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CROSSED-BEAM LIQUID CHROMATOGRAPH–MASS SPECTROMETER COMBINATION

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SUMMARY

A new approach to combined liquid chromatograph–mass spectrometry is described which uses laser vaporization of the liquid chromatographic (LC) effluent and molecular beam techniques to transport and ionize the sample. A key feature of this approach is that samples eluting from the LC are vaporized, ionized, and mass analyzed with minimal contact with solid surfaces. Results are presented on the performance of the crossed-beam system for relatively volatile aromatic hydrocarbons and for components of nucleic acids —bases, nucleosides, and nucleotides— which provide an interesting series of biologically important compounds of decreasing volatility and corresponding increasing difficulty for mass spectrometry. Mass spectra are presented which were obtained by laser vaporization of samples from the LC effluent under both reversed-phase and ion-exchange conditions.

INTRODUCTION

The suggestion has been made that only 20% of the known organic compounds are suitable for gas chromatography (GC) without further derivatization[1]. High-performance liquid chromatography (HPLC), on the other hand, is not limited by volatility or thermal stability of the samples and its ability to separate and analyze complex mixtures is becoming widely appreciated[2]. Advances in instrumentation and column technology have made HPLC even more effective recently, both as a research tool and for routine analyses. Currently a most serious limitation on the technique is the lack of a truly universal detector. The mass spectrometer is an obvious potential solution to the LC detector problems, but interfacing a mass spectrometer with a liquid chromatograph is a significantly more difficult problem than with a gas chromatograph.

There are several approaches currently being studied for liquid chromatograph–mass spectrometer interfacing. These include a simple split (*ca.* 1/1000) of the LC effluent[3–8], use of atmospheric pressure ionization (API) mass spectrometry (MS)[9–11], enrichment of the LC effluent with a membrane separator[12–15], use of mechanical transport of the sample with solvent evaporation in vacuum lock chambers[16–19], and the approach involving laser vaporization combined with molecular beam technology developed in this work.

Our approach employs laser vaporization to rapidly vaporize both the solvent and the sample and molecular beam techniques to transport and ionize the sample with minimal contact with solid surfaces. The rationale for our approach is based in part on the work of Buehler *et al.*[20] and Gaffney and Friedman[21], in which it was shown that quite involatile samples, *e.g.* underivatized peptides, can be vaporized intact by employing rapid heating and vaporizing the sample from weakly interacting surfaces such as PTFE.

A key feature of our approach to interfacing the liquid chromatograph with the mass spectrometer is that solute molecules eluting from the liquid chromatograph are vaporized, ionized and mass analyzed with minimal contact with solid surfaces. The defining characteristic of molecules which cannot be analyzed by GC appears to be their instability with respect to solid surfaces. On contacting a surface, involatile molecules may either adhere or be decomposed. By using the laser vaporization and crossed-beam ionization techniques, contact with solid surfaces is avoided.

An obvious potential problem area for any LC–MS technique applied to involatile solids is the possibility of deposition of solid contaminants on critical surfaces in the instrument. This may be particularly troublesome in the mass spectrometer if the deposited materials are electrical insulators; surfaces covered by insulating layers may be charged by ion or electron bombardment causing erratic performance or complete loss of the ion beam. Disassembly and cleaning is often required before satisfactory performance can be restored. Contamination problems may be particularly severe in the combination of MS with ion-exchange chromatography, where the mobile phase typically contains macroscopic quantities of dissolved salts as buffers. Unfortunately, many of the most promising biomedical applications of LC employ ion-exchange separation techniques.

The crossed-beam liquid chromatograph–mass spectrometer interface possesses the characteristics necessary to cope with potential contamination problems. Since the sample molecules, which are ionized and eventually detected, are transmitted through the apparatus in a molecular beam with little contact with surfaces, the critical surfaces may be heated quite hot to prevent deposition of solids, without causing pyrolysis of the sample. Thus the critical beam defining apertures, *e.g.* the sampling orifice, skimmer and collimator, are heated electrically. While the contamination problems cannot be avoided entirely, these techniques are sufficient to extend the mean operating time between cleanings to the point that down time is not a serious impediment to the application of the instrument.

In developing the crossed-beam LC–MS system the following design goals were adopted:

(1) Chromatographic performance should not be degraded.

(2) The system should be applicable to all of the separation techniques presently employed in modern HPLC including ion exchange.

(3) Up to 1 ml/min of LC effluent should be converted as quickly as possible to high temperature vapor.

(4) The sample analyzed by the mass spectrometer should be vaporized and ionized without contacting solid surfaces.

(5) The system should operate for extended periods without shutdown for cleaning or replacement of components (*e.g.* filaments).

(6) The vacuum system should be capable of maintaining high vacuum (*ca.*

10^{-6} torr) in the mass analyzer with liquid injections into the vaporizer of up to 1 ml/min.

(7) The sensitivity and resolution should be comparable to that currently obtained in GC–MS.

(8) Both positive and negative ion mass spectra as well as normal chemical ionization (CI) and electron impact (EI) mass spectra should be measurable.

