to evaluate the effect of an internal standard to compensate for instrumental variations.

The variation for benzo[a]pyrene was somewhat less than either the corrected or uncorrected values of other compounds. This phenomenon probably reflects the fact that less sample loss occurred during solvent stripping. It evidences the need for accurate control of LC interface setting. The use of internal standards will not adequately compensate for day-to-day variation in stripper and volatilization temperatures.

CONCLUSIONS

PNA sensitivity is, to an extent, a direct function of compound volatility. Losses incurred during eluent stripping appear to be a significant factor in the case of lower molecular weight compound sensitivity. Volatilization temperatures are critical for the recovery of high molecular weight compounds. The lower limit of detectability for polynuclear aromatics in the ion-selective mode is approximately 10 ng.

The precision observed on corrected raw data appears somewhat better at higher PNA molecular weights. This again probably reflects losses during solvent stripping and prevolatilization.

LITERATURE CITED

(1) Tal'rose, V. L.; Karpov, G. V.; Gordoershii, I. G.; Skurat, V. E. *Russ. J. Phys. Chem.* **1968**, *42*, 1658.
(2) Tal'rose, V. L.; Grishin, V. D.; Skurat, V. E.; Tantsyrev, G. D. "Recent Developments in Mass Spectrometry"; Ogta, K., Hagakawa, T., Eds.; University Park Press: Baltimore, MD, 1970.
(3) Scott, R. P. W.; Scott, G. C.; Munroe, M.; Hess, J. *J. Chromatogr.* **1974**, *99*, 395.
(4) Scott, R. P. W. *J. Chromatogr. Libr.* **1977**, *11*.
(5) McFadden, W. H.; Schwartz, H. L.; Evans, S. *J. Chromatogr.* **1976**, *122*, 389.
(6) McFadden, W. H.; Bradford, D. C.; Games, E. E.; Gower, J. L. *Am. Lab (Fairfield, Conn.)* **1977**, *9*, 55.
(7) Dark, W. A.; McFadden, W. H.; Bradford, D. C. *J. Chromatogr. Sci.* **1977**, 15, 454.
(8) Dark, W. A.; McFadden, W. H. *J. Chromatogr. Sci.* **1978**, *16*, 289.
(9) McFadden, W. H.; Bradfor, D. C.; Eglinton, G.; Haglbrahim, S.; Dark, W. A.; Nicolaides, N., 26th Annual Conference of Mass Spectrometry and Allied Topics; May 1978, St. Louis, MO.

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CORRESPONDENCE

Ionization and Mass Analysis of Nonvolatile Compounds by Particle Bombardment Tandem-Quadrupole Fourier Transform Mass Spectrometry

Sir: Recent advances in the development of new ionization techniques have now made it possible to generate $(M + H)^+$, $(M - H)^-$, and $(M + \text{cation})^+$ ions as well as structurally significant fragment ions from many polar biological molecules having molecular weights up to as high as 13 000 (*1–3*). Techniques such as fast atom bombardment (FAB) or liquid secondary ion mass spectrometry (L-SIMS) in which sample ions are ejected from a liquid matrix such as glycerol under bombardment with kilovolt atoms or ions, respectively, are presently in widespread use on both commercial quadrupole and magnetic sector instruments.

Unfortunately, advances in the design of instrumentation for mass analysis and structural characterization of the ejected ions have not kept pace with developments in new ionization methodology. Large magnetic sector instruments operating at full accelerating potential provide molecular weight information up to 12 000 for samples in the 0.05–5 nmol range. Ion current in the mass range greater than 3000 daltons is usually too weak, however, to allow determination of molecular structure from fragmentation patterns. Collision-activated dissociation provides a mechanism for enhancing the structural information obtained in particle bombardment experiments, but kinetic energy released during the fragmentation process limits the resolution achieved by using tandem four sector instruments on samples at the 50 pmol level to ca. 2000. Considerably higher resolution can be achieved if the quantity of sample is increased by a factor of 10–100. Above mass 2500–3000, the combination of weak sample ion current and low ion transmission at unit resolution following the collision cell severely restricts the analytical utility of tandem sector instruments. Presently available tandem quadrupole instruments provide structurally rich collision-activated dissociation (CAD) spectra at unit resolution (*4*, *5*), but inefficient ion transmission above mass 1000 makes these instruments unsuitable for trace level analysis of high molecular weight biological materials.

