^2).

B. Interpolate the COD of each sample using the least-squares standard curve equation. For A_{600}, either subtract the mean blank absorbance value from each sample absorbance or include the coordinate (0, blank A_{600}) in the least-squares equation. For A_{440}, include the coordinate (0, blank A_{440}) in the equation.

$$\text{COD (mg/L)} = (m)(A_{600} \text{ or } A_{440}) + b$$

where m = regression coefficient (i.e., slope)
b = y-intercept

Typical values are:

A_{600}	m =	0.00032
	b =	-0.00291
A_{440}	m =	-0.00193
	b =	0.51936

C. If the sample solution has been diluted, multiply the result by the appropriate dilution factor.

V. Waste Disposal

A. Carefully decant the contents of each tube into the waste carboy. The silver from the catalyst solution can be reclaimed by precipitation with NaCl.

Protocol prepared by: B.M. Jones, G.W. Langlois, R.H. Sakaji, and C.G. Daughton

User Instructions

1 AUTOTITRATION PROGRAM 2

COD

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	LOAD PROGRAM: Calculator in RUN mode, Print in MANUAL position.			
2	PRESS B		B	
3	ENTER mL DICHROMATE	Value		
4	PRESS R/S: Prints mL Dichromate		R/S	Value
5	ENTER DICHROMATE NORMALITY	Value		
6	PRESS R/S: Prints Dichromate Normality		R/S	Value
7	ENTER VOLUME OF SAMPLE	Value		
8	PRESS R/S: Prints volume of sample and stores it in corresponding memory.		R/S	Value
9	REPEAT STEPS 7-8 FOR ALL 6 SAMPLES (the first four samples consist of 2 for the determination of FAS normality and 2 blanks). Following input of last sample volume, display will go to -1.00.			
10	PLACE SAMPLES IN AUTOSAMPLER TRAY AS FOLLOWS: 2 FAS samples, 2 blanks, 6 samples.			
11	START AUTOTITRATOR: with controller in AUTO mode, press start switch. Data printout will be as follows:			
	Sample 1: prints mL titrant, FAS normality.			
	Sample 2: prints mL titrant, FAS normality, and $\bar{x}$ FAS normality.			
	Sample 3: prints mL titrant.			
	Sample 4: prints mL titrant, $\bar{x}$ mL titrant for blanks.			
	Samples 5-10: prints mL titrant, COD value.			

AUTOTITRATION PROGRAM

COD

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
12	Autotitration stops after 10th sample, display goes to 0.00. To continue, repeat steps 7-8 for 6 additional samples. (NOTE: 6 values must be entered).			
13	PRESS START SWITCH ON CONTROLLER: prints mL titrant and COD value for each sample.			

STEP	KEY ENTRY	KEY CODE	COMMENTS
001	*LBL3	21 13	
002	CF3	16 22 03	
003	DSP2	-63 02	
004	P≠Q	16-51	
005	R/S	51	
006	PRTX	-14	
007	STO1	35 01	
008	R/S	51	
009	PRTX	-14	
010	STO2	35 02	
011	P≠Q	16-51	
012	*LBLa	21 16 11	
013	R/S	51	
014	PRTX	-14	
015	STO4	35 04	
016	R/S	51	
017	PRTX	-14	
018	STO5	35 05	
019	R/S	51	
020	PRTX	-14	
021	STO6	35 06	
022	R/S	51	
023	PRTX	-14	
024	STO7	35 07	
025	R/S	51	
026	PRTX	-14	
027	STO8	35 08	
028	R/S	51	
029	PRTX	-14	
030	STO9	35 09	
031	SPC	16-11	
032	*LBLE	21 15	
033	CF0	16 22 00	
034	CF1	16 22 01	
035	1	01	
036	CHS	-22	
037	STOI	35 46	
038	CF3	16 22 03	
039	R/S	51	
040	*LBLA	21 11	
041	CF3	16 22 03	
042	STOA	35 11	
043	ISZI	16 26 46	
044	P≠Q	16 51	
045	RCLA	36 11	
046	1	01	
047	0	00	
048	÷	-24	
049	FRC	16 44	
050	1	01	
051	0	00	
052	×	-35	
053	10^x	16 33	
054	RCLA	36 11	
055	1	01	
056	0	00	
057	0	00	
058	÷	-24	
059	10^x	16 34	
060	x≠y	-41	
061	÷	-24	
062	DSP2	-63 02	
063	PRTX	-14	
064	STOB	35 12	
065	1	01	
066	ENT↑	-21	
067	RCLI	36 46	
068	X≠Y?	16-35	
069	GSBb	23 16 12	
070	3	03	
071	X≠Y	-41	
072	X≠Y?	16-35	
073	GSBc	23 16 13	
074	GSB1	23 01	
075	RCLI	36 46	
076	9	09	
077	X=Y?	16-33	
078	GSBa	23 16 14	
079	CF3	16 22 03	
080	R/S	51	
081	GTOA	22 11	
082	*LBL1	21 01	
083	RCLD	36 14	
084	ENT↑	-21	
085	RCLB	36 12	
086	-	-45	
087	RCLC	36 13	
088	×	-35	
089	8	08	
090	0	00	
091	0	00	
092	0	00	
093	×	-35	
094	RCLI	36 46	
095	÷	-24	
096	PRTX	-14	
097	SPC	16-11	
098	RTN	24	
099	*LBLb	21 16 12	
100	P≠Q	16-51	
101	RCLI	36 01	
102	ENT↑	-21	
103	RCL2	36 02	
104	×	-35	
105	RCLB	36 12	
106	÷	-24	
107	PRTX	-14	
108	P≠Q	16-51	
109	Σ+	56	
110	RCLI	36 46	
111	1	01	
112	X=Y?	16-33	

REGISTERS

0	1	2	3	4 SAMPLE VOLUME	5 SAMPLE VOLUME	6 SAMPLE VOLUME	7 SAMPLE VOLUME	8 SAMPLE VOLUME	9 SAMPLE VOLUME
S0	S1 mL DICHROMATE	S2 DICHROMATE NORMAL.	S3	S4 SUM x_i	S5	S6	S7	S8	S9 n_x

A	B	C	D	E	I
CODED DATA	DECODED DATA (mL titrant)	x̄ FAS NORLMALITY	x̄ DIGESTION BLANK		COUNTER

Program Listing

CHAPTER VI

May 1984

STEP	KEY ENTRY	KEY CODE	COMMENTS	STEP	KEY ENTRY	KEY CODE	COMMENTS
113	GSB3	23 03					
114	SPC	16-11		170			
115	CF3	16 22 03					
116	R/S	51					
117	GTO4	22 11					
118	*LBL3	21 03					
119	X	16 53					
120	PRTX	-14					
121	STO0	35 13					
122	F#S	16-31					
123	0	00					
124	STO4	35 04		180			
125	STO9	35 09					
126	F#S	16-31					
127	RTN	24					
128	*LBL4	21 15 13					
129	ROLB	36 12					
130	Z+	56					
131	ROLT	36 46					
132	3	03					
133	#=0?	16-33					
134	GSB3	23 03		190			
135	SPC	16-11					
136	CF3	16 22 03					
137	R/S	51					
138	GTO4	22 11					
139	*LBL3	21 03					
140	X	16 53					
141	STO0	35 14					
142	PRTX	-14					
143	RTN	24					
144	*LBL4	21 15 14		200			
145	CLX	-31					
146	R/S	51					
147	PRTX	-14					
148	STO4	35 04					
149	R/S	51					
150	PRTX	-14					
151	STO5	35 05					
152	R/S	51					
153	PRTX	-14					
154	STO6	35 06		210			
155	R/S	51					
156	PRTX	-14					
157	STO7	35 07					
158	R/S	51					
159	PRTX	-14					
160	STO8	35 08					
161	R/S	51					
162	PRTX	-14					
163	STO9	35 09					
164	SPC	16-11		220			
165	3	03					
166	STO1	35 46					
167	RTN	24					
168	R/S	51					

