for all fibers from a single exposure to be eliminated or permanently retained in tissue. A single day's ingestion of water contaminated with amphibole fibers was observed to result in a urine amphibole fiber concentration approximately 10^{-5} of the number of fibers ingested, with fibers detectable in urine at least 10 days after ingestion. Subject G, after drinking filtered water for 2 months, had a 90 percent reduction in the urine amphibole fiber concentration. Subject A, 13 months after a change to filtered water, had a greater reduction in the urine amphibole fiber concentration.

The data suggest that some ingested mineral fibers are accumulated in body tissue but do not allow a prediction of whether fibers are permanently retained. Further study of ingestion exposure and urine clearance should allow calculation of steady-state body concentrations of those fibers subject to eventual elimination in urine. Furthermore, the electron microscope analysis of urine samples for inorganic particles such as asbestos fibers can provide a valuable index of past exposure.

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18. P. M. Cook, G. E. Glass, J. H. Tucker, *Science* **185**, 853 (1974). Amphibole minerals are members of a class of hydrated silicates having a double-chain crystal structure. These minerals can crystallize during rock formation in an asbestiform habit (bundles of individual fibers). Asbestiform amphibole minerals used commercially as asbestos are amosite (fibrous grunerite), crocidolite, anthophyllite, tremolite, and actinolite. Most amphibole particles in western Lake Superior water fall in the cummingtonite-grunerite series. A fiber in a water sample is defined as any particle with a length-to-width ratio equal to or exceeding 3:1. Some nonasbestiform amphibole mineral crystals, when crushed, form cleavage fragments that are microscopically very similar, or identical, in morphology, crystal structure, and chemistry to small fibers which result from the crushing of amphibole asbestos. Approximately 95 percent of the asbestos used in North America is chrysotile, a fibrous serpentine mineral with a distinct tubular microstructure.
19. Distilled water, filtered successively through membrane filters with mean pore diameters of 0.45 and 0.1 μm, was used for all glassware cleaning, sample preparation, and blank sample analysis.
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21. Analysis of a sample with a measured addition of amphibole fibers indicated a 29 percent underestimate of amphibole fibers, due probably to interference of urine sediment and failure to completely disperse clumps of ashed sample. Similar measurements on air samples containing amphibole fibers ashed at low temperatures have produced equivalent results. These measures of accuracy only relate to sample loss caused by low-temperature ashing and refiltration. Inability to see and identify all fibers is a problem common to the electron microscope analysis of any particle sample [D. Beaman and D. File, *Anal. Chem.* **48**, 101 (1976); (*15*)].
22. Unfiltered Duluth drinking water ingested by subjects A, B, and G is estimated by combined x-ray diffraction and electron microscope analysis (*20*) to have contained approximately 50 × 10^6 amphibole fibers per liter, significantly less, as a result of seasonal effects on Lake Superior water circulation, than the annual average concentration of 120 × 10^6 amphibole fibers per liter. Subjects I and J drank unfiltered water from both the Duluth and Two Harbors, Minnesota, water supplies and probably had the greatest daily intake of amphibole fibers. Subject K had very little exposure to unfiltered water except for the ingestion of Duluth water with 8 × 10^9 amphibole filters per liter 2 days before the urine sample reported in Table 1 was collected. Filtered drinking water generally contained less than 1 percent of the amphibole fiber concentration of unfiltered drinking water. The entire Duluth municipal water supply has been filtered since January 1977.
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25. The primary source of blank sample chrysotile fiber contamination is the membrane filters which are ashed at low temperature along with the sample. Careful analysis of different lots of filters makes it possible to select filters with minimum chrysotile contamination. Chrysotile fiber concentrations in urine, based on five samples analyzed with low chrysotile contamination filters, may often exceed 1000 fibers per milliliter. A further complication in the evaluation of the measured chrysotile fiber concentration in urine is the possible breakdown of ingested chrysotile fiber bundles into many very small unit fibrils either during fiber migration through the body or during sample preparation procedures. We find that chrysotile fibrils subjected to simulated stomach acidity conditions (stirred for 3 hours in filtered distilled water with *p*H 1.8 at 38°C) retained their structural integrity and gave diagnostic electron diffraction patterns. Some magnesium is probably leached as observed in fibers isolated from lung tissue [F. Pooley, *Br. J. Ind. Med.* **29**, 146 (1972)] or in dissolution studies [J. Thomassin, J. Goni, P. Baillif, J. Touray, M. Jaurand, *Phys. Chem. Minerals* **1**, 385 (1977)].
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Extraction and Analysis of Organic Cations from Acid Solution with Strong Electric Fields and Mass Spectrometry