Fourier transform mass spectrometry (FT-MS) offers an attractive alternative to the above instrumental methods (*6–10*). It can provide a mass range in excess of 10 000 daltons in principle and can function as an ion storage device to accumulate ions produced in low abundance. Mass analysis is accomplished in Fourier transform mass spectrometry without destruction of the ions, and mixture components can be separated by using the double resonance technique. Consecutive collision-activated dissociation (*11*, *12*) and photodissociation (*13–16*) experiments can be performed on the same ion population. Long ion storage times and ultrahigh mass resolution have been demonstrated, but only when the pressure in the analyzer cell is below 10^{-8} torr. Glycerol and other liquid matrices employed in the particle bombardment ionization methods all have vapor pressures in excess of 10^{-6} torr and are therefore difficult to use in conjunction with Fourier transform mass spectrometry.

One way to overcome the problem associated with high gas flow during the ionization step is to use a differentially pumped, two compartment cell within a conventional Fourier transform mass spectrometer. An instrument containing such a cell has recently become available from Nicolet Instruments. Another approach utilizes a tandem-quadrupole Fourier transform mass spectrometer (QFT-MS) in which sample introduction and ionization are carried out in a differentially pumped quadrupole ion source and only the ions of interest

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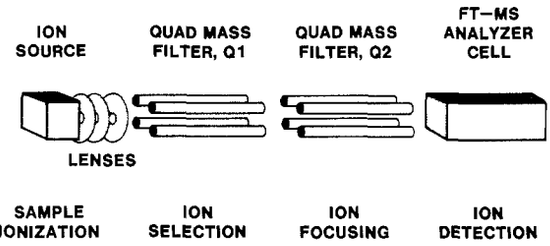

Figure 1. The tandem-quadrupole Fourier transform mass spectrometer.

are then transferred to the ion cyclotron resonance cell for mass analysis. Two of the present authors demonstrated the feasibility of injecting ions from a quadrupole ion source through the intense fringing fields of a superconducting magnet (*17*) and recently showed that daughter ions produced by collision-activated dissociation could be detected at a mass resolution of 140 000 (*18*, *19*). Here we report initial results obtained with a cesium bombardment ion source (*20*) on a second quadrupole Fourier transform mass spectrometer constructed at the University of Virginia.

EXPERIMENTAL SECTION

Tandem-Quadrupole Fourier Transform Mass Spectrometer. This instrument, shown schematically in Figure 1, consists of a standard Model 4500 ion source, lens system, and quadrupole mass filter from the Finnigan-MAT Corp., San Jose, CA, a second set of 86.25 cm long, quadrupole rods termed the guiding quadrupole, an 8.75 × 2.8 × 2.8 cm ion cyclotron resonance cell, and a 7-T superconducting magnet with a 15 cm diameter bore from Oxford Instruments, Oxford, England. The guiding quadrupole and the cyclotron resonance cell are separated by 0.95 cm. Ions enter the ion cyclotron resonance cell through a 90% transmission, 1.75 cm by 1.75 cm, nickel screen which serves as both the ion entrance aperture and one of two trapping plates. The system is currently pumped with Varian VHS4, HS2, and VHS4 oil diffusion pumps placed at the ion source, quadrupole 1, and the entrance to the guiding quadrupole, respectively. The first two pumps have water-cooled baffles and operate with estimated pumping speeds of 500 and 175 L s^{-1}, respectively. The third pump contains a liquid nitrogen baffle and has an estimated pumping speed of 550 L s^{-1}. At present no effort is made to decelerate the ions as they enter the ion cyclotron resonance cell. We assume that the ions observed in the experiments described here become stabilized as a result of collisions with residual gas molecules in the ion cyclotron resonance cell. The electronics and data system to operate the Fourier transform mass spectrometer were obtained from Ionspec, Irvine, CA. Ionization of nonvolatile samples was accomplished by using glycerol as the liquid matrix and a cesium ion gun from Antek, Inc., Palo Alto, CA (*20*). The Antek gun was mounted directly on the Finnigan Model 4500 ion source.

