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LIQUID CHROMATOGRAPH–MASS SPECTROMETER–COMPUTER ANALYTICAL SYSTEMS

A CONTINUOUS-FLOW SYSTEM BASED ON ATMOSPHERIC PRESSURE IONIZATION MASS SPECTROMETRY

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SUMMARY

Atmospheric pressure ionization (API) mass spectrometry is a novel form of mass spectrometry in which ions are generated in a reaction chamber external to the low-pressure region of a quadrupole mass spectrometer. Using this ionization technique a liquid chromatograph–mass spectrometer analytical system was constructed. The entire effluent stream from the chromatograph is vaporized through the API source. The primary ionization source for this work was a corona discharge. The very high ion densities generated by the corona discharge extend the linear range of the API source into the microgram region.

INTRODUCTION

The development of a liquid chromatograph–mass spectrometer–computer (LC–MS–COM) analytical system requires the solution of problems somewhat analogous to those faced in the design of early gas chromatograph–mass spectrometer (GC–MS) combined instruments. The separation step (LC or GC) provides an effluent stream which contains the components of the original sample, but this stream is usually not compatible with the sample introduction requirements of mass spectrometers. In the case of GC–MS instruments and GC–MS–COM analytical systems, the phase change from liquid to gas occurs before the separation process, and the interfacing problem involves reduction in the amount of carrier gas through the use of "molecule separators" or modification of MS source conditions to accept a GC effluent stream without mass-flow change. In a system involving LC separation, the phase change occurs at the interface, with the generation of relatively large amounts of organic solvent vapor. There are several ways in which sample introduction into the MS source might be accomplished. For example, the "molecule separator" concept might be used; this would require devices to reduce the mass flow of solvent vapor without undue loss of sample components. A two-stage transition might be used; solvents could be vaporized and removed in a first stage, and a second stage of heating

could be used to vaporize the sample components. The entire stream could be directed through the MS source; this would be analogous in a conventional mass spectrometer to the use of chemical ionization conditions, but the use of organic solvents with a heated filament source is not entirely practicable. The ability of the atmospheric pressure ionization (API) mass spectrometer[1–3] to accept organic solvents suggests that it should be possible to design a LC–MS–COM system in which the interface requirement is solely that of accomplishing a change of phase from liquid to gas, and directing the gas stream through the API source. This has been accomplished; a functional LC–MS–COM analytical system has been designed and the principles of operation have been demonstrated.

The chief problems at this time lie in reconciling solvent requirements for useful LC separations with those involved in ionization reactions occurring in the API source. All ordinary solvents (isooctane, benzene, ethyl acetate, chloroform, methanol, ethanol, isopropanol) are tolerated by the API source, so that the choice of solvent(s) is not particularly restricted. The ions produced in the source, however, depend upon the chemical properties in the gas phase of the sample components, and upon the nature of the reaction conditions in the source. The present work was carried out with compounds that are protonated by proton transfer from reagent ions derived from ethanol, and with compounds that form negative ions. Both positive and negative ions can be detected with the API instrument.

EXPERIMENTAL

Liquid chromatograph

The liquid chromatograph was a Waters Model ALC-202 high-pressure unit with a standard UV detector (254 nm). The column was a 30-cm Waters column with a packing of Corasil II. Samples were injected by syringe injection. The usual solvent was commercially available chloroform containing about 0.75% of ethanol; experiments were also carried out with isooctane–chloroform mixtures. The flow-rate was 0.5 ml/min at a pump pressure of 400 p.s.i.

A short length of narrow-bore stainless-steel tubing was used to connect the effluent line of the chromatograph to the vaporizer–source assembly.

API mass spectrometer

Details of the design and operation of an API mass spectrometer have been published[1,2]. Two types of primary ionization have been used. Subpicogram sensitivity in detection has been achieved with a low-volume reaction chamber containing a ^{63}Ni foil[2]. A larger reaction chamber with a corona discharge was constructed for use in current studies; details will be published separately. Samples are accepted in the same way for both source designs.

The vaporizer–source assembly is exterior to the low-pressure region of the mass spectrometer. A glass liner was used in the vaporizer and a low flow (8–10 ml/min) of preheated (in the vaporizer body) nitrogen was used to aid in the vaporization process. The assembly was maintained at 200° when samples were injected by syringe. During LC–MS operation, the assembly was held at 280–300°. The temperature profile within the vaporizing region was not determined. The use of a low flow of preheated carrier gas aided in the smooth conversion of the liquid effluent stream

to a gas stream. A condensation vessel was added to the gas exit line in order to avoid discharge of solvent vapors into room air.