This rather demanding set of design goals was used to guide a number of critical design decisions required in developing the instrument described in this paper. As may be seen from the data presented in subsequent sections, not all of these goals have been realized; however, sufficient progress has been made to suggest that, with further refinement and development of the basic techniques developed in this work, these goals may be achieved.

The crossed-beam liquid chromatograph–mass spectrometer interface

The crossed-beam liquid chromatograph–mass spectrometer interface initially developed in this work is shown schematically in Fig. 1. The liquid chromatograph–mass spectrometer interface consists of three essential elements: (1) the laser beam vaporizer; (2) the differentially pumped, free-jet, molecular beam forming apparatus; and (3) the crossed-beam CI–EI ionizer.

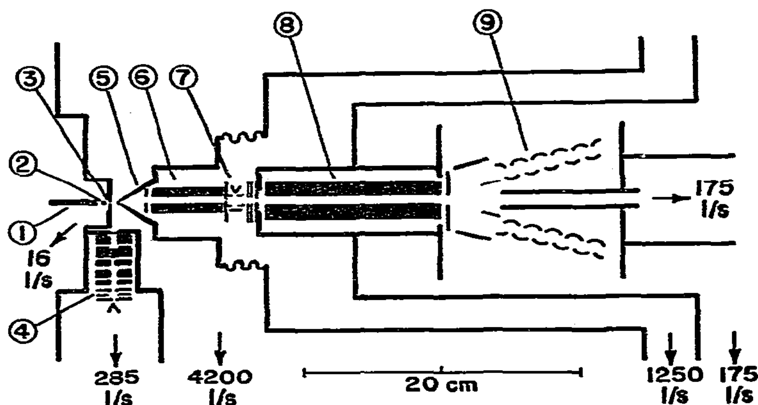


Fig. 1. Schematic diagram of the crossed-beam liquid chromatograph–mass spectrometer interface. 1, Liquid nozzle; 2, laser beam; 3, sampling aperture; 4, CI electron gun; 5, skimmer; 6, quadrupole with RF only; 7, EI ion source; 8, quadrupole mass analyzer; 9, multipliers.

The effluent from the liquid chromatograph enters the vaporization region through a 0.015-cm I.D. stainless-steel capillary tube, and the liquid jet emerging from the capillary is intersected by the focused beam from a 50-W CO_2 laser (Coherent Radiation Model 42). Using a 12.5-cm focal length zinc selenide lens the beam of 10.6-μm radiation is focused to a diameter of 0.015 cm at the intersection with the jet, corresponding to a power density of 10^5 W/cm^2 at the intersection. This radiation intensity is sufficient if entirely absorbed to vaporize up to 1 ml/min of water in the approximately 10^{-4} sec required for the liquid jet to pass through the laser

beam. The absolute positions of the liquid jet and the laser beam are adjustable from outside the vacuum housing to an accuracy of better than ±0.001 cm using micrometer screws. The laser output beam is adjustable between 5 and 50 W and is monitored by a built-in power meter. Since water and many other potential solvents are essentially opaque to 10.6-μm radiation, laser vaporization of the entire LC effluent is feasible over a wide range of flow-rates.

The rapid vaporization converts the liquid jet into a vapor jet which is sampled by a small orifice in a thin molybdenum plate located a short distance downstream. Solvated involatile molecules are carried along by the vapor jet, and by careful control of the laser beam intensity, it is possible to strip all of the solvating molecules from the involatile species without rupturing chemical bonds. The sampling orifice serves as the nozzle for the supersonic molecular beam system employed in the crossed-beam ionizer. In the region between the nozzle and skimmer the vapor undergoes adiabatic expansion. By proper choice of the nozzle to skimmer distance and the skimmer diameter, the transition from viscous flow to free molecule flow can be made to occur near the skimmer orifice, *i.e.*, the mean free path at the skimmer is approximately equal to the skimmer orifice diameter. A special worm gear drive assembly allows the skimmer to nozzle distance to be adjusted from outside the vacuum to a precision better than 0.001 cm while maintaining accurate axial alignment. The skimmer and nozzle orifices are mounted so that they may be removed and replaced with minimal disassembly.

In the region between the nozzle and the skimmer, the molecular beam is intersected by a focused electron beam, which produces ionization, mainly of the solvent molecules. As a result of frequent collisions occurring in this region, the ionization is preferentially transferred to the solute molecules by ion–molecule reactions. In this way, CI of the solute is produced with the solvent as the reagent During the adiabatic expansion, the temperature of the vapor is substantially reduced and some condensation may occur.

A second electron beam intersects the molecular beam in the region between the skimmer and collimator. In this region, the beam is characterized by free molecule flow, *i.e.*, molecular collisions in the beam are negligible. Thus, ionization of the beam in this region produces the normal EI spectra of both the solvent and the solute. By operating one or the other electron gun, or both, we may produce either CI spectra or EI spectra, or the combination of the two.