LABELS

A DATA INPUT	B DICHROMATE CONSTANTS	C	D	E RESET COUNTER
a SAMPLE VOLUMES	b FAS NORMAL	c SUM OF DIG BLANK	d RESET VOL COUNT	e
0	1 COD CALCULATION	2 $\bar{x}$ FAS NORMALITY	3 $\bar{x}$ BLANK CALCULATION	4
5	6	7	8	9

FLAGS

0	
1	
2	
3	

SET STATUS

FLAGS	ON	OFF	TRIG		DISP	
0	☐	☐	DEG	☐	FIX	☐
1	☐	☐	GRAD	☐	SCI	☐
2	☐	☐	RAD	☐	ENG	☐
3	☐	☐			n ____	

Chapter VII

MICROBIAL BIOMASS: QUANTITATION AS PROTEIN

C.G. Daughton, B.M. Jones, and R.H. Sakaji

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INTRODUCTION

Microbial Biomass

The quantitation of microbial growth (i.e., time-dependent increase in biomass concentration in conjunction with cell numbers) is necessary for the accurate assessment of biological waste treatment performance and is essential for execution of valid biodegradation experiments (i.e., to correlate substrate disappearance with biological processes rather than physicochemical activities such as volatilization). The increase in biomass at stationary phases is an indirect measure of organic solute degradation; biomass determinations therefore can be used to validate the actual solute removal values that are determined by other means. Values for solute removal and biomass production can then be used to calculate growth yields.

The quantitation of microbial growth in liquid medium can be accomplished by direct and indirect methods. Direct methods include (i) direct counts (microscopy, electronic particle counting, colony counts), (ii) light scattering (turbidimetry, nephelometry), (iii) most probable number (MPN), (iv) biomass (wet weight, dry weight, suspended solids, volatile suspended solids), (v) nonspecific cellular components (total cellular C, N, P), and (vi) specific cellular constituents (nucleotides, DNA, RNA, protein, muramic acid, lipopolysaccharides). Indirect methods include those that reflect metabolic activity (e.g., manometry, calorimetry, metabolite production, substrate consumption, BOD, chloroform-lysis-incubation).

Few of the direct methods distinguish viable from nonviable cells (e.g., epifluorescence microscopy, ATP, and MPN), and many of these methods are estimators only of cell numbers; various corrections must therefore be performed to convert these values to biomass (e.g., cell volume and moisture content must be known in conjunction with direct counts). Many indirect methods, in contrast, directly reflect viability, or at least cell function. Methods for determining biomass, cell numbers, viability, and function are discussed extensively by Gerhardt (1981), Hanson and Phillips (1981), Karl (1980), Koch (1981), and Paul and Voroney (1980).

Oil shale process wastewaters have several characteristics that preclude the facile measurement of microbial biomass by the more often used methods such as dry cell mass, turbidity, or protein.

Dry cell mass. Dry cell mass is usually measured after collection of cells on membrane- or glass-fiber filters or after centrifugation and separation of the cells from the supernatant fluid. Wet- or dry-mass can then be determined gravimetrically, or "volatile suspended solids" can be determined by difference. Accurate gravimetric procedures require extensive replication and multiple blanks for obtaining reliable background values (e.g., filter extractables such as wetting agents, humectants, and particulate debris can lead to significant losses in filter mass; see: Cooney 1980). There are two major problems with the gravimetric approach. (1) The volume of the sample to be filtered must contain at least several milligrams of dry cell mass for accurate gravimetric determination. Since bacterial cells are about 80% moisture by weight, this requirement poses the dual problems of consuming excessive amounts of experimental treatment samples for dry mass determinations and of filtering oil shale process wastewaters. These waters are not conducive to filtration; we

have found that membranes become clogged before sufficient cell material has been retained by the filter. (2) Separation of cells from the extracellular fluid also effects removal of nonbiological material such as particulates, colloids, and dissolved compounds which are then measured indiscriminately as biomass. The endogenous levels of abiotic particulates and tarry materials in many oil shale wastewaters also yield high and nonreproducible background levels. Membrane fouling by retort waters results not only in low filtrate yields, but also possibly in removal of solutes from the filtrate (i.e., solubilized compounds) by their partitioning into the tars immobilized on the membrane (Daughton, Jones, and Sakaji 1981) or by precipitation (e.g., of carbonates; Fox 1980) on the filter surface. The chemical sorption or precipitation of solutes by electrostatic (Zierdt 1979) or chemical interactions of the solution with the membrane surface can also effect removal of dissolved solutes. The filtrate, therefore, is not necessarily representative of soluble or dissolved material, and likewise, the retentate cannot be considered solely as representative of suspended matter. A further complication is that these filtration phenomena are partially dependent on the membrane or filter type (e.g., depth filters such as glass-fiber, or screen-type filters such as polycarbonate, mixed cellulose esters, Teflon, nylon, etc.; see: Chapter III).

Turbidity. Turbidity, a measure of the light-scattering properties of a mixture, is commonly used as a measure of suspended solids. Cellular material is easily quantitated by measurement of turbidity when the suspended cells are dispersed, but not when they are flocculent. Turbidimetric measurements of nondispersed mixed bacterial cultures can be difficult and inaccurate; flocs, however, can often be disrupted by sonication. The arbitrary units of this approach (e.g., nephelometric turbidity units; NTU) must be standardized against a direct measure such as dry cell mass, because the amount of both scattered and transmitted light is affected by particle shape and size (e.g., bacterial species and growth phase). Even then, abiotic particulates will interfere with turbidity when measured by absorbance or nephelometric techniques. The highly chromophoric character of most oil shale wastewaters prevents direct absorbance measurements. The abiotic particulates of these waters are inherently dark, and bacterial cells grown in these waters sorb and concentrate colored material on their surfaces. This precludes the measurement of turbidity as "optical density" (i.e., by measuring pseudoabsorbance or transmitted light), unless every sample is referenced to its homologous particle-free extracellular fluid. Nephelometry is invalid because much of the scattered light is subsequently absorbed by the chromogenic particulates and fluid. The great advantage of turbidimetric measurements for determining cell concentrations results, however, from its rapid and nondestructive nature; this is especially valuable for small experimental samples that must be conserved for time-course studies.