Two detection procedures were employed. Selective ion monitoring was used to detect specific compounds in the effluent stream. Scanned mass spectra were taken for peaks detected by the UV detector of the chromatograph. Ion masses were determined by voltage measurements after calibration of the mass analyzer.

Computer

The computer was a PDP 8/E with laboratory interface. Operating details have been published[1].

Samples

Solutions of reference compounds were prepared and used as described earlier[1,2].

RESULTS AND DISCUSSION

Design of the system

A flow diagram of the system is shown in Fig. 1. A high-pressure liquid chromatograph, Waters Model ALC-202, was used with a standard 30-cm column packed with Corasil II. A short length of narrow-bore tubing served to connect the chromatograph to the heated source assembly of the API mass spectrometer. A low flow (8–10 ml/min) of carrier gas (nitrogen) was used to aid the vaporization process. The entire effluent stream was vaporized and directed through the external source of the API mass spectrometer; it was condensed after leaving the source to prevent room air contamination.

The ion source in an API mass spectrometer is external to the low-pressure region of the quadrupole mass analyzer, and is maintained at atmospheric pressure. The vaporization step does not involve large pressure changes, but the increase in

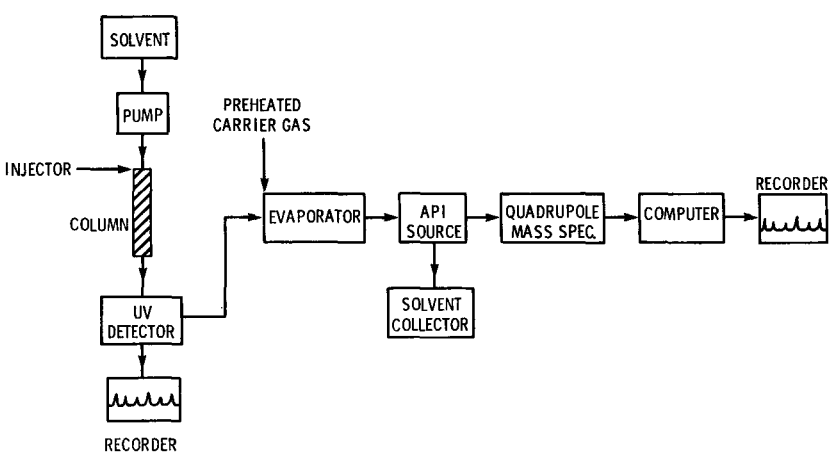

Fig. 1. Schematic diagram showing the flow pattern and physical relationships of component units of the LC–MS(API)–COM analytical system.

volume accompanying the phase change is such that very large liquid flow-rates are to be avoided. The flow-rate used in this work was 0.5 ml/min.

The introduction of ions into the mass analyzer was through a small aperture (25-μm diameter in most of our studies) in a nickel disc that formed one wall of the source; in effect, the mass analyzer and the detection circuitry provided a continuous record of the ions present in the source throughout the period of analysis. Analyses were carried out with selective ion detection, to indicate the presence of a single compound, or by scans which provided a scanned mass spectrum. A UV absorption detector was used in serial fashion.

Two types of sources were used. A ^{63}Ni source with a small reaction chamber, leading to subpicogram sensitivity in detection, has been described[2]. This work was carried out with a slightly larger reaction chamber, and with a corona discharge to initiate the ionization process.

Mechanisms of ionization

In the absence of a sample, the ions present in the source chamber are those derived from the carrier gas (GC or direct injection mode of operation) or the solvent(s) vapors which are the equivalent of a carrier gas flow when LC is used. The positive ions present in nitrogen have been described[1,2]; when an organic solvent is introduced, additional reactions will normally occur. For example, if methanol or ethanol is vaporized in the source assembly, cluster ions of the general structure $(ROH)_nH^+$ are formed. The distribution of n depends upon the temperature of the reaction chamber and the concentration of the alcohol. We have not observed unsolvated protons. Cluster ions with two or three solvating molecules are formed at 100–200° when small amounts of alcohols are introduced in solution in chloroform or methylene chloride. Fig. 2 shows a scanned positive ion mass spectrum observed when chloroform is injected into the source assembly. The ethanol present in the solvent (about 0.7%) is ionized at 200° to form a cluster ion with $n=2$ as the principal ion product. A higher concentration of ethanol leads to ions with $n=3$ or 4; cluster ions with $n=6$ have been observed under some conditions.

