
QUANTITATION OF ACRYLAMIDE (and Polyacrylamide):
Critical review of methods for trace determination/formulation analysis
&
Future-research recommendations

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SUMMARY

During the last three decades, polyacrylamides (especially those converted to polyelectrolytes) have gained wide usage in water treatment (as flocculants/coagulants), tertiary oil recovery, and various other applications such as sewer grouts. Unreacted, residual acrylamide monomer [2-propenamide: $\text{CH}_2=\text{CH}-\text{C}(=\text{O})-\text{NH}_2$] is usually present in the various bulk commercial formulations (and is an additive in certain commodities such as polymeric grouts) at low fractional percentages. Although the polymers are relatively nontoxic, acrylamide can elicit severe neurotoxicity and genotoxicity. For health concerns, use of polyacrylamides in drinking water has been subjected to closer evaluation during the last decade. Currently, dosage standards are indirectly based on the maximum concentration of acrylamide that would result from use of a commercial formulation of known acrylamide content (0.05% acrylamide in the formulated polymer is usually specified as a maximum). Although numerous methods of chemical analysis exist for determining the acrylamide content of a polyacrylamide formulation (mainly polarographic techniques), no standardized method has been adopted for directly determining "trace" concentrations of acrylamide in water (e.g., at the sub-parts-per-billion level: nanograms to micrograms per liter, ng- $\mu\text{g/L}$).

This report represents the first in-depth literature review of methods for determining acrylamide; over 100 references have been reviewed, and those that deal specifically with acrylamide determination have been annotated. The approach was to unify the general chemistry of acrylamide (and amides) with the published methods for quantitation. The published literature has spanned many indirectly related fields (e.g., pharmacology, water research, agricultural chemistry, analytical chemistry, organic chemistry); many of the ongoing lines of research have seemingly been unaware of the existence or relevance of others. This fragmentation is somewhat responsible for the lack of a standard method and for various misconceptions of acrylamide chemistry. Although recommendation of a trace-analysis method for acrylamide in waters was a major objective of this review, areas of future research that could lead to new methods were also considered.

The findings can be easily summarized. Initial isolation/concentration of acrylamide from water has been the most difficult task in analysis. Nearly all researchers have relied on aqueous-phase ionic bromination of the acrylyl double bond (at low pH) to form the 2,3-dibromopropionamide derivative, which has a much higher partition coefficient into organic solvents; unfortunately, the chemistry of this reaction and the chemical reactivity of the derivative are poorly understood. Not surprisingly, for subsequent trace determination, two major separation methods have been evaluated -- gas chromatography (GC) and high-performance liquid chromatography (HPLC). For HPLC, the derivative is amenable only to non-selective UV detection (at about 200 nm) under reverse-phase separation conditions; direct determination of acrylamide itself has also been attempted. These approaches, however, have only achieved low-ppb ($\mu\text{g/L}$) detection limits because it has not been possible to sufficiently preconcentrate the dibromo derivative or acrylamide itself. For GC, 2,3-dibromopropionamide gives excellent detectability with electron-capture detection (ECD); various problems are attendant, however, with the thermal/chemical lability of the derivative (e.g., the dibromo derivative will form quaternary-amine adducts, leading to loss of the β -bromine). Various other methods have been used over the years, including polarography, derivatization/spectrophotometry, and titrimetry. Since none of these methods or HPLC can approach the detection limit of GC-ECD (sub- $\mu\text{g/L}$), the latter is the current method of choice for trace analysis; further improvements are inevitable (e.g., use of bonded-phase capillary columns and standardization of the bromination technique). Polarography or HPLC seem to be the methods of choice for formulation analysis.

With respect to derivatization chemistry, little has been developed specifically for amides. Further research into amide derivatization is strongly recommended. Formation of various fluorinated derivatives would lower the detection limit for GC-ECD (e.g., perfluorobenzoyl imide derivatives), while creation of various chromophores/fluorophores or conversion to an electrochemically active derivative (e.g., amine) would greatly increase the utility of HPLC. The use of newer-generation, silanol-deactivated reverse-phase columns could also improve the performance of any HPLC method. ■

is moderate (LD_{50} values range from 150 to 180 mg/kg for rats, guinea pigs, and rabbits). Because of the lack of a validated, routine method for determining "trace" levels of acrylamide in water, health standards are usually set by limiting the dosage of polymer based on the maximum concentration of acrylamide monomer in the raw polymer as determined by various methods of formulation analysis. The "final" acrylamide concentration in the treated water is thereby deduced, as opposed to actually being determined via direct trace analysis; indeed, acrylamide resulting from unregulated sources, such as grouts, would make the concentrations higher than believed. Currently, the U.S. EPA has only proposed a Recommended Maximum Contaminant Level (RMCL) for acrylamide in potable waters of zero (because of its genotoxicity) (Federal Register 1985); an RMCL, however, is a non-enforceable health goal, not a regulation.

Acrylamide has two centers of potential reactivity: (1) the amide group that undergoes reactions characteristic of an aliphatic amide, and (2) a double bond that is electron deficient because of its conjugation (α,β -position) with the amide group. The amide group is relatively inert for a "functional group" since the amino group (an excellent electron donor) contributes its electrons to the carbonyl carbon (normally an electron-deficient group); neither moiety therefore displays its "normal" range of reactivity.

Acrylamide has been demonstrated as biodegradable when supplied as a sole source of nitrogen to *Nocardia rhodochrous* (DiGeronimo and Antoine 1976); evidence exists that it is not very persistent in native waters (Brown, Rhead, Bancroft, and Allen 1980; Brown, Rhead, Hill, and Bancroft 1982; Croll, Arkell, and Hodge 1974). In waters having low concentrations of microorganisms and therefore low biodegradative potential (e.g., potable waters), however, acrylamide has not surprisingly been shown to be persistent (Brown, Rhead, Bancroft, and Allen 1980; Brown, Rhead, Hill, and Bancroft 1982); presumably this would mean that acrylamide would be fairly persistent in drinking water. In soils, acrylamide has a half-life of less than two days (25 ppm at 22°C) (Lande, Bosch, and Howard 1979). Other aspects of environmental fate are presented by Davis et al. (1976). Acrylamide is also not amenable to sorption by ion-exchange or hydrophobic bonding; even activated carbon shows a limited sorptive capacity (Brown, Bancroft, and Rhead 1980). This property has played a role in development of certain clean-up methods for water analysis (e.g., Brown, Rhead, and Bancroft 1982).

Polyacrylamide $[-CH(C(=O)-NH_2)-CH_2-]_n$

Polyacrylamide (Molyneux 1983): various other names are also used, including poly(acrylamide), polyacrylic amide, poly(1-carbamoylethylene) (IUPAC); acronyms include PAm, PAAm, and PAM; trade names include Cyanamer (American Cyanamid), Hercosfloc (Hercules Chemical), Percol (Allied Colloids), Purifloc (Dow Chemical), and Separan (Dow Chemical).

Polyacrylamide is unusual in having an extremely high molecular weight (e.g., 3 to 15 million number-average MW) coupled with being very hydrophilic while also being nonionic. Its solubility in nonaqueous solvents is restricted to those that are very polar (e.g., glycerol, formamide, and ethylene glycol). It is insoluble in most other organic solvents (e.g., diethyl ether and aromatic hydrocarbons), including those that are miscible with water (e.g., methanol, ethanol, acetone); this property forms the basis of many schemes of formulation analysis (i.e., via extracting unreacted acrylamide monomer from the polymer).

Since the main industrial application of PAM is for flocculation of aqueous particle suspensions, its nonionic character is often modified by chemical conversion to cationic and anionic forms. The latter are formed by hydrolysis of the amide group to a carboxylic group (as an alternative to production of the anionic polymer by hydrolysis of the homopolymer, acrylamide/acrylic acid copolymer can be synthesized directly); the degree of hydrolysis varies immensely among polymers (ranging up to 50%), even within the same manufacturer's lot (Scoggins and Miller 1979). Carboxylic groups can be converted back to amide groups by treatment with thionyl chloride [$S(=O)Cl_2$] and ammonia (Griot and Kitchener 1965). PAM used for gel chromatography and electrophoresis is actually a crosslinked form prepared using methylene-bis-acrylamide as the cross-linking agent. Acrylamide polymerization does not always lead to the primary amide,

INTRODUCTION

This review focuses not only on published methods of water analysis suitable for determination of trace acrylamide concentrations, but also on approaches that either have not been evaluated for trace analysis (but have been used for formulation analysis) or that have never received consideration. The chemistry of amides (in particular that of acrylamide) will first be discussed, with emphasis on those aspects that have potential utility in chemical analysis. This will be followed by a chronological annotated bibliography of published methods for the determination of acrylamide, polyacrylamide, and amides in general. Finally, recommendations for potential future research will be presented. This review does not focus on polyacrylamide (PAM) polyelectrolytes, although methods for their determination were included in this review. Various aspects of the use of polyelectrolytes in water and wastewater treatment and in enhanced oil recovery have been thoroughly reviewed (e.g., AWWA 1983; Davis et al. 1976; Glass 1986; Life Systems, Inc. 1985).

Acrylamide C_3H_5NO $CH_2=CH-C(=O)-NH_2$

Acrylamide or 2-propenamamide (IUPAC); various other names are also used, including propenamamide, acrylic acid amide, acrylic amide, acrylamide monomer, ethylenecarboxamide, akrylamid (Czech.), or rarely acrylamid or 2-propeneamide; acronyms: AA, AAm; CAS Registry No. 79-06-1; NIOSH # AS 3325000.

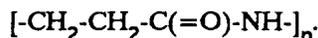
An odorless, white crystalline solid; MW 71.08; density at 30°C 1.122 g/mL; vapor pressure at 25°C 0.005 mm Hg (Kanno 1985); melting point $84.5 \pm 0.3^\circ C$; boiling point 125-136°C at 25 mm Hg; infrared and ultraviolet absorption spectra are given in American Cyanamid Co. (1969) and Bikales (1970); available from various suppliers such as Pierce Chemical in electrophoresis grade (Sequanal) with a mp=84-85°C and acrylic acid content of less than 0.001%. In pure form, acrylamide readily polymerizes at its melting point, 84.5°C (Carpenter and Davis 1957), or under UV irradiation, but otherwise is very stable for a vinyl monomer. Aqueous solutions can be stabilized (even at elevated temperature) with iron complexes of cyanogen or thiocyanogen (American Cyanamid Co. 1969, p.6).

Some representative solubilities (g/L, 30°C): water (2155-2215), methanol (1550), dimethylsulfoxide (1240), dimethylformamide (1190), ethanol (862), acetone (631), pyridine (619), acetonitrile (396), ethylene glycol monobutyl ether (310), dioxane (300), ethyl acetate (126), chloroform (26.6), 1,2-dichloroethane (15.0), benzene (3.46), carbontetrachloride (0.38), and n-heptane (0.068) (American Cyanamid Co. 1969; Carpenter and Davis 1957). From these solubilities alone, it is apparent that acrylamide would not have favorable partitioning coefficients from water into any water-immiscible organic solvent; indeed, acrylamide has an extremely low 1-octanol/water partition coefficient, about as negative as that of methanol (Fujisawa and Masuhara 1981). Such high water solubility, coupled with a very low vapor pressure, indicate that acrylamide could be removed from aqueous samples only via chemically or biochemically mediated molecular alteration; indeed, the "half-life" for acrylamide volatilization from water is about 500 years (Davis et al. 1976).

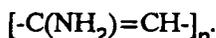
Acrylamide was first prepared and described by C. Moureau in 1893 (Carpenter and Davis 1957), who slowly added dry ammonia to saturate a benzene solution of acrylyl chloride at 10°C. After boiling and filtration to remove the ammonium chloride, acrylamide precipitated upon cooling; it had a melting point of 84-85°C after several recrystallizations from benzene. Acrylamide has been commercially available in the U.S. for a little over 30 years (Bikales 1970). A thorough review of commercial manufacturing data and commercial uses is presented by Davis et al. (1976).

Unreacted acrylamide monomer occurs as a contaminant at various concentrations in polyacrylamides (e.g., polyelectrolytes used in water treatment). The major health concerns for acrylamide have been neurotoxicity (first observed in laboratory animals over 30 years ago) (Davis et al. 1976; Life Systems, Inc. 1985) and, more recently, reproductive effects, genotoxicity, and carcinogenicity (Dearfield et al. 1988); acute toxicity

PAM; with Grignard reagents in the presence of a free-radical polymerization inhibitor or with alkali metal alkoxides, the secondary amide, poly(β -alanine) (also known as Nylon-3), is formed:



Conversion of PAM to ionic forms begins to occur in aqueous solution at neutral pH at about 67°C, where the amide groups are hydrolyzed to carboxylic groups. PAM also undergoes the Hofmann rearrangement, where on treatment with alkaline hypochlorite, the carbonyl group is eliminated, yielding vinylamine groups along the chain:



Other reactions that PAM shares with low-molecular-weight amides include partial methylation of the amide-N's with formaldehyde under alkaline conditions (see: Formation of N-methylol derivatives):



and further reaction with sulfite or bisulfite to give sulfomethylol groups ($-\text{NH}-\text{CH}_2-\text{SO}_3^-$); the methylol groups of the former reaction can, in turn, be reacted with an amine via the Mannich reaction to give secondary amines ($-\text{NH}-\text{CH}_2-\text{NHR}$).

Nonaqueous polyacrylamide (PAM) dispersions are used as flocculating agents (settling aids) in water treatment facilities (e.g., potable and industrial waters such as coal washery streams) and as mobility-control aids in secondary oil recovery. Acrylamide monomer itself is used in various grouts (e.g., Injectite-80[®] for sealing sewer leaks; this has been a documented source of acrylamide poisoning, e.g., see: Lande et al. 1979) and soil stabilizers, as well as in various adhesives and textiles. Only recently has acrylamide received attention as a possible toxicological problem because of its presence as a formulation residue in water-treatment polyacrylamide-based polyelectrolytes; its presence in these polymers (from trace to percentage levels) results from its use as the reactive monomer during their synthesis. Acrylamide residuals are not only environmentally unacceptable in discharge waters, but in industrial reuse operations they can be deleterious to down-stream processes (e.g., affecting the flotation of coal fines).

Aqueous polyacrylamides or emulsion homo- or co-polymers of acrylamide with comonomers such as ethylenic-unsaturated carboxylic acids or ethylenic-unsaturated quaternary amines are used as flocculants, filter aids, mobility control agents for wastewater treatment and oil-field water flooding. The chemistry of acrylamide polymerization has been reviewed by Davis et al. (1976).

Amide Nomenclature

Amides are characterized by the carbonyl-amine linkage (also called the carboxamido group): $\text{R}-\text{C}(=\text{O})-\text{NR}'\text{R}''$ or $-\text{C}(=\text{O})\text{N}<$. They can be regarded as acylated derivatives of ammonia (primary amides) or of amines (secondary and tertiary amides), or alternatively as esters of carboxylic acids with amines (for secondary and tertiary amides) or with ammonia (for primary amides), where the amine/amino group takes the place of the alkoxy (alcohol) group. The term "amide" is derived from *ammonia* and the suffix "ide", which is used in oxide, chloride, etc., as proposed by Berzelius who noted the similarity among NaNH_2 , Na_2O , and NaCl (Nickon and Silversmith 1987). The scientific literature is often confusing because some investigators loosely refer to "amides" as "amines"; this use should be discouraged.

"Unsubstituted" amide refers to amides having no substituents on the nitrogen (i.e., primary only, not *N*-substituted: $\text{R}-\text{C}(=\text{O})-\text{NH}_2$ vs. $\text{R}-\text{C}(=\text{O})-\text{NHR}$). Acrylamide, therefore, is an unsubstituted, primary amide. The acrylyl (or acryloyl) radical refers to 1-oxo-2-propenyl: $\text{CH}_2=\text{CH}-\text{C}(=\text{O})-$; when the amino group of the amide is attached, the C-N bond is referred to as the "acyl" bond of the amide. ■

OVERVIEW OF AMIDE CHEMISTRY

The following is simply an overview of some of the major general reactions in which amides are formed or in which they participate as reactants. The reactions for amide formation are simply for informational purposes, although some of them may have bearing on the *de novo* formation of acrylamide in situ by means other than introduction of polyacrylamide electrolytes. Likewise, the reactions in which they serve as reactants do not necessarily have any relevance to the analytical determination of acrylamide (although it is noted when these reactions do form the basis of reported methods of determination) because (1) some of these reactions occur only under special conditions (e.g., conditions requiring pressure reactors or in the strict and total absence of water or air); (2) others are not sufficiently specific (i.e., compounds with other functional groups undergo the same reactions, sometimes preferentially, necessitating some form of chemical-class separation prior to reaction); (3) multiple reaction paths may coexist resulting in a mixture of products (and therefore multiple analytes); and (4) some reactions require rather exotic (or highly toxic) reagents, unsuitable for a routine analytical method. Reactions relevant exclusively to N-substituted amides (i.e., only secondary and tertiary amides) will be ignored.

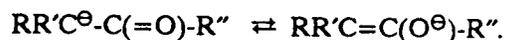
This review of reaction chemistry is required to ensure that the published literature has indeed addressed the problem of chemical derivatization from all obvious angles. Derivatization methods that have been developed specifically for amides will be covered in a later section (see: Derivatization Chemistry, under METHODS). Despite much having been published on amide chemistry (e.g., see: Zabicky 1970), the major impetus behind this published work has been the importance of the amide functionality in proteins (i.e., the secondary amide or "peptide" bond); most of this work has little relevance, however, to the determination of acrylamide.

TAUTOMERISM

For amides (among many compounds) two or more structurally distinct forms of the compound coexist in rapid equilibrium (March 1985, p.66). *Tautomerism* is the rapid shifting back and forth of these forms. In almost all instances, the shifting involves the migration of a proton from one atom to another. The distinct structures are called *tautomers*. For amides, the equilibrium shifting is referred to as *keto-enol* tautomerism, involving the carbonyl group (*keto* form) possessing an α -hydrogen (" α " refers to the adjacent position) and its *enol* form (where the α -hydrogen shifts to the carbonyl oxygen, forming the hydroxylated derivative):



For simple structures, the equilibrium favors the keto form since the sum of its bond energies is higher. Enolization is affected greatly by solvent, concentration, and temperature (e.g., water reduces the enol form by hydrogen bonding with the carbonyl, thereby reducing the propensity for "internal" hydrogen bonding). In the presence of a strong base, both the keto and enol forms can lose a proton. The resulting anion is called an *enolate* ion, and it is identical for both tautomers since it differs only in placement of the electrons (i.e., they are *canonical* forms, with the charge distributed between the carbonyl oxygen and the atom to which the α -hydrogen is bonded):

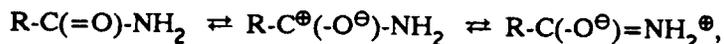


For amides, the tautomers can be shown as:



Tautomerism plays an important role in determining whether amides react via the amido nitrogen or oxygen. In general, most reactions that amides undergo proceed from one of two mechanisms (Challis and Challis 1970, p.733): (1) the most common is nucleophilic attack by the oxygen or nitrogen on electrophilic centers, being either positive or neutral (N-substituted products are common and result from rearrangement of the O-substituted precursor), and (2) less common is nucleophilic addition to the carbonylic carbon. The more notable features of amide reactions (i.e., elimination, dehydration, deamination, and others) are usually side results of these reactions.

Amides can also be considered as resonance hybrids (Davidson and Skovronek 1958):

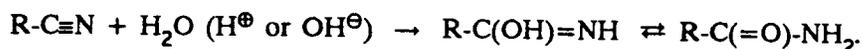


giving the carbonylic oxygen partially anionic character and the amide nitrogen partially cationic character. Because of the delocalized, unshared electron pair on the nitrogen, amides are only weakly basic.

FORMATION/SYNTHESIS OF AMIDES

There are numerous synthetic routes to amides, almost all of which have no commercial relevance (e.g., Bikales 1970). None of these is of relevance to this discussion other than those that could lead to in-situ, *de novo* formation of acrylamide (i.e., other than by introduction of parent acrylamide occurring in polyacrylamide) and those that have relevance in the manufacture of acrylamide (because of by-product contaminants).

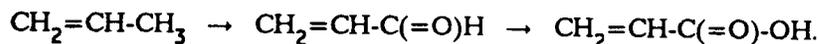
Hydrolysis of nitriles (Beckwith 1970, p.119; March 1985, p.788): Nitriles are hydrated under acid or base conditions to give amides, but since the requisite conditions also lead to the subsequent hydrolysis of these amides, carboxylic acids are usually the end product. A number of reagents is available, however, for stopping the reaction at the amide, such as concentrated sulfuric or hydrochloric acid (ammonium salts are by-products):



Hydration under basic conditions involves attack of hydroxide at the $\text{C}\equiv\text{N}$ triple bond, while attack under acid conditions is via the protonated nitrile. Note that the amide is in equilibrium with its tautomer, which is the initial addition product. This is the commercial route of acrylamide synthesis (i.e., hydrolysis of acrylonitrile in the presence of acid using various copper catalysts; MacWilliams 1978, p.303). If the hydrolysis is allowed to continue, acrylic acid (2-propenoic acid) results:

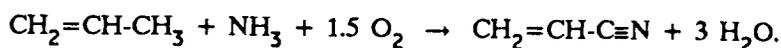


The major commercial synthetic route for acrylic acid is based on a two-stage reaction process involving oxidation of propylene to acrolein (2-propenal), which is then oxidized to acrylic acid:



Acrylic acid can also be a biogenic product. For example, it is produced by marine algae (e.g., *Phaeocystis* and *Polysiphonia lanosa*) as a result of hydrolysis of dimethyl- β -propiothetin.