An effective means of determining turbidity in highly colored waters involves calculating the ratio of transmitted light intensity to the average of two values of scattered light intensity measured at 90° to the incident radiation. This avoids the problems associated with chromophoric solutes and colored suspended solids. Correction curves still need to be calculated, however, because the nephelometric idiosyncrasy of higher sensitivity at higher turbidity (resulting from secondary scattering) will give overestimations of biomass. An instrument that employs this technique is the DRT-100 (HF Instruments, Bolton, Ontario, Canada).

Protein. Cellular components such as organic carbon or organic nitrogen are nonspecific estimators of biomass. Standard methods for these techniques are time-consuming, and organic carbon is susceptible to bias because of the possibility of storage of large quantities of carbon as intracellular polymers (e.g., polyhydroxybutyrate). Less direct but more specific measures include nucleic acids and protein. Methods for determining protein include UV absorbance, amino acid analysis (reduced ninhydrin reaction), biuret (cupric-peptide chelation), Lowry (aromatic amino acid-phosphomolybdate reduction), and dye-binding (Coomassie Blue-protein binding). Many of these methods are useful only for relatively pure protein solutions. UV absorbance (280 nm) of proteins mainly results from the aromatic amino acids, tryptophan and tyrosine; nearly all proteins contain these two amino acids, which are present in a rather constant proportion to other amino acids (Hanson and Phillips 1981). This approach, however, is prone to many interferences. Total nitrogen (e.g., by Kjeldahl) is very time consuming. The Lowry method, as well as the dye-binding method, has a relatively low minimum detection limit, but it is prone to interferences (e.g., by most phenols). A low detection limit, however, does present difficulties with obtaining representative subsamples from nonhomogeneous samples, because the subsample must be initially small or subsequently diluted. Although the biuret method has a higher minimum detection limit which necessitates large sample sizes, it is easy to perform. Since a method suitable for research purposes must have a minimum detection limit sufficiently low to avoid consuming excessive quantities of experimental samples and because sample throughput is critical, only the Lowry and dye-binding assays were considered for the experimental work reported here.

THEORY

Determination of Biomass as Protein

The three major steps in determining cellular protein are (i) cell harvest, (ii) cell digestion (i.e., protein liberation), and (iii) quantitation of whole-cell protein. Cell harvest serves to concentrate the cells when they are present at low concentrations and to separate them from solutes in the extracellular fluid (e.g., free protein, colored substances, and phenols) that may interfere with the subsequent detection step. Cell harvest is usually done by centrifugation (e.g., 10,000 x *g*, 15 min) followed by collection and washing of the pellet. We have employed a more convenient method that uses filtration, washing, and collection of the retentate. Since the quantitative removal of the retained cells is difficult, it is necessary to digest the cells while they remain on the filter. Basic problems with these steps include representative subsampling of flocculent bacterial cultures, loss of cells during sampling because of sorption to pipette tips and membrane filter holders, cell lysis during washing, and retention by the filter of compounds that interfere with the protein assay. Whole-cell protein is solubilized by digestion in alkaline solution; the filter must be able to withstand the digestion and not interfere with the detection of protein.

Protein quantitation can be done by numerous methods, most of which involve formation of a reaction product that absorbs light. Two of the most prevalent methods are the Bradford and Lowry assays. The Bradford assay employs the protein-binding capability of Coomassie Brilliant Blue dye; the absorption maximum of the dye shifts from the red anionic form to the blue form when bound to amino groups of amino acids. The Lowry assay (Lowry et al. 1951) ("reducing

phenol method") makes use of the chemical reduction of phosphotungstic-phosphomolybdic acid (Folin phenol reagent) by phenols, as well as many other substances, to yield heterophosphomolybdate blue ("molybdenum blue"). Of the amino acids used in biosynthesis, apparently only tyrosine, and to a lesser extent tryptophan, can reduce the reagent. The addition of cupric ions in alkali gives a peptide-cupric biuret reaction, which produces a more reproducible, enhanced color development with the Folin reagent. The biuret reaction results from the chelation of cupric ions by the carbonyl groups of carbamylurea (biuret); cupric ligands are formed similarly by the carbonyl moieties of the peptide amide groupings, but not all peptide linkages react, and the stoichiometry of the ligand is not consistent, being dependent on the type of protein. Nonetheless, proteins give very consistent biuret values. This serves to modulate the more erratic absorbance values obtained with the Folin reaction.

The disadvantages of the dye-binding assay include (i) interference by alkali, which necessitates neutralization of the digested samples prior to dye addition, (ii) the necessity of subsampling the digestate, and (iii) the particulate nature of the protein-dye complex, which causes erratic absorbance readings when a micro-flow-through cell is used in place of cumbersome cuvettes in a spectrophotometer. Although the Lowry assay has an inherently higher minimum detection limit than the dye-binding assay, procedural stipulations (e.g., dilutions are not required) allow the use of much smaller sample sizes during cell harvest. Sample sizes are generally smaller than 1 mL; cultures can therefore be sampled repetitively during time-course studies with minimum volume disruption. A major advantage of using the Lowry assay is that the entire procedure can be performed in a single tube, thereby minimizing dilution errors and glassware usage. For these reasons, the Lowry assay has a lower minimum detection limit, in practice, for the application described here. A disadvantage is that the color of retort water, in volumes as small as one microliter, can give positive interference; this necessitates thorough rinsing of all extracellular fluid entrained by the filter after cell harvest.

Cell harvest can be achieved with many different filters that have nominal pore diameters of 0.2 to 0.45 μm. Glass-fiber filters and cellulose ester and polycarbonate membranes release silicates, particulates, and phenols during digestion that interfere with the Lowry assay; polycarbonate membranes can be used, however, for the dye-binding assay. Nylon membranes cannot be used for the Lowry assay because of irreversible sorption of the reduced phosphomolybdate complex from solution. The chemical inertness of Teflon membranes has allowed their use in the protocol described here. Their hydrophobicity, however, necessitates their wetting by methanol to allow passage of aqueous sample; they must also be physically restrained under the surface of the digestion solution to ensure contact of the retained cells with the alkali. Inert hydrophilic membranes (chemically modified polyvinylidene fluoride) that have recently been marketed by Millipore Corp. (Durapore) may not have these disadvantages and should be evaluated. For a thorough discussion of membrane filtration and chemistry, see Brock (1983).

The protein assay described here was developed specifically for the facile and accurate quantitation of microbial biomass in oil shale wastewaters. This method is an adaptation of the modified Lowry method of protein measurement as described by Herbert, Phipps, and Strange (1971) using the Folin phenol reagent. It is important to emphasize that the sensitivity of response will vary with

bacterial species because different proteins give widely different absorbances. The method must therefore be standardized against another measure such as dry mass or organic nitrogen for each type of microbial assemblage.