The mass analyzer is a high-performance ELFS quadrupole (Extranuclear Model 4-162-5 with Model 011-1 RF power supply) with a mass range of 1–1000 a.m.u. After exiting the quadrupole, ions may be detected by either of two bakeable electron multipliers. These multipliers are displaced laterally from the quadrupole exit to minimize noise due to photons or excited neutrals emerging from the analyzer and to minimize contamination by involatile substances in the molecular beam. Deflectors are provided to direct the ions into the active surface of one or the other multiplier. The anode of each multiplier is connected to the amplifier outside the vacuum envelope through a high-voltage triaxial feedthrough allowing either one to be wired for detecting either positive or negative ions. In normal operation the deflectors and multiplier bias voltages are set up so that one multiplier detects positive and the other negative ions. The ion intensities may be measured either by ion counting or by current amplification.

Carefully designed differential pumping is required to cope with the large ratio of molecular density in the liquid (*ca.* 3×10^{22} molecules per ml) to that in the high vacuum required for the mass analyzer (*ca.* 3×10^{10} molecules per ml). The pumping speeds employed on the various regions of the apparatus are indicated on Fig. 1. The vaporization region is evacuated by a 1000-l/min mechanical pump; the adiabatic expansion volume between the nozzle and skimmer is evacuated by a 4000-l/sec diffusion pump backed by a 1000-l/min mechanical pump; the region between the skimmer and the quadrupole which contains the EI source and ion lens is evacuated by a 1200-l/sec diffusion pump backed by a 300-l/min mechanical pump; and the quadrupole is evacuated by a 175-l/sec diffusion pump backed by a small mechanical pump. Separate pumping is also provided for the electron gun producing the CI ionizing beam which is isolated from the nozzle-skimmer except for a 1-mm aperture for the electron beam and it is pumped by a 285-l/sec diffusion pump backed by a 100-l/min mechanical pump. The apertures in the ion lens are arranged so that the final aperture in front of the quadrupole serves as the collimating aperture for the molecular beam with all other apertures being sufficiently large that they are not struck by the molecular beam. Furthermore, the quadrupole and its exit aperture are sufficiently large that the entire molecular beam transmitted through the collimating aperture passes unimpeded to the beam trap, which is evacuated by a 175-l/sec diffusion pump backed by a small mechanical ump. The molecular beam entering the trap impinges on a liquid nitrogen cooled surface so that the effective pumping speed of the trap for condensibles is much higher. A gate valve is provided between the trap and the remainder of the vacuum system so that the trap can periodically be warmed to ambient to allow the more volatile components to be pumped away. The pumping systems are fully valved to allow rapid cycling when it is necessary to break vacuum for servicing, and the system is fully protected against power or cooling water failures.

A high-performance liquid chromatograph (Perkin-Elmer Model 601) is equipped with a variable-wavelength detector. This system provides pulse-free reproducible flows from 0.05 ml/min to 6 ml/min at head pressures to 3000 p.s.i. The system is operated with the UV detector prior to, but in series with the mass spectrometer to allow direct comparisons of the two detectors.

Two alternate systems are provided for measuring the ion intensities transmitted by the quadrupole mass analyzer. One of these consists of a 100-MHz ion counting system followed by a digital-to-analog convertor; the other is a 10-kHz current amplifier with a dynamic range of 10^4. The ion counting system may be used for the measurement of the intensities of either positive or negative ions, but is routinely used on the negative ion channel.

The output of either data channel may be connected to the visicorder for recording spectra; alternatively, both channels may be connected to the computer through digital-to-analog convertors and pseudo-simultaneous measurement of both positive and negative ion spectra may be accomplished using the modulation technique described previously by Hunt and coworkers[22]. The computer system is the Finnigan/Incos Model 2300.

RESULTS

An example of the performance of the crossed-beam liquid chromatograph–mass spectrometer run in the EI mode on a mixture of aromatic hydrocarbons is shown in Fig. 2, where the output of the UV detector (254 nm) is compared to mass chromatograms on the parent masses and the reconstructed liquid chromatogram obtained by summing all of the ions detected. This data was obtained using 5-sec repetitive scans from 60 to 360 a.m.u., and the chromatographic conditions are given in the caption of Fig. 2. Under these conditions the widths of the peaks at half-height are approximately 30 sec and no difference is detectable between the widths of the peaks on the UV trace and those in the mass chromatograms. Thus, at least at the LC resolution displayed in this example, there is no apparent degradation of effective LC efficiency as the result of using the mass spectrometer as a detector. For the *ca.* 2-μg samples shown in Fig. 2 the peak sample elution rate is about 70 ng/sec, and except for mass 128 (naphthalene) no background signal was detected at the parent mass with the detector sensitivity used in these measurements. The minimum detectable signal per a.m.u. for these detector conditions is about 0.4% of the maximum signal observed for biphenyl at mass 154. We conclude from this result, and a series of similar measurements that the minimum detectable effluent rate using the mass chromatogram is about 1 ng/sec corresponding to a total sample injection of about 30 ng for these admittedly favorable compounds. The detection