Abstract. *High electric fields have been used to extract organic cations from solutions of small organic molecules in polyphosphoric acid. The cations have been analyzed by mass spectrometry. Production of ions by this method is shown to be related to ease of protonation by acid rather than to ionization potential.*

The presence of well-defined amounts of protonated solute organic molecules in solutions composed of organic compounds and mineral acids is well established. This report presents experimental results indicating that cationic species in such solutions may be sampled directly into a vacuum system by using high electric fields and subsequently analyzed by mass spectrometry. In these experiments a solution is prepared in situ in the ion source of a mass spectrometer from the combination of polyphosphoric acid supported on a tungsten wire and organic samples admitted into the instrument in the gas phase. Extraction potentials of 3000 to 12,000 V applied between the solution and a counter electrode provide detectable ion currents.

Experiments using techniques of field desorption mass spectrometry (*1*) have shown that in the presence of proton sources or alkali metal salts, the ion beams observed may consist of protonated or metal-complexed organic sample molecules (*2*). In addition, it has been shown that these processes are enhanced by the emitters that lack the

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dendritic microstructure (3, 4) necessary for field ionization (5). Field-induced ion fragmentations are diminished under these conditions (6). Such observations are difficult to explain in terms of conventional field ionization processes (2).

Analogous protonated and metal-complexed molecules are also observed during electrohydrodynamic ionization (7) of sample molecules from glycerol–sodium iodide solutions (8). Electrohydrodynamic ion beams are known to originate from highly pointed field-induced deformations of the liquid surface (7). The ions themselves are considered to arise from simple electric field extraction of preexisting ions from solution (8).

Experiments have been described by Röllgen and Ott (9) in which field desorption emitters are coated with polymerically saponified lithium salts. Organic samples touching or dissolving in these solutions are complexed with lithium and the complex is extracted by the field. These experiments differ from organic electrohydrodynamic ionization only in that the supporting liquid phase has such a high molecular weight that its metal complexes are not field extracted. Accordingly, it is reasonable to assume that with respect to dissolved sample molecules these processes have a common mechanism. It must also be suspected that this same mechanism can compete with field ionization under conditions of conventional field desorption mass spectrometry. In both organic electrohydrodynamic experiments (8) and field desorption experiments exhibiting cation extraction (2), the applied voltages were below those necessary for classical field ionization.

I endeavored to design an experiment that eliminates field ionization processes and specifically generates solutions in a high-field region that can reasonably be expected to be rich in preexisting protonated organic molecules. Experiments were carried out with a Varian MAT 731 mass spectrometer equipped with a combined electron impact, field ionization, field desorption ion source operated electronically in the field ionization mode. Solutions were prepared on a bare 10-μm tungsten wire mounted on a standard emitter base. The wire was treated outside the mass spectrometer with neat polyphosphoric acid so that droplets of acid were deposited randomly along the wire. Polyphosphoric acid was chosen as the mineral acid component because of its low volatility and good electrical conductivity. The organic second component of the solutions was admitted to the mass spectrometer in the gas phase from a reservoir via a gold leak. The pressure of the organic component in the vicinity of the treated wire was estimated to be about 5×10^{-6} torr. A potential of +8 kV was applied to the wire and a potential of −4 kV was applied to the extraction element (counter electrode). These potentials were chosen to provide optimum ion extraction and are fixed by the ion source geometry. It is important to note that such high potentials are not necessary to observe significant total ion currents, and signals have been observed with potentials between the wire and the extraction element as low as 3000 V. The emitter was operated at room temperature, and a useful lifetime of about 2 hours was observed.

The results obtained to date are summarized in Table 1. The most important features of these data are the following. First, the bare wires used as support for the polyphosphoric acid produce no detectable ion current before application of the acid. Second, molecular ions of the kind obtained by field ionization are completely absent. In their place are molecules ionized by simple protonation. Third, the fragmentations normally associated with field ionization are absent. This is clearly demonstrated in Fig. 1, where it can be noted that the (M − 15) ion normally present in the field ionization spectrum of isopropanol has been replaced by a small $(M + H - H_2O)$ ion at mass 43. The methyl radical loss in the field ionization spectrum is a high-energy odd electron → even electron process, whereas the acid-induced ions decompose by a lower-energy even electron → even electron process. For

Table 1. Molecules studied and summary of results obtained.

