High-Performance Liquid Chromatography–Mass Spectrometry

Marvin L. Vestal

Summary. Techniques for on-line coupling of high-performance liquid chromatography with mass spectrometry are reviewed with particular emphasis on those suitable for application to nonvolatile samples. The present status of various techniques is summarized and the strengths and weaknesses of the various approaches are assessed. The potential for future application of recently developed techniques for combined liquid chromatography and mass spectrometry is discussed.

The potential benefits to be derived from on-line coupling of mass spectrometry to liquid chromatography have been recognized for at least 15 years. Unfortunately, the problems associated with accomplishing this union in a practical way are substantial, and only recently have techniques emerged which appear to offer feasible solutions. This recent progress has been extensively reviewed (*1–3*), including an exhaustive review of the literature through 1982 (*4*).

Mass spectrometry (MS) is widely recognized as a powerful analytical tool which can provide both qualitative and quantitative data that may not be readily available by other techniques. In particular, it can provide molecular weight, empirical formula (through precise mass measurement), isotope ratios, detection of functional groups and other substituents, and elucidation of structure, including in some cases stereochemical features. For most of these determinations by classical electron impact techniques it is important to vaporize a relatively pure sample into the ion source of the mass spectrometer without causing decomposition. Production of a vapor of intact neutral molecules from nonvolatile samples is (by definition) impossible. Chemical manipulations which produce a more volatile derivative sometimes offer a way around this difficulty, but application of these techniques to trace components in complex mixtures is often unsuccessful. Recently developed ionization techniques which involve direct production of ions from the condensed phase, thus bypassing the neutral intermediate, have largely overcome this volatility barrier. These new techniques very often provide ions characteristic of the intact molecule, but frequently do not provide the stable, reproducible fragmentation pattern required for structural elucidation.

During the past decade tremendous advances have been made in high-performance liquid chromatography (HPLC) column technology and in the development of improved HPLC instrumentation. These improvements have led to very rapid growth in applications to almost all areas of science requiring chemical analyses. An important component of the HPLC system is the device used to monitor the effluent to detect, quantify, and (ideally) identify the eluting components. Detectors usually involve measurement of a bulk property of the effluent which is sensitive to the presence of sample (such as refractive index); measurement of a sample property not possessed by the mobile phase (such as optical density at a suitable wavelength); or measurement of a sample property after elimination of the mobile phase. In gas chromatography (GC) the properties of the samples of interest are often sufficiently different from suitable mobile phases that the second approach can be used with negligible interference. In liquid chromatography (LC) the properties of the samples and the mobile phases are often more nearly the same, and a nearly universal detector comparable to flame ionization in GC does not exist (*5*). The most widely used detectors in modern HPLC are photometers based on ultraviolet and visible light absorption. These detectors have high sensitivity for many solutes, but samples must absorb in the spectral region where the mobile phase is essentially transparent (typically 200 to 600 nm). This is a serious restriction since the strongest optical absorption bands occur for most samples (and mobile phases) at shorter wavelengths.

The analytical power of combined GC-MS is now widely recognized despite the fact that only about 20 percent of the known organic compounds are suitable for gas chromatography without derivatization (*6*). Liquid chromatography suffers from no such limits, but mass spectrometry is not so readily compatible with LC. The basic incompatibility between MS and LC stems primarily from two factors. First, the mass spectrometer requires ions in the gas phase at rather low background gas pressures ($\sim 10^{-6}$ torr) while LC employs liquid solutions often containing nonvolatile solutes and buffers at atmospheric pressure and flow rates of ~1 ml/min. This mass flow corresponds typically to about 100 times the amount that can be accommodated by the mass spectrometer vacuum system. Thus, it is necessary to vaporize the LC effluent and to modify either the LC or the MS in such a way as to overcome this basic mass flow incompatibility. The second major problem is that in LC, unlike GC, sample volatility is not an issue; therefore, to be generally useful the mass spectrometer must utilize an ionization technique which produces the required gas phase ions without necessarily requiring thermal vaporization of the sample.

Most of the early work on LC-MS interfacing focused on taking a standard, commercial mass spectrometer with either electron or chemical ionization and attempting to find a means of overcoming the mass flow incompatibility without significantly modifying the standard instrument. This approach led to the successful development of useful LC-MS interfaces such as the direct liquid introduction technique pioneered by McLafferty and co-workers (*7*), and the transport systems developed originally by Scott (*8*) and further refined and improved by McFadden (*9*). LC-MS interfaces based on these concepts are commercially available at present from several manufacturers.

Despite the obvious successes of these approaches, they have not been fully accepted by the potential users of LC-MS and the literature on applications to real problems is still rather sparse (*10*). There may be many reasons for this lack

Marvin L. Vestal is a professor in the Department of Chemistry, University of Houston, Houston, Texas 77004.