Operation of the Tandem-Quadrupole Fourier Transform Mass Spectrometer in Conjunction with a Particle Bombardment Ion Source. Samples were prepared by adding 1 μL of a 1 nmol μL^{-1} solution of peptide in 5% acetic acid to 1 μL of glycerol on a stainless steel probe. The resulting mixture was then subjected to particle bombardment using 4–6 keV cesium ions. Quadrupoles 1 and 2 were operated in the rf only mode. For Met-Arg-Phe-Ala, mol wt 523; Leu-Trp-Met-Arg-Phe-Ala, mol wt 822; angiotensin, mol wt 1295; and renin tetradecapaptide, mol wt 1757; the rf power on Q1 and Q2 was set so as to transmit only those ions above mass 280. The ion cyclotron resonance cell was operated with a trapping potential of 1.5–2.0 V. Mass spectra were produced by using the computer to apply a sequence of pulses to the cell while the sample was being continually bombarded with particles from the cesium ion gun. The first, quench pulse, is applied to the trapping plates to clear the cell of all charged particles. After a delay time set between 100 and 4000 ms, an rf pulse containing a range of frequencies is applied to the two opposite transmitter plates to accelerate the ions trapped in the cell and to cause them to move coherently at their cyclotron frequency. Ion image currents induced on the other two opposite side plates, receiver plates, are then detected, amplified, and digitized. The resulting time-averaged signal is then Fourier transformed to produce the desired mass spectrum.