Molecule	Ion observed	Relative sensitivity* (acetone = 1.0)	Ionization potential† (eV)
Hexane	None	0.	10.18
Carbon tetrachloride	None	0.	11.47
Benzene	$(M + H)^+$	0.2	9.24
Isopropanol	$(M + H - H_2O)^+$	0.39	10.15
	$(M + H)^+$		
	$(2M + H)^+$		
Acetone	$(M + H)^+$	1.0	9.69
	$(2M + H)^+$		
Acetonitrile	$(M + H)^+$	1.5	12.22
	$(2M + H)^+$		
Nitromethane	$(M + H)^+$	1.6	11.08
Pyridine	$(M + H)^+$	55.0	9.23

*Calculations were based on the $(M + H)^+$ ion. The acetone signal used for normalization was about 6 percent of the signal normally obtained under the same conditions with field ionization. †Measurements were made by photoionization (13).

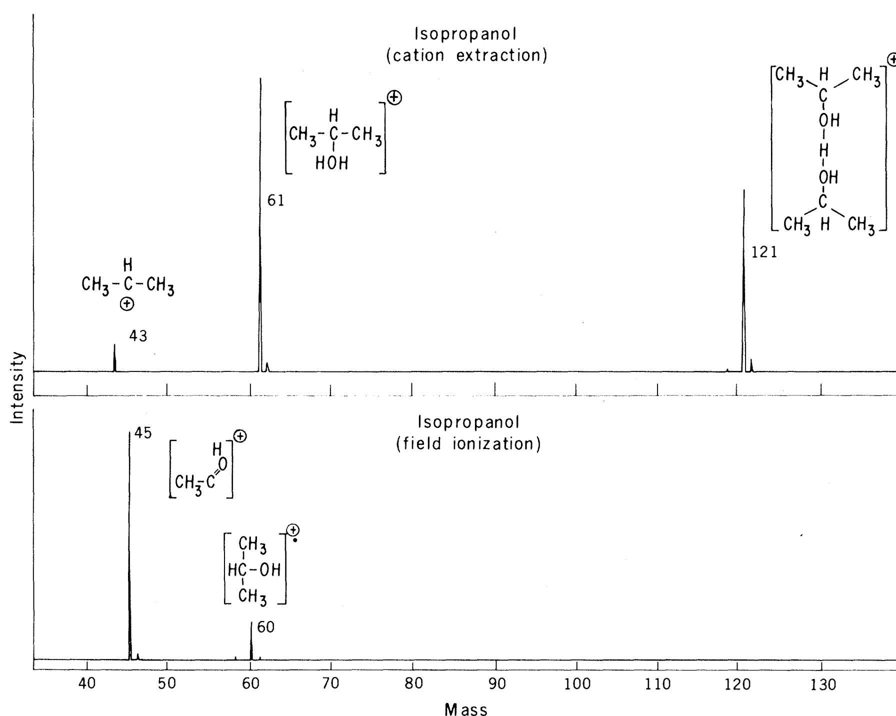

Fig. 1. Field ionization and cation extraction spectra of isopropanol.

the other molecules studied, fragmentation, if present, was below the detection limits of the instrument (<<1 percent). Odd-electron ions have not been observed. These results suggest an unusually mild ionization process. Fourth, the relative signal strengths observed are roughly proportional to the ease of protonation of the molecule in solution. Hexane and carbon tetrachloride are readily ionized under field ionization conditions but are not ionized at all under these conditions, whereas easily protonated compounds such as pyridine give strong signals. It is of interest that the sensitivities observed are not related to ionization potentials. For example, Table 1 shows that the sensitivity for acetonitrile (ionization potential, 12.22 eV) is far better than that for benzene (9.24 eV). Finally, the presence of cluster ions of the form $(2M + H)^+$ suggests that ionization occurs from the liquid phase, since pressures in the gas phase are too low for significant dimerization. When mixtures of materials are examined, both homogeneous and heterogeneous cluster ions can be observed and the relative intensities can be related to relative basicity.