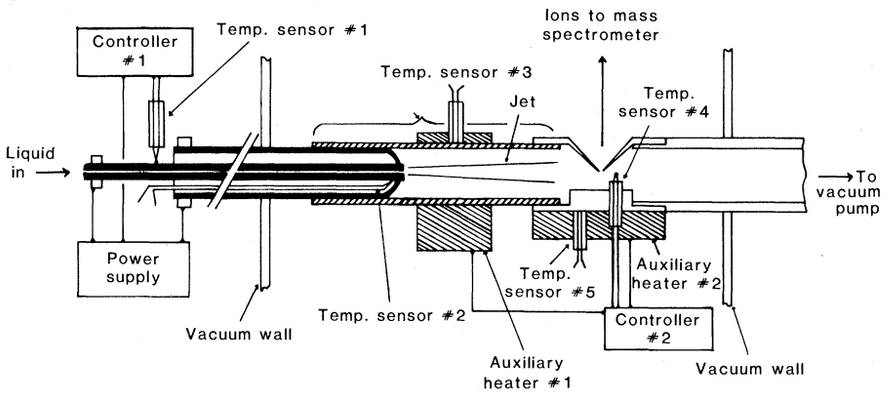


Fig. 1. Schematic diagram of thermospray LC-MS interface, using direct electrical heating of the capillary. Liquid effluent from the LC is forced through the capillary tube and heated sufficiently to produce a supersonic jet of vapor carrying entrained droplets of unvaporized solvent and sample. Most of the solvent vapor exits through the pumping line opposite the vaporizer; this interface can accommodate vaporization of water or other liquids at up to 2 ml/min directly into the ion source.

of acceptance, but a major one appears to be the volatility limit imposed by the conventional electron ionization or chemical ionization mass spectrometer. While these LC-MS interfaces have extended the volatility limit significantly beyond that imposed by GC, they are simply not applicable to the vast majority of samples which are nonvolatile.

During the past few years several ionization techniques have been developed for MS which provide intact molecular ions from nonvolatile molecules (*11*). A common feature of all these methods appears to be the "direct" production of ions from a condensed phase without formation of a neutral gas-phase molecule as an intermediate. These techniques produce relatively stable even-electron species similar to those present in solution rather than the odd-electron species produced directly by gas-phase electron ionization. Recently, several groups have reported on attempts to employ some of these new ionization techniques in LC-MS interfaces which might be applicable to a much wider range of samples. The scope of the present review is limited to these attempts to develop LC-MS interfaces which are not limited by sample volatility.

Direct Coupling with Ion Evaporation

Many of the techniques for so-called soft ionization are not simply adaptable to on-line coupling with LC. The earliest of these, and still one of the most successful, is the field desorption (FD) technique developed by Beckey and co-workers (*12*). For many years this was essentially the only technique available for obtaining molecular weights on large, nonvolatile, or thermally labile molecules. To obtain an FD spectrum, the sample is deposited on the wire emitter in liquid solution, which is then dried. The emitter, which is usually mounted on a direct insertion probe, is then inserted into the mass spectrometer, and the mass spectrum recorded (if the operator has the right combination of skill and luck). Schulten and co-workers (*13*), in particular, have been very successful at using FD-MS in an off-line combination with LC, but no practical approach to an on-line combination of FD-MS with LC has been suggested.

For many years Loeb and his students and co-workers studied the processes involved in static electrification (*14*), and it is now apparent that many of these early results are pertinent to the problem of producing gas-phase ions from nonvolatile samples. In the 1930's Chapman (*15*) reported results from ion mobility analysis which indicated that singly charged ions containing at most a few solvent molecules could be obtained by pneumatically spraying aqueous solutions at atmospheric pressure and room temperature. More recently, Iribarne and Thomson (*16*) reproduced the results of Chapman with a mobility analyzer, and then proceeded to analyze the ions with a quadrupole mass spectrometer. In the latter experiments solutions of electrolytes are pneumatically sprayed into air at atmospheric pressure, the resulting droplets are charged by induction by using an auxiliary electrode at high potential, and an electric field across the plume of evaporating spray extracts small ions, which are drawn through a small orifice into the vacuum chamber of the mass spectrometer. A dry air "curtain" is used to suppress clustering during the free jet expansion downstream of the sampling orifice. Mass spectra have been recorded from a number of inorganic and organic electrolytes sprayed in aqueous solution, typically at concentrations of about $10^{-3}M$. The spectra generally show that the ions expected to exist in solution are observed with approximately the degree of hydration expected for equilibrium in moist air at room temperature and atmospheric pressure. While some details of the mechanism of ion production from charged liquid droplets are still unresolved, it appears clear that field-assisted ion evaporation, as suggested by Thomson and Iribarne, is responsible for the observed molecular ion production. This mechanism is apparently quite similar to that involved in FD, except that instead of applying an external electrical field to a liquid or solid surface, one uses the electrical field generated by the droplet or particle itself as the result of its large charge and small radius.