RESULTS AND DISCUSSION

Figure 2A shows the mass spectrum obtained from 1 nmol

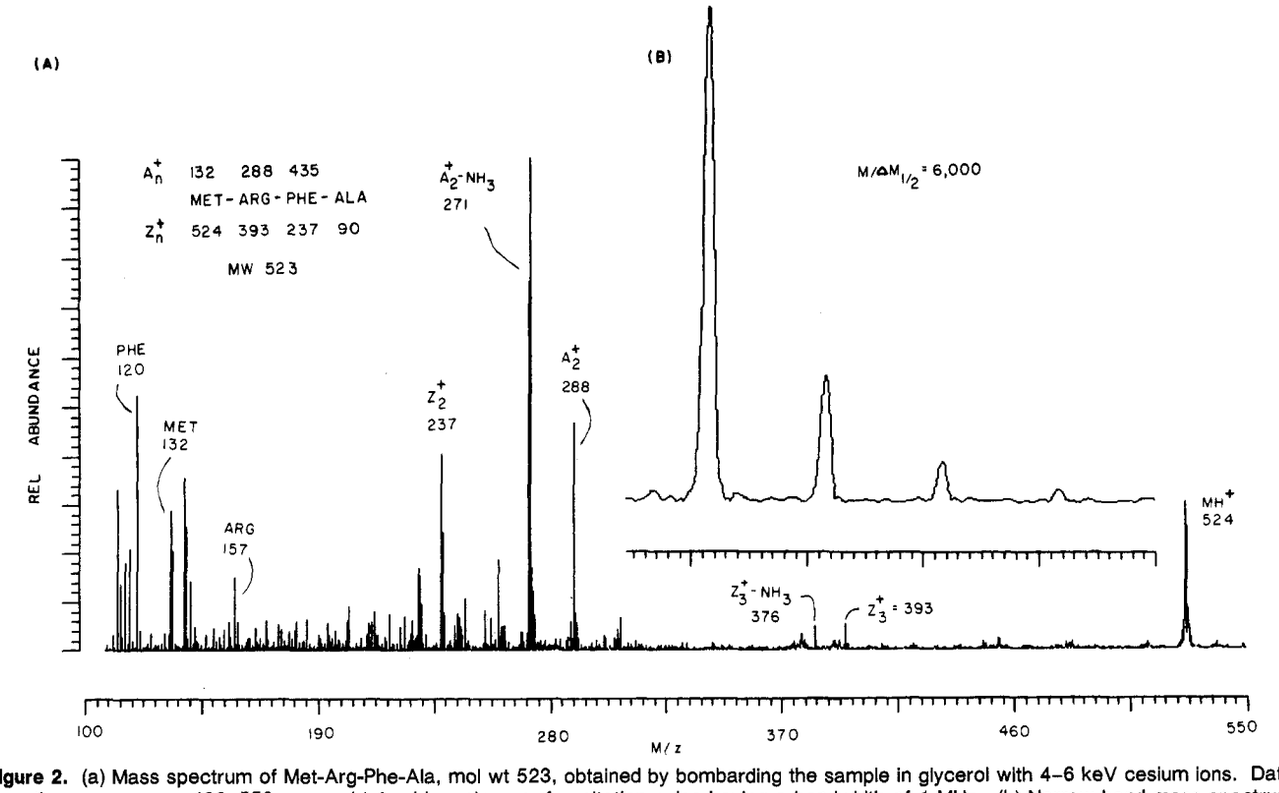

Figure 2. (a) Mass spectrum of Met-Arg-Phe-Ala, mol wt 523, obtained by bombarding the sample in glycerol with 4–6 keV cesium ions. Data over the mass range, 100–550, were obtained by using an rf excitation pulse having a bandwidth of 1 MHz. (b) Narrow band mass spectrum recorded over the mass range, 520–530, by using an rf excitation pulse of 10 kHz.