Acrylonitrile was first made commercially in Germany in the 1930s (Wiseman 1986, p.76). Until 1959, two processes were used -- one based on acetylene and hydrogen cyanide and the other (less important) based on ethylene oxide and hydrogen cyanide. In 1959, the current process was developed, based on the oxidation of propylene in the presence of ammonia (*ammoxidation*):



This reaction proceeds through the aldehyde-ammonia complex, $\text{CH}_2=\text{CH}-\text{C}(\text{OH})\text{H}-\text{NH}_2$, which is then dehydrated and dehydrogenated to acrylonitrile.

Hydration (hydrolysis) of nitriles by microorganisms: The analogous routes of conversion of acrylonitrile to acrylamide are performed by various microorganisms. Nitrile hydratase catalyzes the hydration (sometimes erroneously called hydrolysis) of acrylonitrile to acrylamide (Mathew et al. 1988):



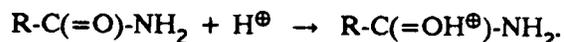
This reaction has been recently used for the low-temperature production of acrylamide with minimal by-products (e.g., 400 g/L of acrylamide in 7.5 hours with only traces of acrylic acid) (Asano et al. 1982) and represents the first use of biotechnology in the petrochemical industry (Watanabe 1987). An amidase can then hydrolyze the acrylamide to acrylic acid and ammonia; since the specific activity of the amidase is significantly lower than that of the hydratase, little acrylic acid is produced. This has been suggested as a production/synthesis method for acrylamide since the enzyme is active with acrylonitrile concentrations up to 1.25% (Hwang and Chang 1987). In contrast, an alternative route exists for the direct cleavage of nitriles to their corresponding acids and ammonia, without the amide intermediate; this route is catalyzed by nitrilases (Mathew et al. 1988). The former route has possible significance in terms of an alternative source of acrylamide in water (i.e., production *de novo* from acrylonitrile by autochthonous bacteria) when the second step involving the amidase is for some reason nonoperative. Microbial genera known to effect these reactions include *Nocardia*, *Brevibacterium*, *Arthrobacter*, *Rhodococcus*, and *Pseudomonas* (Asano et al. 1982; DiGeronimo and Antoine 1976; Watanabe 1987).

REACTIONS UNDERGONE BY AMIDES

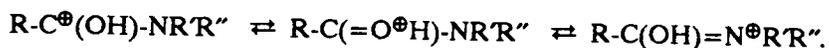
For the purposes of chemical derivatization, acrylamide possesses two functional groups at which chemical modifications can be easily (and possibly selectively made) -- the carboxamido group itself and the olefinic (acrylyl) double bond, which happens to be conjugated with the amide carbonyl group (the α,β -position). The reactions in which each of these groups can participate will be summarized, and notation will be made when these reactions serve as the basis of current methods of analysis or whether they have the potential to be exploited for new methods. For cyclic compounds, the line structures use an asterisk (*) after each of the two ring atoms that are normally joined by a single covalent bond; as with conventional line-structure notation, moieties in parentheses are covalently bonded to the atom that directly precedes (i.e., they are out of the line). Reaction equations have not necessarily been balanced, and repetition of certain aspects of amide chemistry has been purposefully made to add emphasis and continuity. The mention of trade names does not imply endorsement, but rather is intended to give the reader rapid access to possible sources of materials. This overview does not pretend to be a thorough investigation of amide reactions; this can best be gained from other sources (e.g., Zabicky 1970).

REACTIONS OF THE AMIDE GROUP (also called carboxamido)

Protonation (March 1985, p.222): Although both the oxygen and nitrogen are potential sites, protonation occurs on the carbonylic oxygen:

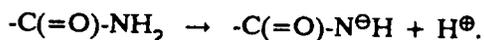


This subject has received an enormous amount of attention because of the importance of hydrogen bonding in inter- and intra-molecular interactions of proteins. Although the amine function would appear at first to be the site of protonation (since it is more basic than the carbonyl group), the carbonyl group receives additional stability through its resonance with two other canonical forms (Homer and Johnson 1970, p.188):



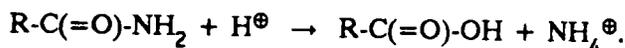
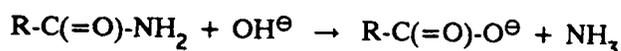
Nevertheless, amides are relatively weak bases (their pK_a 's are lower than those of the corresponding amines by about 10 units) and would be expected to protonate only under relatively acid conditions.

Deprotonation (Homer and Johnson 1970, p.238): Despite the influence of the carbonyl group, the amide-amino group is a very weak acid, and will lose a proton (yielding the carboxamido anion) only under very alkaline conditions:

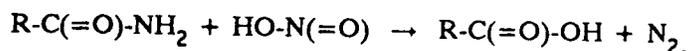


Because of the mild acid and base characteristics of amides, they can be expected to remain neutral under all but extreme conditions.

Hydrolysis (hydroxy-deamination) (Challis and Challis 1970, p.816; March 1985, p.338): Sometimes referred to as saponification, hydrolysis yields the corresponding carboxylic acid and amine from cleavage of the acyl-N bond. Water alone is insufficient to hydrolyze most amides since NH_2 is a poor leaving group; prolonged heating, even with catalysis, is usually necessary (indeed, amides can be recrystallized from water). Unsubstituted (primary) amides can be hydrolyzed under either acidic or basic catalysis (secondary and tertiary amides require more drastic conditions); a traditional standard method for indirectly determining primary amides involves hydrolysis in 10N HCl at 100°C for 5 hours and determination of the distilled, liberated ammonia by titration. The respective products are the free acid (acrylic acid results from acrylamide) and the ammonium ion or the salt of the acid and ammonia (the production of salts makes these reactions irreversible); secondary and tertiary amides yield the respective alkylamine. In alkaline hydrolysis (March 1985, p.239), which is more efficient than acidic hydrolysis, the rate-determining step is attack of the hydroxide ion at the carbonylic carbon. For base-catalyzed hydrolysis, simple amides are about 1000 times less reactive than simple esters (in 0.5N NaOH at 100°C, approximately 90% of the acrylamide in a 10% solution is hydrolyzed in 12 minutes); in contrast, the acid-catalyzed rates are equivalent for simple amides and esters (Mabey and Mill 1978):



A reaction much faster than ordinary hydrolysis of primary amides is mediated by nitrous acid to yield molecular nitrogen and the corresponding carboxylic acid (side reactions are minimal); hypochlorous and hypobromous acids can also be used. The reaction is about 10^6 times faster than acidic or basic hydrolysis (Ellis and Holland 1976):



Nitrous acid hydrolysis has formed the basis of several analytical methods for primary amides, including gasometric methods (for the evolved molecular nitrogen) and indirect determination via colorimetric quantitation of unreacted nitrite ions after amide hydrolysis by nitrous acid in excess (e.g., Ellis and Holland 1976).

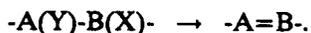
Dehydration

Dehydration of amides is one of the major problems that confronts the formation of stable derivatives for analytical determination (e.g., see: Derivatization Chemistry).

Dehydration of amides to ketenimines (March 1985, p.902): Secondary amides can be dehydrated with P_2O_5 , pyridine, and Al_2O_3 to give ketenimines:



Dehydration of unsubstituted amides to nitriles (N,N-dihydro-C-oxo-bi-elimination) (March 1985, p.932): The most common dehydrating agent is phosphorus pentoxide (P_2O_5), but many others have been successfully used (e.g., acid halides or anhydrides) (see: N-Acylation of amides). The reaction can be formally viewed as a β -elimination of water from the enol form (β -elimination refers to loss of two groups, one from each of two adjacent atoms) to form a new double or triple bond:

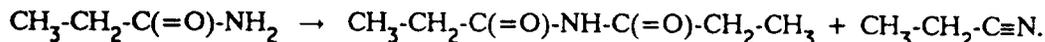


Dehydration of amides to nitriles is one of the oldest known reactions in organic chemistry (first accomplished by Wohler and Leibig for the dehydration of benzamide to benzonitrile; Bieron and Dinan 1970, p.274):

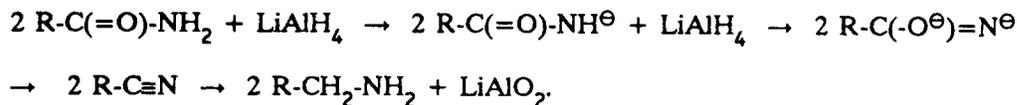


Reduction of primary amides to the amine with metal hydrides proceeds through the nitrile as an intermediate (see: Reduction of amides to amines).

Pyrolytic dehydration to nitriles (Bieron and Dinan 1970, p.279): Pyrolysis to nitriles is an uncontrollable process that yields numerous other products, including ammonia, and probably proceeds through an imide intermediate, as shown for propionamide:



Reduction of amides to amines (or nitriles) (Challis and Challis 1970, p.795; March 1985, p.1099): Amides can be quantitatively reduced to the corresponding amines with metal hydrides (especially $LiAlH_4$ and BH_3) but other functional groups (esp. those containing active hydrogen, that is, hydrogen not bonded to carbon) usually are reduced more easily and therefore have precedence; nitriles, imides, and nitro compounds, however, are among the only ones that will yield amines. Nitriles are intermediates in the reduction of unsubstituted amides:



This reduction is commonly performed in ether. Primary amides are reduced slowly, and reactant ratios are important; reduction of acrylonitrile, in particular, is reported to be difficult (Siggia and Hanna 1979, p.200), indicating that this approach may not be suitable for acrylamide determination. In diglyme, primary amides can be quantitatively reduced and stopped at the nitrile (Challis and Challis 1970, p.798).

A newer and safer reducing agent is available: Vitride[®], $NaAlH_2(OCH_2CH_2OCH_3)_2$; available from Hexcel Corporation (technical brochure available; 215 North Centennial St., Zeeland, MI 49464; 616-772-2193). Advantages over aluminum alkyls are advertised as its being nonpyrophoric at ambient conditions, being extremely soluble in a number of solvents, and having unlimited shelf life when dry.

Still other reducing agents known to work with amides and having several advantages over lithium aluminum hydride (LAH) are borane complexes, in particular, borane-tetrahydrofuran ($BH_3 \cdot THF$) and borane methyl sulfide [$(CH_3)_2S \cdot BH_3$] (Lane 1977). With boranes, all amides are rapidly reduced under reflux. The major

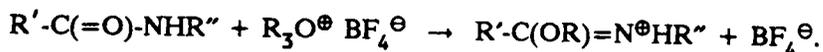
advantage of boranes over LAH is that they are not as powerful at reducing, making them more selective; LAH will debrominate α -bromoamides (an important consideration for use with dibromopropionamide). Since borane is electron-deficient, it attacks at electron-rich centers; the hydrides, in contrast, are nucleophiles. Borane reductions occur at relatively low temperatures (minimizing side reactions), and the inorganic by-product is usually an inert, water-soluble borate salt.

Reduction to the corresponding amines presents several possibilities with respect to trace analysis since amines are conducive to formation of derivatives that are extremely electron-capturing and that give intense fluorescence (e.g., Beale et al., in press 1988).

Alkylation

Amides have three potential sites for undergoing alkylation: the carbonyl-O, the amide-N, and the α -carbonyl-C (Beckwith 1970, p.159). Since amides are very weak nucleophiles, however, they undergo alkylation only with the strongest alkylating agents (e.g., alkyl sulfates, oxonium salts, and diazoalkanes); alkyl halides usually require a high temperature (e.g., 150°C) or heavy-metal catalysts (which polarize the halide, enhancing its reactivity). The major determinants of the alkylation site are pH followed by temperature. Neutral conditions yield mixtures of O- and N-alkyl derivatives; O-alkyl derivatives are favored at ambient room temperature, whereas both substitutions occur in various proportions at temperatures above 60°C, probably because of rearrangement of the O-alkylimidate to the N-alkylamide (a type of Chapman rearrangement). Amide:reagent ratios are also critical (Challis and Challis 1970, p.735, 744).

O-Alkylation (March p.359): The carbonyl oxygen of amides can be alkylated by alkyl sulfates or oxonium salts to yield salts of N-alkylimino esters (alkoxymethyleniminium salts):



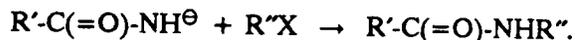
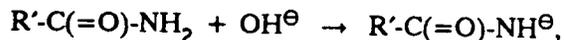
These ions, in turn, can be reacted with various nucleophiles. For example, they can be reduced to amines with $NaBH_4$:



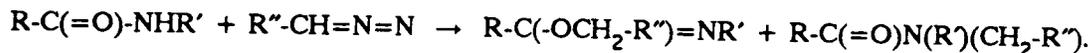
O-Alkylation has also been accomplished with alkyldiphenylsulfonium salts ($Ar_2SR^+ BF_4^-$) and with ethyl chloroformate (Challis and Challis 1970, p.742):



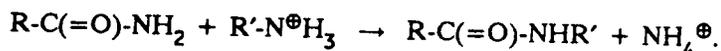
N-Alkylation (Beckwith 1970, p.159; Challis and Challis 1970, p.748): Under strongly basic conditions, the amide anion is alkylated at the nitrogen, probably via abstraction of an amide-N hydrogen to form the carboxamide anion:



Diazoalkanes (like many alkylating agents) give a mixture of N- and O-alkylated products but are unique in that the N-alkyl derivatives are produced at low temperatures (in the absence of metal catalyst), probably by direct attack at the nitrogen (0-20°C) (Challis and Challis 1970, p.740, 748):

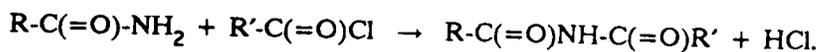


N-Alkylation of amides by amines (N-Acylation of amines by amides: alkylamino-deamination) (Beckwith 1970, p.116; March 1985, p.376): Usually carried out by the salt of the amine (usually primary). This is an exchange reaction where the leaving group is NH_2 ; BF_3 can be added to complex with the leaving ammonia:



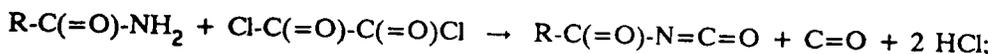
This reaction has wide applicability and can take place by heating at 60-70°C for several minutes; it may be a potential means of generating a secondary amide from acrylamide, but addition to the α,β -double bond probably has precedence (see: Reactions of the Olefinic Group: α,β -Unsaturated alkenes).

N-Acylation of amides (acylamino-dehalogenation) (March 1985, p.379): Attack of amides or their salts on acyl halides (or acyl anhydrides, esters, or acids) gives imides (diacylamines):

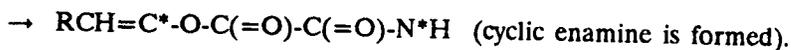


When the molar ratio of acyl chloride to primary amide is 1:2, the products are N,N-diacylamides (triacylamines): $\text{R-C(=O)N[C(=O)-R]}'_2$; the best synthetic method for acyclic imides is reaction of amide with an anhydride at 100°C catalyzed by H_2SO_4 .

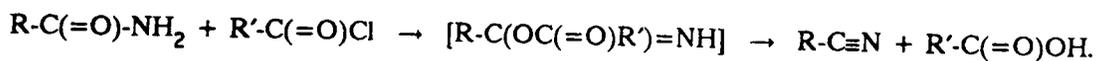
An exception to this scheme is the reaction of a primary amide with oxalyl chloride where an acyl isocyanate or cyclic enamine is formed depending on whether the α -carbon has a hydrogen (Challis and Challis 1970, p.770):



where R=t-alkyl, aryl, etc., the isocyanate is formed (but is easily hydrolyzed); otherwise:



Of all reactions, acylation presents the most promise with respect to derivatization for chromatography. For primary amides, unfortunately, a competing reaction is dehydration of the amide to the nitrile (with formation of a carboxylic acid from the acyl halide) via a mixed anhydride intermediate, a species that plays a central role in amide chemistry (Challis and Challis 1970, p.759; Davidson and Skovronek 1958) (also see: Miscellaneous Reactions of the Amide Group):

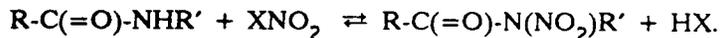


Dehydration is exacerbated by more powerful acylated agents. Because of the relatively complex reaction mechanism, acylation can therefore yield three products (from primary amides): diacylamines, triacylamines, and nitriles. The relative proportions depends on the type of acyl halide, the temperature, and catalyst (Challis and Challis 1970, p.762). To optimize the yields of N-acyl derivatives, it is best to use a basic catalyst (e.g., pyridine; or 4-dimethylaminopyridine is superior; Katritzky, Nowak-Wydra, and Rubio 1984) at about 0°C, and use a relatively weak acylating reagent.

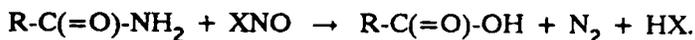
Ehrsson and coworkers (e.g., Ehrsson and Mellström 1972) have done extensive work on amide benzoylation. Reaction of secondary amides with pentafluorobenzoyl chloride (PFB chloride) requires trimethylamine as a catalyst. Compared with trifluoroacetic anhydride (TFA; e.g., Kawahara 1976), the derivatives form much more slowly but they are also more stable and respond to electron-capture detection with extreme sensitivity; the TFA-derivatives are extremely susceptible to hydrolysis by trace amounts of water (TFA derivatives decompose simply upon dilution with more solvent that happens to contain trace amounts of water). The PFB-derivatives are made in aprotic organic solvent (with acetone added to solubilize the amide).

Benzamide, the only primary amide studied, indeed yielded benzonitrile upon benzylation; the usefulness of PFB-derivatives for primary amides is therefore unknown.

N-Nitration & -Nitrosation (Challis and Challis 1970, p.780; March 1985, p.573): Amides (but preferentially amines) can be N-nitrated with nitric acid, N_2O_5 , or NO_2 ; the reaction has been studied mainly with secondary amides:



With certain weak bases, nitrous and nitric acid form the electrophiles, nitrosonium ion (NO^{\oplus}) and nitronium ion (NO_2^{\oplus}), respectively, which cause N-nitroso-deamination to the corresponding carboxylic acid (Challis and Challis 1970, p.780) (also see: Hydrolysis):



Deamination also occurs for the nitrated products but higher temperatures are required. For primary amides, however, the N-nitro derivative is very unstable, leading to rapid deamination.

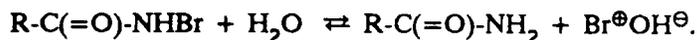
N-Halogenation (N-halo-dehydrogenation) (Challis and Challis 1970, p.775; March 1985, p.574): Sodium hypochlorite or hypobromite react with unsubstituted and N-substituted amides. For the N-substituted amides, the resulting N-halo-N-alkyl amides or N-halo imides are quite stable (this is how the reagent N-bromosuccinimide is made):



For the unsubstituted (primary) amides, however, the N-halogenated product is rarely isolated because of its instability. Molecular halogens also yield N-halogenated derivatives, but the hypohalites (equimolar sodium hydroxide and molecular halogen) are preferred because of a lack of C-halogenation (which is promoted by the presence of aromatic substituents):



In neutral aqueous solution, N-bromoamides are in equilibrium with the hypobromous acid; the equilibrium is influenced by pH and solvent (polar solvents such as water favor the N-haloamide). This reaction forms the basis of certain indirect spectrophotometric methods for amides that make use of iodide oxidation by hypobromous acid (e.g., Scoggins and Miller 1975):



In basic solution, the N-halogenated product rearranges with carbon elimination to a nonhalogenated amine with one fewer carbon atom (see: The Hofmann rearrangement); since equimolar concentrations of base and amide are difficult to arrange (and required for hypohalous formation), this is the usual outcome of halogenation. The unsubstituted amides can be N-brominated or -dibrominated with dibromoisocyanuric acid; uncontrolled mixtures of both C- and N-fluorination can be accomplished with F_2 via a free-radical process. In acidic solution, N-haloamides are again unstable. The halogen is usually released (as hypobromous acid -- "positive" bromine) to yield the parent amide, and then it is available to oxidize another portion of the molecule, such as a double bond (Challis and Challis 1970, p.775); this is the outcome in those methods of acrylamide determination that use acidic bromination (i.e., formation of 2,3-dibromopropionamide). It should be noted that the ionic form of bromine in these reactions has been the subject of much controversy (e.g., Farook, Viswanathan, and Ganesan 1984).

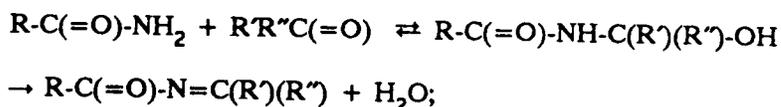
gem-Difluorination (March 1985, p.808): Amides react with sulfur tetrafluoride (SF_4) to give the 1,1,1-trifluorides; aldehydes and ketones (and even CO_2) also undergo the reaction, however. The initial product is the acyl fluoride, which then undergoes gem-difluorination (*gem*- is the abbreviated form for *geminate*, sometimes called *geminal*, and refers to two identical groups bonded to the same carbon atom):



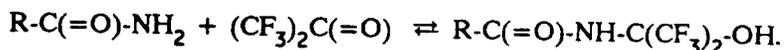
Reactions with SF_4 require a stainless-steel pressure reactor. Regular glassware and atmospheric pressure can be used with selenium tetrafluoride (SeF_4); also of possible use is the commercially available DAST (diethylaminosulfur trifluoride; $[\text{CH}_3\text{CH}_2]_2\text{NSF}_3$; e.g., available from Aldrich Chemical: #23,525-3); DAST, however, is best for fluorination of simple alcohols, aldehydes, and ketones (see: Aldrichimica Acta 1988, 21(1), p.27.).