An LC-MS interface patterned after the original design of Thomson and Iribarne and applicable to nonvolatile samples is commercially available (*17*). It is now clear that other approaches to direct coupling of LC with MS which are applicable to nonvolatile molecules also make use of the ion evaporation mechanism. Some of the approaches are the "liquid ionization" developed by Takeuchi and co-workers (*18*), electrospray by Whitehouse and Fenn (*19*), and our own thermospray process. These various approaches differ in the way in which the liquid is vaporized or nebulized, in the techniques used to produce charged droplets, and in the details of handling the charged droplets and extracting ions into the mass spectrometer, but all appear to depend on the ion evaporation mechanism as an important step in the production of molecular ions from nonvolatile molecules. A complete discussion of the details of all of these techniques is beyond the scope of this review, which will be limited to a brief overview of the technique with which we are most familiar.

Thermospray

The thermospray technique has emerged from efforts to develop an LC-MS interface suitable for efficiently analyzing samples dissolved in aqueous mobile phases at typical analytical flow rates on the order of 1 ml/min. In contrast to other techniques, this is accomplished by application of heat to the capillary tube connecting the LC to the MS. Thermospray may be defined as the production of a supersonic jet of vapor with entrained particles or droplets by application of sufficient heat to a capillary to effect controlled partial vaporiza-

tion of a liquid as it passes through the capillary. This approach has evolved from the original use of a focused 50-watt CO_2 laser (*20*), through oxy-hydrogen torches (*21*), to the use of simple electrical cartridge heaters embedded in a copper block brazed to the end of the capillary (*22*). In the most recent work the "indirect" electrical heating has been replaced by "direct" electrical heating in which the power required to vaporize the liquid is supplied by passing an electric current through the capillary tube itself (*23*).

A schematic diagram of a recent version of the thermospray LC-MS interface is shown in Fig. 1. This interface and ion source are much simpler than the earlier versions and require only minor modifications of a commercial mass spectrometer. Until recently the thermospray interface had been restricted to quadrupole mass spectrometers, but similar interfaces are now available for magnetic instruments as well (*24*). As shown in Fig. 1, the thermospray vaporizer is mounted in a probe which may be introduced into the mass spectrometer by use of the direct insertion probe lock normally used for introducing solid samples. The vaporizer produces a supersonic jet of vapor containing a mist of fine droplets or particles, as indicated in Fig. 2, where a flash photograph of the thermospray jet is contrasted with that obtained under unheated conditions. As the droplets travel at high velocity through the heated ion source they continue to vaporize due to rapid heat input from the surrounding hot vapor.

In the thermospray interface ions may be produced by conventional chemical ionization techniques, but for application to nonvolatiles the use of ion evaporation techniques, as described above, is necessary. The fact that such a mechanism is possible in the thermospray interface was discovered accidently in some earlier experiments in which the electron-emitting filament normally used to initiate ionization burned out, but the ion beam persisted. At first it may seem surprising that an apparatus in which vaporization and ionization occur in a region entirely free of external electrical fields can produce ions by a field desorption mechanism requiring electrical fields on the order of 10^8 to 10^9 volt/m. However, the symmetric charging mechanism studied by Dodd (*25*) (also a student of Loeb) can easily provide droplets with the necessary properties.

When a liquid surface is disrupted by spraying or bubbling, the droplets produced are often electrically charged. For liquids containing substantial concentrations of dissolved ions the charging mechanism (in the absence of an applied electrical field) appears to be dominated by the statistical mechanism described by Dodd. If the separation of the droplet from the bulk liquid occurs sufficiently rapidly that conduction in the liquid can be neglected, then the net charge is determined by statistical fluctuations. In this case the resulting charge distribution on the droplets is Gaussian, with zero mean and a standard deviation equal to the square root of the total number of ions with the volume of the droplet. The absolute mean charge (of either sign) is given by

$$<|q|> = \left(\frac{2}{\pi}\right)^{1/2} (2VN)^{1/2} = \left(\frac{4VN}{\pi}\right)^{1/2} \quad (1)$$

where V is the volume of the droplet and N is the number of ions of each sign per unit volume. In thermospray, solutions of ~0.1M ammonium acetate are frequently used and the initial droplet sizes are on the order of 1 μm or less. In this case the mean field strength at the surface of the droplet is initially about 10^7 volt/m and increases rapidly as the droplet vaporizes. At sufficiently high surface fields the evaporation of ions, or small clusters, becomes thermodynamically competitive with evaporation of neutrals, and ion evaporation occurs as a natural part of the evaporation of the droplets.