PROTOCOL SUMMARY

Determination of Biomass as Protein

See the detailed protocol for whole-cell protein quantitation. A Teflon membrane filter (25-mm diameter; 0.45-μm pore diameter) is placed in a glass filtration unit and wetted with 1 mL of methanol. Immediately upon vacuum filtration of the methanol, an appropriate, accurately measured volume of dispersed culture is applied to the filter. The retained cells are rinsed with phosphate buffer followed by methanol (to remove entrained wastewater and tarry materials, respectively). The membrane is pushed to the bottom of a screw-capped Pyrex culture-tube by a glass rod that rests on the retentate side of the membrane. The glass rod is left inside the tube to hold the membrane under 0.5 mL of aqueous 1N NaOH. The tubes are sealed with Teflon-lined screw caps and placed in a fluidized sand bath at 100°C for 10 minutes. A sand bath has the advantage over a water bath in that the tops of the tubes remain cool. This encourages refluxing and prevents the possible leakage of steam through the cap seals which would lead to irreproducible losses in the volumes of the digestates. To each of the cooled digestates, 2.5 mL of a solution prepared by addition of 25 parts of 5% sodium carbonate to one part of a mixture of 0.25% $CuSO_4 \cdot 5H_2O$ and 0.5% sodium tartrate is added. After mixing, followed by incubation for 10 minutes at ambient temperature, 0.5 mL of 1.0N Folin-Ciocalteu phenol reagent is added, and the solutions are immediately mixed; immediate and thorough mixing is essential because although reduction of the phosphotungstic-phosphomolybdic acid by tyrosine occurs only in sufficiently alkaline solutions, the Folin reagent is also unstable at high pH (Folin and Ciocalteu 1927). After 30 minutes of incubation at ambient temperature, the absorbance (750 nm) of the samples is read against a reagent blank. Protein values are determined by interpolation from a standard curve prepared from reference standards (e.g., bovine serum albumin) that are developed in parallel with the digested samples.

Validation of Method

A typical standard curve (Fig 1.) was constructed from a dilution series of bovine serum albumin; the coefficient of determination (r^2) was 0.999 for the range from 30 to 150 μg of protein per assay. This corresponds to a limit of quantitation of about 15 μg per assay. The standards for this curve were evaluated on four different days. The applicability of this adapted form of the Lowry assay for quantitation of whole-cell protein in oil shale wastewater was evaluated by comparison of results with the relative percent concentration of cells in a solution that was made by resuspending bacteria in filtered Oxy-6 retort water (i.e., abiotic particulates had been eliminated prior to resuspension). Serial dilutions were made of this culture, and protein concentrations were determined and regressed against the relative bacterial concentration (percent) (Fig. 2), volatile suspended solids (VSS) (Fig. 3), dry mass (Fig. 4), turbidity (absorbance at 660 nm) (Fig. 5), and nephelometric turbidity (Fig. 6). Turbidity measurements at A_{660} were zeroed against homologous cell-free filtrates. Nephelometric turbidity was obtained from an HF Instruments DRT-100 turbidimeter. Dry-mass determinations were performed using 0.2-μm pore diameter polycarbonate membranes, which were dried at 105°C for

0.5 h or until the weight was constant. The VSS of each sample was determined by drying the residue of glass-fiber (Whatman GFC) filtration at 103°C for 1.0 h followed by igniting the sample at 550°C for 15 minutes; the difference in filter mass was defined as VSS.

The regressions of protein versus relative bacterial concentration, dry mass, VSS, turbidity as absorbance, and nephelometric turbidity, had r^2 values of 0.996, 0.979, 0.978, 0.995, and 0.984, respectively. The excellent correlation of protein with relative bacterial concentration demonstrated that protein accurately reflected biomass concentration. Since protein concentration was shown to be linearly correlated with bacterial concentration, deviations from linearity for the other regressions must have resulted from nonlinearity of the alternate method. Indeed, dry mass, VSS, and nephelometric turbidity appeared to be the least accurate, although they too exhibited excellent linearity. These deviations probably resulted from volatile abiotic particulates, inadequacies of gravimetric analysis, and secondary light scattering. Turbidity as absorbance yielded excellent results, but its use as a routine tool is limited because each sample must be zeroed against its own filtrate necessitating excessive sample consumption and preparation. The slope of the regression line for protein versus dry cell mass (Fig. 4) indicated that the cells used in this study were 53% protein on a dry weight basis; this agrees extremely well with published values for protein content (Stickland 1951), although variations should be expected because of compositional differences in species and because of intercomparison of results from different methods normalized to different protein reference standards. This method has been used routinely for following the exponential growth of batch cultures of acclimated bacteria in retort waters. Representative results are presented by Jones et al. (1982), where protein concentration is correlated with substrate removal and with phosphate concentration.

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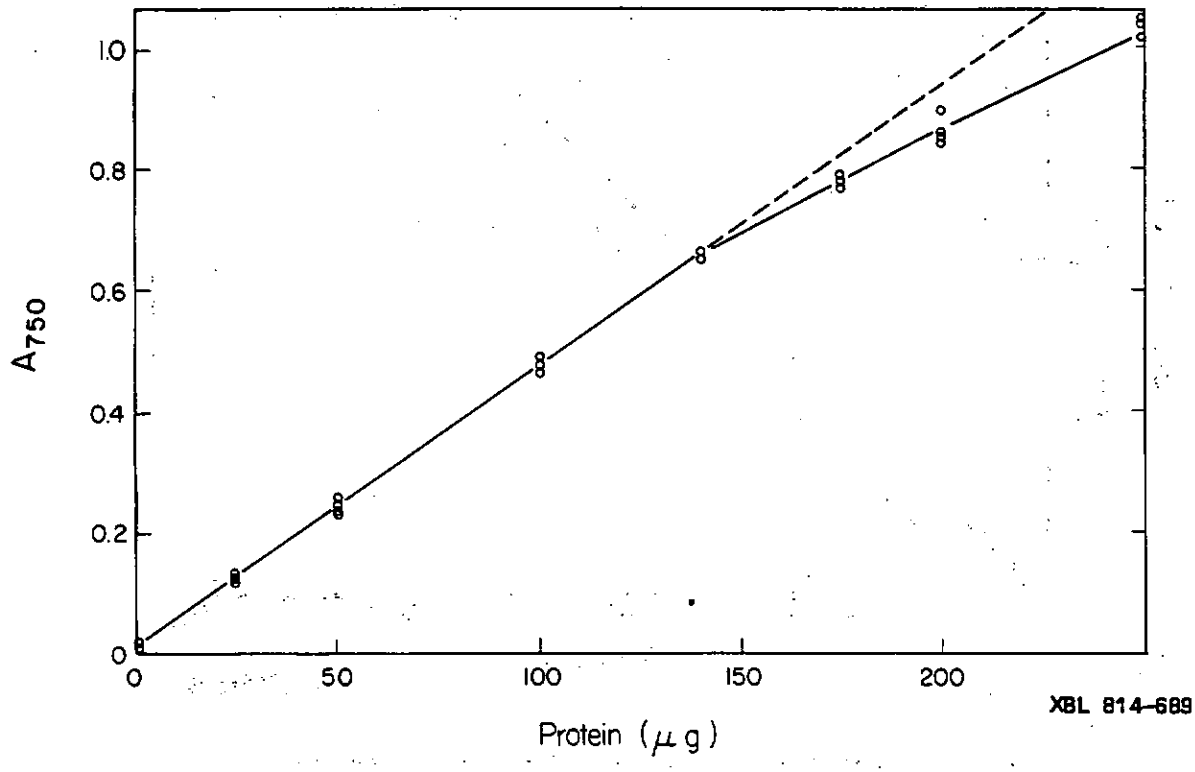