Conversion of carbonyl to thiocarbonyl (March 1985, p.794): The carbonyl group, $>\text{C(=O)}$, of amides can be converted to the thiocarbonyl group, $>\text{C(=S)}$, by treatment with 2,4-bis(4-methoxyphenyl)-1,2,3,4-dithiadiphosphetane-2,4-disulfide; the carbonyl group of carboxylic esters is also converted.

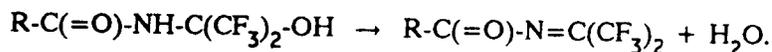
Addition of amides to aldehydes or ketones (Challis and Challis 1979, p.754; March 1985, p.798): The amide nitrogen of primary and secondary amides can reversibly add to the carbonyl group of activated aldehydes and ketones (the carboxamide anion, $\text{R-C(=O)-NH}^\ominus$, is the nucleophile under basic conditions) to yield the initial adduct, acylated amino alcohols (N-acylcarbinolamines), which are stable in neutral and basic conditions but which undergo dehydration/coupling under acidic conditions:



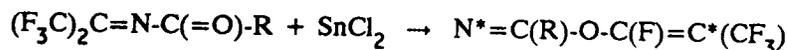
acrylamide and formaldehyde therefore yield N-methylolacrylamide. O-Substituted products are notably absent, probably because of the high temperature (100-150°C) required, which causes the Chapman-like rearrangement (see: O-Alkylation). Ketones are generally less reactive than aldehydes, but hexafluoroacetone (1,1,1,3,3,3-hexafluoro-2-propanone; CAS Registry No. 684-16-2) or sym-dichlorotetrafluoroacetone condense with primary amides at 50°C to yield 1:1 adducts (Challis and Challis 1970, p.755); this reaction was first reported by Newallis and Rumanowski (1964) to occur for primary amides (but not secondary amides) in isopropyl ether or tetrahydrofuran:



Dehydration of the hexafluoroacetone-amide adduct gives the 4,4-bis(trifluoromethyl)-1,3-oxazolydiene derivative:

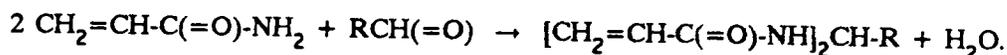


This reaction serves as the basis of new methods for synthesis of trifluoromethylated 1,3-azole heterocycles (Burger 1987); for amides, the result would be 1,3-oxazoles (thioamides, selenoamides, and amidines yield the respective thiazoles, selenazoles, and imidazoles). With SnCl_2 , a nearly quantitative cycloadduct results (Sn bridges the amide carbonylic oxygen and the bis-trifluoromethyl-methylene carbon). Subsequent ring opening at elevated temperature polarizes the oxygen-tin bond, resulting in elimination of one fluoride ion, which is scavenged by oxidation of the tin. This gives a heteropentadienylic anion, which undergoes ring closure with expulsion of the tin and another fluorine to give the 5-fluoro-4-trifluoromethyl-1,3-oxazole:

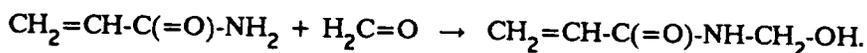


With the 4-substituted trifluoromethyl group, this compound is susceptible to nucleophilic substitution at the 5-position, resulting in displacement of the remaining ring-fluorine. Numerous compounds are possible. This reaction scheme (i.e., trifluoromethylation and cyclization) currently has unknown utility in the determination of acrylamide, but derivatives so formed could presumably allow for electron-capture or fluorometric detection.

Formation of N-methylol derivatives (N-hydroxymethylation) (Challis and Challis 1970, p.756; MacWilliams 1978, p.299): A specific case of addition to aldehydes. Amides easily form stable (under neutral/mildly basic conditions) N-methylol derivatives (hydroxymethylamides) upon reaction with formaldehyde; under acid conditions, the carbinolamine is often dehydrated and other products formed (e.g., alkylidene or arylidene bisacrylamides) (American Cyanamid Co. 1969, p.13):

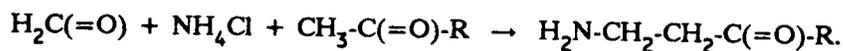


The reaction of acrylamide with formaldehyde under aqueous conditions (pH 7-9) yields N-methylolacrylamide, an extremely important industrial starting material for higher polymers (the N-hydroxymethylamides condense readily with compounds having active hydrogen):

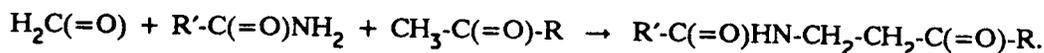


For primary amides, further heating with excess aldehyde yields the bis-hydroxymethylated derivatives.

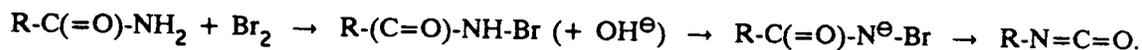
Mannich Reaction (March 1985, p.800): Strictly, the reaction of active methylene compounds with formaldehyde and ammonia or primary or secondary amines to yield β -aminocarbonyl (aminoethylcarbonyl) compounds (the Mannich base):



Primary amides can serve in place of ammonia, yielding a secondary amide with an isolated carbonyl group:

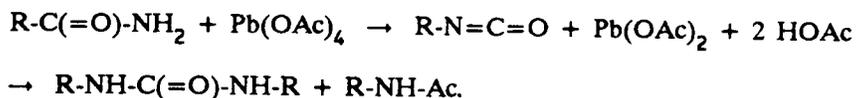


The Hofmann rearrangement (Hofmann reaction) (March 1985, p.983): Conversion of an unsubstituted amide to a primary amine with one fewer carbon atoms than the parent amide upon treatment with sodium hypobromite (or sodium hydroxide and bromine); the reaction proceeds through the intermediate isocyanate, which usually undergoes facile hydrolysis to R-NH₂ under the reaction conditions:

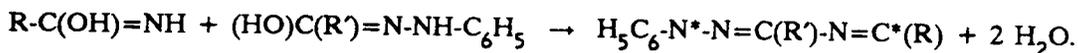


The transient formation of the N-bromo derivative forms the basis of many spectrophotometric titration methods for indirectly determining amides (e.g., Siggia and Hanna 1979; Scoggins and Miller 1975, 1979; Wimer 1970, p.340). A possible severe limitation in applying this reaction to quantitation is that the amine is not always produced quantitatively, especially for acrylamide in aqueous medium (Siggia and Hanna 1979, p.212). Acrylamide continues to react with hypobromite past the theoretical formation of N-bromoacrylamide (Post and Reynolds 1964); this problem can possibly be circumvented by the use of N-chlorination with hypochlorite (Post and Reynolds 1965). Another reagent that effects the conversion is I₂-bis(trifluoroacetoxy)iodobenzene [PhI(OCOCF₃)₂].

Oxidation by lead tetraacetate (Challis and Challis 1970, p.793; March 1985, p.983): In a reaction having results similar to the Hofmann rearrangement, amides (notably, only primary amides) react with lead tetraacetate to form initially an isocyanate (which is stable only under basic, aprotic conditions). The isocyanate reacts to form the amine, which in turn reacts with the acetate to give acetyl amides and ureas:

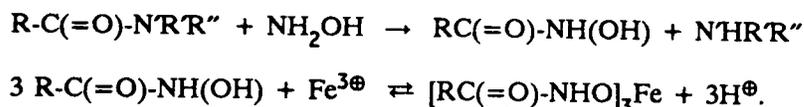


Condensation of amides and acyl hydrazines to form substituted 1,2,4-triazoles (Pellizzari reaction; Windholz 1976, p.ONR-66):



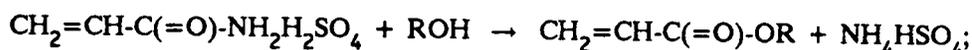
When the acyl groups of the amide and of the acylhydrazine differ, interchange of acyl groups may occur with formation of a mixture of triazoles. These derivatives would have unknown utility in detection by HPLC.

Formation of hydroxamic acids (Wimer 1970, p.340): Amides react with hydroxylamine in aqueous solution to form hydroxamic acids, which form characteristic, deeply colored (red-violet) chromophores with ferric ion (primary amides react the fastest, forming ferric hydroxamates):



First discovered for amides (i.e., acetamide) in 1889 by Hoffmann. Lactones, esters, anhydrides, acid chlorides, and nitriles also react with hydroxylamine, some at a much faster rate (i.e., minutes versus hours), others at a slower rate (e.g., nitriles); indeed, the same reactions have been traditionally used for determination of carboxylic acid derivatives such as esters and anhydrides. Among the amides, molar absorptivities vary widely; hydrolysis of the amide is a competing reaction since the reaction is performed under alkaline conditions at elevated temperature. Optimal temperatures for color development vary with the compound (Bergmann 1952). This reaction was first developed as a nonspecific colorimetric method for amides by Bergmann (1952) and by Soloway and Lipschitz (1952); reaction times can vary from 1 to 6 hours.

Formation of esters (MacWilliams 1978, p.299): By reacting acrylamide with potassium tert-butoxide in tert-butyl alcohol with sulfuric acid, its sulfate salt is formed. Reaction of the sulfate salt with alcohols yields the corresponding esters of acrylic acid:

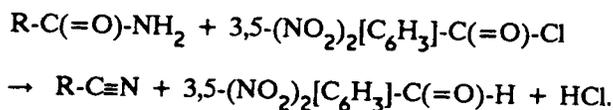


sometimes the alcohol adds instead to the double bond to give 3-alkoxy-N-alkylpropionamides as a by-product (American Cyanamid Co. 1969, p.9).

MISCELLANEOUS REACTIONS OF THE AMIDE GROUP

There are numerous reactions that the primary amide group is known to undergo, many of which yield chromophoric species, that may (or may not) be amenable to HPLC. Few of these have ever been evaluated for use with UV detection. The following examples are taken from Wimer (1970), and others can be found in American Cyanamid Co. (1969): oxalic acid forms oxalate salts, phthalyl chloride forms N-acyl phthalimides, mercuric oxide forms N,N-bismercuriacyl amides, xanthinol forms N-xanthyl amides, benzhydrol forms mono- and bis-N-benzhydrol amides, and chlorination followed by treatment with aqueous pyridine solution of potassium cyanide and 1-phenyl-3-methyl-2-pyrazolin-5-one (or barbituric acid) forms a red chromophore that turns blue (0.1 μg can be detected on TLC plates).

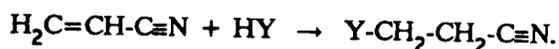
Primary amides react with 3,5-dinitrobenzoyl chloride in pyridine; the amide is dehydrated to the nitrile, and the acid chloride is converted to its free acid (see: N-Acylation of amides):



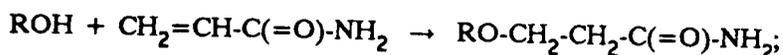
Benzoylation is an important means of derivatization for GC and HPLC. The dinitrobenzoyl derivatives absorb strongly at 254 nm, and they also are strong electron-capturing agents. Benzoylation is intended for amino and hydroxylated compounds. In this example, the amide is dehydrated, simply resulting in conversion of the benzoyl chloride to its aldehyde. This is an indirect method of determination and is of no use for analysis unless the amide is known to be the only reactant present. In a normal benzoylation derivatization, the functional group is benzoylated (e.g., alcohols form benzoate esters) in an appropriate solvent (e.g., pyridine), and the excess reagent is hydrolyzed to the benzoic acid.

REACTIONS OF THE OLEFINIC GROUP

Electron-donating groups increase the reactivity of a double bond toward electrophilic addition, and electron-withdrawing substituents, such as halo and cyano, result in a reduced reactivity since they reduce the electron density of the double bond (March 1985, p.671); so in the absence of electron-withdrawing groups, simple olefins do not react by the nucleophilic mechanism. When the double bond is conjugated with a group, such as with -C(=O)-NH_2 , it almost always reacts by nucleophilic addition (March 1985, p.664), with the nucleophile bonding to the carbon away from the conjugated group (e.g., as in the cyanoethylation of a nucleophile by acrylonitrile):



Formation of ethers from amides (MacWilliams 1978, p.300): In another example of nucleophilic addition, hydroxy compounds add readily to acrylamide in the presence of base to give 3-alkoxypropionamides (or 3-aryloxypropionamides from phenols):



primary aliphatic alcohols are the most reactive, but the presence of water will compete via hydrolysis of the amide. This reaction is the basis of acrylamide reaction with cellulose and other polyalcohols (American Cyanamid Co. 1969). The analogous 3-(alkylthio)ethers are formed from thiols (American Cyanamid Co. 1969, p.11).

Halogenation of double bonds (dihalo-addition) (March 1985, p.724): Most double bonds are easily halogenated with Br_2 or Cl_2 (or interhalogen compounds; the relative reactivities for some of these compounds are: $\text{BrCl} > \text{ICl} > \text{Br}_2 > \text{IBr} > \text{I}_2$):

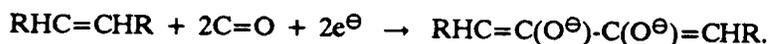


The Herzfelder rule may apply here, depending on whether the two bromine atoms come from two molecules: the introduction of a second halogen into a monohalogen compound always takes place at that carbon atom situated adjacent to the carbon already substituted with the halogen. Reaction with Br_2 is very rapid at room temperature (for this reason, bromine is often used as a test for unsaturation), and other substituents (e.g., carbonylic and amino groups) usually do not compete. A convenient reagent for adding Br_2 to a double bond under laboratory conditions is the commercially available pyridinium bromide perbromide ($\text{C}_5\text{H}_5\text{NH}^{\oplus} \text{Br}_3^{\ominus}$), which is made from pyridine, hydrobromic acid, and bromine.

For acrylamide, gaseous chlorination or bromination yields the respective 2,3-dihalopropionamides (MacWilliams 1978); indeed, this may be the immediate fate of acrylamide residuals in drinking water subjected to gaseous chlorination under non-alkaline conditions (i.e., 2,3-dichloropropionamide; Davis et al. 1976, p.43). Aqueous bromine yields 2,3-dibromopropionamide (α,β -dibromopropionamide: $C_3H_5Br_2NO$). Hydrochloric and hydrobromic acids add to yield β -halopropionamides (thermally reversible adducts).

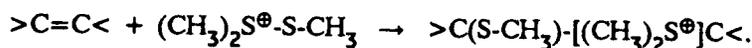
Other additions to double bonds: Hydrogenation of acrylamide to propionamide can be done with borohydride, nickel boride, or rhodium carbonyl among others (MacWilliams 1978, p.301).

A newly documented reaction (unprecedented in organic or organometallic chemistry) inserts two carbon monoxide molecules into a carbon-carbon double bond by use of an organosamarium complex (Evans and Drummond 1988): bis(pentamethylcyclopentadienyl) samarium(II)bis(tetrahydrofuranate). This converts the structure to a bis-enolate that is complexed with two $[(CH_3)_5C_5]_2Sm$ groups:



This reaction has possible utility in the derivatization of alkenes but the complex is extremely air- and moisture-sensitive.

Formation of sulfonio cations (Mazurkiewicz 1985): Conversion of acrylamide to an ionic form could be advantageous for electrophoretic techniques. Ethylenic double bonds will undergo addition to dimethylsulfoniomethylthio cations to form cationic species:



The cation reagent is produced from dimethylmethylthiosulfonium fluorosulfonate. Relative to the dimethylsulfonium cation, the mobility (using paper electrophoresis) for derivatized acrylamide was 0.53, the same as for crotonaldehyde, so it is not clear as to whether sufficient resolution could be obtained (although capillary electrophoresis would be promising).

α,β -Unsaturated alkenes

These compounds, of which acrylamide is a representative, have the carbon-carbon double bond located in the 2,3-position (α,β -position) with respect to a functional group. They are susceptible to the normal halogenation reactions (discussed above) as well as to two reactions specific for α,β -unsaturates: bisulfite addition and amine (esp. morpholine) addition (Siggia and Hanna 1979, p.452-467).

The sodium bisulfite (or sulfite) addition method reacts the alkene with sodium bisulfite to form the substituted sodium sulfonate (for acrylamide, sodium- β -sulfopropionamide [3-sulfopropionamide] is formed):

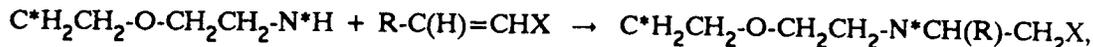


where X is a strong electron-attracting group.

The reaction is affected strongly by pH (optimum pH = 5.5-6.5). Competing substitution reactions can occur when carbon-halogen bonds are present, and addition to carbonyls can occur. Since this reaction is rapid at room temperature with sodium sulfite, it has become the established method for scavenging acrylamide monomer from PAM (MacWilliams 1978, p.300). Sulfite, incidentally, is used also to scavenge excess bromine from bromine-derivatization reaction solutions (e.g., see: Croll and Simkins 1972).

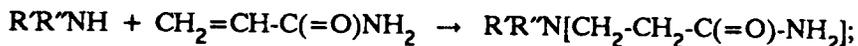
The morpholine-addition method makes use of the well-characterized reactivity (nucleophilic addition) of ammonia, and primary and secondary amines, with α,β -unsaturated compounds (e.g., Beesing et al. 1949;

Belcher and Fleet 1965b; Critchfield, Funk, and Johnson 1956). The method uses an excess of the secondary amine, morpholine, which reacts in the presence of acetic acid (catalyst) with the unsaturated bond, forming the tertiary amine adduct, β -N-morpholinopropionamide (3-morpholinopropionamide):

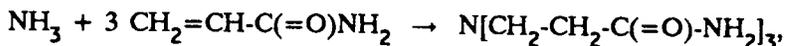


where X is a strong electron-withdrawing group; aldehydes and ketones do not react.

For acrylamide, reaction with primary and secondary amines yields the bis- and mono-adducts, respectively (MacWilliams 1978, p.300):



these reactions are thermally reversible. Reaction with ammonia yields 3,3',3''-nitrilotrispropionamide:



a compound frequently found as a contaminant in crystalline acrylamide (MacWilliams 1978, p.300). Similarly, aqueous acrylamide quickly reacts with sodium sulfide to form the insoluble 3,3'-thiobispropionamide (American Cyanamid Co. 1969, p.11).

These amine-addition reactions have been identified as problematic in acrylamide gel-electrophoresis where amines are used as buffers (Geisthardt and Kruppa 1987); of all the amino buffers investigated, only trimethylamine reacted faster than morpholine (the reaction was completed in less than 10 minutes as opposed to 1 day). Acrylamide also covalently binds to proteins via the ϵ -amino group of lysine residues. Even tertiary amines are known to react (only those without N-methylation are not reactive), forming quaternary ammonium salts (see: Geisthardt and Kruppa 1987; Katritzky, Nowak-Wydra, and Rubio 1984); the free, unprotonated tertiary amine base reacts at the vinylic bond, and the hydrogen is abstracted from the solvent. This adduct-formation reaction is the basis for the catalytic properties of certain pyridines in acylation reactions (Katritzky et al. 1984); for example, with 4-dimethylaminopyridine the β -N-pyridinoacrylamide adduct is formed. ■

METHODS OF DETERMINATION

Amides in general (not carbamates) have played a relatively minor role in environmental toxicology. The analytical literature therefore is very limited with respect to environmental applications. This is borne out by the absence of attention to amides in the "Master Analytical Scheme (MAS) for Organic Compounds in Water" developed by Pellizzari et al. (1985), as well as by the dearth of information on the derivatization of amides for chromatography (e.g., Blau and King 1978; Frei and Lawrence 1981; Knapp 1979; Pierce 1968). Amides have played a far more important role in analysis of pharmaceuticals and other biochemicals.

The problem of trace analysis can be attacked using two approaches, often in combination: (1) analyte preconcentration (using various means of solvent reduction such as evaporation or liquid- or solid-partitioning), and (2) selective and sensitive means of detection. For the latter, if the analyte is not directly amenable to selective detection, it may be suitable to chemical modification (derivatization). These two approaches are discussed below with respect to acrylamide. Throughout this discussion, the following standard acronyms are used: HPLC (high-performance liquid chromatography), GC (gas chromatography), ECD (electron-capture detection), and MS (mass spectrometry).

DERIVATIZATION CHEMISTRY

"Derivatization" of a compound for subsequent determination (the analyte) refers to its chemical modification (formation of a "derivative") for the purpose of improved detection or quantitation. The objectives may be several, including (1) permitting separation of the analyte from possible interferences prior to chromatography (e.g., facilitating liquid extraction of an otherwise polar compound from aqueous solution), (2) making the analyte less reactive (e.g., less susceptible to thermal alteration during gas chromatography), (3) making the derivative more volatile by eliminating hydrogen bonding (e.g., for gas chromatography of polar/ionic compounds) or less volatile (e.g., for low-molecular amines), (4) improving the chromatographic separation or efficiency (e.g., masking of amine groups to prevent chromatographic tailing), (5) lowering the detection limit (preferably selectively) by incorporating a more appropriate chemical group (e.g., an electrochemically active or fluorescent functionality [fluorophore] for HPLC or a halogenated, "electron-capturing" functionality [electrophore] for GC with electron-capture detection), or (6) increasing the number of characteristic ions (while decreasing the total number of noncharacteristic ions) imparted by fragmentation during mass-spectrometry (if GC-MS is used) or during selected-ion monitoring (SIM; also known by various other names such as mass fragmentography or multiple-ion monitoring or detection). Probably all of these objectives are relevant to the determination of acrylamide by various HPLC or GC methods.