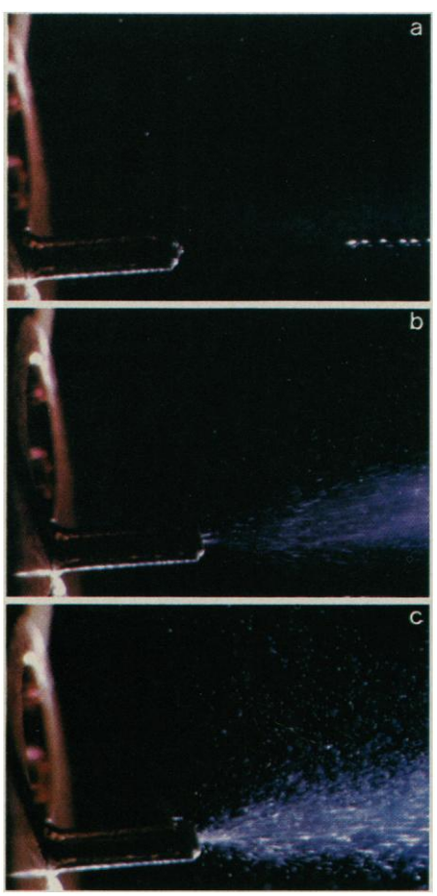
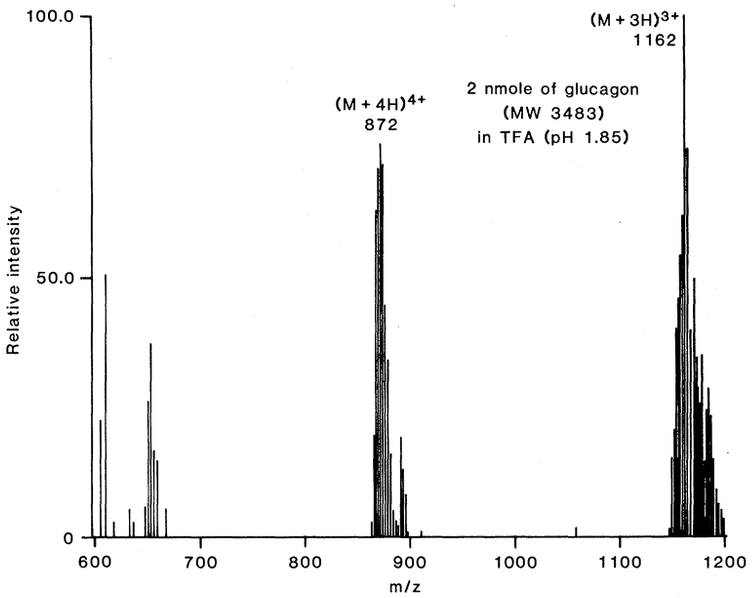


Fig. 2. Flash photographs of jets issuing from a thermospray vaporizer: (a) heated to just below the threshold for formation of the thermospray jet; (b) just above the onset of thermospray; and (c) fully developed thermospray jet corresponding to ~98 percent vaporization. [Photograph by G. J. Fergusson]

Thermospray interfaces have only recently become available commercially from several manufacturers; thus, the number of published accounts of applications of LC-MS to nonvolatile samples is still rather small. An excellent example of the potential of the technique is provided by its recent application to on-line sequencing of proteins and peptides (*26*). With large peptides containing a number of basic residues it is possible to obtain multiple protonation of the molecule, particularly at low pH of the buffer. As an example, a thermospray spectrum of glucagon (molecular weight 3483) is shown in Fig. 3. This result was obtained on a Biospect quadrupole configured for a maximum mass range of about 1300 atomic mass units (amu). The singly and doubly protonated ions, if present, cannot be observed because of the mass range limitation, but prominent peaks are observed corresponding to the triply and quadruply protonated molecule. The resolution available ($m/\Delta m \approx 600$) was insufficient to resolve the isotope peaks, which are spaced nominally 1/4 and 1/3 amu apart. The additional peaks at higher mass are due to contributions from partial substitution of Na^+ and K^+ for the protons.

The presence of multiply charged ions can sometimes be used to extend the effective range of molecular weights which can be analyzed by the thermospray LC-MS. An example of the reversed-phase separation and thermospray detection on a mixture of three peptides is shown in Fig. 4. This mixture was separated on a 4.6 mm by 15 cm C_{18} column, using a gradient of 30 to 50 percent acetonitrile with 0.1M ammonium acetate, pH 5.5, at a total flow rate of 1.0 ml/min. The computer-generated mass chromatograms correspond to the doubly protonated molecular ions for α-MSH and renin substrate at (mass-to-charge ratio) m/z 833 and 881, respectively, and the protonated molecule for (Sar^1, Ala^8) angiotensin II at 927 amu. The mass spectrum was scanned repetitively from 400 to 1000 amu in 3 seconds.

Mass spectra obtained from peptides by the direct ion evaporation mechanism generally show little or no fragmentation, so no structural information is obtained directly. However, it is possible to use this fact to advantage by employing on-line cleavage with selected en-