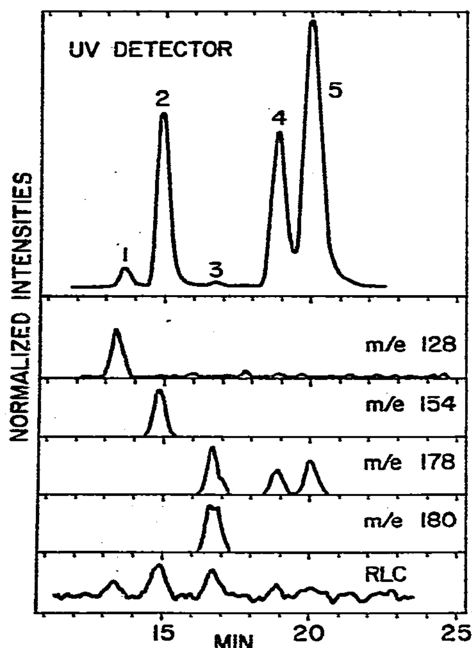


Fig. 2. UV (254 nm) detector trace and reconstructed mass and liquid chromatograms for an aromatic mixture. 1 = 2.4 μg naphthalene; 2 = 5.4 μg biphenyl; 3 = 4.8 μg 9,10-dihydroanthracene; 4 = 2.0 μg phenanthrene; 5 = 2.4 μg anthracene. The mixture was separated using a 25 cm × 4.6 mm Partisil-10-ODS-2 column with a 20% water in acetonitrile isocratic mobile phase at 0.5 ml/min.

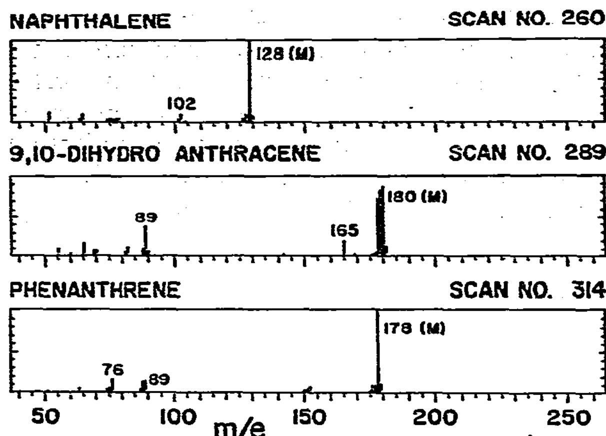


Fig. 3. EI mass spectra obtained for some of the aromatics shown in Fig. 2.

limit using the reconstructed liquid chromatogram corresponds to about 1 μg of sample as the result of base-line noise caused by flow fluctuations and variations in the molecular beam intensity. An advantage of the mass spectrometer over the UV detector is illustrated by 9,10-dihydroanthracene, which, due to its weak UV adsorption at 254 nm, is barely detectable in the UV trace, even though it is a major component in the mixture. The mass chromatograms show nearly the same sensitivity for all five components.

Examples of the mass spectra obtained for some of these aromatic hydrocarbon samples are shown in Fig. 3. Except for differentiating between anthracene and phenanthrene which have very similar EI spectra, all of these components were easily identified by automatically searching the computer library file of some 23,000 spectra.

Evaluation of the performance of the instrument has primarily employed samples of biological significance with special emphasis on the components of nucleic acids —bases, nucleosides and nucleotides— which provide an interesting series of biologically important compounds of decreasing volatility and corresponding increasing difficulty for MS. The EI mass spectrum of the purine base adenine, ob-

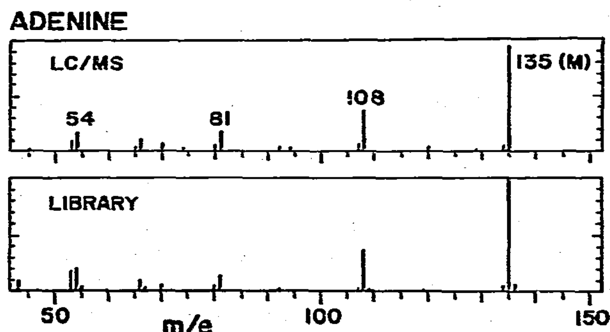


Fig. 4. Comparison of an EI spectrum of adenine obtained on the LC–MS instrument with that on file in the computer library.

tained using the LC–MS, is compared with the spectrum retrieved from the computer library in Fig. 4. This spectrum corresponds to a 5-μg injection using pure water as the LC solvent at a flow-rate of 0.2 ml/min and represents the average of twelve scans from 20 to 320 a.m.u. of 5 sec each; the background has been automatically subtracted. As can be seen from Fig. 5 the EI spectrum obtained by laser vaporization from aqueous solution is in good agreement with the library spectrum which was presumably obtained by thermal vaporization from the direct insertion probe.

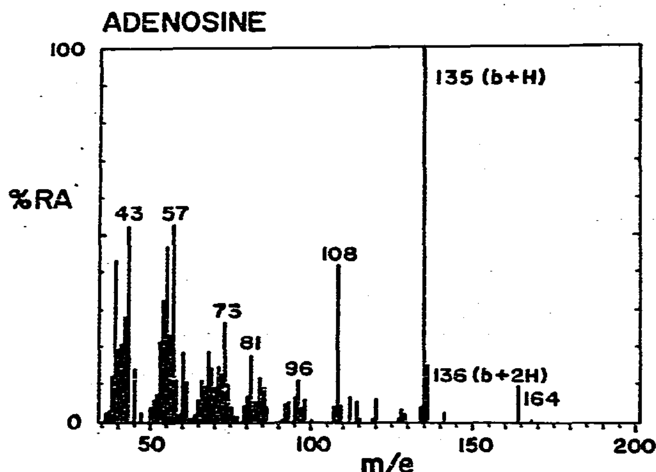


Fig. 5. EI mass spectrum of adenosine under conditions in which partial pyrolysis occurs.