Figure 1. Lowry assay standard curve for bovine serum albumin

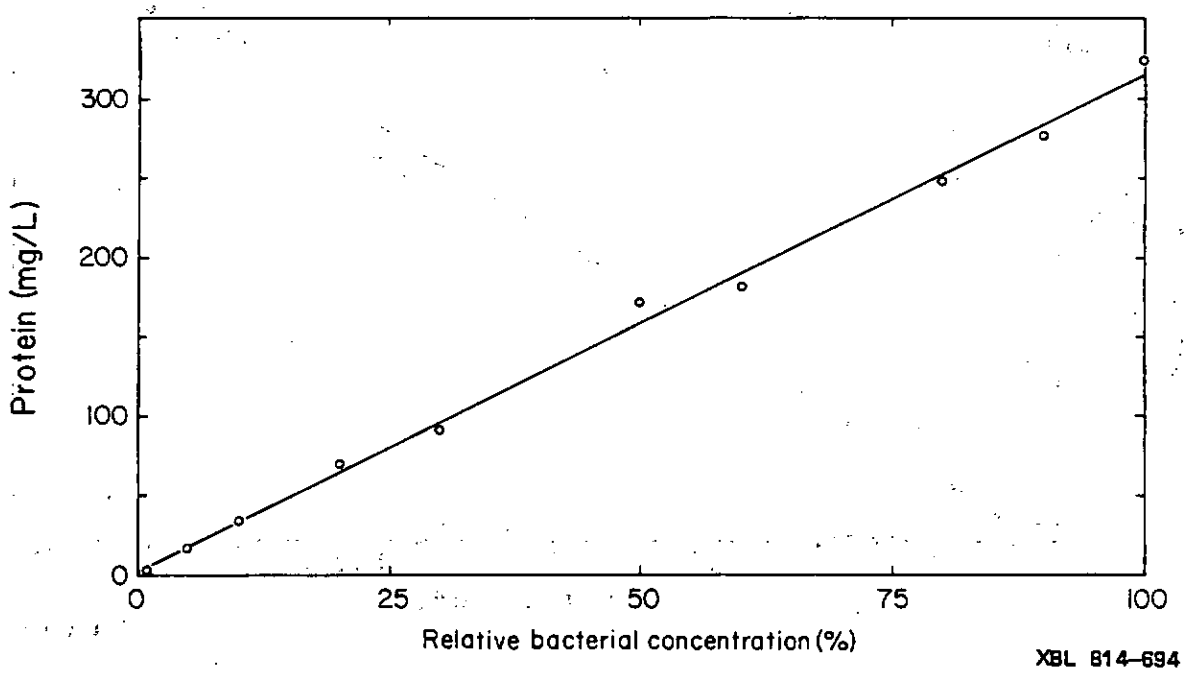

Figure 2. Correlation of a dilution series of bacteria in Oxy-6 retort water with whole-cell protein

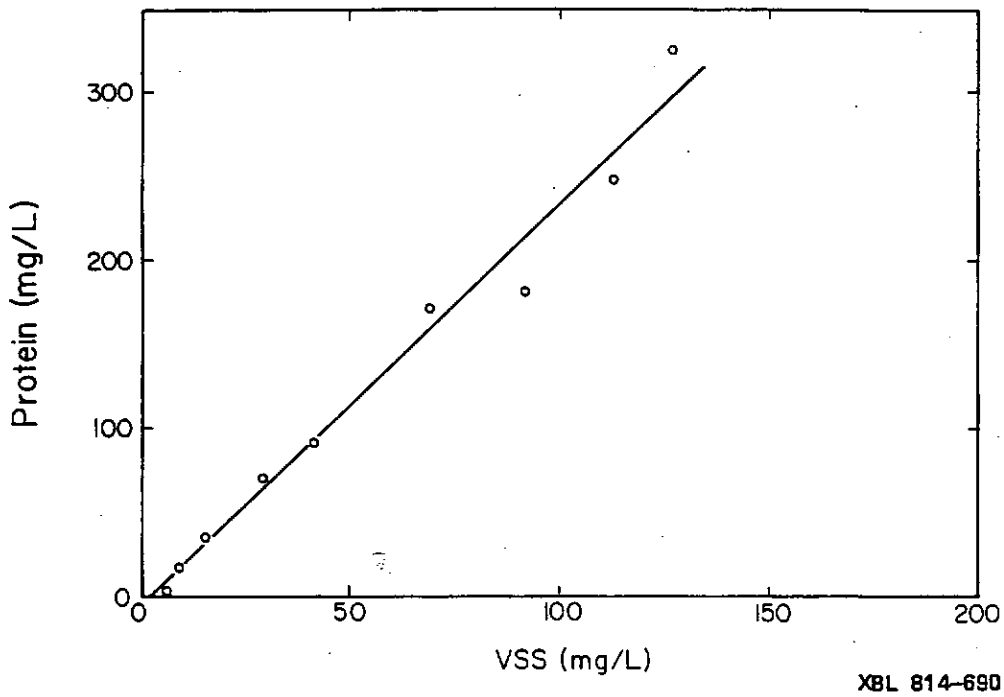

Figure 3: Correlation of volatile suspended solids in Oxy-6 retort water with whole-cell protein