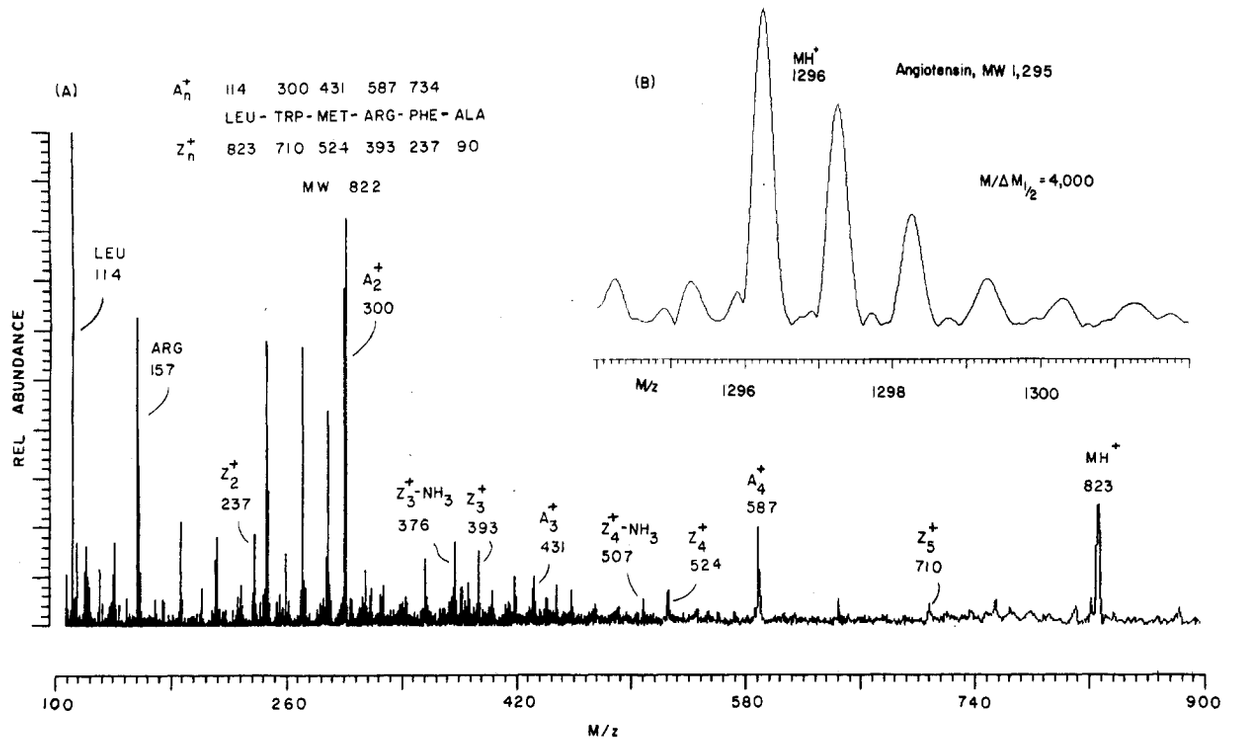

Figure 3. (a) Mass spectrum of the hexapeptide, Leu-Trp-Met-Arg-Phe-Ala, mol wt 822, obtained by bombarding the sample in glycerol with 4–6 keV cesium ions. Data over the mass range, 100–900, were recorded by using an rf excitation pulse having a bandwidth of 1 MHz. (b) Narrow band mass spectrum of angiotensin, mol wt 1295, recorded over the mass range, 1295–1305, by using an rf excitation pulse of 10 kHz.

of Met-Arg-Phe-Ala, mol wt 523, dissolved in 1 μL of glycerol using the combination of cesium ion bombardment and the newly constructed tandem-quadrupole Fourier transform mass spectrometer. The spectrum of the hexapeptide, Leu-Trp-Met-Arg-Phe-Ala, mol wt 822, appears in Figure 3A. To acquire these two sets of data, quadrupoles 1 and 2 were operated in the rf only mode and were set so as to reject all ions below mass 280. Ions above mass 280 were allowed to accumulate in the cell for 500–2000 ms. No effort was made to decelerate the ions as they entered the cell so only a small fraction, less than 0.1%, of the transmitted ions is actually stored in the present experiments. These presumably fall into stable orbits after undergoing stabilizing collisions with residual gas molecules in the cell. This stored ion population was irradiated with rf having a bandwidth of 1 MHz. Image currents so produced were then detected, amplified, digitized, and Fourier transformed. Fifty such scans were summed together to generate the spectrum in Figure 2A. Ten scans were accumulated for the spectrum in Figure 3A.

Figure 2B shows the spectrum of Met-Arg-Phe-Ala recorded in the narrow band mode using a frequency bandwidth of 10 kHz. Again, ions above mass 280 were allowed to accumulate in the cell for 3000 ms and then mass analyzed. Fifty scans, taken over the mass range, 520–530, were then summed together to produce the data shown. Transient lifetime was measured at 52 ms. Isotope peaks corresponding to the presence of one, two, and three carbon-13 atoms appear in this spectrum at close to the expected theoretical abundance. The resolution at m/z 524 is 6000.

Figure 3B shows the mass spectrum of angiotensin, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu, recorded in the narrow band mode by using a frequency bandwidth of 10 kHz. Ions were allowed to accumulate in the cell for 1000 ms and then mass analyzed. Twenty scans were summed together to produce the spectrum shown. Resolution at the MH^+ ion, m/z 1296, is 4000. A spectrum of renin tetradecapeptide, mol wt 1757, recorded in the narrow band mode showed an MH^+ ion having a signal to noise of 5/1.

Without dc/rf voltage on the rods, the efficiency of ion transmission through the quadrupole filters is greater than 90% and only ions above m/z 280 enter the ion cyclotron resonance cell. Conventional FAB or L-SIMS spectra are usually dominated by glycerol MH^+ and its solvated oligomers plus low mass fragment ions produced from both glycerol and the sample under the particle bombardment ionization conditions. If allowed to enter the ion cyclotron resonance cell, these ions would quickly exceed the capacity of the cell and seriously impair the capability of the instrument to detect weak signals due to sample MH^+ ions. Quadrupole filters 1 and 2 thus provide a convenient mechanism for extending the dynamic range of the ion cyclotron resonance cell. In its final configuration the tandem quadrupole Fourier transform instrument will operate with a low mass cutoff that can be set anywhere below mass 2000.