The EI mass spectrum of the nucleoside adenosine, obtained using the same conditions, is shown in Fig. 5. The peaks at 135, 108 and 81 a.m.u. characteristic of the adenine base are obtained with approximately the same relative intensity as are observed for the free base. In addition b + 2H (mass 136), b + 30 (mass 164), and fragments of the sugar are observed. The parent ion was not detected and no other significant peaks were observed at higher masses. Comparison of the spectrum with published spectra of adenosine[23] indicates that partial pyrolysis of the sample occurred prior to ionization.

EI mass spectra of uracil, uridine, and 5′-UMP are shown in Fig. 6; the conditions used are the same as those for adenine. The spectrum of uracil is in good agreement with published spectra[23], although again slightly more fragmentation is observed. The uridine spectrum shows substantially more fragmentation than published spectra obtained by careful vaporization from the solids probe[23]. Very weak ion intensities were observed at masses 141, 155, 171 and 226, but the molecular ion peak was not detectable. The spectrum obtained for 5′-UMP is essentially the same as that of uridine; the small apparent differences are within the range of the variations found in repeated runs of these compounds. We have also obtained spectra of the uridine disphosphate and triphosphate and these compounds also produce spectra which are characteristic of the nucleoside. None of the nucleotide spectra show any significant peaks indicating the presence of the phosphate moiety.

Most of our work has employed reversed-phase LC separation with water,

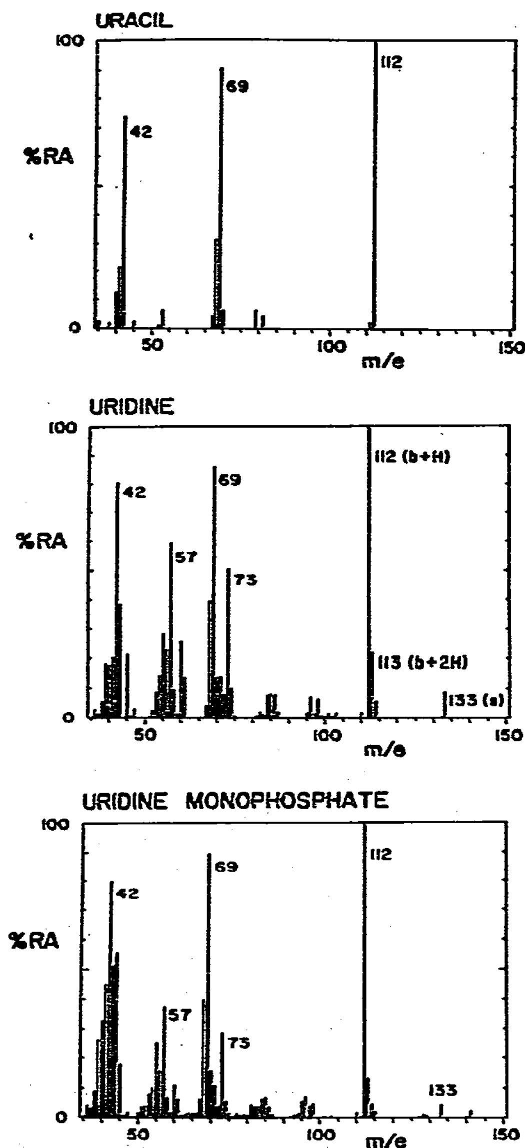


Fig. 6. EI mass spectra of uracil, uridine, and 5′-uridine monophosphate.

acetonitrile, and water–acetonitrile mixtures as solvents; however, we have also done some work using ion-exchange chromatography. The first such work employed gradient elution between pure water and buffer consisting of 0.05 *M* $NH_4H_2PO_4$ adjusted to pH 3.6 with phosphoric acid. The EI mass spectrum of the vaporized buffer above mass 35 is shown in Fig. 7. The base peak is mass 81 ($H_2PO_3^+$) and significant intensities are observed at mass 98 ($H_3PO_4^+$) and mass 63 (PO_2^+). We are aware of no previous measurement of the EI spectrum of phosphoric acid, but it appears that these ions are reasonable and correspond to the following fragmentation mechanism:

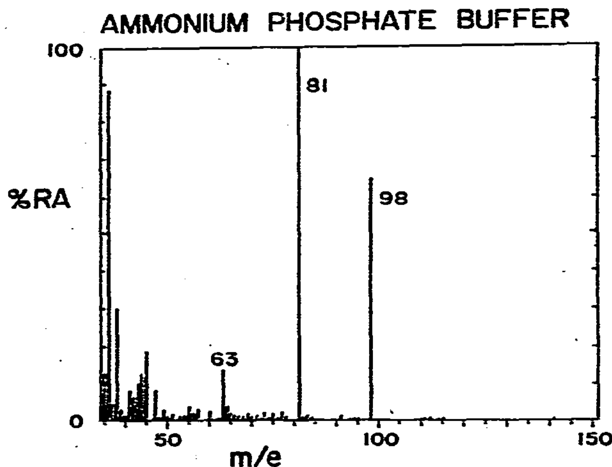
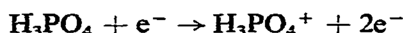