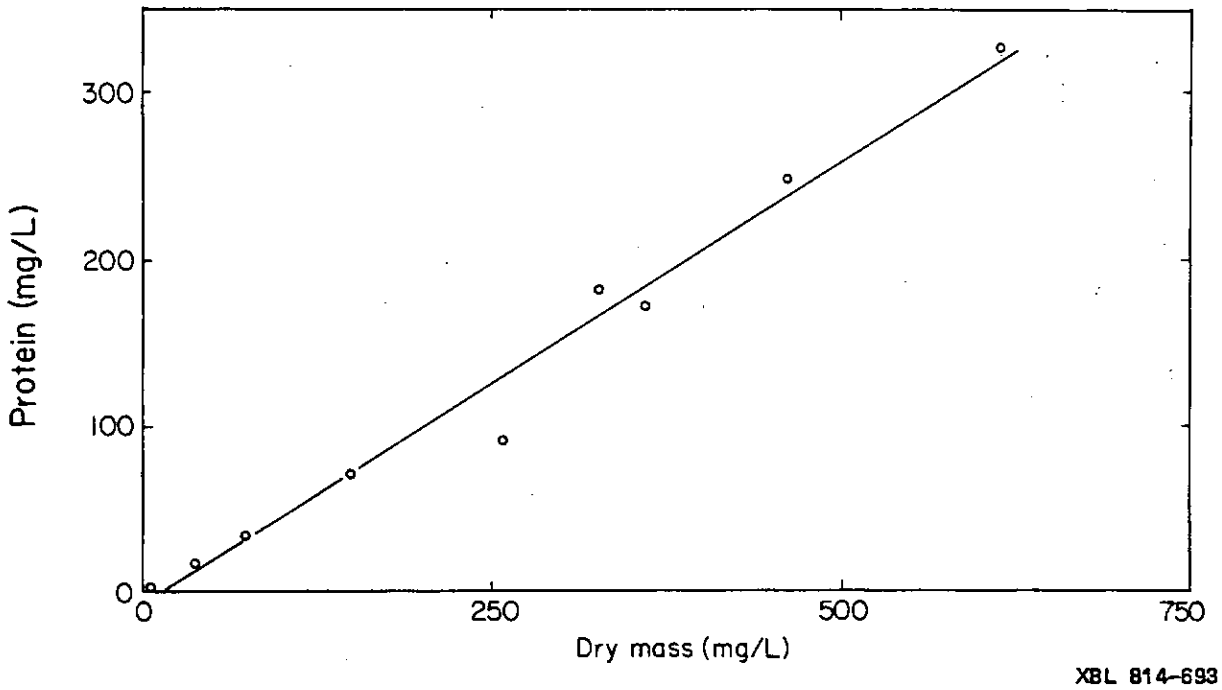

Figure 4. Correlation of dry cell mass in Oxy-6 retort water with whole-cell protein

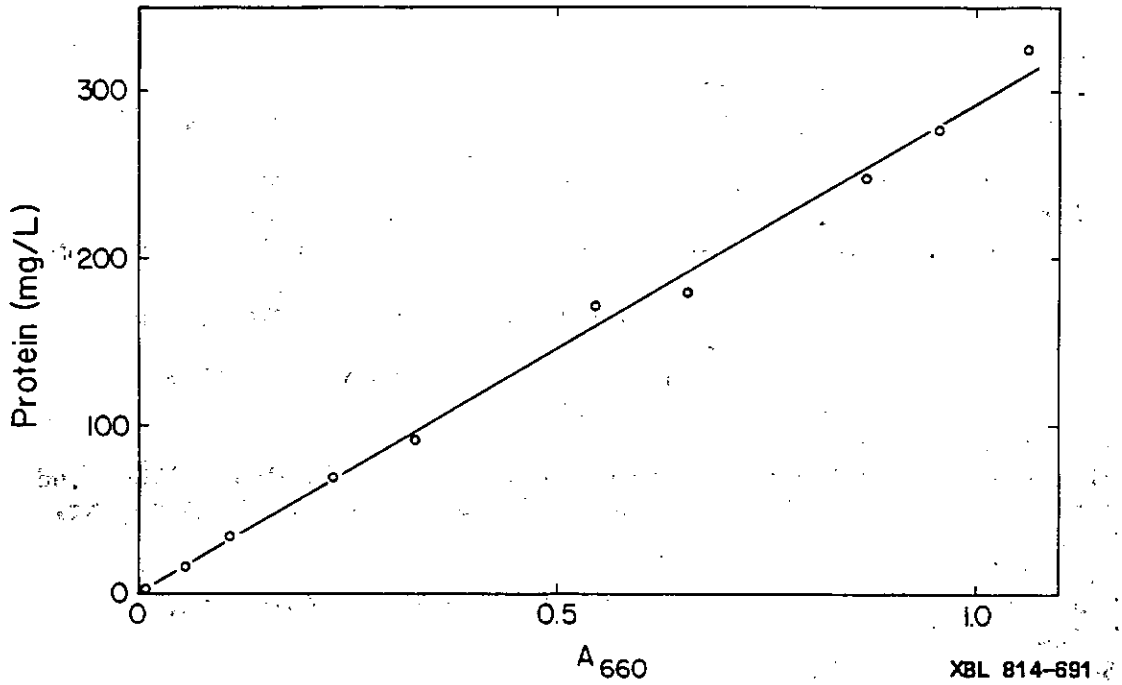

Figure 5. Correlation of turbidity (measured as absorbance at 660 nm) in Oxy-6 retort water with whole-cell protein

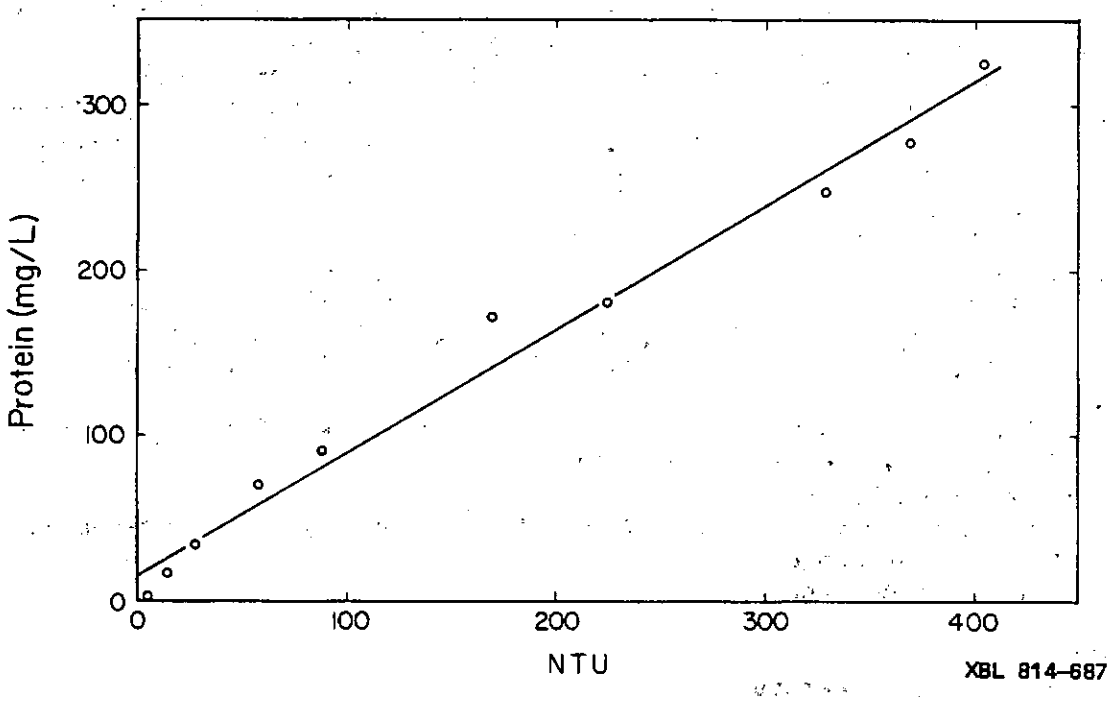

Figure 6. Correlation of turbidity (measured as nephelometric turbidity units) in Oxy-6 retort water with whole-cell protein

PROTOCOL: PROTEIN ASSAY

I. Apparatus
   A. Glassware (one each per sample)
      1. Culture tubes: Pyrex (16-mm O.D. X 150 mm) with Teflon-lined screw caps
      2. Glass rods: 3-mm O.D. X 145 mm
   B. Filtration apparatus
      1. Vacuum filtration flask connected to water aspirator or vacuum pump.
      2. Microanalysis filtration unit (e.g., Millipore, catalog # XX1002500 or XX1002530).
      3. Filters: Teflon, 25.0-mm diam., 0.45-μm diam. pore size (e.g., Sartorius, catalog #11806)
      4. Forceps (2 pairs).
   C. Pipettes (calibrated): air displacement (e.g., Gilson, P-1000) and positive displacement (e.g., S.M.I. Micro/Pettors, digital adjust, 20-100 μL)
   D. Vortex mixer
   E. Heating unit: Tecam fluidized sandbath SBL-1 (Techne, Inc., Princeton, NJ)
   F. Sonicator bath (125 watts)
   G. Turbidimeter: DRT-100 (HF Instruments Ltd., Bolton, Ontario, Canada)
   H. Spectrophotometer: (e.g., Bausch & Lomb Spectronic 88 with micro flow-through cell, 1-cm path length; ensure that the EPR O-rings are used)

II. Reagents

NOTE: All reagents are made from Analytical Reagent Grade chemicals; when used as a reagent, "water" refers to ASTM Type I quality.