For a compound to be a successful candidate for a derivatization method it must (1) have at least one "functional" group capable of undergoing relatively facile reactions (acrylamide essentially has three groups: an olefinic linkage, a carbonyl group, and an amino group -- the last two compose the amide or carboxamido group), (2) undergo a "clean" derivatization reaction (i.e., only one product [derivative] should result), and preferably reaction of other types of functional groups from other compounds present should be minimal (i.e., the reaction should be as specific as possible), (3) give good yields of derivative while also undergoing reproducible conversion under controlled time and reaction conditions, (4) yield a product stable to the conditions of analysis (e.g., thermal or hydrolytic stability), and (5) yield reaction by-products that do not interfere with detection or foul chromatographic apparatus. Finally, the reaction should ideally proceed quickly and be easily performed under mild conditions. No single method will meet all of these requirements for a particular analyte. Furthermore, since derivatization adds at least one additional step to sample manipulation, it increases analysis time and also imparts more error into any analytical method, adversely affecting accuracy and precision.

During the early development of methods for chemical derivatization, procedures were merely adopted from the field of synthetic organic chemistry that were used for "blocking" or "protecting" functional groups during

various stages of synthesis. Since these groups would necessarily require removal during later stages of a synthesis, their inherent instability imparted undesirable properties for use in analytical detection schemes (e.g., thermal instability during gas chromatography). The design of derivatives specifically for chromatography has been accorded attention only within the last two decades, and therefore methods that have been developed for particular functional groups are not necessarily the best that may be possible. This may be especially true for amides, for which a large literature exists on reaction chemistry (e.g., see Zabicky 1970) but little has been applied to methodologies for trace analysis. Much basic research is still required to develop derivatization methods specifically tailored for amides. This is a major shortcoming in analytical chemistry, and it is reflected by the lack of any specific trace methods for amides.

In general, among the most common derivatization methods are esterification (e.g., for carboxyl groups), etherification, acylation, silylation, cyclization, and formation of numerous chromophores and fluorophores. Amides in general do not undergo any selective derivatization reactions because, in many respects, they can be considered to already be derivatives (i.e., derivatives of the amine and carboxylic functions). The reactivity of the three amido-chain atoms, O-C-N, is diminished since the π -electrons are delocalized along the chain; the carbonyl reactivity is reduced as well as the nucleophilic properties of the amine-N (Challis and Challis 1970, p.733); because of the three resonance hybrids of amides, the carbonyl oxygen has partial anionic character, and the amide nitrogen has partial cationic character. This is why many reactions occur only under strongly basic or acidic conditions, where the reactive species are the carboxamide anion $[R-C(=O)-NH^{\ominus}]$ or the conjugate acid $[R-C^{\oplus}(OH)-NH_2]$, respectively. A major problem regarding derivatization of the amide functionality is that mixtures of O- and N-derivatives are almost always obtained, and their ratios are difficult to control.

For amides, only acylation and several miscellaneous methods have relevance. Perhaps the first well-defined derivatization approach was that of silylation (Pierce 1968). In general, silyl derivatives (e.g., R_3Si -, especially trimethyl derivatives -- TMS) are easily formed, but they do little for improving detection via the most sensitive, commonly available GC detector (the ECD). For amides, silylation can yield mixtures of single (N-silyl, the more stable tautomer) or double (N,O-bis-silyl) silyl derivatives (Pierce 1968, p.244), depending on the reaction solvent and molar ratios of analyte and silylating reagent. The Si-N bond is very unstable hydrolytically (Blau and King 1978, p.26, 179); many N-TMS derivatives are themselves silylating agents. For functional groups amenable to silylation, ease of reaction is in the decreasing order: alcohols > phenols > carboxylic acids > amines > amides (Blau and Kin 1978, p.157); obviously, silylation is not a very favorable approach for amides.

Acylation (introduction of the acyl group, $R-C(=O)-$, by substitution for a replaceable hydrogen: $R-C(=O)-X + HYR' \rightarrow R-C(=O)-YR' + HX$) appears to be a more promising approach to derivatization of amides; it is most commonly used, however, for compounds containing hydroxyl, thiol, or amino functionalities. To obtain maximum sensitivity (i.e., the lowest possible detection limit), the acyl group should be electron capturing (for GC) or strongly chromophoric or fluorophoric (for HPLC). In general, sensitivity in electron-capture detection increases in the order $F < NO_2 \approx Cl < Br < I$ (Blau and King 1978, p. 108). Since volatility is also a consideration, however, it is usually better to have perfluorinated derivatives rather than those with multiple Cl, Br, or I; when a carbon atom is multiply substituted with halogens, the electron-capturing effect is synergized (Knapp 1979, p.3). Acylated derivatives of amides still retain a polar nature and still tend to tail during chromatography; moderately polar stationary phases are therefore recommended.

The main classes of acylating agents are acyl anhydrides, acid halides, and various other acyl derivatives such as acyl imidazoles, amides, and phenols. One of the most commonly used halogenated acylating agent is trifluoroacetic anhydride (TFAA). As with trimethylsilyl derivatives, however, TFA-derivatives of amides are not very stable (Blau and King 1978, p.128), being very susceptible to hydrolysis (e.g., by trace amounts of water in organic solvent; Ehrsson and Mellström 1972). To date, the most successful derivatization reagents seem to be those based on the pentafluorobenzoyl moiety, generally obtained via pentafluorobenzoyl chloride; this agent provides N-perfluorobenzoyl derivatives in better yield and also that are less sensitive

to hydrolysis (Ehrsson and Mellström 1972). Dehydration of primary amides to the corresponding nitriles, however, is always a possible side reaction that can lessen yields (see: N-Acylation of amides). This is a result of the well-known dehydration reactions of acid chlorides (as well as phosphorus pentoxide) and primary amides (Challis and Challis 1970, p.814). Conversion of the primary amide to a secondary amide (e.g., see N-Alkylation of amides by amines) may possibly make the reaction more feasible. Although pyridine has traditionally been used as an acylation catalyst, it has recently been recognized that 4-dimethylaminopyridine is superior (Katritzky et al. 1984).

Despite these potential problems with benzoylation, this derivatization method has been successfully employed for the analysis of amides -- mainly formation of pentafluorobenzoyl derivatives of secondary-amide pharmaceuticals (e.g., Ehrsson and Mellström 1972; Wallace et al. 1979). The resulting pentafluorobenzimides (or amides if an amine is the analyte) give an extremely strong response to electron-capture detection. This response far exceeds that expected of highly electronegative pentafluorobenzyl derivatives (e.g., those obtained from pentafluorobenzyl chloride) and has been shown to result from the conjugation of the adjacent carbonyl group, $-C(=O)-$ [or $-C(=N)-$], with the perfluoroaromatic ring. The carbonylic carbon is polarizable and thereby enhances the electron deficiency (Moffat et al. 1972); this results in ECD responses from pentafluorobenzoyl derivatives that are 20 to 60 fold better than those from the pentafluorobenzyl derivatives (e.g., Kawahara 1976). An example of the utility of pentafluorobenzoyl chloride is the derivatization of carbamazepine, a primary amide; the resulting product is the N-bis-pentafluorobenzoyl derivative (Schwertner, Hamilton, and Wallace 1978). Another advantage of these derivatives is their distinctive mass spectra (Coutts et al. 1987). It is also possible that simultaneous extraction/derivatization using pentafluorobenzoyl chloride (and organic solvent such as toluene) in alkaline medium may be possible (Coutts et al. [1987] successfully employed this approach with the primary amine lidocaine in rat urine); this would bypass the necessity of first forming and extracting the dibromo derivative.

For extensive information on derivatization, the reader is referred to several handbooks that deal specifically with derivatization (Blau and King 1978; Frei and Lawrence 1981; Knapp 1979; Pierce 1968); a good introduction to derivatization for environmental analyses was published by the Research Triangle Institute (RTP Analytical Sciences Division 1985, Chapter 6). It should be noted, however, that historically, derivatization has been developed for detection of biochemicals (including pharmaceuticals) that occur in either simple matrices or in complex matrices with few other compounds. Little research has been done in the application of derivatization methods to complex, environmental samples.

METHODS FOR FUNCTIONAL-GROUP SEPARATION

The fractionation of complex chemical-class mixtures into individual groups is an ideal way to approach the problem of determining compounds that do not undergo unique, characteristic reactions or that cannot be separated during analytical chromatography. Little work has been accomplished on functional-group class-separation methods, although functional-group analysis is an established approach (e.g., Ma and Ladas 1976; Siggia and Hanna 1979), since only during the last decade have investigators focused their attention on the analysis of complex mixtures, such as industrial wastes. Most fractionation schemes rely on multiple combinations of pH adjustment, liquid-liquid partitioning, and purge/trap (RTP Analytical Sciences Division 1985, Chapter 5).

Other than the MAS (Pellizzari et al. 1985), which did not address amides, one of the most extensive "fractionation" schemes to date was developed for the separation of the numerous chemical classes that occur in oil shale process waters (Leenheer 1981). This method involves the differential retention/elution of chemical classes on hydrophobic and ion-exchange resins. Complex mixtures are separated into six fractions using this method: hydrophobic (lipophilic) and hydrophilic acids, bases, and neutrals. In a subsequent publication (Leenheer, Noyes, and Stuber 1982), this approach was used to identify the polar constituents in these wastewaters; among the classes identified were aliphatic amides, which occurred in the hydrophilic neutral class. The complete fractionation procedure is very involved and laborious. For the polar compounds, the authors truncated the procedure so that the amides would be separated from the acid,

volatile base, and volatile neutral solutes; these are substances that could interfere with many potential derivatization schemes (e.g., amines, nitriles, and carboxylic acids). A 20-mL sample is passed through a 3-mL column of hydroxide-saturated anion-exchange resin (acids are removed here). The eluate is pooled with a 50-mL distilled-water rinse and concentrated down to 10 mL (volatile base and neutrals are removed here; e.g., amines, nitriles). The pH is neutralized with nitric acid and the volume further reduced to 1 mL (additional volatile neutrals are removed). The sample is not taken to dryness since amides will sublime. Indeed, because of sublimation, air-filtration has been deemed inappropriate for work-place monitoring of acrylamide vapors; with an air flow of 30 L/min, loss of acrylamide from filters at 22.5°C and 26°C was 90 µg/min and 207 µg/min, respectively (Kanno 1985). This resin-fractionation approach may have utility in the analysis of waters for acrylamide (especially by HPLC). Several methods have used simplified versions of this for sample clean-up prior to chromatography (e.g., Brown, Rhead, and Bancroft 1982; Freshour et al. 1985).

ANNOTATED BIBLIOGRAPHY OF METHODS FOR ACRYLAMIDE DETERMINATION

Each of the major references on determination of acrylamide in water, polymers, and other matrices is summarized in the following annotated bibliography; selected references have also been reviewed on the determination of polyacrylamides. After the authors' names and publication date are listed their affiliations, and notation is made if only an abstract was available for review. The references are presented in chronological order to maintain an historical perspective, which is important since the literature is so fragmented. Note that the annotations are not completely written in full, grammatically correct sentences to make the summaries less wordy.

TRACE ANALYSIS OF WATERS

Notes on Bromination of Alkenes

Most of the methods that have been developed specifically for trace analysis (i.e., sub-ppb concentrations) of acrylamide in water rely on the aqueous-phase ionic bromination at acid pH of the α,β -unsaturated bond to yield 2,3-dibromopropionamide (α,β -dibromopropionamide; CAS Registry No. 15102-42-8). Although nearly all researchers have synthesized their own standard reference compound, 2,3-dibromopropionamide is now available commercially (from TCI American [American Tokyo Kasei] Organic Chemicals, 9211 North Harborside St., Portland, OR 97203; 800-423-8616; cat. no. D1378; mp=133°C; available in lots of 25 g [\$46.55] or 500 g [\$391.55]). It is unstable under alkaline conditions. Any trace-analysis method that uses preconcentration from solvent must consider the possibility of hydrolysis of this dibrominated derivative if the sample is taken to dryness. The rationale for forming this derivative is two-fold: (1) greatly enhanced sensitivity (and much lower detection limits) when using halogen-specific or halogen-sensitive detectors (e.g., electron capture), and (2) greatly decreased polarity, enhancing solvent extraction from water (acrylamide itself cannot be extracted from water with solvents that can be further concentrated by evaporation). A side benefit of bromination is that thermal polymerization of acrylamide during gas chromatography is avoided.

Although numerous methods have used this approach, none has mentioned the competing reaction -- bromination of the amide-N. N-Halogenation is easily accomplished, and the N-halo derivative can have two different fates, depending on the pH of the reaction medium (cf. N-Halogenation vs. The Hofmann rearrangement). Under alkaline conditions, bromination results in transient formation of the N-bromoamide, which then decomposes to an amine with loss of one carbon atom (The Hofmann rearrangement). In contrast, under acidic conditions, the nitrogen loses the bromine to hypobromous acid, returning the parent amide; the bromine is then available to attack the double bond. The latter condition is employed in nearly all of the bromination reactions that are used to facilitate extraction. Neutral conditions yield an equilibrium mixture of the N-bromoamide and hypobromous acid, although one recent method reports the formation of N,N-dibromo derivatives after bromination of neutral solutions (presumably with molecular bromine) (Aizenberg, Goryunova, and Dvornikov 1987).

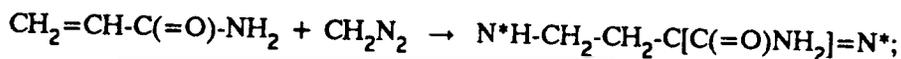
Essentially none of the publications on trace analysis has addressed the issue of the mechanism of ionic bromination (i.e., by bromide ion). The mechanisms of alkene bromination are extremely complicated and have yet to be fully elucidated. A recent study (Farook, Viswanathan, and Ganesan 1984) has provided data indicating that most of these studies have probably overlooked side reactions that would adversely affect the recovery of the dibromo derivative as well as lead to less reproducibility. They showed that hypobromous acid does not play a role in the reaction (100-fold variations in the concentration of perchloric, sulfuric, or nitric acids had no effect on rate constants); they also discounted the role of bromine cation ("positive" bromine) since they used such high concentrations of bromide anion. They concluded that the reaction was effected mainly by Br_2 and Br_3^- ; the relative roles of each of these reactants is profoundly affected by the ionic strength of the aqueous phase; the rate constant for Br_2 increases with increasing ionic strength (and that for Br_3^- decreases). Multiple products occur depending on the ionic strength and Br^- concentration. These include, in addition to the 2,3-dibromopropionamide, two isomeric bromohydrins: 2-bromo-3-hydroxy- and 3-bromo-2-hydroxy-propionamide; the percentage of bromohydrins increases dramatically as the concentration of Br^- decreases. For example, with acrylamide, the resulting bromohydrin:dibromide ratio is 0.85 at a bromide concentration of 0.50M and is 4.2 at 0.10M. The reaction with molecular bromine is summarized as double-bond attachment with decomposition of an intermediate complex to a carbonium ion (Br^- is expelled), which rearranges to a bromonium ion. The bromonium ion readily forms the dibromide (by reaction with bromide) or, alternatively, forms bromohydrin or bromo nitrates by reaction with water or nitrates, respectively. The tribromide anion reacts in a parallel manner. These bromohydrin products may explain some of the anomalies reported in the various bromination/trace-analysis literature. As opposed to most investigators, Farook et al (1984) extracted the dibromo derivative from the aqueous phase with ethyl ether after sample-saturation with NaCl; the ethereal phase was dried over Na_2SO_4 and concentrated by evaporation.

The dibromo derivative itself can also participate in other reactions such as formation of the β -triethylammonium adduct salt by elimination of the β -bromine: $[\text{CH}_3\text{-CH}_2\text{-}]_3\text{N}^+\text{-CH}_2\text{-CHBr-C(=O)-NH}_2\text{-Br}^-$ (e.g., see: Katritzky et al. 1984); the α -bromine, however, is not amenable to elimination. The 2,3-dibromopropionamide can also react with primary amines or ammonia in the presence of barium hydroxide to form aziridine-2-carboxylic acids via cyclocondensation (e.g., Kitagawa, Yokoi, and Saito 1986); this is a starting point for the preparation of intermediates for various pharmaceuticals and agrochemicals.

The confusion over bromination of acrylamide is evident from the various conflicting opinions put forth by different researchers as to whether acrylamide is even susceptible to olefinic bromination. This controversy cannot be resolved in this report.

• **Mattocks 1968** (Toxicology Research Unit, Medical Research Council Laboratories, Surrey, England)

Few colorimetric methods have been developed specifically for acrylamide. This work was an early colorimetric method for acrylic amides (and esters) as well as for the intermediate reaction products (pyrazolines) themselves. Diazoalkanes react with α -unsaturated carbonyl compounds to yield pyrazolines (five-membered heterocycles containing the -N-N= \rightleftharpoons -N=N- tautomeric group). Mattocks applied this reaction to acrylamide:



this reaction is rapid in methanol-ether, and the pyrazoline product has a melting point of 97°C. Acidic Ehrlich's reagent (4-dimethylaminobenzaldehyde) forms a strongly yellow product (presumably a Schiff's base) with the pyrazoline ($A_{\text{max}} = 440 \text{ nm}$; molar absorptivity = 47,600); 4-dimethylaminocinnamaldehyde gave a stronger and more stable purple product ($A_{\text{max}} = 538 \text{ nm}$; molar absorptivity = 92,200). These colored products formed rapidly at room temperature and were stable for hours.

Samples must contain acrylamide in the range 2-20 μg in dry form (moisture not exceeding 0.1 mL). TRIS (1 mL) is added (stabilizing the results for an unknown reason) followed by 2 mL of ethereal diazomethane

(10-30 min reaction time); the pyrazoline derivative is very volatile, so a concentration step cannot be used here. One of either 4-dimethylaminoaldehyde reagents is then added, and the absorbance is measured against a standard curve.

The method is subject to numerous interferences, including pyrroles, indoles, aromatic amines, hydrazines, pyrazolines, and carbonyl compounds in general. *N*-Substitution on amides does not inhibit pyrazoline formation, but substituents on the unsaturated carbons do inhibit reaction. With respect to adapting this method to HPLC, the major problem is that the colored product is highly susceptible to solvent composition; for example, acetone almost completely discharges the color, probably because it is a carbonyl compound. It is not known whether this method could be adapted for trace analysis in complex mixtures; perhaps chromatography of just the pyrazoline would be sufficient.

The author apparently was unaware of work done by MacWilliams, Kaufman, and Wailing (1965) (see: Formulation Analysis).

• Croll and Simkins 1972 (The Water Research Association, Medmenham, England)

This was the first major method reported for determining trace amounts of acrylamide monomer; it allowed detection of 0.25 $\mu\text{g/L}$ (ppb; with *rsd* of 20%) in river water by GC-ECD (detection limit of 0.1 $\mu\text{g/L}$) after bromination of the double bond via irradiation with UV light; according to Hashimoto (1976), this method suffered from low yields of brominated product (because of the free-radical nature of reaction), from the necessity of a UV light source, interference from ammonium ions, and inability to eliminate interferences by clean-up methods.

This is a relatively involved and cumbersome method. At the time of its development, however, it was the only method available for determining sub-ppb levels of acrylamide in waters. The method involves the very tricky bromination of acrylamide with bromine water at pH 1.0 catalyzed by UV light; the method for preparing 2,3-dibromopropionamide standards by reacting acrylamide in water (in the dark) with KBr in aqueous 6N H_2SO_4 and destroying the excess bromine with sodium sulfite was found to be unsuitable for acrylamide concentrations of less than about 30 ppb (yields were less than 3%); it is unclear why this was true. It was found that the intensity of UV radiation and especially pH were extremely important (pH 0.5, 1.0, 2.0, and 5.0 gave yields of 49, 62, 41, and 13%, respectively); yield was at a maximum at pH 1.0. The bromine water also had to be added very rapidly; use of sodium sulfite instead of sodium thiosulfate for stopping the bromination gave fewer chromatographable interferences. Note that sulfite can add to the double bond of any remaining, unreacted acrylamide to form β -sulfopropionamide; in fact, this is the method used to scavenge residual acrylamide from PAM (see: Reactions of the Olefinic Group: α,β -Unsaturated alkenes); sulfite can also dehalogenate and react with carbonyls.

UV irradiation proved tricky to optimize (too much presumably resulted in photodegradation of the resultant dibromopropionamide), and any resulting optimized conditions gradually changed as the operational hours of the lamp increased. Furthermore, the optimized yields of the brominated product were a function of acrylamide concentrations, with yields decreasing as the acrylamide concentration decreased (yields of 50-66% at 1-500 ppb and a yield of 34% at 0.25 ppb); precision was much better than accuracy (*rsd* values ranged from ± 5 to $\pm 10\%$ at 50 and 0.25 $\mu\text{g/L}$, respectively). The type of water being analyzed also influenced recovery. This would have necessitated the use of standards made up in the candidate water, so a rather laborious "internal standardization" method was developed that involved analyzing each sample twice (thereby increasing the *rsd*).