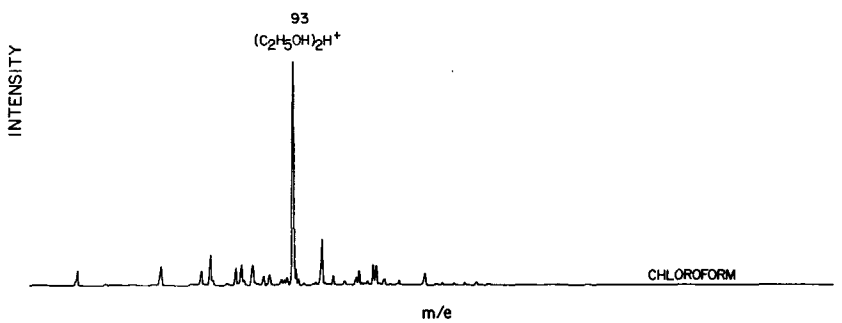

Fig. 2. Ion profile (mass scan) showing positive ions present in the API source after the injection of 2 μl of chloroform. The major positive ion at 200°, obtained with carrier gas (nitrogen flow), from the ethanol normally present in commercially available chloroform is $(C_2H_5OH)_2H^+$. When chloroform is vaporized through the source assembly in continuous fashion with a liquid flow-rate of 0.5 ml/min, under the usual conditions of operation of the LC–MS–COM system, the major ion is $(C_2H_5OH)_3H^+$.

When benzene is introduced into the source, the principal ions are $C_6H_6^+$ and $C_{12}H_{12}^+$; these ions are available for charge transfer. Isooctane is not ionized under normal operating conditions, but traces of water are usually present and $(H_2O)_nH^+$ ions are available for proton transfer. Chloroform and methylene chloride do not yield positive ions under ordinary conditions, but the addition of methanol or ethanol provides cluster ions as discussed earlier.

Negative ions are formed from some solvents. Chloroform, for example, provides Cl^- and cluster ions of the structure $(CHCl_3)_nCl^-$; at 200°, n is usually 1 or 2. Superoxide ions, O_2^-, are produced when oxygen is present. Free electrons are also available for electron attachment.

Organic compounds introduced into the source as sample components may be ionized in a variety of ways. Proton addition to form MH^+ and proton loss to form $(M-H)^-$ are common routes of ionization for gas phase bases and gas phase acids, respectively[1,2]. Molecular ions, M^+, are formed under a variety of conditions; the ionization potential required for this conversion depends upon the structure of M. The direct formation of M^- is also possible for some molecules.

Separation and detection by ionization to MH^+

Many organic compounds are stronger gas-phase bases than ethanol or methanol. For example, α,β-unsaturated ketones and esters, as well as amines, are protonated in an API source in the presence of ethanol.

The general reaction can be written as:

$$B + AH^+ \rightarrow BH^+ + A$$

Reaction to form BH^+ will occur whenever B is a stronger base than A.

It is possible to carry out LC separations of gas-phase bases with isooctane–chloroform mixtures, or with chloroform, and to detect the passage of the sample through the source by monitoring for MH^+ or $(MHC_2H_5OH)^+$. Fig. 3 shows a

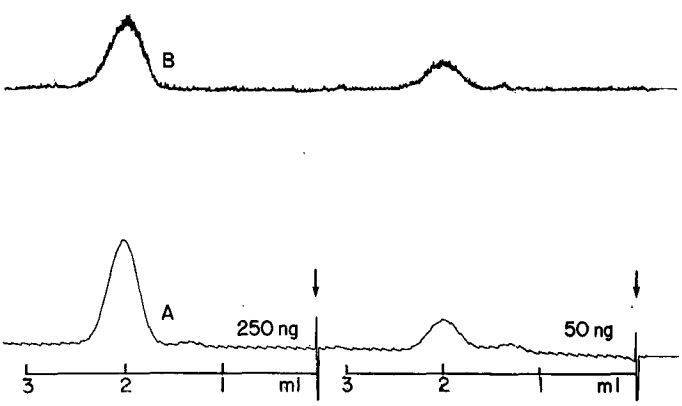

Fig. 3. Chart records showing the detection of progesterone in a LC–MS–COM system. (A) Response of the UV detector to samples of 50 and 250 ng eluted from the column with chloroform at a flow-rate of 0.5 ml/min. (B) Response of the MS(API) in selective ion detection mode for MH^+. The ions formed from the sample were determined in separate experiments to be MH^+ and $(MHC_2H_5OH)^+$.

comparison of UV detection and API detection for progesterone. Chloroform was the LC solvent; ethanol in the chloroform provided $(C_2H_5OH)_nH^+$ ions, and MH^+ was monitored. A mass scan, taken as the progesterone peak was eluting, showed MH^+ and $(MHC_2H_5OH)^+$ ions for the steroid.