   A. Stock Protein Standard: bovine serum albumin (BSA) stock solution (2.800 g/L). Slowly dissolve (to prevent foaming) 0.2800 g of BSA in a 100.00-mL volumetric flask with 70 mL of water and bring to volume. Freeze 5-mL portions of stock in small, polyethylene reagent bottles for later use.
   B. Lowry Stock Reagents:
      1. 5.0% Na_2CO_3 in water (w/v). Make up 500 mL for stock.
      2. 1.0% $NaKC_4H_4O_6 \cdot 4H_2O$ in water (w/v). Make up 100 mL for stock.
      3. 0.5% $CuSO_4 \cdot 5H_2O$ in water (w/v). Make up 100 mL for stock.
      4. Folin-Ciocalteu phenol reagent, 2N. (e.g., Sigma Chemical Co.)
   C. Digestion Reagent:
      1. 1.0N aqueous NaOH (make up 100.00 mL for stock solution and store in polyethylene reagent bottle).
   D. Filter-Rinsing Reagents:
      1. Phosphate buffer: KH_2PO_4-K_2HPO_4 buffer (pH 7.4; ionic strength = 0.1; buffer capacity = 3.1)
      2. Anhydrous methanol

III. Protocol
   A. Preparation of apparatus
      1. Turn on sandbath. Adjust temperature setting and air flow rate to stabilize temperature at 100°C.
      2. Set up filtration unit.
      3. Turn on spectrophotometer.

4. Use only culture tubes without chipped rims (to ensure a gas-tight seal). Wash tubes and glass rods in a 35% nitric acid bath, and rinse with water. Dry thoroughly. Using forceps, put one glass rod in each test tube. Cap tubes loosely.

B. Preparation of assay solutions
1. Lowry assay reagent solutions (prepare fresh on day of assay).
   a. **Solution A:** (Volume required depends on the number of samples). To twenty-five parts of 5.0% Na_2CO_3, add one part of cupric-tartrate reagent (v/v). Cupric-tartrate reagent is made by combining equal volumes of 0.5% $CuSO_4 \cdot 5H_2O$ and 1.0% $NaKC_4H_4O_6 \cdot 4H_2O$.
   b. **Solution B:** (Volume required depends on the number of samples). Dilute the 2N Folin-Ciocalteu phenol reagent to 1.0N with water.
2. Protein standards
   a. thaw (to ambient temperature) one bottle of BSA stock solution.
   b. dilute BSA stock solution (2.800 g/L) so that 0.50 mL of a diluted standard contains the following quantity of protein:
      0.140 g: 1.000 mL of stock and dilute to 10.00 mL
      0.125 g: 0.890 mL of stock and dilute to 10.00 mL
      0.100 g: 0.715 mL of stock and dilute to 10.00 mL
      0.070 g: dilute 0.140-g standard with equal vol. water
      0.050 g: dilute 0.100-g standard with equal vol. water
      0.025 g: dilute 0.050-g standard with equal vol. water

NOTE: Use positive displacement pipettes whenever possible.

C. Preparation of standard curve
1. Pipette (positive displacement) 0.500 mL of each BSA standard into a screw-capped culture tube (glass rods not necessary.)
2. Prepare reagent blank by pipetting 0.500 mL of water into a tube.
3. To each tube, add 0.500 mL of 1.0N NaOH.
4. Assay with digested samples. (Do not heat-treat these standards.)

D. Preparation of samples
1. Disperse the culture by sonic treatment for 1 minute.
2. Take turbidity reading. Determine (from NTU) an appropriate amount of culture to filter so that the amount of protein in each sample will yield an absorbance at 750 nm that will fall mid-range on the standard curve (e.g., filter 0.50 to 0.75 mL for a culture of 300 to 600 NTU).
3. Filter samples. Each culture should be assayed in duplicate.
   a. place the Teflon membrane on the filtration apparatus.
   b. turn on the vacuum.
   c. using positive displacement pipette, sample the predetermined volume of culture.
   d. prewet the Teflon filter with 1 mL of methanol.
   e. dispense the sample immediately after the methanol has been drawn through the membrane. If possible, avoid contact of the sample with the filter-holder funnel. Record the applied sample volume.
   f. wash membrane retentate with 1 mL of phosphate buffer.
   g. following the phosphate buffer wash, rinse the membrane with 1 mL of methanol (to dissolve tarry materials).
   h. place the filter on top of the test tube (retentate side facing up) and push the filter to the bottom of the tube using

the glass rod. This can be accomplished by holding the rod with a pair of forceps in one hand while removing the filter from the filter holder with forceps using the other hand. Be sure that the glass rod is on top of the membrane and that the membrane is not folded.

NOTE: Do not touch the membrane with your skin.

i. if necessary, samples now may be stored in sealed tubes at -20°C for assay at a later date.

E. Sample digestion
1. Add 0.500 mL of 1.0N NaOH to each sample tube.
2. Seal tubes tightly with Teflon-lined screw caps.
3. Place sample tubes in sandbath. Heat at 100°C for 10 minutes.
4. Remove sample tubes and cool in waterbath.
5. Add 0.500 mL of water to digested sample.

F. Color development
1. Add 2.50 mL of Solution A to each tube containing sample or standard.
2. Vortex.
3. Incubate at ambient temperature for 10 minutes.
4. Add 0.500 mL of Solution B and vortex **IMMEDIATELY**.
5. Incubate the mixture at ambient temperature for 30 minutes (color is stable for at least one hour).
6. Read the absorbance of the standards and samples at 750 nm against the reagent blank.

IV. Data Reduction

A. Determine the linear regression equation by "least squares" analysis for the protein standard curve. Calculate the coefficient of determination (r^2).

B. Using the regression equation for the standard curve, interpolate the mass of protein collected on each membrane, e.g.,
mass of protein = $(m)(A_{750}) + b$

C. Calculate the concentration of protein (μg/mL) for each culture, e.g.,
concentration of protein (μg/mL) =
mass (μg) of collected protein/filtered sample volume (mL).