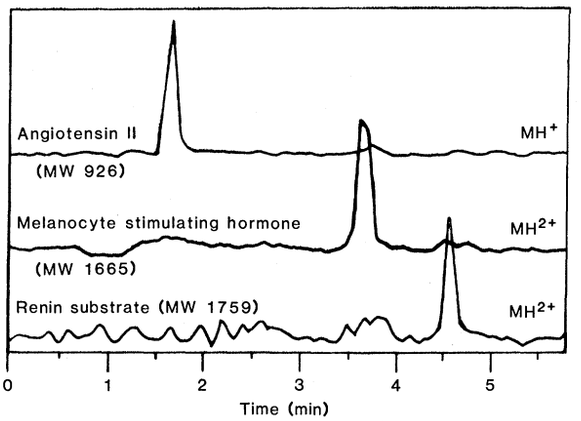


Fig. 3 (left). Mass spectrum obtained by ion evaporation in thermospray interface from injection of 2 nmol of glucagon in trifluoroacetic acid at *p*H 1.85. [From (*26*)] Fig. 4 (right). Separation of peptides by reversed-phase HPLC and detection by thermospray MS. A linear gradient from 30 percent acetonitrile in 0.1*M* ammonium acetate, *p*H 5.5, to 50 percent acetonitrile was used at a flow of 1.0 ml/min. The mass chromatograms correspond to the mass region of interest selected by computer program from full mass scans. The sample consisted of 2 nmol of each peptide in 20 μl of water. [From (*26*)]

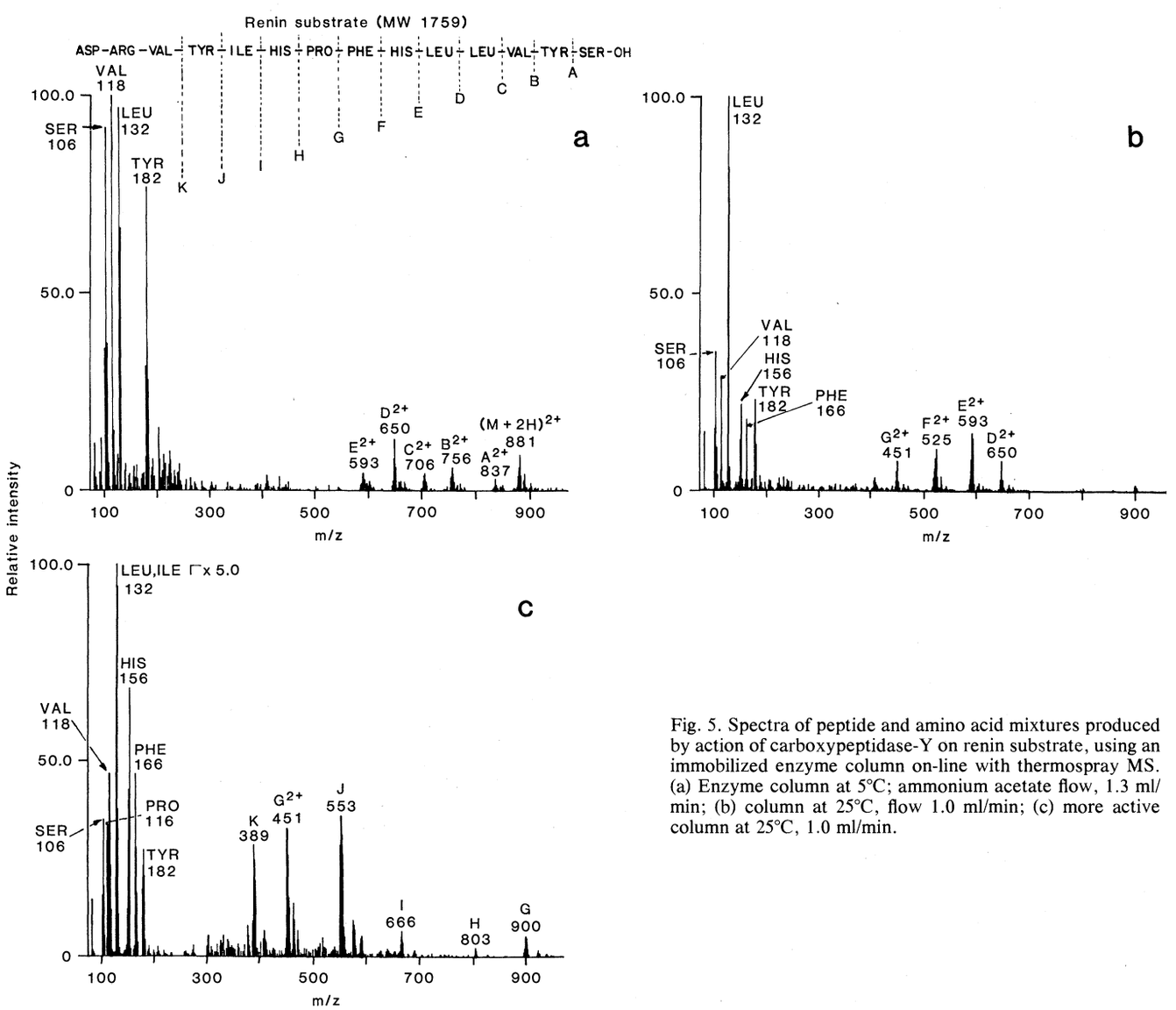


Fig. 5. Spectra of peptide and amino acid mixtures produced by action of carboxypeptidase-Y on renin substrate, using an immobilized enzyme column on-line with thermospray MS. (a) Enzyme column at 5°C; ammonium acetate flow, 1.3 ml/min; (b) column at 25°C, flow 1.0 ml/min; (c) more active column at 25°C, 1.0 ml/min.

zymes. Immobilized enzymes can be packed in a column which may be employed alone or in series with an HPLC column. An example of results obtained with a carboxypeptidase-Y (CPY) immobilized enzyme column directly coupled to the thermospray interface is shown in Fig. 5.