At the 0.25-ppb level in river water, interferences could not be eliminated with different GC stationary phases or with alumina, silica, or Florisil clean-up (this chromatographic separation problem could probably be solved with modern capillary columns, e.g., see: Andrawes, Greenhouse, and Draney 1987). The interferences were partially reduced after bromination by the use of a pre-extraction with diethylether at pH 1.0 (where the amide carbonyl group is protonated) followed by the extraction of 2,3-dibromopropionamide

at pH 3.0; the technique used for addition of hydroxide is also critical because localized concentrations of hydroxide ion can destroy the dibromo analyte. Diethylether was chosen as the extraction solvent, even though ethyl acetate gave a larger partition coefficient, because of the importance in being able to concentrate the extract (e.g., 100 fold) to increase the concentration of analyte; ethyl acetate also contributed interferences when concentrated. Final samples were chromatographed on a 1-m X 3-mm i.d. column packed with 60/80 mesh acid-washed DMCS-treated Chromosorb W coated with 10% FFAP ("free fatty acid polyester": Carbowax 20M terminated with 2-nitroterephthalic acid). An additional problem was the occurrence of materials with longer retention times than the analyte (6 min.); this would necessitate the use of a back-flush or column-switching device.

The author's laboratory has since applied this method to the determination of acrylamide in effluents and river water down-flow from several industries (e.g., Croll, Arkell, and Hodge 1974). For example, effluent from a clay pit was found to contain acrylamide at 16 $\mu\text{g/L}$, giving a concentration of 1.2 $\mu\text{g/L}$ in the receiving stream; at a water-works intake downstream, the concentration was 0.3 $\mu\text{g/L}$. Crude sewage from a manufacturer using acrylamide contained 1.1 mg/L, which was reduced to 0.28 mg/L at a sewage treatment facility.

- **Arimitsu 1974** (Japan) (no copy available)

Neither this reference nor its abstract could be located, but it is cited by Hashimoto (1976) as one of first methods (but postdating Croll and Simkins 1972) for determining trace amounts of acrylamide monomer; it is also cited by Arikawa and Shiga (1980), Fujiki et al. (1982), and Suffet et al. (1975). Essentially, it appears to have been another rendition of the Croll and Simkins (1972) paper. It allowed detection of 0.25 $\mu\text{g/L}$ by GC-ECD after bromination of the double bond via irradiation with UV; the author had suggested that ethyl acetate was best solvent for extraction of brominated product but could not sufficiently purify it (the same problem reported by Croll and Simkins 1972).

- **Hashimoto 1976** (Kitakyushu Municipal Institute of Environmental Health Sciences, Japan)

Derivatization based on bromination of the double bond by an ionic mechanism using potassium bromide and concentrated hydrobromic acid (under acid conditions in the dark) as opposed to a free-radical mechanism (yields over 80%), salting-out extraction of the resulting 2,3-dibromopropionamide with ethyl acetate, and clean-up with Florisil. Rate of bromination in water with bromine water is very slow because of the electron-withdrawing effect of the carbonyl group; bromide ion is therefore needed. Detection limit for an aqueous standard is 0.32 $\mu\text{g/L}$; instrument detection limit (GC-ECD) is ca. 1.0 pg. Percentage recoveries from spiked samples of river water, sewage effluent, and sea water (at 4 $\mu\text{g/L}$) were 99.4 (± 2.5), 101.3 (± 3.0), and 98.0 (± 3.2), respectively. As opposed to the UV-bromination method, ammonium ion (8%) did not interfere. This bromination method is also referred to as the bromate-bromide method (Siggia and Hanna 1979, p. 383) and can be enhanced by use of mercuric sulfate as a catalyst; all compounds containing unsaturated sites will be brominated.

Used 5% FFAP (free fatty acid polyester) on Chromosorb W; Croll and Simkins (1972) used 10% FFAP but interferences in the potassium bromide (almost all of which have longer retention times) could not be resolved. Used ethyl acetate as suggested by Arimitsu (1974) and by Croll and Simkins (1972), but also used salting out with sodium sulfate to reduce the volume of solvent required. Obtained essentially 100% recoveries for the extraction step at spiking levels of 10 $\mu\text{g/L}$. Analyte retention time was 4 minutes.

Cutié and Kallos (1986) claim that they tried derivatization with Br_2 with GC-ECD, but the method suffered from problems with the detector and interferences. Brown and Rhead (1979) also contend that ECDs are difficult to maintain uncontaminated.

- **Nakamura 1977** (Japan) (abstract only)

An example of one of the various miscellaneous publications in non-English journals that has explored the ionic-bromination GC-ECD method. This particular one claims to use the "Florisil clean-up method" (presumably like that of Arikawa and Shiga [1980]). Presumably, the 2,3-dibromopropionamide is extracted, the organic-phase liquid removed and exchanged for a less-polar solvent, which is then passed over Florisil; interferences are washed off and then the analyte is possibly eluted into a small volume. The sample was then chromatographed on an ethyleneglycol succinate (EGS) column (15%). The detection range was notable in that it was reported to be 0.1-1,000 ng/L (0.1 ppt to 1 ppb); this is the lowest detection limit reported for any method. For municipal water, artificial seawater, and sewage sludge, the accuracy was reported to be 86-93% with an rsd less than 2%.

- **Brown and Reid 1979** (Plymouth Polytechnic, Plymouth, Devon, England)

Essentially an improvement on the HPLC method of Husser et al. (1977) (see: Formulation Analysis) by improving the extraction/concentration procedure. Tried anionic, cationic, and hydrophobic (XAD-2) resins and solvent extraction with hexane, diethyl ether, and ethyl acetate among others, all without success for underivatized acrylamide. Used the bromination method of Hashimoto (1976) to form the α,β -dibromopropionamide and thereby effected efficient extraction with ethyl acetate. The organic extract was concentrated to dryness (this critical step removed the gross interference that any residual ethyl acetate will display at 196 nm), and the residue was redissolved in a small quantity of water, allowing an 800-fold concentration factor; Poole et al. (1981), however, claim that evaporation to dryness results in partial and uncontrolled loss of the derivative. The concentrated sample was then chromatographed on a Spherisorb 10- μ m ODS HPLC column (retention time ca. 4 min.); a bonded cyano-phase column could not effect sufficient resolution.

Collected samples were boiled if storage was to be for longer than 16 hours (to prevent biodegradation); this may be an ill-advised method of preservation, possibly resulting in acrylamide hydrolysis (aseptic membrane-microfiltration would be better). Preextractions were done with ethyl acetate and hexane (for sewage) to minimize interferences by extraneous organic materials. Samples (100-mL) were prepared according to the bromination method of Hashimoto (1976). The extracted and dried residue was redissolved in 120 μ L of distilled water, and up to 100 μ L was chromatographed with a mobile phase of distilled water and detector set at 196 nm (A_{max}). They synthesized their own α,β -dibromopropionamide reference material; standard curve was prepared through the range 0.02-8.0 μ g/L, and the slope was not affected by the type of UV detector lamp or by changing to other Spherisorb ODS columns. Chromatograms from environmental samples spiked with acrylamide show the analyte peak on the shoulder of a large, unresolved envelope. In contrast to Hashimoto's 100% recoveries for spiked samples, only 85% recoveries were obtained, probably because the step requiring complete removal of the ethyl acetate also involves partial loss of the analyte to sublimation. For acrylamide-spiked estuarine waters, sea water, sewage effluent, china clay process waters, and potable waters, the recoveries averaged 70% over the range of concentrations from 0.2 to 8.0 μ g/L.

Noted that the HPLC method has several advantages over the GC-ECD method of Hashimoto (1976), including speed of analysis and stability of the standard curve. The method in general, however, appears to definitely suffer from gross interference since the analyte peak does not display baseline resolution. An instrument detection limit of about 2 ng compares unfavorably with 1 pg for GC-ECD (Hashimoto 1976).

Cutié and Kallos (1986) stated that LC-UV was tried to avoid problems encountered by GC-ECD (Hashimoto 1976), but the procedure still suffered from interferences.

- **Brown, Rhead, and Bancroft 1982** (Plymouth Polytechnic, Plymouth, Devon, England)

This paper has rarely been cited by subsequent investigators, possibly because the detection limits are still too high for trace analysis. They maintained that the previous methods had high detection limits (e.g., 100-1000 ppb), which were inadequate for samples, such as sewage, that contain many interferences. Avoided the use of bromination to effect preconcentration of acrylamide (e.g., Brown and Rhead 1979); instead, they used a hydrophobic/mixed-bed ion-exchange resin clean-up step coupled with HPLC to give a rapid method that could detect down to 5 $\mu\text{g/L}$ of underivatized acrylamide. Maintained that derivatization via bromination was tedious and time-consuming and that it was difficult to achieve reproducible bromination; only 16 samples could be analyzed by one person in two days (32 samples per person-week). This work is an extension of that by Skelly and Husser (1978) (see: Formulation Analysis) and antedates the work of Freshour et al. (1985) (see: Trace Analysis of other Matrices).

Samples were membrane-filtered and stored at 4°C prior to analysis; no acrylamide loss was noted after 1 week of storage. The aqueous sample was passed through the mixed-bed resin that consisted of Amberlite XAD-2 (hydrophobic resin) followed by anion and cation exchange resins; the first effluent volume was discarded. Acrylamide is totally unretained under these conditions; this step serves not as preconcentration, but rather to separate the acrylamide from numerous potential interferences. Of the second volume, a 100- μl portion was injected onto a Hypersil ODS column using water as mobile phase and detection at 202 nm (at maximum sensitivity: 0.004 AUFS); the wavelength of maximum acrylamide absorbance (197 nm) was not used because the remaining inorganic ions, which eluted immediately before acrylamide, absorbed even more strongly. For all other reverse-phase columns tested (Spherisorb ODS; Partisil PXS ODS-2; μ Bondapak ODS), a major contaminant always eluted directly in front of acrylamide; when the Hypersil ODS column was used, however, the acrylamide eluted before the contaminant. The pressure peak and inorganic ions eluted at 1-2 minutes, followed by acrylamide (3 minutes) and the major unidentified interference (4-10 minutes). The method barely provides resolution of acrylamide from these interferences.

The use of the newer, more-efficient reverse-phase columns that are also deactivated may make this method more usable. Although acrylamide is not strongly basic, these columns would probably give better peak symmetry and resolution; the addition of a trialkylamine modifier to the mobile phase could also help. The most recent addition to this new generation of columns is DuPont's ZORBAX Rx; this column supposedly provides better peak symmetry than Supelcosil-DB, which is more suited to this type of analysis than is Hypersil ODS (a much more acidic column).

The resin clean-up step allowed for this direct determination of underivatized acrylamide in river water, sewage, china clay effluents, and paper-mill process waters. The small analyte peak was bracketed by two large peaks for samples that were spiked with 5-10 $\mu\text{g/L}$ of acrylamide. For untreated wastes, the interferences were too great, and the standard additions method was used; detection limits were then increased to 20-100 $\mu\text{g/L}$. The method is therefore suitable only for rapid screening.

- **Andrawes, Greenhouse, and Draney 1987** (American Cyanamid Co., Stamford, CT)

One of the most recent articles on trace determination. Its most significant finding is that all bromination-GC methods (e.g., Croll and Simkins 1972; Hashimoto 1976) have been flawed in assuming that the species detected by ECD has been the α,β -dibromopropionamide derivative; they maintain that this oversight has resulted from the prior verification of structure using standard reference material and direct-introduction MS as opposed to GC-MS. By using GC-MS, they found that 2,3-dibromopropionamide is not always stable, being dehydrobrominated at the front end of a packed FFAP column to give the monobrominated species, 2-bromopropenamide: $\text{H}_2\text{C}=\text{C}(\text{Br})-\text{C}(=\text{O})-\text{NH}_2$; the possible conversion to either cis- or trans-3-bromopropenamide was ruled out by ^1H NMR. This conversion is perhaps reproducible on a given column, but it is an unknown function of column activation; significantly, the conversion occurs at higher efficiencies as the analyte concentration decreases. Dehydrobromination is probably a function of basic sites in the column packing; it did not occur on a fused-silica FFAP-bonded capillary column. Andrawes et al. (1987)

were unaware of the study by Poole et al. (1981), who were the first to notice that the dibromo derivative was thermally unstable (they recommended not exceeding 180°C and using FFAP as the stationary phase). The authors seemed unaware of the work by Cutié and Kallos (1986) who also had earlier pointed out that the dibrominated derivative was not thermally stable. Bromination (adopted from Hashimoto 1976, for acrylamide) has also been used for the derivatization/extraction of acrolein from water (Nishikawa, Hayakawa, and Ikeda 1986); these authors also suspected that the brominated derivative had limited stability in water, probably forming HBr (and possibly a bromohydrin, but not suggested by the authors).

For MS with EI fragmentation, the dibromo derivative does not yield molecular ions (m/z 229, 231, and 233, for the three combinations of Br-79 and Br-81), but rather an m/z of 150 (and 152) from the loss of one bromine atom; in contrast, the monobromo (dehydrobrominated) derivative yields a molecular ion at m/z 149 (and 151) (because of the additional loss of the hydrogen). While any molecular fragment retains a bromine, characteristic m/z ion pairs result (separated by two mass units) because of the two equally abundant, natural bromine isotopes (79 and 81). Because the dehydrobromination process is not controllable, they recommended that the dibromo derivative not be analyzed, but rather that it first be dehydrobrominated. This is especially necessary if a capillary column is to be employed.

Dehydrobromination of the 2,3-dibromopropionamide to 2-bromopropenamide can be easily effected under mildly basic conditions by adding triethylamine to the initial dibromo derivative (50 μ L to 1 mL of solution). The reaction appears to be quantitative, reproducible, almost immediate, and occurs at room temperature. Although not mentioned by the authors, this reaction is probably effected by way of formation of the β -triethylammonium adduct salt: $[\text{CH}_3\text{-CH}_2\text{-}]_3\text{N}^{\oplus}\text{-CH}_2\text{-CHBr-C(=O)-NH}_2\text{-Br}^{\ominus}$ (e.g., see: Katritzky et al. 1984) (acrylamide itself is unreactive towards triethylamine; Geisthardt and Kruppa 1987); the α -bromine, however, is not amenable to elimination. Using a DB-5 capillary column (mildly polar), a linear response on ECD was obtained for the concentration range of 330 ppt (ng/L) to 660 ppb (μ g/L). The capillary column may make cleanup of environmental samples unnecessary, and since it has more plates than the packed columns that have been used to date, the reduced ECD signal from the monobromo derivative is thereby compensated. This publication is the first in which 2,3-dibromopropionamide was not synthesized, but rather it was commercially purchased.

- Gortseva and Dregval 1987 (USSR) (abstract only)

Another GC-ECD method for acrylamide based on formation of the dibromo derivative. The detection limit in water is reported as 0.2 μ g/L; this is slightly lower than that reported by Andrawes et al. (1987) probably because the analyte is the dibromo derivative. The article is in Russian.

- Thompson and Karasek 1987 (Univ. Waterloo, Canada)

Not a method per se for acrylamide, but rather a method for trace analysis of organic microcontaminants in inorganic water-treatment chemicals. For aqueous samples, they used a three-fold extraction with dichloromethane followed by drying over sodium sulfate. Since salts have a low solubility in dichloromethane, the organic extract was passed through an activated silica column to remove the salts; their accumulated deposition at the head of GC columns creates active sites that result in peak tailing. Samples were analyzed by MS using both electron ionization (EI) and positive ion chemical ionization using methane. Acrylamide was found in one batch of polyelectrolyte.

TRACE ANALYSIS OF OTHER MATRICES

- McLean, Mann, and Jacoby 1978 (Dow Chemical Co., Midland, MI)

Applied the differential-pulse polarographic formulation-analysis method developed by Betso and McLean (1976) (see: Formulation Analysis) for the determination of acrylamide in air samples and wipe samples (for use in industrial hygiene monitoring). Detection limit of 0.5 mg/L with rsd of 3.7%.

- **Arikawa and Shiga 1980** (Ebara-Infilco Co., Tokyo, Japan) (in Japanese; English abstract)

One of the many papers published by Japanese researchers over the years using the bromination GC-ECD technique. Determined acrylamide in various agricultural crops. Sample was ground and extracted with water. The filtrate was adjusted to 500 mL, and the pH of a 100-mL sub-sample was adjusted to 1.0 with sulfuric acid; 30 g of KBr and 10 mL of 0.1 M potassium bromate was added; bromination efficiency was found to be optimal at pH 1 and began to fall off precipitously above pH 3. The solution was extracted twice with 50 mL of ethyl acetate (at 10 ppb, one extraction gave 90% recovery), and the organic phase was removed by evaporation. The residue was redissolved in benzene and passed through a Florisil column. The column was washed with 2% ethanol in hexane, and the dibromo derivative was eluted with acetone:benzene (2:3, v/v). The eluate was concentrated by evaporation, and a portion was injected on a 10% DEGS packed column (with 2% KBr) at 170°C; detection was by GC-ECD (pulsed). The authors also evaluated columns packed with 10% FFAP and 15% EGS and found that these two gave retention times greater than 11 minutes, whereas the DEGS column gave a retention time of 8 minutes with better analyte separation from potential interferences. The method detection limit was about 0.5 µg/L. Overall recoveries varied from 96-98% (for 5 ppb) to 80-94% (for 0.5 ppb); rsd values were less than 10%.

- **Poole et al. 1981** (Wayne State University; University of Houston; and Albert Einstein College of Medicine)

Claim to have improved the ionic bromination method of Hashimoto (1976) for application to biological tissue homogenates. To 50 mL of distilled water was added 0.5 mL of tissue homogenate, 7.5 g of potassium bromide, and sufficient hydrobromic acid to give a pH of 1-3. Saturated bromine water was added (in the dark), and the reaction was allowed to continue for 2 hours at 0°C; excess bromine was then decomposed by adding sodium thiosulfate, and sodium sulfate (15 g) was added to promote salting out during solvent extraction (salting out is required since the dibromo derivative still has significant water solubility). The reaction mixture (250 mL) was transferred to a separatory funnel and extracted with 15 mL and then 10 mL of ethyl acetate. The pooled organic phase was dried over sodium sulfate, centrifuged, and evaporated to 0.5 mL under nitrogen at 67°C (note that no sample concentration has been effected). A 2.0-µL portion was injected on a Gas-Chrom Q 5% FFAP packed column (6 ft. X 2-mm i.d.) held at 155°C; a constant-current pulse-modulated Ni⁶³ ECD was maintained at 350°C.

Found that the overall recovery of 2,3-dibromopropionamide is 80-90% for acrylamide concentrations in the 10- to 1000-ppb range. Since the solvent-extraction step was shown to have an efficiency of 96-100%, they surmised that the main loss occurred because of incomplete bromination and possibly because of the solvent-reduction step. Indeed, they maintain that methods necessitating complete removal of solvent (e.g., the reverse-phase HPLC technique of Brown and Rhead 1979) or solvent-reduction temperatures in excess of 80°C will show loss of the dibromo derivative; more than a 70% loss was demonstrated when the solvent was completely removed at 70°C and the residue redissolved in water. Like most published reports on bromination of the double bond, these authors also erroneously thought that the acidic pH is required because acrylamide is hydrolyzed under basic conditions; instead, acidic pH promotes double-bond halogenation as opposed to N-halogenation (e.g., see: The Hofmann rearrangement) and C-dehalogenation.

This was the first study (apparently unknown to Andrawes et al. 1987) that demonstrated that the dibromo derivative was thermally labile. They found significant decomposition on Carbowax 20M and STAP columns at 150°C as well as on all-nickel columns. They found that silanized glass was necessary (for packed columns). In addition to FFAP, OV-225 also gave good results; column temperatures had to be held below 180°C.

They also evaluated the Hall detector in the halogen mode, but found that the minimum detection limit was 100 ng (this was expected since they have found the Hall detector to be 1000 times less sensitive to bromine than to chlorine); the Hall detector also showed a dramatic temperature dependence, whereas the

ECD did not. For acrylamide itself, the minimum detection limit by ECD was 15.3 ng as opposed to 9.5 pg for the dibromo derivative. The response for the dibromo derivative was linear in the ranges 10-100 pg and 50-170 pg. The derivative was found to elute from the FFAP column at about 4.5 minutes and barely showed baseline resolution from a pre-eluting "impurity" that occurred in all samples (at 4 minutes), including the blank. The method limit of detection was about 4.75 $\mu\text{g/L}$ (ppb) in the tissue homogenate; this limit was high because no concentration of the derivative was effected (i.e., the derivative was essentially transferred from the 0.50-mL sample to 0.5 mL of ethyl acetate). Presumably, much lower method detection limits could be achieved by preconcentration.

- Fujiki, Asada, and Shimizu 1982 (Institute of Community Medicine, University of Tsukuba, Japan)

Another paper by Japanese researchers on the use of the bromination/GC-ECD method. Although they applied the method for the first time to fish samples (in a study of bioaccumulation), Poole et al. (1981) antedated them by applying the method first to tissue samples. Study differed from most others in its evaluation of different packed columns for the dibromo derivative and in its investigation of optimal bromination conditions.