Protocol prepared by: K. Yu, B.M. Jones, R.H. Sakaji, and C.G. Daughton

Appendix I. Origins of Oil Shale Process Wastewaters Used in Study

process water	water type	retort/process	shale source	retorting atmosphere	maximum retorting temp. (°C)	operator/ collection date
		Field In-Situ Retorts				
Oxy-6 RW	RW[a]	retort #6/MIS[b]	Logan Wash, CO	air/steam	unknown[c]	Occidental Oil Shale Inc., 1979
Oxy-6 GC	GC[d]	retort #6/MIS	Logan Wash, CO	air/steam	unknown	Occidental Oil Shale Inc., 1979
Geokinetics	RW	retort #9/TIS[e]	Book Cliff, UT	air	unknown	Geokinetics Inc., 1978
Omega-9	RW	site #9/TIS	Rock Springs, WY	air	unknown	LETC, 1976
Rio Blanco sour	RW	retort #0/MIS	Tract C-a, CO	air/steam	unknown	Rio Blanco Oil Shale Co., 1980
		Field Surface Retorts				
Paraho	RW	Paraho direct mode	Anvil Points, CO	air/re-cycle gas	750	Development Engineering, Inc., 1977-1978
TOSCO	RW	TOSCO HSP[f]	Colony Mine, CO	inert gas	500	TOSCO, March 1981
		Simulated In-Situ Retorts				
150-Ton	GC & RW	LETC 150-ton, run 13	Anvil Points, CO	air	800	LETC, 1976
S-55	GC & RW	LETC 10-ton, run 55	Anvil Points, CO	air/steam	650	LETC, 1978

[a] Retort water. [b] Modified in-situ. [c] Retorting temperatures for MIS field retorts are not accurately known; temperatures may reach 1000°C. [d] Gas condensate. [e] Horizontal true in-situ. [f] Hydrocarbon solids process.

APPENDIX II. Summary of Data Obtained for Oil Shale Wastewaters (Chapters I & III-VI)[1]

wastewater	TOC high-temp	TOC UV-persulfate	DOC (direct) high-temp	DOC (direct) UV-persulfate	POC (HpF-DOC)	(POC/DOC) X 100	DIC
Paraho	43 415 (0.55)	42 680 (0.66)	41 809 (1.4)	42 066 (1.1)	35 358	84	210 (1.3)
150-Ton (R-13)	4 857 (0.44)	5 060 (0.53)	3 147 (0.58)	3 259 (0.46)	1 153	35	1 932 (1.8)
Oxy-6 retort water	3 817 (1.2)	3 952 (0.54)	2 829 (0.80)	2 942 (0.40)	1 349	46	985 (1.0)
Geokinetics-9	3 674 (1.3)	3 682 (0.45)	1 627 (1.1)	1 656 (0.55)	529	32	1 994 (0.67)
TOSCO HSP	3 370 (0.59)	3 486 (0.97)	2 651 (0.23)	2 726 (0.61)	1 106	41	825 (0.54)
Oxy-6 gas condensate	2 735 (0.63)	2 866 (0.47)	652 (2.6)	641 (0.51)	110	17	2 213 (0.38)
S-55	2 595 (1.8)	2 633 (0.51)	2 213 (0.40)	2 285 (0.34)	926	41	340 (1.6)
Omega-9	2 119 (2.1)	2 174 (0.29)	695 (0.34)	718 (0.44)	300	42	1 387 (1.3)
Rio Blanco sour water	556 (0.25)	548 (0.47)	206 (1.4)	207 (1.3)	57	28	364 (1.4)

	Total N		Organic N			Inorganic N		
	TN[2]	TKN	NPON[2] (LpF-TN)	NVON[2] (NOGD-TN)	ONN	NH_3 titrimetric	NH_3 colorimetric	PN[2] (HpF-TN)
Paraho	28 805 (3.5)	29 661 (1.9)	645 (5.7)	4 839 (1.1)	4 299 (1.8)	26 385 (1.3)	24 689 (1.0)	30 101 (0.7)
150-Ton (R-13)	10 084 (1.3)	10 453 (1.2)	295 (2.7)	587 (3.7)	541 (3.4)	10 838 (1.3)	10 662 (0.7)	11 047 (1.8)
Oxy-6 retort water	1 313 (1.8)	1 349 (0.9)	159 (1.7)	218 (0.5)	183 (3.0)	1 161 (0.5)	1 127 (0.9)	1 197 (0.7)
Geokinetics-9	1 844 (2.1)	1 826 (0.5)	133 (3.2)	242 (4.1)	194 (3.4)	1 991 (1.0)	1 905 (0.7)	1 880 (1.4)
TOSCO HSP	2 826 (2.6)	2 809 (1.7)	236 (1.9)	470 (1.9)	305 (1.2)	2 401 (0.1)	2 292 (1.2)	2 671 (3.8)
Oxy-6 gas condensate	6 886 (3.5)	6 985 (1.9)	79 (1.2)	30 (17.9)	21 (1.4)	7 157 (0.1)	7 202 (0.7)	7 201 (1.9)
S-55	4 196 (2.1)	4 379 (2.3)	172 (0.5)	414 (1.5)	372 (0.9)	4 079 (0.08)	4 047 (1.4)	4 561 (0.6)
Omega-9	3 574 (1.9)	3 698 (1.3)	81 (2.0)	100 (2.9)	112 (0.9)	3 690 (0.4)	3 583 (1.1)	3 959 (1.9)
Rio Blanco sour water	1 133 (3.4)	1 074 (1.5)	21 (2.9)	17 (2.3)	14 (4.8)	1 118 (0.3)	1 065 (0.9)	1 136 (1.6)

	COD			Oil and Grease		
	titrimetric	colorimetric	specific COD	unfiltered	filtered	pH
Paraho	151 600 (0.79)	141 379 (4.5)	3.60	nd[3]	nd	8.4-9.0
150-Ton (R-13)	11 048 (1.1)	11 662 (2.4)	3.39	641 (58)	448 (37)	8.8-9.1
Oxy-6 retort water	8 967 (0.52)	8 990 (0.85)	3.05	242 (40)	219 (39)	8.7-8.8
Geokinetics-9	7 191 (0.81)	7 578 (1.5)	4.34	160 (24)	162 (8)	8.6-8.8
TOSCO HSP	9 193 (0.47)	9 459 (2.3)	3.37	276 (20)	175 (18)	9.2-9.4
Oxy-6 gas condensate	2 308 (1.0)	2 074 (6.4)	3.60	86 (48)	71 (35)	8.7-8.8
S-55	9 414 (0.35)	9 781 (2.4)	4.12	334 (78)	178 (20)	8.1-8.4
Omega-9	3 596 (1.2)	3 729 (1.2)	5.01	58 (33)	56 (44)	8.2-8.5
Rio Blanco sour water	912 (0.33)	925 (0.74)	4.41	nd	nd	8.6-9.0

[1] all boldface values are means (mg/L); values in parentheses are rsd percentages except for oil and grease, where they are ranges. See ACRONYMS for definitions of column headings. Ranges for pH were obtained in a separate study; [2] determined by C/CL; [3] not determined.

A MANUAL OF ANALYTICAL METHODS FOR WASTEWATERS

(Oil Shale Retort Waters)

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