Fig. 7. EI mass spectrum of a buffer solution consisting of 0.05 *M* ammonium dihydrogen phosphate adjusted to pH 3.6 with phosphoric acid.



$$H_3PO_4 + e^- \rightarrow H_3PO_4^+ + 2e^-$$

$$H_3PO_4^+ \rightarrow H_2PO_3^+ + OH$$

$$H_2PO_3^+ \rightarrow PO_2^+ + H_2O$$

A mixture of uracil, dimethyl uracil, dimethyoxypyrimidine, thymine and dimethylthymine was successfully separated and mass spectrometrically identified by ion exchange on a Bondapak CX–Corasil column using the phosphate buffer. However, the sensitivity was relatively poor due to the rather large relative intensity of the buffer ions and some instabilities in the molecular beam. The instrument was operated for several hours with continuous injection and vaporization of the buffer at a flow-rate of 0.2 ml/min, and while an unsightly gray deposit (presumably phosphates) built up on cooler surfaces in the vaporization and expansion chamber, no degradation in performance was observed. After these experiments were completed, the instrument was operated for several days under reversed-phase conditions before it was shut down for reasons unrelated to the phosphate deposits. The deposited material was readily soluble in water and easily removed. No deposits were observed downstream of the skimmer.

More recently some additional experiments on ion-exchange LC–MS have been carried out using an ammonium acetate–acetic acid buffer. With this buffer no visible deposits were formed and the performance of the mass spectrometer was comparable to that obtained with pure water at similar flow-rates.

All of the work described in the preceding section employed ordinary EI ionization with the ion source located immediately adjacent to the quadrupole entrance as shown in Fig. 1. Prior to the installation of the strong-focusing quadrupole lens, the intensity of the ion beam produced by the crossed-beam CI source was rather low, but following this modification the CI spectra were obtained with high-pressure gases injected through the jet nozzle. However, when the molecular beam was formed by laser vaporization of liquids, nearly 100% modulation of the ion beam

was observed at frequencies in the range of a few kHz. Similar oscillations had sometimes been observed in the ion beams produced by the EI ionization source, but the degree of modulation was significantly smaller. At sufficiently low flow-rates of relatively volatile liquids (*e.g.*, hexane at 0.3 ml/min) the modulation could be made to disappear and stable CI operation with reasonable sensitivity could be obtained. As an example, the EI and CI spectra obtained for N,N′-dimethyl-N,N′-dicarbomethoxyethylenediamine under these conditions is shown in Fig. 8. This is a good example of a molecule for which the molecular ion is undetectable under EI, while CI (using hexane as the reagent) gives a strong M + 1 ion as well as the major fragment ions observed in EI.

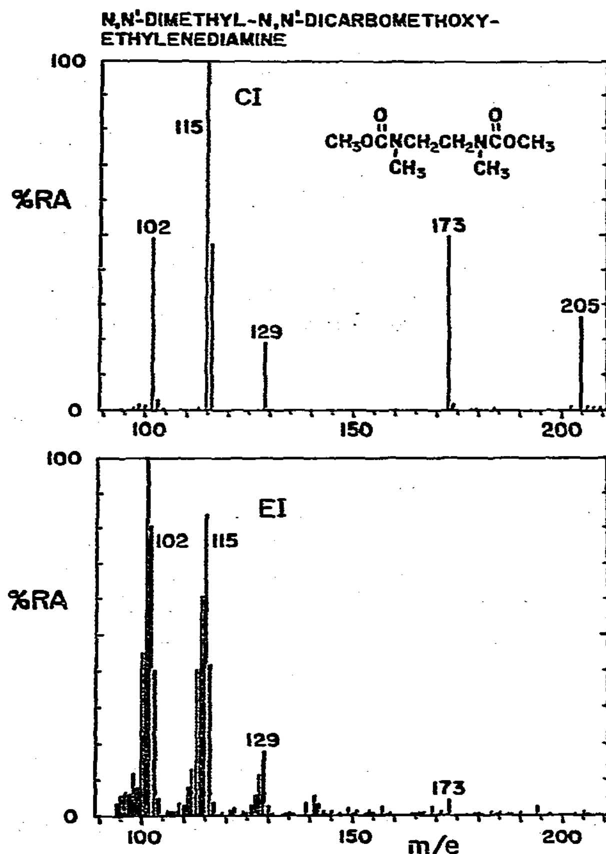


Fig. 8. EI and CI mass spectra of N,N′-dimethyl-N,N′-dicarboxyethylenediamine using hexane as solvent and reagent ion.

In these experiments it was also observed that the onset of the oscillations in the CI ion beam corresponded to the formation of a visible mist in the beam in the region between the supersonic nozzle and skimmer. Very recently we have made some additional modifications to the instrument and have conducted some additional experiments which have led to a much better understanding of some of the results cited

above. Before discussing these changes let us summarize the performance of the system in the configuration shown in Fig. 1 and compare the present status with our initial design criteria.