As opposed to most other investigators, they found DEGS and EGS stationary phases to be unacceptable because of rapid deterioration (especially when using ethyl acetate). They found the best peak symmetry and column stability with OV-275 (2%). Showed that increased amounts of potassium bromide (up to 10 g) increased the recovery of 2,3-dibromopropionamide; in contrast, recoveries stayed constant when increasing the potassium bromate concentration. These results are in accordance with Farook et al. (1984), who also demonstrated that a high bromide ion concentration was necessary to inhibit the side reaction of bromohydrin formation (Fujiki et al. [1982] used 2.5 M Br^{-1} , well above the 0.5 M used by Farook et al. [1984]). The minimum acidic bromination time for maximum recovery was 20 minutes.

An extractive cleanup step sometimes was used, involving bromination, extraction with benzene, back-extraction with water, and aqueous-phase extraction with water. Recoveries with this method were poor (ca. 12% with 7.5% *rsd* vs. 82% with 1.7% *rsd* with the direct ethyl acetate extraction). This is not surprising since the dibromo derivative is not very soluble in benzene. Furthermore, the recoveries (from fish homogenates spiked with 10 ppm acrylamide) were low because only one 1:1 partitioning was done. The detection limit was not investigated.

- Freshour et al. 1985 (Dow Chemical, Midland, MI)

This method represents the most sophisticated direct HPLC method published to date; essentially an extension of the work by Brown, Rhead, and Bancroft (1982). Portions of tissue-culture solutions are subjected to initial separation (0.001N aqueous sulfuric acid mobile phase) on a C_{18} column (Waters Radial-Pak), and the effluent corresponding to acrylamide is automatically switched to an Aminex 50W-X4 column (Bio-Rad Laboratories) for final separation; although not claimed by the authors, the ion-exchange column essentially served in place of the mixed-bed resin cleanup step of Brown, Rhead, and Bancroft (1982). Acrylamide was detected at 210 nm. The absolute method detection limit (in aqueous standards) was 5 $\mu\text{g/L}$ (ppb); the limit of quantitation in spiked tissue-culture solutions was higher (10 $\mu\text{g/L}$) because the acrylamide eluted on the tail of one of at least three major pre-eluting peaks; because of the lack of base-line resolution, peak heights instead of areas were used for quantitation. Average recoveries of acrylamide from spiked samples of 10-1000 $\mu\text{g/L}$ was 96.7%; the *rsd* at the low level was 2.3%. Sample analysis time was greater than 20 minutes. Acrylamide in spiked samples was stable for about a week at 1°C or several days when frozen. Again, the use of newer, more advanced deactivated, reverse-phase HPLC columns would probably improve the resolution problems that were encountered in this study. Next to the methods of Brown and Rhead (1979), this paper reported the lowest detection limit for any traditional HPLC method described to date.

- Cutié and Kallos 1986 (Dow Chemical, Midland, MI)

Polyacrylamide is used in the sugar industry as a flocculant. Naturally occurring amides in sugars are hydrolyzed at high temperatures in the presence of lime to give soluble lime salts and ammonia. The lime is then flocculated with polyacrylamides and other water-soluble polymers. Presence of the acrylamide monomer in the final product is therefore of concern.

Multidimensional LC/thermospray MS method for determining parts-per-trillion of acrylamide in refined sugar. Acrylamide is derivatized with bromine (hydrobromic acid and bromine water), separated by multidimensional reverse-phase LC (using heart cutting and column switching from one Partisil 10 ODS-2 column to another to enhance the separation of 2,3-dibromopropionamide from interferences), and detected by MS coupled to a thermospray interface; the derivative, dibromopropionamide decomposes in water; it was found to be very stable in acetonitrile. Direct determination of underivatized acrylamide (by selected ion monitoring using the protonated molecular ion at m/z 72 and the water adduct at m/z 90) was not possible in the complex matrix of sugar. Use of original LC methods encountered interferences by numerous nitrogenous compounds including asparagine and glutamine (ppm levels). Therefore, they decided to brominate the acrylamide so that it could be more selectively extracted from the sugar matrix (using purified ethyl acetate). The thermospray interface was found superior to the moving-belt interface since dibromopropionamide was found to be thermally unstable (EI yielded only a weak molecular ion at m/z 229; CI yielded a protonated molecular ion at m/z 230 but also a strong protonated acrylamide ion at m/z 72, indicating significant decomposition); in contrast, the thermospray (TSP) interface using chemical ionization showed a very intense protonated molecular ion at m/z 230 and a less-intense m/z 72 peak (for dibromopropionamide); when trifluoroacetic acid was added postcolumn, the additional protonated molecular ions at m/z 232 and 234 were obtained (from the protonated forms of Br-79/Br-81 and Br-81/Br-81). Recovery data for acrylamide spiked into sugar (corrected for background in water blank) ranged from 15% to 45% for concentrations of 0.2 to 2.0 ppb. Precision for dibrominated derivative standards was 3.2% at one standard deviation; the method was linear for over three orders of magnitude.

- Farkas and Tekel 1987 (Czechoslovakia) (abstract only)

Method for determining trace amounts of acrylamide in sugar. Sample is subjected to ionic bromination to form 2,3-dibromopropionamide, which is extracted into ethyl acetate. The organic extract is passed over silica gel for further purification. The eluate is analyzed by GC with alkaline flame ionization detection (nitrogen-selective). This is one of the only GC methods using bromination that does not resort to the ECD. Used a cross-linked OV-1 glass capillary column (14 m X 0.3 mm). Recoveries for samples containing 20 and 100 $\mu\text{g}/\text{kg}$ were 77.1% and 70.0%, respectively. The detection limit in beet sugar was 1 $\mu\text{g}/\text{kg}$ (1 ppb).

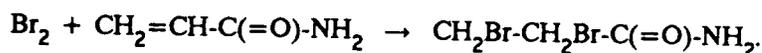
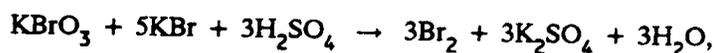
FORMULATION ANALYSIS

Formulation methods (i.e., determination of impurities in a formulated product) are used for determining unreacted acrylamide concentrations from 0.01% (100 ppm) to 20% for purposes of manufacturing process quality control. Such methods are generally not applicable to trace determination in water, but were and still are used to set the maximum permissible monomer contents of the polymer that would yield particular target monomer concentrations in the treated water. Numerous publications have appeared in obscure foreign journals on formulation analysis for acrylamide by polarography; only a few are summarized in this section.

- Narita, Uchino, and Machida 1964 (Univ. Kyoto, Japan) (abstract only)

This work represents one of the first methods to use bromination of the acrylamide double bond. Studied the addition of Br to acrylamide. Used a bromate-bromide mixture (KBrO_3 and KBr) in H_2SO_4 ;

quantitation was by iodometric titration of the excess Br remaining after reaction with acrylamide. The addition was found to proceed quantitatively without side reactions with good reproducibility:

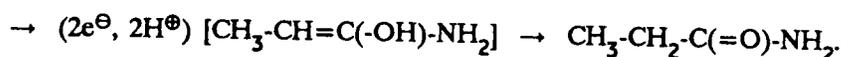
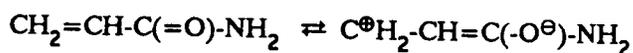


- **MacWilliams, Kaufman, and Waling 1965** (Dow Chemical, Midland, MI)

Presented two methods for determining acrylamide in polyacrylamide based on selective extraction of the monomer from the polymer and quantitation by polarography or UV spectrophotometry. Methanol/water (80/20 v/v; this ratio was critical) is used to solubilize the acrylamide while minimizing the polyacrylamide solubility. For UV analysis, acrylic acid, which has a nearly identical UV absorption spectrum, was removed by passage through ion exchange resin. The polarographic method was much more specific for acrylamide but also less sensitive (0.01-0.5%). The UV method (using a baseline extrapolation method at 240 nm) gave a working range of 0.005-0.1%.

- **Vajda 1967** (Mining Research Institute, Budapest, Hungary)

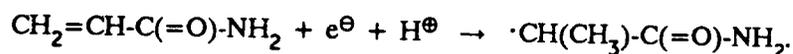
Investigation of the mechanism of electrode reduction of acrylamide and application of the method to determining acrylamide in polymers. In neutral solutions of tetramethylamine chloride or lithium chloride, acrylamide was reduced at -1.87 V (S.C.E.; standard calomel electrode). Found that the double bond becomes saturated by addition of two protons and two electrons. Maintained that neither the double bond nor the amide group would be reducible by themselves, and that the electronic conjugation of the two brings about a mesomeric electron shift (towards the carbonyl oxygen, producing a keto-enol rearrangement) allowing the reduction to propionamide:



In strongly alkaline solution, the enol imide form is more stable than is the enol hydroxyl form in acidic/neutral solution. The electronegative nitrogen accepts a proton more readily than the carbonyl group in neutral solutions, and therefore the molecule is reduced at a higher potential (-1.70 V). Used the latter method to determine the acrylamide content in Sedosan. Acrylamide monomer contents of less than 0.1% could be determined in 10-15 minutes.

- **Gorokhovskaya and Markova 1968** (Tashkent. Tekst. Inst., Tashkent, USSR) (abstract only)

Polarographic determination of acrylamide in anhydrous methanol in the presence of $(\text{CH}_3\text{CH}_2)_4\text{NI}$ gives sharp polarographic waves with a half-wave potential of -1.72 V. The reduction is a one-electron process:



- **Croll 1971** (The Water Research Association, Buckinghamshire, England)

Extensive study of methods for quantitative extraction of acrylamide from polyacrylamides. Compared cold aqueous-methanolic extraction with Soxhlet extraction for 20 polyacrylamide polymers or copolymers of acrylic acid (nonionic, anionic, and cationic). Essentially verified the conclusion of MacWilliams, Kaufmann, and Waling (1965) by showing that the optimum extraction solvent was methanol/water (80/20), which causes the polymer to swell without dissolving; inferior recoveries of acrylamide were obtained using other aqueous-methanol concentrations, methanol, chloroform, or dimethylformamide. Extraction conditions involved cold shaking for 24 hours. Soxhlet extraction proved inferior without the addition of 2% glacial acetic acid,

which presumably neutralized base that was being extracted from the polymer and then serving as a reactant to create another product (not acrylic acid; hypothesized to be a methoxylated derivative or acrylamide adduct with itself or acrylic acid). Extracts were chromatographed on Chromosorb W with 20% Carbowax 20M using an FID. Acrylamide eluted in 4.5 min; buildup of nonvolatile contaminants was noted to decrease column performance. For some polymers, an interference was noted. This required a cleanup step but ion-exchange resins, silica gel, alumina, and Florisil were not successful; thin-layer chromatography was required to effect the separation prior to GC. The calculated detection limit was 0.0004% of acrylamide in polymer; routine determinations of 0.05% gave rsd values of 4%. Incorrectly cites Vajda (1967) as a UV-spectrophotometric method when it is actually a polarographic method.

• Schmötzer 1971 (Germany)

Obsolete method for determining acrylamide in polyacrylamide by using standard, liquid column-chromatography. The polymer was dissolved in water, and its viscosity was decreased by adding electrolytes and by mechanical shearing. The mixture was separated on a Dowex 50W-X8 resin in the protonated form; the polymer eluted first, and the acrylamide (and acrylic acid) eluted later and were detected at 200 nm. Detection limits were $1\mu\text{g}/0.5\text{ mL}$ (2 ppm).

• Rapaport and Ledovskikh 1972 (Vses. Inst. Gig. Toksiokl. Pestits., Kiev, USSR) (abstract only)

Method for detecting acrylamide in (presumably) aqueous polymer extracts by heating an aqueous extract at 95-100°C for 1 hour with a solution of iodine chloride (0.001N HCl). The reagent added to the double bond to give 2-chloro-3-iodopropionamide: $\text{CH}_2\text{I}-\text{CHCl}-\text{C}(=\text{O})-\text{NH}_2$. The absorbance of the resultant colored solution was determined at 350 nm. Detection limit was 0.5 mg/L.

• Betso and McLean 1976 (Dow Chemical Co., Midland, MI)

Improved on the polarographic method of MacWilliams et al. (1965). Using more advanced instrumentation (differential pulsed polarograph instead of the older direct-current instrument), they were able to greatly lower the detection limit to less than 1 ppm. The acrylamide reduction peak was well-defined and resolved from the background. They applied the method to determination of acrylamide in PAM, but the method should be suitable to other matrices. Acrylamide is reduced at the dropping mercury electrode at about -2.0 V (S.C.E.). Extracted PAM with methanol/water (80/20); also noted that poorly defined polarograms were obtained in an all-aqueous system. The methanolic extracts were exposed to mixed-bed ion exchange resin for 20 min. to eliminate ionic species; no loss of acrylamide at 1 ppm was noted at this step. Of the resin-treated sample, 10 mL was added to the polarographic cell together with 0.5 mL of 1 N tetra-n-butylammonium hydroxide (supporting electrolyte). Quantitation was done by known addition of acrylamide.

The recovery of acrylamide through the PAM-extraction resin-treatment step was greater than 90% with overall precision of 5%. At -2 V, major interferences are alkali cations; these were removed by the ion-exchange resin. Other potential interferences evaluated included acrylic acid (protonated), which was reduced at -1.7 V and did not interfere; acrylate esters do interfere; acrylonitrile also interferes but can be easily removed because of its high relative volatility compared with acrylamide (purging with nitrogen gas for 30 min. removes more than 90% of the acrylonitrile but no acrylamide); acrolein does not interfere but formaldehyde does (although it gives a response 1/20th that of acrylamide). Polarography seems ideally suited to formulation analysis, especially since the instrumentation is less expensive than GC or HPLC.

• Husser et al. 1977 (Dow Chemical, Midland, MI)

Two methods for determining residual acrylamide monomer in aqueous and nonaqueous dispersed-phase polymeric systems (i.e., in the formulated product itself, not in water samples). Both procedures used an internal standard of benzamide and an extraction-precipitation step to separate the monomer from the polymers. Two LC methods were developed: (1) nonaqueous normal-phase separation (detection limit of

10 ppm), and (2) aqueous ion-exclusion separation (detection limit of 0.1 ppm). Cites several spectrophotometric methods and says that polarographic methods are particularly prone to interferences, especially by substituted acrylamides (such as *N,N'*-methylenebis[acrylamide] and *n*-methylol acrylamide cross-linkers) and acrylates (such as ethyl and butyl acrylates), which are all indistinguishable. When analyzing aqueous dispersed-phase systems using the method of Croll (1971), they found excellent specificity and sensitivity but very poor recoveries of spikes (although reproducible) (this comment agrees with Hashimoto [1976]).

(1) Nonaqueous method: 10 g of nonaqueous dispersed polymer is added dropwise to 50 mL of methanol containing the internal standard benzamide; after stirring for 2 h, it is centrifuged. Five μL of the supernatant fluid (which contains the acrylamide) is injected into a normal-phase column (cyano-bonded silica) with a mobile phase of 15% methanol and 85% dichloromethane. The benzamide and acrylamide have near-baseline resolution (at about 4 minutes); subsequent chromatograms show acrylamide at 8 mins with resolution from acrylic acid/ethylacrylate (3 min), *N,N'*-methylenebis(acrylamide) (5 min), and *n*-methylol acrylamide (9.5 min). The detection limit is 10 ppm (20 ng absolute) since the detector wavelength cannot be set below 240 nm (because of excessive absorbance by dichloromethane).

(2) Aqueous method: used an "extraction-coagulation" procedure, where five grams of dispersed aqueous polymer was added dropwise to a stirring mixture of 10 mL of dichloromethane, 40 mL of water, and 0.5 mL of concentrated HCl; stirring is continued for 30 min., and the mixture is then centrifuged (1 h). The supernatant fluid (ca. 40 mL of aqueous fluid) is concentrated to 10 mL by evaporation with air, and if the concentrate is not clear, it is filtered (10- μm pore diameter). Samples (0.5 mL) are injected on a Dowex resin 50W-X4 column with a mobile phase of 0.01N H_2SO_4 and detector set at 225 nm (acrylamide absorbs 100 times more strongly at 225 nm than at 254 nm). This is the ion-exclusion mode, where separation occurs via porous-polymer stationary-phase partitioning together with ionic interactions depending on the pK_a of the analyte (if the analyte is charged, i.e., has a pK_a less than the mobile phase pH, it is disassociated and excluded from the stationary phase by repulsion from the sulfonated surface). The retention time for acrylamide was around 29 min (significantly longer than with the nonaqueous system), but the detection limit was about 0.1 ppm (2 orders of magnitude lower). In the presence of sample, however, the acrylamide peak occurs on the trailing shoulder of the polymers.

Different polymer preparations (e.g., ethylacrylate-based vs. acrylonitrile-based) responded differently to the extraction-coagulation step. Both methods, however, displayed high recoveries of acrylamide spikes (80 to 100%), a major advantage over the GC-ECD method.

• **Skelly and Husser 1978** (Dow Chemical, Midland, MI)

First method reported that did not involve further sample preparation following extraction. Method designed for "trace" analysis of wipe samples and of impingers. Direct analysis of underivatized acrylamide by reverse-phase HPLC using a Whatman Partisil-10 ODS-2, 250-mm X 4.6-mm i.d. column with water as mobile phase (apparently no organic modifier was used to allow for a low detection wavelength; this would make the routine cleanup of the column with increasing concentrations of methanol a necessity, the frequency depending on the quantity of other solutes present that have higher capacity factors); the detection wavelength was 208 nm (probably chosen as being slightly above the wavelength at which water begins to absorb). Acrylamide standards were prepared in water, but acrylamide in polyacrylamide had to be prepared in 80/20 methanol/water (this was a stronger solvent than the mobile phase and therefore gave broader, tailing peaks). Response to acrylamide was found to be linear from 1 to 500 ppm. For 20- μL injections, the amount detected would correspond to 0.02 to 10 μg ; the limit of detection was approximately 0.1 ppm (although as noted, either a lower wavelength, decreased attenuation, or sample preconcentration could lower this value). The retention time for acrylamide was longest for C_{18} columns of intermediate loadings (5.2-5.8 min. for 10-15% loading), and lower or higher loadings gave shorter retention times (2.6-3.6 min for 5 or 25% loading). Excellent separation was noted for compounds that would probably be expected as co-contaminants in polyacrylamides. Representative compounds and their retention times

(minutes) are: acrylic acid (1.4), β -hydroxypropionamide (2.1), acetamide (3.0), acrylamide (5.4), propionamide (7.3), acrylonitrile (11.8), methacrylamide (18.0), butanamide (20.8), and methacrylonitrile (46).

The method was compared with the ion-exclusion HPLC method of Husser et al. (1977) for acrylamide in polyacrylamide, and the results compared well. For acrylamide recovery from wipes, the average percent recovery was 101% with an rsd of 2.9%.

• Ludwig, Sr. and Besand 1978 (Petrolite Corporation, Tretolite Div., St. Louis, MO)

For residual acrylamide in water-in-oil emulsion copolymers of acrylamide and cationic comonomers. Claimed that the methods of Croll (1971), Croll and Simkins (1972), Hashimoto (1976), and Husser et al. (1977) were all unsuitable. Despite this claim, the method is essentially a minor modification of Husser et al. (1977). Their objective was to minimize the time required for polymer-acrylamide separation to facilitate the study of kinetics; found the GC method to have retention times that were too long, presumably because of late-eluting polymeric materials. To 100 mL of acetone (with benzamide internal standard) was added dropwise 4-6 g of polymer emulsion/solution (for cationic emulsions); methanol was used for anionic emulsions. After stirring for 15 mins, 1-2 mL of the supernatant fluid (no centrifugation was required compared with the precipitation method of Husser et al. 1977) was filtered (0.5- μ m pore diameter membrane). Samples were analyzed by the nonaqueous normal-phase HPLC method of Husser et al. (1977). Benzamide eluted at 6 min. and acrylamide at 7 min., both on the badly trailing tail of the solvent peak. Although they investigated other internal standards that would elute after acrylamide (e.g., esters, ketones, alcohols, amines, and carboxylic acids), none was found. Recoveries for acrylamide spiked into polymer emulsions were all greater than 80%, but it must be kept in mind that these are high levels (greater than 100 ppm).

• Onuoha, Chaplin, and Wainwright 1979 (University of New South Wales, Kensington, Australia)

Two HPLC methods were reported. One (process-control analysis) was developed for simultaneously and directly determining water, acrylonitrile, and acrylamide during copper-catalyzed acrylamide synthesis. The other (quality-control analysis) was intended for determining impurities in the reaction. The authors were among the few who pointed out the possibility of acrylamide polymerization during direct gas chromatography of the underivatized amide (i.e., at temperatures above 150°C); they maintained that polymerization is indicated in GC when the peak is badly tailing and broad. Therefore, another advantage in brominating acrylamide is prevention of polymerization during GC analysis. Previous methods were not suited to determining the two synthesis reactants. The proposed method was suitable for on-line synthesis-process monitoring.

The first method used a reverse-phase (C_{18}) column with a mobile phase of methanol/water (30/70 v/v). Detection was by refractive-index monitoring. The second quality-control method used an all-water mobile phase (so that water in the aqueous mixture gave no peak). The process-control method gave three peaks (approximate retention times in minutes): water (3), acrylamide (3.5), and acrylonitrile (4.5); the relative molar responses were 0.03 (water), ca. 3.3 (acrylamide), and 1.0 (acrylonitrile). The quality-control method is one of few reported that addresses possible interfering contaminants/impurities resulting from the synthesis process. It gave reasonable, baseline resolution for the following (approximate retention times in minutes): acrylic acid impurity (3.5), β -hydroxypropionitrile (4), acrylamide (5), acrylic acid (7.5), and acrylonitrile (8.5); acrylonitrile tailed badly. A major advantage of this method is purported to be the elution of β -hydroxypropionitrile (ethylene cyanohydrin, an acrylamide isomer and low-level contaminant of copper-catalyzed acrylamide synthesis) before the acrylamide, allowing for its detection; using RI detection, its detection limit is about 0.02%, but could be lowered by using a UV detector.