An example of a separation of three methylthiohydantoin derivatives, from glycine, valine and phenylalanine, is shown in Fig. 4. The UV detector record shows the elution pattern obtained with chloroform as the solvent. Selective ion detection for MH^+ for the glycine derivative was employed with the API mass spectrometer; the chart record is shown in Fig. 4. The ions present in the API source during the elution of the three compounds are shown in Fig. 5. The principal reactant ion was $(C_2H_5OH)_3H^+$, and each methylthiohydantoin derivative was ionized to MH^+ and $(MHC_2H_5OH)^+$ ions.

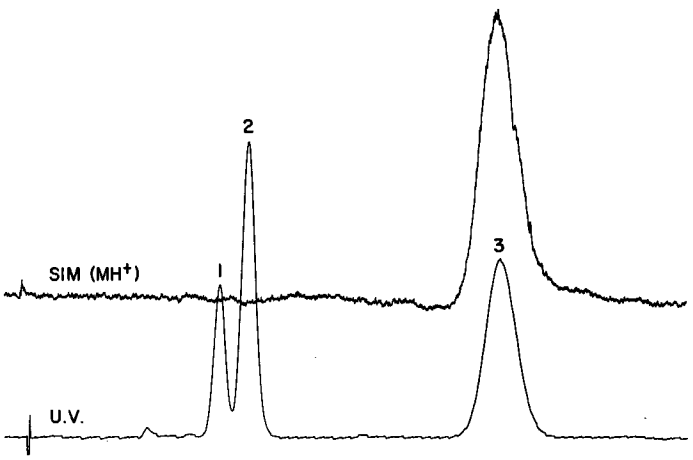

Fig. 4. Chart records showing the detection of methylthiohydantoin (MTH) derivatives of three amino acids. The UV detector record shows the elution pattern for all three compounds: 1 = MTH-phenylalanine; 2 = MTH-valine; 3 = MTH-glycine. The MS detection was by selective ion monitoring for MH^+ for the glycine derivative.

In these instances chloroform, containing a small amount of ethanol, was a suitable solvent for the LC separation, and suitable reagent ions of the structure $(C_2H_5OH)_nH^+$ were generated when the effluent stream was vaporized through the API source. It seems likely that solvent systems of graded polarity, based on isooctane–chloroform–ethanol, or isooctane–methylene chloride–methanol, will prove to be useful eluting solvents for LC–MS(API)–COM analytical systems, in instances where MH^+ ions are formed by the sample components under study.

One of the difficulties often encountered when UV or other relatively non-specific detection procedures are employed is that peak overlap results in peak shapes that are difficult to quantify. When mass spectrometric techniques are employed, specific detection methods can be used to avoid this difficulty. For example, if ten or twenty methylthiohydantoin derivatives of amino acids had been present in the original sample, separated as in Fig. 5, it would have been possible to use a repetitive scan procedure, and by programmed analysis a separate elution chart record could have

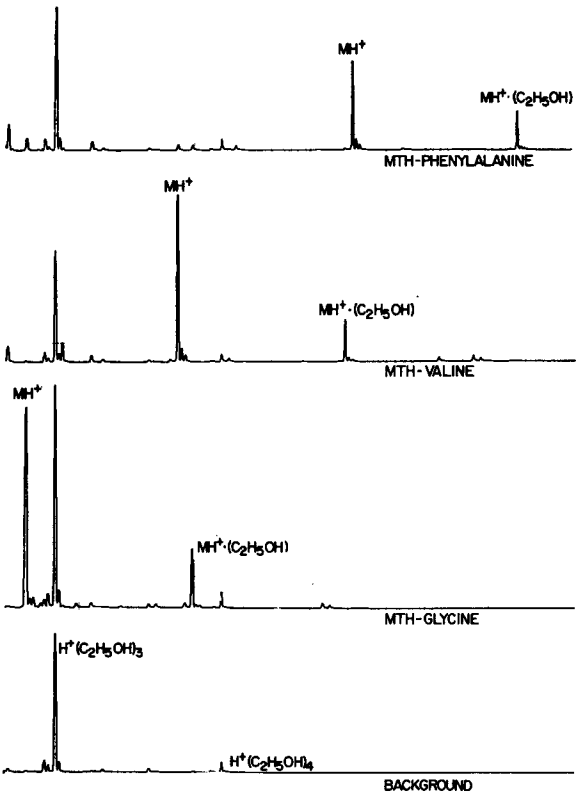

Fig. 5. Ion profile (scanned mass spectrum) showing the positive ions present in the API source for the methylthiohydantoin derivatives of three amino acids. The product ions were MH^+ and $(MHC_2H_5OH)^+$.

been obtained for each MH^+ ion. Techniques of this sort are now used with GC–MS–COM analytical systems.