This series of results was obtained by increasing the extent of reaction by raising the reactor temperature, decreasing the liquid flow rate, or increasing the enzyme activity. The upper spectrum shows the intact, doubly protonated renin substrate at *m/z* 881, and the doubly protonated peptide fragments corresponding to cleavages A, B, C, D, and E from the COOH-terminus. The corresponding amino acids serine, tyrosine, valine, and leucine are detected in the low-mass portion of the spectrum. The sequence can also be correctly deduced by quantitating the amino acids by comparison with a standard mixture, but this approach may incorrectly assign leucine because of its repetition in the sequence. The peptide masses, on the other hand, allow the sequence to be assigned unambiguously, except that this approach cannot distinguish between leucine and isoleucine.

As the reactor temperature is increased and the flow rate decreased, the reaction of the CPY proceeds further along the chain, as shown in Fig. 5b. The doubly protonated F and G are observed along with D and E and weak signals corresponding to singly protonated G and H at *m/z* 900 and 803, respectively. When the extent of reaction is increased still further, as in Fig. 5c, singly protonated ions corresponding to fragments G, H, I, J, and K are observed along with doubly protonated G. It is interesting to note that after cleavage of the proline to leave the residual hexapeptide containing an arginine and the COOH-terminal histidine, only singly protonated ions are observed. In all cases the amino acids produced are detected as the MH^+ ions and confirm the sequence determined from the peptide masses. These results were obtained from individual injections of 2 nmol of renin substrate, and each experiment required less than 2 minutes of operation of the LC-MS system.

Similar results have been obtained recently with endopeptidases (*27*). In this case the immobilized enzyme (for instance, trypsin) may be used in series with an HPLC column. A large peptide can then be injected, cleaved adjacent to the arginine and lysine residues, and the fragments separated by HPLC and identified by thermospray LC-MS. If necessary, the CPY or other exopeptidase column can be inserted downstream of the HPLC column to allow terminal sequencing of each peptide as it elutes. Work is being done to combine these techniques into an effective system which will allow a large amount of sequence information to be obtained from single injections of large peptides or small proteins. Each such experiment requires only a few minutes of operation and can generally be done on about 1 nmol of sample.

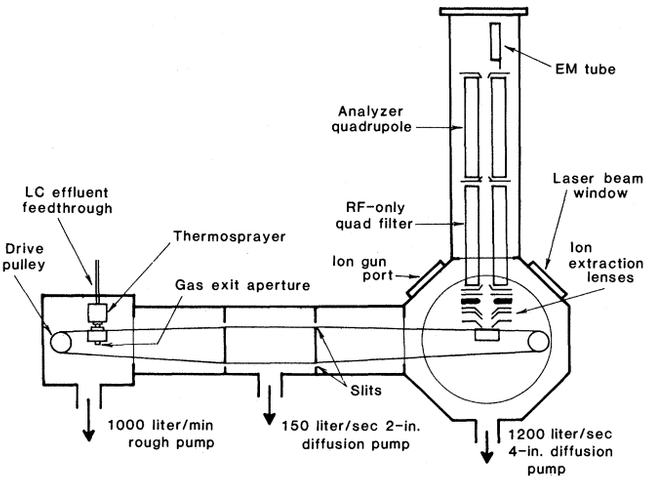


Fig. 6. Schematic diagram of a transport LC-MS interface employing thermospray deposition and both laser and fast ion desorption ionization. The laser and ion beams enter ports on the vacuum housing and can be focused on the same spot on the surface of the moving belt. [From (*37*)]

Applications such as these are still quite new, and a great deal of work remains before their utility can be fully established. An exciting aspect of this work is that it illustrates the potential of the thermospray LC-MS interface for on-line monitoring of reactions in solution as well as its potential for direct LC-MS analyses of mixtures. One of the difficulties is that it is necessary to find a set of operating conditions which is simultaneously compatible with the enzyme chemistry, the HPLC separation, and the thermospray interface. For the peptide work this has been a nuisance but has not presented any insurmountable problems. Other potential applications may present more difficulty. In these cases the decoupling that is possible with transport devices may offer some advantages.

Desorption Ionization with Transport Interface

In the present version of the thermospray interface the solvent vapor enters the ion source along with the sample. In some cases this may make simultaneous optimization of both the LC conditions and the mass spectrometer difficult or impossible. It may be possible to remove the solvent vapor and replace it with an inert gas, as done in some other direct coupling techniques (*19*), but such interfaces have not yet been developed for thermospray. An alternative approach to LC-MS interfacing is the use of a mechanical transport device in which the sample is deposited on a moving solid surface and the solvent is removed before the sample reaches the ion source of the mass spectrometer.