Although only ions of mass greater than m/z 280 enter the ion cyclotron resonance cell in the present experiments, Figure 2A clearly shows a substantial number of signals at masses lower than this cutoff. In the absence of evidence to the contrary, we assume that these ions, and some of the higher mass fragments as well, result from decomposition of vibrationally excited MH^+ ions having lifetimes greater then ca. 1 ms, the time required to traverse the two quadrupoles, and less than 3000 ms, the maximum time the ions spent in the cell prior to mass analysis.

Fragmentation observed in Figures 2A and 3A is consistent with that expected from conventional fast atom bombardment, liquid secondary ion mass spectrometry, or low energy collision-activation dissociation experiments. Ions of type A_n^+ and $\dot{A}_n^+$, corresponding to the general formula, $H(NHCHRCO)_n^+$ and $H(NHCHRCO)_nNHCHR^+$, respectively, define the sequence of amino acids from the amino terminus. Ions of type Z_n^+, corresponding to the general formula $^+H(NH_2CHRCO)_nOH$, define the sequence of amino acids from the carboxyl terminus of the peptide. Ions indicating the presence of individual amino acids in the peptide are of the type, $H_2NCHRCO^+$ or H_2NCHR^+.

As was indicated earlier, transient times exceeding 100 ms and resolution in excess of 100 000 at m/z 1000 can easily be achieved if the pressure in the ion cyclotron resonance cell is kept below 10^{-8} torr. In the present experiments the cell pressure could only be maintained at 2×10^{-7} torr, with or without sample in the ion source, because the system is still under construction and is now operating with temporary pumps of marginal capacity on the cell. In its final configuration the pumping system on the tandem-quadrupole Fourier transform instrument will maintain the cell pressure at 10^{-9} or below.

Initial results presented in this and four earlier papers (*16–19*) provide us with considerable encouragement in our effort to develop a new approach to biopolymer sequencing using the tandem-quadrupole Fourier transform mass spectrometer. Previous work demonstrated that ions produced in a quadrupole ion source could be transmitted with high efficiency through the fringing fields of a superconducting magnet using rf only quadrupoles and then stored in an ion cyclotron resonance cell (*17–19*). Here we have shown that the tandem-quadrupole Fourier transform instrument can also be employed for mass analysis of ions produced by cesium ion bombardment of nonvolatile sample dissolved in a volatile liquid matrix. Laser photodissociation of peptide MH^+ ions in an ion cyclotron resonance cell has recently been found to proceed with high efficiency and yield excellent sequence information (*16*). Thus it now seems reasonable to assume that large oligopeptides produced by either chemical or enzymatic cleavage of proteins can be completely sequenced at the low picomole level by using a pulsed cesium ion gun to minimize sample consumption during the ionization process, rf only quadrupoles to transmit selectively only the high mass ions of interest, an ion cyclotron resonance cell operated at low pressure to accumulate and store MH^+ ion produced in low abundance, laser photodissociation to generate fragment ions characteristic of the amino acid sequence, and Fourier transform mass spectrometry to produce a mass spectrum of the daughter ions at high resolution. This same approach should also work well for the characterization of oligonucleotides, oligosaccharides, and glycoproteins.

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Registry No. Met-Arg-Phe-Ala, 67368-29-0; Leu-Trp-Met-Arg-Phe-Ala, 53214-98-5; angiotensin, 484-42-4; renin tetradecapeptide, 64315-16-8; cesium ion, 18459-37-5.