Separation and detection by ionization to $(M-H)^-$

Gas phase acids of varying acid strength will ionize to $(M-H)^-$ in the API source whenever ions of sufficient basicity are present. The general reaction:

$$HA + B^- \rightarrow A^- + HB$$

will occur whenever HA is a stronger acid than HB. Decades of experimental observations have resulted in measurements of dissociation constants for many organic acids in a variety of solvents. These observations have almost no relevance for gas-phase work. For example, picric acid is a very strong acid in the gas phase. Malononitrile is a stronger acid than acetic acid, and phenobarbital is a stronger acid than hydrochloric acid.

When a chloroform stream is vaporized through the API source, the negative ions that are formed are Cl^- and the cluster ions $(CHCl_3)_nCl^-$; the distribution of

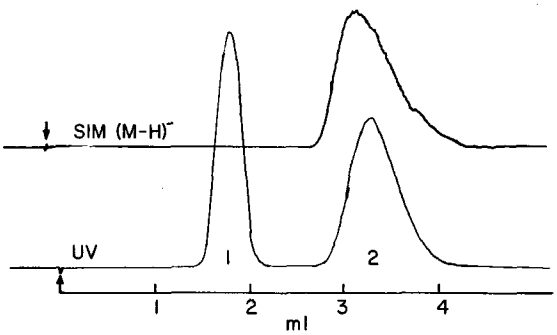

Fig. 6. Chart records showing the detection of trinitrotoluene (1) and trinitrophenol (2). The UV detector record shows the elution pattern for both compounds. The MS detection was by selective ion monitoring for $(M - H)^-$ for trinitrophenol.

ions depends upon the concentration of chloroform and the temperature of the source. If a gas phase acid is introduced, ionization to $(M - H)^-$ will occur if MH is a stronger gas phase acid than HCl. While ordinary carboxylic acids are weaker than hydrochloric acid, and will not ionize under these conditions there are many compounds that are stronger acids which will ionize. Fig. 6 shows a LC separation of trinitrotoluene and picric acid (trinitrophenol) in chloroform solution, with a comparison of UV detection and API selective ion detection of $(M - H)^-$ for picric acid. Fig. 7 shows a mass scan of the ions present in the source during the elution of this compound. Two solvated chloride ion species were present; the picric acid was present as $(M - H)^-$.

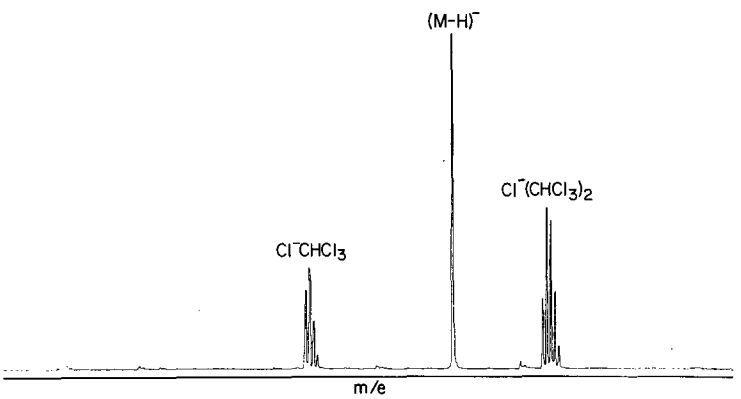

Fig. 7. Ion profile (scanned mass spectrum) showing the negative ions present in the API source during the ionization of trinitrophenol. The reactant ions are derived from chloroform; trinitrophenol is a strong gas-phase acid, and is ionized by proton transfer to form $(M - H)^-$.

Directions of further work

Many low-resolution separations can be carried out rapidly and effectively by LC techniques. It is likely that most applications of LC–MS(API)–COM systems which should be investigated will fall into this category. For example, separations of methylthiohydantoin derivatives of amino acids should be investigated in greater

detail. These methods are limited to compounds which are volatile, or which can be volatilized after derivative formation.

High-resolution LC separations are generally slow. While speed of operation is not always a major factor in an analytical problem, it seems unlikely that slow-speed analyses with LC–MS(API)–COM systems will become highly useful.

Current studies are directed to evaluating these methods for possible use in drug monitoring during anticonvulsant therapy, and in metabolic profile studies of urinary and plasma bases.

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