(1) Chromatographic performance should not be degraded

As shown in Fig. 2, the LC peaks observed by the mass spectrometer are the same width and shape as those form the UV detector. Since the time scale for transmission of samples through the crossed-beam liquid chromatograph–mass spectrometer is very short (10^{-3} sec) compared to even the most narrow peaks produced by the liquid chromatograph, we would not expect any degradation in LC performance so long as we avoid the obvious problems of dead space in the connection to the liquid chromatograph.

(2) The system would be applicable to all of the separation techniques presently employed in modern HPLC including ion exchange

Ion exchange is obviously the technique which presents the most potential difficulties. We have demonstrated successful operation using a phosphate buffer such as commonly used in ion-exchange chromatography on biological molecules; however, deposits build up inside the vacuum system which eventually will cause some problems. On the other hand, the use of an acetate buffer presented no difficulties, and we anticipate that organic buffers such as acetates, formates, and citrates can probably be substituted for the inorganic buffers commonly used without serious loss of chromatographic performance.

(3) Up to 1 ml/min of LC effluent should be converted as quickly as possible to high-temperature vapor

We have successfully measured EI mass spectra of a number of compounds injected in liquid solution at flow-rates of up to 1 ml/min for a number of common solvents such as methanol, acetonitrile, hexane, and chloroform; however, the maximum flow-rate of water that we have been able to vaporize stably is about 0.5 ml/min. Also as discussed above, we have recently encountered problems with mist formation in the region between the nozzle and skimmer of the free jet expansion. This is thought to be due either to incomplete vaporization or to condensation occurring during the adiabatic expansion.

(4) The sample analyzed by the mass spectrometer should be vaporized and ionized without contacting solid surfaces

The system was originally designed to accomplish this goal; however, it now appears that final vaporization of the mist described above may have occurred as the result of contact with hot surfaces in the vicinity of the EI source. This effect probably accounts for some of the apparent pyrolysis observed in the case of the nucleosides and nucleotides.

(5) The system should operate for extended periods without shutdown for cleaning or replacement of components (e.g. filaments)

During the approximately nine months the instrument has been operating we have yet to be forced to shut down to replace a filament or because the system re-

quired cleaning. However, the system has been shut down relatively frequently to make modifications, *e.g.* to change aperture sizes, and routine replacement of a filament or cleaning of components has sometimes been done in conjunction with these changes. Often the system has operated for several hundred hours between modifications and from our limited experience to date it appears that the use of molecular beam techniques successfully minimizes the potential contamination problems.

(6) The vacuum system should be capable of maintaining high vacuum (ca. 10^{-6} torr) in the mass analyzer with liquid injections into the vaporizer of up to 1 ml/min

The mass transfer in the molecular beam has turned out to be somewhat higher than initially calculated. As a result at flow-rates of 1 ml/min the pressure in the quadrupole mass filter is often above 10^{-5} torr in the configuration shown in Fig. 1. We have recently modified the instrument to the transverse geometry shown in Fig. 9 in which the pressure in the quadrupole mass analyzer is maintained at 10^{-6} torr at liquid flow-rates in excess of 1 ml/min.

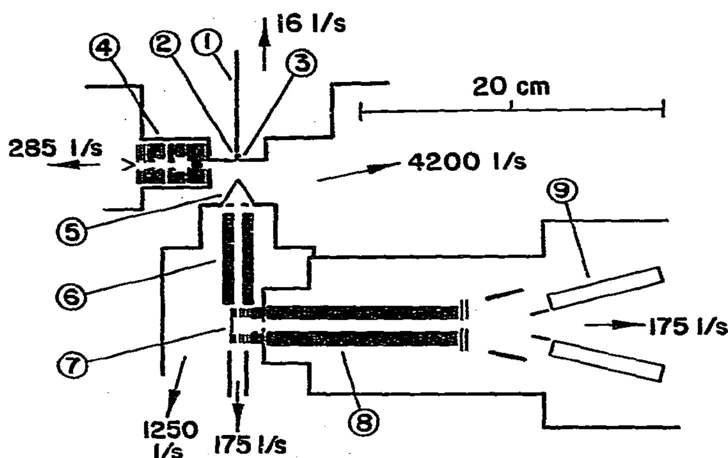


Fig. 9. Schematic of LC–MS with transversely mounted quadrupole. 1, liquid nozzle; 2, laser beam; 3, sampling aperture; 4, CI electron gun; 5, skimmer; 6, quadrupole with RF only; 7, EI ion source and transverse extraction region; 8, quadrupole mass analyzer; 9, multipliers.

(7) The sensitivity should be comparable to that currently obtained in GC–MS

This has not yet been achieved; the present sensitivity is about two orders of magnitude poorer than that achieved in a properly functioning GC–MS system. However, we have not yet completed the development and optimization of the system and this still appears to be a reasonable goal.