The early work on transport interfaces employed direct deposition of the liquid effluent onto a wire or belt and thermal desorption of samples into either electron ionization or chemical ionization sources. These approaches have been successful for a variety of samples in relatively volatile solvents, but do not give good results for nonvolatile samples, and difficulties have been experienced with volatile samples in polar solvents, especially water. In addition to the obvious limitations imposed by the use of ionization techniques suitable only for volatile molecules, problems are encountered with rapidly vaporizing large quantities of polar solvents from the surface. If sufficient heat is supplied to cause the water, or other polar liquid, to vaporize before it reaches the ion source, then a nonequilibrium boiling mode is likely rather than smooth equilibrium vaporization. As a result, a significant fraction of the liquid may depart as small droplets carrying some of the sample along in solution. Several approaches have been developed to overcome this difficulty, including the use of a segmented flow reactor system (*28*), spray deposition (*29*), and micro LC (*30*). Of these, the spray deposition technique appears to be the most generally applicable, since it allows the sample to be deposited on the moving surface with essentially complete vaporization of the solvent in the spray.

Beginning with the pioneering work of

Macfarlane and co-workers (*31*), using ^{252}Cf fission fragments, techniques for producing ions from nonvolatile organic molecules on surfaces have been developed with extraordinary rapidity (*11*). These techniques involve bombarding with particle beams ranging from mega-electron-volt fission fragments (*31*) and dust particles (*32*) to kiloelectron-volt ions (*33*) and neutrals (*34*). Irradiation with focused, pulsed lasers with energy densities in the range of 10^7 to 10^8 W/cm^2 has also proved effective for producing molecular ions from nonvolatile molecules (*35*). While details of the resulting spectra depend on the operating conditions and properties of the primary excitation technique, as well as on the sample matrix, all of these "desorption ionization" methods have produced ions characteristic of the intact molecules from a variety of nonvolatile materials. These techniques are particularly well suited for use with transport LC-MS interfaces.

Jungclas and co-workers (*36*) have described an LC-MS interface suitable for use with ^{252}Cf desorption ionization, and preliminary work on both laser and kiloelectron-volt ion desorption has been reported by others. A schematic diagram of one approach to an on-line LC-MS system with either laser or particle desorption (*37*) is shown schematically in Fig. 6. In this apparatus the sample is deposited on a moving stainless steel belt with a thermospray vaporizer, and sample ions may be desorbed into a quadrupole mass spectrometer by using either a Q-switched Nd:YAG laser or a beam of 3-keV xenon ions. Thermospray sample deposition is superficially similar to other spray deposition techniques, but appears to have some important advantages for some applications. In particular, it allows rather complete solvent vaporization in the spraying process while maintaining good sample transfer efficiency as long as the difference in boiling point between sample and solvent is at least 50°C or so. Virtually any LC mobile phase can be accommodated (including aqueous buffers) at virtually any flow rate up to at least several milliliters per minute. Since only a very small fraction of the solvent (~0.1 percent) is actually in contact with the belt as liquid, the optimum belt speed is determined by the required chromatographic performance and the properties of the mass spectrometer and ionization technique rather than the requirement for a large liquid capacity on the surface. This allows rather low belt speeds to be used (~10 cm/min for a 1-second nominal response time), which also keeps the local concentration of sample on the surface relatively high.

Recently this apparatus has been applied to direct comparisons of laser desorption (LDMS) and kiloelectron-volt ion desorption (secondary ion mass spectrometry, SIMS) (*38*). Experiments have also been aimed at elucidating the effect of sample matrix on the mass spectra obtained by these two techniques (*39*). In almost all cases, intense molecular ions have been obtained for a variety of nonvolatile organic and inorganic samples, but the sensitivities and spectral quality depend very strongly on operating conditions and details of the sample matrix and surface condition, which are not yet well understood. The mass spectra obtained are often qualitatively quite dissimilar, particularly in the positive ion mode, and qualitatively different from those obtained by ion evaporation techniques such as direct thermospray. As an example, positive ion spectra obtained by LDMS and SIMS for histidine are shown in Fig. 7. LDMS gives a modest MH^+ peak and strong peaks corresponding to Na^+ and K^+ addition to the neutral histidine. Peaks are also observed corresponding to double alkali addition to the deprotonated histidine anion, and very little fragmentation is observed. In contrast, the SIMS spectrum shows extensive specific fragmentation which is easily reproduced and can be useful for structural identification, but the intensity of the protonated molecular ion is quite dependent on sample coverage and matrix. The result shown in Fig. 7 was obtained with relatively thick sample coverage (~10 μg/cm^2); at lower sample coverages the MH^+ ion disappears but can be restored by codepositing an appropriate proton-donating matrix such as tartaric acid. Ion evaporation normally gives only the MH^+ ion from histidine with little or no fragmentation. The negative ion spectrum of histidine is dominated by the deprotonated molecular ion $(M - H)^-$ in all three ionization techniques.