The experiment described here is a simple, reliable, and unusually mild method for producing ion beams of protonated molecules. To my knowledge, this is the first unambiguous evidence that an ion-generating mechanism independent of classical field ionization can operate under substantially the same conditions. The data suggest a mechanism based on simple field-induced cation extraction from solution—a mechanism previously invoked in organic electrohydrodynamic ionization—and provide further evidence that this mechanism can be operative in conventional field desorption experiments. It should be noted that cation extraction by a high field does not necessarily reflect equilibrium conditions in the bulk liquid since physical parameters such as temperature (*10*) may be very different in the areas of active ion emission.

It will be of considerable theoretical interest to compare ions formed by cation extraction with ostensibly the same ions formed by chemical ionization mass spectrometry (*11*), using mass-analyzed ion kinetic energy spectrometry (*12*).

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Fibrinopeptide B and Aggregation of Fibrinogen

Abstract. *Removal of fibrinopeptide B from human fibrinogen by reaction with the procoagulant enzyme from copperhead snake venom below 25°C resulted in tight aggregation of the fibrinogen, which, in turn, progressively blocked a concomitant but sluggish release of fibrinopeptide A by the enzyme. When the clots obtained at < 25°C were warmed, they dissociated into soluble aggregates and monomers. Release of fibrinopeptide A then resumed, and a secondary coagulation followed. The aggregation induced by release of fibrinopeptide B itself involves a plasmin-susceptible segment located just distal to B in the Bβ chain of fibrinogen, a segment previously shown to be of little importance in the aggregation induced by release of fibrinopeptide A.*

The formation of fibrin in the blood of vertebrates involves a proteolytic reaction in which certain NH_2-terminal segments known (*1*) as fibrinopeptides A and B (hereafter termed A and B) are cleaved from the Aα and Bβ chains (*2*) of fibrinogen by enzymic action of thrombin, with subsequent self-aggregation of the altered fibrinogen into a clot (*3*). The release of A usually precedes the release of B, and suffices to transform fibrinogen into fibrin (*4*). Herzig *et al.* (*5*) observed that coagulation of human fibrinogen by a procoagulant enzyme from the copperhead snake *Ancistrodon contortrix contortrix* depended on the release of A even though the enzyme released B beforehand. This observation focused attention on the release of A as a critical step in coagulation of human fibrinogen. In contrast, Doolittle (*6*) had observed that release of B from lamprey fibrinogen sufficed to elicit clot formation without measurable release of A. With the prospect that the disparate effects of removing B might have been due to differing temperatures rather than to the species used, we reexamined human fibrinogen. Our studies indicate that removal of B from human fibrinogen does yield a self-aggregating and clot-forming derivative that coagulates at low temperatures near 25°C as used in the studies on the lamprey (*6*), but one that dissociates into soluble aggregates and monomers at 37°C, the temperature used in the study of Herzig *et al.* (*5*). More important though, the aggregation accompanying direct removal of B from human fibrinogen provides a basis for reinterpreting an observation (*7*) that plasmin rapidly removes segments containing B without eliciting aggregation. This observation together with qualifying data lead us to conclude that the plasmin-susceptible segments located immediately behind the fibrinopeptide comprise a critical portion of the B-dependent aggregation site. Further, the aggregation effected through release of B hinders release of A while little hindrance to release of both peptides occurs when they are released in opposite order, a condition we view as adding new significance to the usual removal of A before B in the course of blood coagulation.

Materials and methods used were essentially the same as described with the copperhead enzyme (*5*) except for a modification in the processing of the venom to inactivate a fibrinolytic enzyme that had chromatographic and gel filtration characteristics differing only slightly from the procoagulant, and which occasionally destroyed the procoagulant on storage. The inactivation was carried out with 10 μM tosyllysine chloromethyl ketone (TLCK) immediately after DEAE chromatography. After treatment for 30 minutes with TLCK, fractions with procoagulant activity were dialyzed, concentrated, and purified by gel filtration as described (*5*). All reactions between the procoagulant and fibrinogen were carried out in buffer containing 0.135M NaCl, 0.015M tris-HCl, and 0.1 mM EDTA at pH 7.4.

Ultracentrifugation at 25°C indicated a full transformation of fibrinogen to soluble aggregates ($s_{w,20} \simeq 16S$) in association with the preferential release of B by the procoagulant, whereas concomitant but relatively sluggish release of A reached only one-fifth of completion. As

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