- Klyachko and Sladkova 1980 (Russia)

Probably one of the first methods reported that uses an approach requiring no sample preparation. Acrylamide monomer is indirectly determined in polyacrylamide by following the kinetics of oxidation of oxalate by permanganate with Mn^{2+} as a catalyst. In the presence of acrylamide, the reaction rate changes in proportion to the acrylamide concentration; polyacrylamide has no effect. The method is reported to have an impressive detection limit of 10 ng/L (ppt), but the authors also state that technical polyacrylamide containing less than 0.01% acrylamide could be analyzed; so it is unclear which is correct. The former is the lowest detection limit reported for a formulation analysis method; the method has no apparent application, however, for more complex matrices.

- Herzig and Weigel 1987 (University of Texas Medical Branch, Galveston, Texas)

Authors apparently were unaware of the previous work by Klyachko and Sladkova (1980), who presented one of the first accounts of acrylamide determination by permanganate oxidation. This version, however, relies on the direct oxidation of the acryloyl group by permanganate ion; other functional groups are also oxidized such as amines, thiols, carbonyls, and reducing sugars, but the kinetics for acrylamide are much faster. In alkaline solution (sodium carbonate, pH 10), the permanganate ion is purple (absorbance maxima at 311, 507, 525, and 545 nm), and when reduced to the manganate ion, there are comparable reductions in absorbance at 525 and 545 nm; absorbance increases below 480 nm:



The reduction in absorbance at 545 nm was chosen as the indication of reductant concentration; it is linear in the range of 0 to 2 μmol of analyte (per 100 μL of sample). Less than 0.1 μmol of acrylamide can be reliably detected; on the basis of a 100- μL sample, however, this is 1 mM (ca. 80 ppm). The authors point out that decoloration of permanganate by its reduction has been used since the turn of the century as a qualitative test for alkenes (Baeyers test).

ANNOTATED BIBLIOGRAPHY OF METHODS FOR POLYACRYLAMIDE DETERMINATION

TYPES OF PAM

Anionic, carboxylated PAM: Separan MG700 (Dow Chemical Co.); Percol E-10 (Allied Colloids); Hercofloc 1021 (Hercules Chemical Co.).

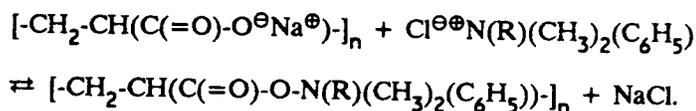
Nonionic PAM: Superfloc 127 (Cyanamid).

Cationic PAM (most often used as primary coagulants, whereas the nonionic and anionic are usually used as coagulant aids; Mallevalle, Bruchet, and Fiessinger 1984): Percol 352 (Allied Colloids).

TRACE ANALYSIS OF WATERS

- Crummett and Hummel 1963 (Dow Chemical Co., Midland, MI)

Evaluated two methods for determining polyacrylamide flocculants in water: (1) distillation-Nesslerization, and (2) a turbidimetric method. With the distillation method, many organonitrogen compounds will be obvious interferences. Analyzed waters containing 0.25-5.1 mg/L of Separan's (e.g., Separan NP10). With the turbidimetric method, they used the quaternary amine, diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride (Hyamine 1622; a large cation) to form a colloidal, light-scattering complex with partially hydrolyzed (anionic) polyacrylamides and measured the turbidity:



The method supposedly cannot be used with nonionic polyacrylamides, which must first be hydrolyzed (Griot and Kitchener 1965). Turbidities could be determined down to 10 mg/L of Separan NP10. Numerous potential interferences are noted; heavy metal ions are particularly problematic. This basic method was later automated by Wimberley and Jordan (1971) for determination of hydrolyzed polyacrylamide down to 5 ppm; they also noted that many anions can interfere (e.g., alkylbenzene sulfonates and high-MW fatty acids).

• **Frankoski and Siggia 1972** (Univ. Massachusetts, Amherst, MA)

Method for determining amide and nitrile functionalities, especially in polymers, based on alkaline hydrolysis. Amides in general are not easily hydrolyzed, although primary amides are more conducive than secondary or tertiary amides (yielding ammonia and the corresponding carboxylic acid salt). By using high-temperature fusion with highly concentrated caustic, the nitrogen is liberated as either ammonia or organic amines. The samples are put in platinum boats in a specially designed fusion chamber. The liberated products are captured in a cryogenic injection loop which is then thermally desorbed to a Chromosorb 103 column in a GC. Each type of polymer gives a characteristic peak (e.g., Nylon 66 yields 1,6-hexanediamine). Polyacrylamide liberated ammonia (98.5% recovery); analysis time was on the order of a half hour.

• **Attia and Rubio 1975** (Imperial College, London, England)

Method developed for nonionic PAM, since the Hyamine-turbidimetric method of Crummett and Hummel (1963) was useful only for anionic PAM (e.g., partially hydrolyzed PAM). Simple method that relies on observation that tannic acid precipitates PAM from aqueous solution at low-ppm concentrations in the presence of NaCl (thereby eliminating the need for hydrolysis). Nephelometric turbidity readings could be obtained on PAM concentrations as low as 0.1 mg/L with reproducibility of 1%. Method was linear for the PAM concentration range of 0.1 to 2 mg/L and useful for the range 2 to 10 mg/L, where the turbidity readings became nonlinear. Aqueous samples were added to 50-mL volumetric flasks containing 5 mL of 0.1% tannic acid and 40 mL of 1 M NaCl; the volumes were adjusted to 50 mL with water and shaken for 1 min. After 1 hour, the turbidities were measured with a nephelometer. No effect of pH was noted for the range of 2.5 to 10; above pH 10, the tannic acid changed to a yellow color. Turbidity slowly increased with time, and therefore the readings were made within 30 minutes. A strongly anionic polymer gave no turbidity; for anionic polymers they therefore recommend the Hyamine method of Crummett and Hummel (1963).

• **Scoggins and Miller 1979** (Phillips Petroleum Co., Bartlesville, OK)

An application of the method that the authors developed for amides (Scoggins and Miller 1975; see: Annotated Bibliography of General Methods for Determining Amides) to the determination of polyacrylamide in oil field brine water. Method involves oxidation of amide-N to the N-bromo derivative with excess bromine, destruction of remaining bromine, oxidation of iodide to iodine by the bromo derivative, and formation of the starch-triiodide complex (whose absorbance is determined at 610 nm). They made various modifications to their original general method, including: (1) Use of pH 3.5 for initial oxidation by bromine (this eliminated interference by chloride ion, which is oxidized by bromine above pH 4); elimination of chloride by anion exchange would have been suitable but this would have also removed anionic, partially hydrolyzed polyacrylamide; interference by endogenous iodide ion could also be eliminated if the bromine oxidation were done in the narrow pH range of 5.2 to 5.7 but this was not as important for these brine samples as was elimination of interference by chloride. (2) Trivalent metal ions interfered by enhancing the subsequent development of the starch-triiodide color (presumably by enhancement of amide oxidation by bromine via decreased steric hindrance); therefore, Al^{3+} was added to the oxidation buffer to standardize this effect (aluminum commonly occurs in oil field brines). (3) Destruction of excess bromine

after amide oxidation was done with formate under carefully controlled, timed conditions to prevent formate reaction with N-bromoamide oxidation product. (4) Because commercial polymers vary in their degree of hydrolysis, standard curves must take this into account. For samples containing 5 to 100 ppm of polymer, the standard deviation was 0.73 ppm.

- **Hawn and Talley 1981** (Alcolac, Baltimore, MD, and GAF Corp, Wayne, NJ)

Extremely indirect, circuitous method involving hydrolysis of polyacrylamide to release ammonia, followed by formation of the dinitro derivative (dinitroaniline) using 1-fluoro-2,4-dinitrobenzene (FDNB or often called Sanger's Reagent; Blau and King 1978, p. 393); the dinitroaniline is then determined by GC.

Polyacrylamide samples (100 mL) are concentrated to 1-2 mL under a stream of nitrogen; for aqueous samples, this would be a slow process. After 3 mL of 10N NaOH is added, the tube is sealed and heated for 15 h at 95°C. Contents are neutralized with HCl and FDNB reagent is added and heated at 60°C for 30 minutes. The solution is basified with 5 mL of 10N NaOH and extracted with 1 mL of toluene. The dinitroaniline partitions to the organic phase, and 2- μ L samples are injected on a 3% OV-225 Gas Chrom Q 2-m glass column. Quantitation of the ammonia is by comparison with derivatized ammonia standards.

- **Beazley 1985** (Marathon Oil Co., Littleton, CO)

Partially hydrolyzed polyacrylamide (PHPA) has been used for decades in enhanced oil recovery for mobility control in surfactant-based processes and polymer-augmented water floods. The amide function of PHPA "strongly" absorbs at 185 nm (molar absorptivity is 2300 at 192.5 nm). Used size-exclusion HPLC to separate the polymer from all of the numerous low-molecular-weight interferences in the production waters. RSD of 1% for 1-100 ppm range with detection limit of 10 ppb. No interferences were observed.

Used Synchropak CPC 100 Å column (a rigid silica packing with smallest pore size available to eliminate peak tailing by the excluded polymer) with diol-bonded phase (to avoid strong adsorption of contaminants); newer columns such as those from Polymeric Labs (true reverse-phase SEC) or those of TSK may be superior (e.g., Leung et al. 1987). Detection wavelength of 190 nm. Polymer had to be mechanically sheared prior to HPLC to avoid column plugging. Used sodium perchlorate (NaClO_4) as buffer since it is soluble in water and methanol and is transparent at 190 nm; without a buffer, adsorption is the dominant separation mechanism. Also needed pentanesulfonic acid (usually used for paired-ion chromatography) to make the excluded peak sharp (unknown as to why this worked). Dilute hydrogen peroxide was found to readily degrade the polymer to fragments sufficiently small to permeate the pores. Method found superior to the Chlorox bleach turbidimetric method because of interference from sulfide-sulfur precipitates.

- **Carns and Parker 1985** (East Bay Municipal Utility District, Oakland, CA)

EBMUD uses two methods for determining polymer residuals in treated effluent - a colorimetric test and a titrimetric method. Both suffer from interferences in Mokelumne raw water.

- **Langhorst et al. 1986** (Dow Chemical, Midland, MI, and Princeton University, Princeton, NJ)

This is a recent example of research aimed at determining the molecular weight distributions of polyacrylamide molecules. These are sophisticated techniques that have little relevance in analysis of total polyacrylamides.

- Leung, Pandey, and Das 1987 (Coal Research Laboratory, Guelph Chemical Laboratory, and Ontario Research Foundation, Canada)

Original methods for polyacrylamide (PAM) residuals in water used turbidimetric techniques (light scattering) (various references cited) that relied on formation of insoluble complexes by reacting anionic PAM with an aliphatic cation. Separation from numerous other interferences was a major problem.

The authors proposed a method for anionic and nonionic PAM by separation of the two types from potential interferences by ultrafiltration (UF) followed by size-exclusion (SEC) HPLC with UV detection. Ultrafiltration (Amicon Model DC2 hollow-fiber dialysis/concentrator with Dialflo hollow fiber cartridge, Type H1P 100-20, nominal MW cutoff of 100,000; humic acids and salts would permeate the membrane) at 110 mL/min achieved a concentration factor of 10 fold (followed by thin-film evaporation of the retentate water at 30°C and dissolution of the dried residue in 0.05 M Na₂SO₄) while at the same time eliminated many interferences. SEC-HPLC used a TSK gel 5000 PW column (MW exclusion limits 3X10⁴ to 8X10⁶; this is a semirigid porous hydrophilic polymer gel whose main surface functionality is hydroxylated polyether, -CH₂-CH(OH)-CH₂O-), with detection at 208 nm (same as used by Skelly and Husser 1978). The MW size exclusion limits ensured that all of the PAM molecules would be excluded from the pores and elute in a single, well-defined peak. Found that the 190-nm detection wavelength used by Beazley (1985) resulted in high background noise and an unstable baseline; 208 nm is therefore a compromise between sensitivity and usability. Ionic strength of the mobile phase (0.05 M Na₂SO₄) was very important in minimizing adsorption to the gel; other mobile phases previously used (e.g., Tris-HCl and ammonium nitrate) were unacceptable because they absorb at 208 nm. Salts in the sample can change the ionic strength sufficiently to disturb the chromatographic separation. This forced a double-pass/triple-wash cycle of UF to ensure that all salts were separated from the PAM (salts would also cause precipitation of PAM during further evaporative concentration).

Recoveries of PAM spiked to distilled water were about 100%, while recoveries were about 30% when spiked into the coal washery thickener feed and passed through only one UF cycle. In contrast, with a two-cycle UF, recovery from distilled water dropped to 76% (because of PAM loss during the subsequent passes) but increased to 50% for the coal washery sample (because of absence of loss to salt precipitation). The critical step in using ultrafiltration for sample preparation seems to be in determining the balance between the amount of PAM lost by repeated UF washing cycles versus the amount of PAM made unavailable for chromatography because of interaction with soluble salts not removed by use of only one UF cycle.

For the anionic Separan MG 700, a single, sharp peak eluted at 10 min. for coal washery effluent spiked with 6.7 ppm (and a large low-MW peak at 20 min.); two other anionic and one nonionic PAM eluted similarly, but the cationic Percol 352 was not resolved (a Gly-CPG packing with water mobile phase was more successful). Precision for 20-ppm standards was $\text{rsd} = 5.8\%$.

This method has many variables in the UF preconcentration/separation step and therefore does not seem to currently lend itself to routine use. No indications were made as to analysis times. It is also possible that the UF step degrades the PAM to lower MW fragments, but the MW of these fragments probably still exceeds the cutoff for the SEC column.

ANNOTATED BIBLIOGRAPHY OF GENERAL METHODS FOR DETERMINING AMIDES

- Scoggins and Miller 1975 (Phillips Petroleum Co., Bartlesville, OK)

One of the first methods for primary amides that used the bromine-oxidation iodide-starch spectrophotometric method; this method is sometimes referred to as *bromatometry*. Excess bromine is used to oxidize the amide-N (pH 5-6) to yield the N-bromo derivative (the first step in the Hofmann rearrangement), and the remaining bromine is selectively reduced with sodium formate (yielding bromide

ion). The bromo-derivative exists in equilibrium with the free, parent amide and hypobromous acid ($\text{Br}^\ominus \text{H}^\ominus$; see: N-Halogenation), the latter of which can oxidize iodide ion to iodine. The iodine is quantified spectrophotometrically as the blue starch-triiodide complex (using the Lambert potato-starch/ CdI_2 method) at 610 nm. Timing and formate concentration are critical since the formate also reacts with the hypobromous acid, although at a much slower rate. Primary and secondary amines are major potential interferences since they yield apparent molar absorptivities that are much higher than those of the amides. The authors later applied this method to the determination of partially hydrolyzed polyacrylamide in oil filed brines (see: Annotated Bibliography of Methods for Polyacrylamide Determination: Trace Analysis of Waters: Scoggins and Miller 1979).

- Hu and Cheng 1983 (Shangai Institute of Organic Chemistry, Peoples' Republic of China) (abstract only)

Converted amides to corresponding nitriles with trifluoroacetic anhydride (i.e., dehydration via acetylation; see: N-Acylation of amides and Dehydration of amides to nitriles); method was evaluated for benzamide, isobutyramide, and acetamide. The nitriles are then determined by GC-FID.

- Ji, Yang, and Wang 1985 (Changchun Inst. Appl. Chem, Peoples' Republic of China) (abstract only)

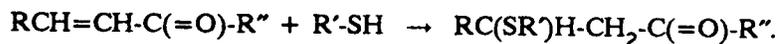
This is essentially another rendition of previously published work (e.g., Scoggins and Miller 1975) that makes use of the initial Hofmann rearrangement product (the N-bromoamide). A spectrophotometric method for acrylamide-acrylic acid copolymer in water that has been tuned for lower concentrations. After adding bromine water, sodium formate, and a color-developing agent (bean starch mixed with potassium cadmium iodide dihydrate, $\text{K}_2\text{CdI}_4 \cdot 2\text{H}_2\text{O}$), the absorbance is measured at 610 nm. Linear standard curves are obtained at concentrations less than 2.4 $\mu\text{g/mL}$; the detection limit is 0.05 mg/L .

ANNOTATED BIBLIOGRAPHY OF GENERAL METHODS FOR DETERMINING α,β -OLEFINIC BONDS

Many thousands of methods have been developed for determination of unsaturation (e.g., see: Belcher and Fleet 1965a). Since many of these are for isolated double bonds, they have no specific relevance to acrylamide, which contains a vinylic double bond conjugated to the electron-attracting amide group (this effectively reduces the bond order to less than two).

- Beesing et al. 1949 (B.F. Goodrich Research Center, Brecksville, OH)

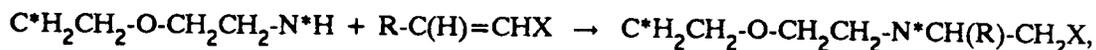
Probably one of the first methods specifically for determining (indirectly) the α,β -unsaturated carbonyl (especially that of esters and aldehydes) or nitrile compounds; ketones react only semiquantitatively. Method does not give a response for other types of unsaturation. Several interfering substances were noted. Developed specifically for acrylonitrile. An appropriate excess (e.g., 25-400%) of n-dodecanethiol or other primary thiol (dodecanethiol was chosen because of its lower volatility while still being soluble) reacts under alkaline conditions with the double bond, and the excess thiol is titrated iodometrically or amperometrically with silver nitrate. Analyzed from 2-200 mg of compound (concentration cannot be lower than 0.5 mg/mL in the reaction mixture). Optimum reaction times varied with the analyte. The reaction involved relies on nucleophilic addition to the carbon-carbon double bond (see: Reactions of the Olefinic Group: α,β -Unsaturated alkenes):



The authors do not specifically mention the use of this method for amides.

- Critchfield, Funk, and Johnson 1956 (Carbide and Carbon Chemicals Co., South Charleston, WV)

Maintained that halogenation methods were inappropriate for α,β -unsaturated compounds when the bond is adjacent to a strong electron-attracting group. Halogenation proceeds nonquantitatively when the adjacent group is a nitrile, carboxyl, amide, sulfonic acid, nitro, or carbalkoxy; this decreased reactivity towards halogens results from increased positive character of the bond (verified by Belcher and Fleet 1965a). Mentioned Beesing et al. (1949) as having the only method available specifically for α,β -conjugated compounds. Developed a method making use of the known reactivity of primary and secondary amines with non-isolated α,β -unsaturated compounds. Method uses an excess of the secondary amine morpholine, which reacts in the presence of acetic acid (catalyst) with the unsaturated bond, forming a tertiary amine at the morpholine ring-nitrogen:



where X is a strong electron-withdrawing group (for acrylamide, R = H and X = C(=O)-NH₂; indeed those compounds found most amenable to reaction were acrylics, i.e., R = H); aldehydes and ketones (isolated double bonds) were not reactive (and therefore they are amenable to halogenation). Upon completion of the reaction, the excess morpholine is acetylated with acetic anhydride in acetonitrile to form the corresponding amide (N-acetylmorpholine) and acetic acid, and the tertiary amine is titrated with alcoholic HCl to an indicator end point (the direct measure of the amount of unsaturated material present). Analysis of pure acrylamide gave 100% purity using this method. This derivative may have utility in HPLC detection.

- Belcher and Fleet 1965b (Univ. Birmingham, England)

Improved the morpholine addition method of Critchfield, Funk, and Johnson (1956) by changing the indicator and solvent system. In addition to acrylamide, nearly quantitative recoveries also were obtained for pure acrylonitrile, ethyl acrylate, and acrylic acid.