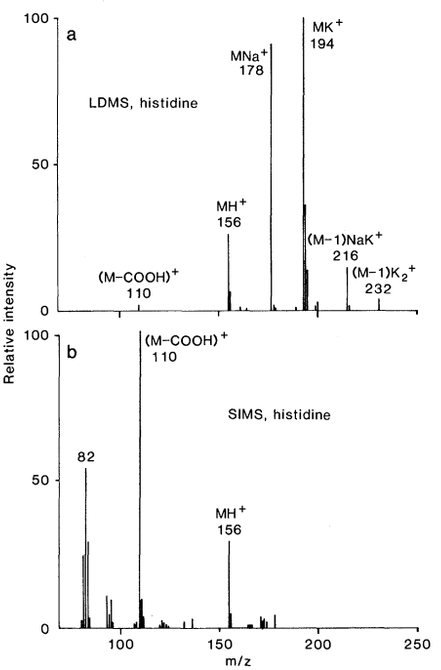


Fig. 7. Mass spectra of histidine obtained with the transport LC-MS interface: (a) laser desorption, positive ion; (b) 3-keV xenon ion desorption, positive ion. [From (*38*)]

The transport device with spray deposition and desorption ionization appears to have considerable potential for on-line LC-MS, but much work remains to fully develop and evaluate this approach. In the preliminary work, detection limits in the low nanogram range have typically been obtained. Generally, the detection limits are set by chemical interferences from the belt rather than detector noise. A major impetus for pursuing this approach is that it allows the sample matrix and ionization technique to be chosen almost independently of the LC solvent and conditions used, while in the case of directly coupled interfaces, such as thermospray, the vaporization and ionization environment is strongly affected by the LC mobile phase and operating conditions.

Present Status of LC-MS Interfacing

The field of LC-MS interfacing has survived infancy and may be entering adolescence, but it is not yet mature. Despite rapid progress in developing apparatus and techniques over the past 10 years, the number of published accounts of applications to real samples is still quite small. In my opinion the present status of LC-MS may be stated as follows:

1) The transport detector with spray deposition of sample still offers great (but as yet largely unrealized) potential, in that it can provide electron ionization, chemical ionization, and desorption ionization spectra and excellent decoupling between the LC and the MS. This technique is likely to be developed further, and it holds great promise for future applications.

2) Direct liquid introduction has made an important contribution to the development of LC-MS, but it is limited to chemical ionization of relatively volatile compounds and is restricted to rather low LC flow rates. These limitations, along with the practical difficulties associated with the use of very small aper-

tures, make it unlikely that this technique will survive, at least in its present form.

3) Thermospray is presently the method of choice for obtaining molecular weight information for a wide variety of samples. It is applicable to a broad range of flow rates and LC conditions (including micro LC with postcolumn makeup) and is reliable and simple to operate.

4) Electrospray is not yet commercially available, but it appears very promising, particularly for use with micro LC.

It is not possible at present to accurately estimate the performance to be expected from any of these techniques in specific applications. With thermospray it is usually possible to obtain reliable molecular weight information on 10 ng or less of components present in relatively complex mixtures even if the LC separation is less than complete. Some structural information may be obtained, but its reliability and utility remains to be established. In the near future, transport systems, particularly with spray deposition and desorption ionization, may provide comparable performance.

Future Directions

LC-MS, with either thermospray or transport interfaces, should provide very powerful techniques for many applications. While these applications have not yet been extensively developed, the apparatus and techniques have evolved to the point that many appear practical. With the available mass spectrometric techniques, molecular weight can be determined for almost any volatile or nonvolatile, organic or inorganic, sample. When combined with the information available from the LC separation, this may be sufficient in some cases. The qualitative identification and structural information available from electron ionization spectra is presently limited to volatile molecules. Future development of the combination of LC with tandem mass spectrometry (MS-MS) may provide similar information for nonvolatile samples. Measurement of isotope ratios and determination of empirical formula (through precise mass measurement) are now practical for nonvolatile samples, and it is now possible, in principle, to perform such measurements with on-line LC separation. The recent development of inductively coupled plasma–mass spectrometry (ICP-MS) makes mass spectrometry competitive for determination of the elemental composition of inorganic samples. It appears that an on-line LC-ICP-MS is very likely in the near future, and it is possible that such an instrument might operate in both elemental and molecular modes to provide both elemental analysis and molecular weight for individual peaks separated by LC.

Very little work has been reported to date on quantitative applications of LC-MS, but it appears that techniques such as thermospray now provide sufficient stability and reproducibility to make precise quantitative measurements feasible. In the peptide work (*28*), amino acids in complex mixtures have been quantitated routinely to a precision of better than 10 percent by using external calibration with injection of standard mixtures before and after each analysis. For higher precision, LC-MS, like GC-MS, will undoubtedly require the use of internal, isotopically labeled standards. Development of quantitative applications for LC-MS may proceed rapidly, since many of the techniques developed for quantitative GC-MS, such as selected ion monitoring, can be readily adapted.

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