LITERATURE CITED

(1) Hunt, D. F. *Int. J. Mass Spectrom. Ion Phys.* **1982**, *45*, 111–123.
(2) Busch, K. L.; Cooks, R. G. *Science* **1982**, *218*, 247–254.
(3) Rinehart, K. L., Jr. *Science* **1982**, *218*, 254–260.
(4) Hunt, D. F.; Bone, W. M.; Shabanowitz, J.; Rhodes, G.; Ballard, J. M. *Anal Chem.* **1981**, *53*, 1704–1706.
(5) Fitton, J. E.; Hunt, D. F.; Marasco, J.; Shabanowitz, J.; Winston, S.; Dell, A. *FEBS Lett.* **1984**, *169*, 25–29.
(6) Comisarow, M. B.; Marshall, A. G. *Chem. Phys. Lett.* **1974**, *25*, 282–283.
(7) McIver, Jr., R. T. *Am. Lab.* (*Fairfield, Conn.*) **1980**, *12* (11), 18–24.
(8) Wilkins, C. L.; Gross, M. L. *Anal. Chem.* **1981**, *53*, 1661A–1676A.
(9) Bowers, W. D.; Hunter, R. L.; McIver, R. T., Jr. *Ind. Res./Dev.* **1983**, *25* (11), 124–128.
(10) Carlin, T. J.; Frieser, B. S. *Anal. Chem.* **1983**, *55*, 955–958.
(11) McIver, R. T., Jr.; Bowers, W. D. In "Tandem Mass Spectrometry"; McLafferty, F. W., Ed.; Wiley: New York, 1983; pp 287–301.
(12) Cody, R. B.; Burnier, R. C.; Cassady, C. J.; Freiser, B. S. *Anal. Chem.* **1982**, *54*, 2225–2228.
(13) Dunbar, R. C. In "Gas Phase Ion Chemistry"; Bowers, M. T., Ed.; Academic Press: New York, 1979; pp 181–220.
(14) Thorne, L. R.; Wright, C. A.; Beauchamp, J. L. In "Ion Cyclotron Resonance II"; Hartmann, H., Wanczek, K. P., Eds.; Springer-Verlag: Berlin, 1982; pp 43–97.
(15) Jasinski, J. M.; Rosenfeld, R. N.; Meyer, F. K.; Brauman, J. L. *J. Am. Chem. Soc.* **1982**, *104*, 652–658.
(16) Bowers, W. D.; Delbert, S. S.; Hunter, R. L.; McIver, R. T., Jr. *J. Am. Chem. Soc.*, in press.
(17) McIver, Jr., R. T.; Hunter, R. L.; Story, M. S.; Syka, J.; Labunsky, M.; Presented at the 31st Annual Conference on Mass Spectrometry and Allied Topics, Boston, MA, 1983.
(18) McIver, R. T., Jr.; Hunter, R. L.; Bowers, W. D. Presented at the "32nd Annual Conference on Mass Spectrometry and Allied Topics", San Antonio, TX, 1984.
(19) McIver, R. T., Jr.; Hunter, R. L.; Bowers, W. D. *Int. J. Mass Spectrom. Ion Phys.*, in press.
(20) Abreth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* **1982**, *54*, 2029–2034.

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Hollow Fiber Postcolumn Reactor for Liquid Chromatography

Sir: A great deal of attention has been focused on postcolumn derivatization for enhanced detection of species eluting from liquid chromatography columns. Frei and Scholten (*1*) have reviewed many of the theoretical and technical aspects of reaction detectors, including the problems associated with their use, while Takata and Muto (*2*) first discussed reaction detectors for uses such as enhanced electrochemical detection. Hollow fiber membranes have been reported for postcolumn reaction in both unpacked and packed hollow fiber suppressors for ion chromatography (*3*, *4*).

The requirements for good postcolumn derivatization can be reduced to three key components: (a) reagent introduction, (b) delay or reaction/mixing loops, and (c) proper detection devices. Both (b) and (c) are essentially fixed by the reaction time/temperature and chemistry chosen. Part a, the reactor itself, allows more flexibility. For example, with reagent mixing, techniques such as air segmentation, pumping reagent into a mixing device, and packed beds of reagent granules have all been used. Unfortunately, each of these methods has its drawbacks. Air segmentation and mixing devices require an additional pump and lead to band spreading and dilution,

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