(8) Both positive and negative ion CI mass spectra and normal EI mass spectra should be measurable

The instrument is set up to do both positive and negative ion CI, but so far no negative ion measurements have been done. At present we see no serious obstacles to accomplishing this goal; however, some simplifications may be realized by designing an instrument for either EI or CI rather than both.

DISCUSSION

Several problems remain to be solved before fully satisfactory operation of the crossed-beam liquid chromatograph–mass spectrometer can be achieved. For the instrument configuration shown in Fig. 1, these include:

(1) The pressure in the mass analyzer is too high, sometimes reaching 10^{-4} torr at high solvent flow-rates.

(2) The sensitivity for relatively involatile molecules is about two orders of magnitude lower than our design goal.

(3) The spectra of relatively involatile, thermally labile molecules show evidence for pyrolysis prior to ionization.

(4) Large oscillations of the ion beam are observed when formed under CI conditions.

(5) A mist is sometimes formed in the molecular beam due to incomplete vaporization or partial condensation.

Many of these problems are related and now it appears that a common solution may be possible. We have recently modified the instrument to incorporate a transverse mounting of the quadrupole as shown schematically in Fig. 9. This modification solves the problem of high pressure in the mass analyzer and leads to improved sensitivity due to reduction in ion losses by small-angle scattering. However, the major loss of sensitivity as well as the other problems noted above appear to be related to mist formation. In recent experiments we have established that the onset of the mist depends on laser power, flow-rate and very critically on the diameter of the liquid jet orifice. By reducing the diameter of this orifice from 0.25 mm to 0.05 mm the flow-rate of heptane at which the onset of mist formation occurs can be increased dramatically from *ca.* 0.3 to above 1 ml/min. We are presently conducting additional experiments designed to establish the conditions for completely vaporizing several important solvents without unduly heating and pyrolyzing labile solutes.

The oscillations observed in the CI ion beam have been definitely related to the formation of fine droplets or mist in the molecular beam, and it now appears that this effect is also responsible for the low sensitivity observed for involatile samples and may be implicated in some of the apparent pyrolysis that has been observed. It appears that involatile sample molecules, which serve as nuclei for condensation, are preferentially contained in the droplets even though most of the solvent is vaporized. These sample-containing droplets may be carried along by the molecular beam and pass through the ion source and mass analyzer without being detected. However, those droplets which strike heated surfaces in the vicinity of the ion source may be vaporized on the surface and sample molecules vaporized, ionized and detected. Since the EI source was operated at *ca.* 350° during the measurements of spectra of nucleosides and nucleotides, this effect probably accounts for the observed pyrolysis rather than excessive heating by the laser beam.

Our most recent results obtained using the transverse mounting of the quadrupole appear to support these conclusions. With the beam defining apertures chosen to prevent the molecular beam from striking the heated ion source electrodes, EI spectra of adenosine and uridine were obtained which showed no evidence for pyrolysis. For example, a spectrum of adenosine obtained using laser vaporization from methanol solution at a flow-rate of 0.5 ml/min is compared to a spectrum re-

trieved from the computer library in Fig. 10. In contrast to the earlier result shown in Fig. 5, in which pyrolysis apparently occurred, this spectrum shows all of the structurally significant peaks with intensities in good agreement with the library and published spectra[23].

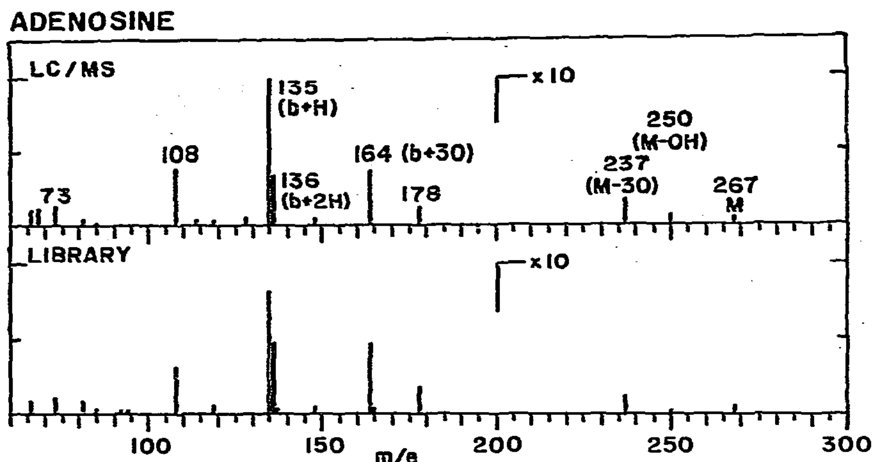


Fig. 10. Comparison of EI mass spectrum of adenosine obtained on the LC–MS instrument with that on file in the computer library. This spectrum was obtained using the transverse geometry shown in Fig. 9 under conditions in which the molecular beam does not contact heated surfaces in the vicinity of the EI source.

Our understanding of all of the effects observed following laser vaporization of LC effluent is not yet entirely satisfactory, and the performance of the crossed-beam liquid chromatograph–mass spectrometer system has not yet matched all of our design goals. Additional studies are presently in progress, and at present none of the problems which has been encountered appears to pose an insurmountable barrier to achieving fully satisfactory performance of a combined LC–MS system using the crossed-beam approach.

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