- Bachmann and Dagon 1972 (Forschungslabor Fribourg, Lonza A.G., Friborg, Switzerland)

Method involves a minor modification of the amine-addition method of Critchfield, Funk, and Johnson (1956). Instead of morpholine, they used pyrrolidine in the presence of acetic acid as catalyst to form the tertiary amine addition compound with the pyrrolidine-N. Detection was made in nonaqueous solvent with an accuracy exceeding 90%. Incorrectly cited by Husser et al. (1977) as a spectrophotometric method. ■

GENERALIZATIONS/LIMITATIONS OF ACRYLAMIDE-DETERMINATION METHODS

- I. Main Methods of Determination: Only three specific approaches and one general approach to acrylamide determination have been addressed in the literature: (1) Polarography, a somewhat specific and direct means of analysis, has detection limits that are not sufficiently low for trace analysis; it is most applicable to formulation analysis (i.e., fractional percentage levels of acrylamide in polyacrylamide electrolytes). Polarography has been recommended by NIOSH for work-place air monitoring since the method detection limit can be lowered simply by sampling larger volumes of air (NIOSH 1976); reduction of the acrylyl double bond occurs at about -2.0 V (S.C.E.). (2) Aqueous-phase bromination of the double bond followed by extraction/concentration into organic solvent and determination of the 2,3-dibromopropionamide by GC-ECD. This is the current method of choice for trace analysis in water because of the low detection limit. The bromination step serves two purposes: (i) greatly increases the partition coefficient by removing the unsaturated site, and (ii) introduces strongly electron-capturing halogens that provide for low detection limits by using the electron capture detector; direct determination of underivatized acrylamide, besides giving poor detection limits, also risks on-column thermally induced polymerization (Onuoha et al. 1979). (3) High-performance liquid chromatography by either of two approaches: (i) direct determination of underivatized acrylamide using UV detection, and (ii) aqueous-phase bromination followed by extraction/concentration and determination of the dibromo derivative by reverse-phase separation and UV detection. (4) Various miscellaneous methods, including both direct and indirect methods, that couple derivatization with spectrophotometry (e.g., Mattocks 1968).
- II. Key Literature: Among the most important references reviewed with respect to an historical perspective and for technical content have been the following (in chronological order). For gas chromatography (Croll and Simkins 1972; Hashimoto 1976; Poole et al. 1981; Andrawes et al. 1987), for HPLC (Brown and Rhead 1979; Brown, Rhead, and Bancroft 1982; Freshour et al. 1985), for polarography (MacWilliams et al. 1965; Betso and McLean 1976), and for miscellaneous methods (Herzig and Weigel 1987; Klyachko and Sladkova 1980). Fewer than ten articles have specifically addressed the problem of trace analysis of waters; other trace-analysis methods have been developed for different matrices such as biological tissues, crops, and sugar (e.g., Poole et al. 1981; Fujiki et al. 1982; these methods differ from water-analysis methods mainly with respect to sample work-up), and these have been ignored in the former literature. Of all the laboratories that have been involved in methods development for acrylamide, the Dow Chemical Laboratories (Midland, MI) have probably published the most papers. Formulation analysis has received more attention, however, than has trace analysis. A large pre-1960 literature is available on general acrylamide chemistry; its relevance towards trace determination is unknown since it has not been applied.
- III. Sample Storage: To prevent the possible microbial degradation of acrylamide in samples stored for future analysis, mercuric chloride has been added, and boiling has been used (possibly risking acrylamide hydrolysis). A preferable method is aseptic microfiltration (using filters with no extractables, e.g., Anopore[®] inorganic membranes, Anotec Separations, NY) with storage at 4°C. The use of iron complexes of cyanogen or thiocyanogen should be investigated for stabilization of aqueous acrylamide (American Cyanamid 1969, p.6). Sulfites and bisulfites will rapidly scavenge acrylamide (esp. at pH 5-7), forming the 3-sulfopropionamide. Acrylamide loss from water by volatilization is not a concern.
- IV. Derivatization: Few methods have been reported that have focused on the large number of possible derivatives that could be formed from the amide group. Nearly all methods have simply relied on aqueous-phase acidic (pH 1) ionic bromination of the double bond, an approach that does not provide for optimal sensitivity with electron capture detection and which also imparts much error because of its nonquantitative nature; bromination was used over twenty years ago in titrimetric techniques for acrylamide (e.g., Narita et al. 1964). Furthermore, the understanding of the chemistry of alkene bromination is incomplete and controversial. There is even argument over whether acidic bromination is appropriate for acrylamide. A major possible side reaction is the formation of isomeric bromohydrins

(hydroxy-bromo-propionamides); high bromide-ion concentrations ($>0.5M$) are required to moderate this side reaction (Farook et al. 1984). Recently, several studies have stated or demonstrated that the dibromo derivative is labile. Apparently, it is easily dehydrobrominated to 2-bromopropenamide, and it is recommended that this reaction be purposefully effected (by treatment with alkaline triethylamine) prior to GC analysis (Andrawes et al. 1987), even though the monobromo derivative will give a reduced ECD response; this reaction probably occurs by formation of the β -triethylammonium adduct salt by elimination of the β -bromine (Katritzky et al. 1984). After derivatization, excess bromine is almost always scavenged by the purposeful addition of sulfite or thiosulfate. Much basic research is still required to develop derivatization methods specifically tailored for amides. This is a major shortcoming in analytical chemistry, and it is reflected by the lack of any specific trace methods for amides. Nearly all investigators have had to synthesize their own 2,3-dibromopropionamide reference standard; this compound is now commercially available.

- V. **Extraction:** Because of the high solubility of acrylamide in water, it can be extracted only via derivatization (it is also not amenable to "solid-phase extraction"). The only method currently used is bromination of the double bond. The best solvent for extraction of the dibromo derivative has generally been ethyl acetate, which gives the highest partition coefficient, but which also must be purified if subsequently concentrated for GC-ECD. Although diethylether gives a lower partition coefficient, it is much easier to concentrate. Salting out is also usually used (e.g., with sodium sulfate). Extraction of the dibromo derivative is essentially quantitative and imparts relatively little to overall method variability; most variability results from the bromination derivatization step.
- VI. **Clean-Up:** Few methods have been used for sample clean-up. The most common approaches have been removal of aqueous-phase interferences from acrylamide by mixed ion-exchange resins and hydrophobic resins (this makes use of the lack of sorption by acrylamide) (e.g., Brown, Rhead, and Bancroft 1982; Freshour et al. 1985). Back-extractions (solvent exchange; e.g., Fujiki et al. 1982) with pH adjustment and complete solvent removal have also been used for the dibromo derivative; evaporation to dryness (e.g., required for certain HPLC methods where the analyte must be dissolved in an aqueous mobile phase; Brown and Rhead 1979), however, results in partial loss of the derivative (Poole et al. 1981). Preextractions (either before or after bromination) have been done at low pH (ca. 1), where the amide carbonyl group is protonated, to remove potential interferences; analyte extraction is then done at pH 3 (the actual technique of alkali addition is critical since it can lead to debromination). The use of high-resolution bonded-phase capillary columns for GC may make sample clean-up unnecessary (Andrawes et al. 1987).
- VII. **Chromatographic Separation:** For gas chromatography, the major phases used for determination of the dibromo derivative have been relatively polar, the main one being FFAP; DEGS, EGS, OV-225, and OV-275 have also been used with success. Of particular note is the preponderance of packed columns; few investigators have used the much more advanced bonded-phase capillary columns (e.g., Andrawes et al. 1987). For HPLC, the main separation method has been reverse-phase, and most methods have used organic modifiers (mainly methanol or acetonitrile); the newer columns that are deactivated for bases have yet to be evaluated.
- VIII. **Detection:** For GC, electron-capture detection has been used almost exclusively for trace analysis. Nitrogen-selective (e.g., AFID; Farkas and Tekel 1987) and FID detection (e.g., Croll 1971) have been rarely used, probably because of their relative lack of sensitivity; lack of sensitivity is also the limitation to other halogen-specific detectors such as the Hall detector (Poole et al. 1981), which is more sensitive to chlorine than bromine. For LC, detection is limited to non-specific UV absorbance (ca. 200 nm).
- IX. **Detection Limits:** With currently available methodologies, bromination/GC-ECD easily provides the lowest method-detection limit (sub-parts-per-trillion: sub-ng/L). Nakamura (1977) has reported the lowest detection limit (0.1 ppt); this detection limit is for the dibromo derivative and is three orders of magnitude lower than that for underivatized acrylamide. In contrast, HPLC with UV detection

currently provides only for low-parts-per-billion (low- $\mu\text{g/L}$) detection limits (e.g., Brown and Rhead 1979; Freshour et al. 1985); gross interferences are a major problem. The instrument detection limits for HPLC-UV and GC-ECD are on the order of 1 ng and 1 pg, respectively. Despite the three orders of magnitude better method-sensitivity of GC-ECD over HPLC-UV, the latter is faster, standard curves are more stable, and the UV detector is more reliable than the electron-capture detector; the method detection limits for HPLC could be lowered if appropriate acrylamide-concentration schemes could be developed, if newer deactivated columns were used (which give better resolution), or if fluorescent derivatives could be formed. For formulation analysis, the lowest detection limit is reported for the permanganate oxidation method (Klyachko and Sladkova 1980); the most common method, polarography, gives only ppm sensitivity.

- X. Accuracy and Precision: For the bromination/GC-ECD method, accuracy generally falls in the range of 80% to mid-90%, with rsd values of usually 1-10%. Most of the error in accuracy occurs because of incomplete/erratic bromination of acrylamide; excessive solvent reduction can also lead to evaporative loss of the dibromo derivative.
- XI. Formulation Analysis: The main methods used in formulation analysis involve monomer extraction from the polymer (Croll 1971; MacWilliams et al. 1965) followed by polarography (e.g., Betso and McLean 1976), GC (Croll 1971), or HPLC (Husser et al. 1977; Skelly and Husser 1978). A totally direct method (requiring no sample preparation) makes use of the oxidation of acrylamide itself (or of an intermediary) by permanganate, an adaptation of Baeyers Test for alkenes (Herzig and Weigel 1987; Klyachko and Sladkova 1980); extremely low detection limits have been reported.
- XII. Interferences to Acrylamide Determination: Few researchers have addressed the issue of interferences, including individual compounds, such as acrylic acid, acrolein, acrylonitrile, β -hydroxypropionitrile (ethylene cyanohydrin), 3,3',3''-nitrotrispropionamide, propionamide, methacrylamide, and methacrylonitrile, or bulk parameters such as TDS; those who have (e.g., Onuoha et al. 1979; Skelly and Husser 1978) discussed them only in terms of formulation analysis. Acrylonitrile can be selectively purged from aqueous solution (Betso and McLean 1976).
- XIII. Polyacrylamide Determination: The "trace" (sub-ppm) determination of polyacrylamides in water has mainly relied on precipitation/nephelometric techniques such as anionic-PAM precipitation with high-MW quaternary amines (e.g., Crummett and Hummel 1963) or neutral-PAM precipitation with tannic acid (Attia and Rubio 1975), and on size-exclusion HPLC (Beazley 1985; Leung et al. 1987). Detection limits are as low as 1 ppm.
- XIV. General Methods for Amides: Several methods have been commonly employed for the general determination of amides as a class. These include bromine oxidation to the N-bromo derivative (the initial Hofmann rearrangement product), followed by iodide oxidation and quantitation of the starch-triiodide complex (e.g., Scoggins and Miller 1975), and dehydration and GC determination of the resulting nitriles (Hu and Cheng 1983).
- XV. General Methods for α,β -Olefins: Thousands of methods have been developed for olefinic bonds, but few have been reported for the α,β -olefins (double bond conjugated with an electron-attracting group, such as with acrylamide). These include thiol addition (to the 3-position) and titration of the excess thiol (Beesing et al. 1949) and secondary amine addition (e.g., morpholine; to the 3-position) and direct determination of the resulting tertiary amine (Belcher and Fleet 1965b; Critchfield et al. 1956). ■

RECOMMENDATIONS

Method for Immediate Use

- (1) Simply on the basis of detection-limit requirements, the method of choice for trace analysis in water is clearly the aqueous-phase acidic bromination GC-ECD method. Although this is apparently a tedious and laborious method, it is the only method currently available with a method detection-limit in the nanogram-per-liter range. The original methods of Croll and Simkins (1972) and Hashimoto (1976) should be modified (after further evaluation) to incorporate the improvements of Andrawes et al. (1987) and Poole et al. (1981); these include dehydrobromination of 2,3-dibromopropionamide to 2-bromopropenamide prior to GC and the use of a bonded-phase capillary column (e.g., J&W DB-Wax or the recently available DB-FFAP).
- (2) Further research is required, however, on the chemistry of aqueous-phase acidic bromination of the acrylyl group. The mechanism needs to be better defined so that side-products can be minimized, production of the dibromo derivative maximized, and stabilization of the product optimized. Even the identity of the proper bromination reagent requires clarification; for example, pyridinium bromide perbromide ($C_5H_5NH^{\oplus} Br_3^{\ominus}$) should be evaluated.

Potential, New Methods for Trace Acrylamide Determination

- (1) The most promising approach to acrylamide detection at low concentrations would be formation of a highly fluorescent derivative combined with HPLC, but to date none has been reported. A possible approach would be the determination of acrylamide as an amine. This would require separation of acrylamide from all amines (e.g., bromination and organic extraction at acid pH where amines will be protonated). Conversion of the extracted dibromopropionamide to its corresponding amine by chemical reduction or rearrangement (e.g., see: Reduction of amides to amines and The Hofmann rearrangement), followed by derivatization of the amine with a fluorophore and quantitation via HPLC with fluorescence detection would give extremely low detection limits. Ultrahigh sensitivity (femtomole levels) has been achieved using new derivatizing reagents (e.g., 3-benzoyl-2-quinolinecarboxaldehyde) for the precolumn formation of highly fluorescent isoindoles and detection by helium-cadmium laser-based detector (Beale et al., in press).
- (2) The newer, deactivated reverse-phase HPLC columns would probably provide better resolution of acrylamide or its dibromo derivative from interferences and thereby offer lower detection limits. These columns (e.g., Supelco's Supelcosil-DB C-18 and DuPont's Zorbax RX, the latter distributed by Mac-Mod Analytical, Inc., Chatsford, PA 800-441-7508) should be evaluated using methods already published (e.g., Brown, Rhead, and Bancroft 1982; Freshour et al. 1985). Both columns have maximum coverage and are end-capped. For the former column, "DB" denotes "deactivated for bases" (treated to minimize the amount of free surface-silanols and metals). The latter column reportedly gives even less tailing; it is manufactured to minimize the acidity of the surface (i.e., active silanols); for this reason, it is suggested that samples be run at $pH < 4$, preferably 2.5 to 3.5, to protonate whatever silanols happen to remain; then potassium salts should be added to the mobile phase to reduce ionic effects; and finally, triethylamine (or dimethyloctylamine) should be added to compete with remaining sites (Stadalius, Berus, and Snyder 1988).
- (3) Evaluate alternative approaches to the preconcentration of acrylamide or of the dibromo derivative.
- (4) Explore the possibility of forming N-derivatives of acrylamide. In particular, simultaneous perfluorobenzoylation/extraction is an attractive possibility (e.g., Coutts et al. 1987; Ehrsson and Mellström 1972) because of the greatly enhanced response when using GC-ECD (Moffat et al. 1972); dehydration to the nitrile during acylation would be a major anticipated problem, however. Dimethylaminopyridine should be evaluated as the catalyst for acylation (Katritzky et al. 1984).

- (5) Explore the possibility of forming other acrylamide derivatives. There are many other possible derivatives (especially for HPLC), including (i) trifluoromethylation and cyclization with hexafluoroacetone and stannous chloride to give a 4-trifluoromethyl-1,3-oxazole (for HPLC or GC-ECD) (Burger 1987); (ii) reaction with diazomethane for formation of a pyrazoline derivative (amenable to GC), which can also be converted to the Schiff's base with a high molar absorptivity (for HPLC) (Mattocks 1968); (iii) β -N-morpholinopropionamide derivative (for HPLC) (e.g., Belcher and Fleet 1965a); (iv) amide-N-fluorination with selenium tetrafluoride or diethylaminosulfur trifluoride (DAST); (v) hydroxylamide derivative (hydroxamic acid), which forms an intense chromophore with ferric ion (ferric hydroxamate) (Wimer 1970); and (vi) aziridine-2-carboxylic acids via cyclocondensation of 2,3-dibromopropionamide and ammonia in the presence of barium hydroxide (Kitagawa et al. 1986).
- (6) Determine the possibility of using new chromatographic methods. Two of the most promising are capillary electrophoresis and GC with selected-ion monitoring (see the following section: Miscellaneous Methods Considered for Acrylamide...). ■

MISCELLANEOUS METHODS CONSIDERED FOR ACRYLAMIDE BUT EXCLUDED BECAUSE OF LACK OF COMMERCIALIZATION OR HIGH COST

The basic problem with acrylamide trace detection resides more with innovative means of detection rather than separation. Despite this, several other currently nonroutine or even experimental instrumentation separation methods were considered. Several less-common commercial instruments were also considered, but all would probably have problems in achieving sufficiently low detection limits.

Capillary GC with Selected-Ion Monitoring

The current generation of dedicated selected-ion monitoring GC detectors provides for very specific and sensitive detection and confirmation. For example, the HP 5971A mass-selective detector (MSD) produces EI spectra with low picogram sensitivity in the 10-650 amu range. Its current price of about \$27,000 (excluding data system) precludes its use for routine work. Furthermore, the acrylamide sample would still require derivatization (e.g., bromination) to provide unique fragments for such a low-molecular-weight compound.

Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is a rapidly emerging chromatographic method that fills both the gap between and crosses over with HPLC and GC. The cost of these systems has been high. Newer, "stripped-down" versions are being marketed. Even these, however, are not inexpensive. For example, Suprex has released lower-priced instruments (SFC/100A and SFC/150A) ranging from \$25,000 to \$29,000; the more expensive, automated SFC/200A costs \$36,750. Computer Chemical Systems makes a unit that converts the HP5890A GC into an SFC unit; it is priced at \$20,000. With the matter of cost aside, to achieve the necessary detection limit for acrylamide, the only detector that could possibly be of use would be any of the various N -thermionic detectors; these have the problem of differential cooling (and therefore varying sensitivity) during SFC density programming.

Capillary Electrophoresis

Capillary electrophoresis (either electrokinetic, based on electroosmosis or hydrostatic, based on gravity) is a newly emerging technique combining the established method of gel electrophoresis with new capillary-column and micro-detection methods. Its tremendous potential is in its ability to separate molecules regardless of size, in providing for enormously high theoretical plates (e.g., millions) with separation times of minutes to an hour, and in its ability to detect certain molecules down to the attomole level; these low detection limits, however, are on an absolute mass basis (not concentration basis), and since the sample size is so restricted (nL), the method detection limits are not that much better than currently available methods. To date, this has been a research tool only. Several manufacturers have units that are under design, although Microphoretic Systems (Sunnyvale, CA) just released the first commercial instrument (Microphore 1000); this instrument can be used to effect separations based on molecular charge via isoelectric focusing, isotachopheresis, or micellar electrophoresis. Its utility for neutral molecules such as acrylamide is not as well established; these compounds must be separated as micelles of ionic detergents. Ethylenic compounds can be derivatized to cations, however, as described by Mazurkiewicz (1985), who separated acrylamide from other ethylenic compounds by electrophoresis of the dimethylsulfoniomethylthio derivatives.

Microcolumn Liquid Chromatography (Novotny 1988)

Microcolumn (or capillary) LC uses various bonded or packed columns having internal diameters of much less than a millimeter (e.g., 3-50 μm for open tubular to 40-200 μm for packed capillary). Microcolumn LC provides for fast analysis times with hundreds of thousands of theoretical plates. Its main current disadvantage is its rigorous requirements for nonstandard LC components capable of delivering very low and steady mobile-phase flow rates and having extremely low internal dead volumes. With the use of laser-

fluorescent tag detection, extremely low detection levels (e.g., low femtograms) can be met. Its low flow rates make it amenable to direct MS interfacing and for the use of traditional (although redesigned) GC detectors. For example, Novotny (1988) has designed a nitrogen-phosphorus specific detector for microcolumn LC. These systems nevertheless remain outside the realm of routine analysis.

HPLC with Electrochemical Detection (ECD)

Electrochemical detection (ECD) offers the lowest detection limit available for routine, direct (i.e., no derivatization) analysis by HPLC. Its sensitivity is a function of the number of electrons that can be transferred during oxidation or reduction; low-picogram levels of aromatic amines and peroxides can be detected. Even though acrylamide is amenable to polarographic analysis (because of its unsaturation), such low negative voltages require a mercury electrode, and -2V is difficult to maintain with an ECD. Indeed, EG&G Princeton Applied Research confirmed that researchers from Pharmacia had tried to use ECD for monitoring acrylamide in PAM without success. Furthermore, with currently available information, acrylamide forms no derivatives that would facilitate the use of HPLC-ECD.

Other Element-Specific GC Detectors

The chemiluminescence nitrogen detector that has been adapted to GC by Antek Instruments (Model 705 Nitrogen Specific Detector; Houston Texas) shows much greater specificity towards nitrogen than any other "N-specific" GC detector (because of its conversion of nitrogen to NO, which is then reacted with ozone to form NO₂ in the excited state, which relaxes with light emission). It also has a very wide linear dynamic range. Its detection limit, however, is somewhere less than 100 pg; this is several orders of magnitude greater than the detection limit for halogenated compounds by electron capture detection.

The Redox Chemiluminescence Detector (RCD; Sievers Research model 207B; Boulder, CO) uses an indirect approach to the NO-O₃ chemiluminescence method to obtain response to any functional group that can reduce NO₂ under various catalytic conditions. Compounds containing oxygen, nitrogen, sulfur, and phosphorus, as well as olefins will react (but not alkanes, water, many gases, and chlorinated solvents). The detection limit is 50 pg for NO (the most sensitive species).

Various electrochemical detectors have been developed over the years (e.g., the Hall detector). All of these detectors are noted for difficulties in routine use in any of their different modes (e.g., halogen-, N-, and S-specific) as well as in not having sufficiently low detection limits.

TLC-FTID

A unique combination of TLC technology and flame-thermionic detection has been incorporated in an instrument called the Iatroscan TH-10 TLC/FTID Analyzer (Iatron Laboratories; distributed by Ancal, Los Osos, CA). This instrument automatically performs TLC separations on miniature normal-phase "rods" that are passed through the flame of an ionization detector; the instrument's main advantage is its ability to accept very small sample sizes (e.g., 1 µg). The method has been widely used for sample matrices not amenable to other methods (esp. high-molecular-weight substances). The detection limit even with the nitrogen/halogen-sensitive flame thermionic detector (1 ng) would not be sufficient since the efficiency of separation is significantly lower than HPLC. ■

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