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Evaluation of an alternating current plasma emission detector for high-performance liquid chromatography

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ABSTRACT

The details of construction and operation of an alternating current plasma (ACP) detector supported with helium gas for reversed-phase high-performance liquid chromatography is described. The system is evaluated as a selective detector for the determination of organomercury compounds. The chromatographic eluent is introduced into the plasma by means of a frit nebulizer and the plasma can tolerate the nebulization of 100% methanol without extinguishment. Detection limits of methylmercury chloride and ethylmercury chloride were found to be 4.5 and 2.2 ng Hg/sec, respectively. In the nanogram to microgram mass range studied the precision was found to be less than 10% (relative standard deviation). The detection of organomercurials in complex sample matrices illustrates the selectivity of the ACP detector.

INTRODUCTION

The advantages of high-performance liquid chromatography (HPLC) in combination with plasma emission detectors has been described during the last decade[1–4]. One attractive feature of this type of detectors is their capability for elemental speciation permitting the determination of organic molecules containing a specific heteroatom in a very complex sample matrix, where resolution of a desired compound and matrix interferences may affect its detection. The choice of a suitable detector is one area that should be considered for the utilization of HPLC for speciation purposes[5].

There are three principal plasma sources that have been evaluated as specific detection modes in HPLC: direct current plasma (DCP), inductively coupled plasma (ICP) and microwave-induced plasma (MIP). Most studies have focused on the combination of HPLC with ICP and many applications have been reported[6–11]. The introduction of the direct injection nebulizer (DIN) has made the technique more attractive[12,13]. The extensively reported fact that the DCP is compatible with a wide variety of solvents has facilitated its combination with HPLC[14–17]. However, the interface of HPLC and MIP has been more challenging because of the low tolerance of the MIP towards organic solvents typically used in HPLC although this situation has

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been addressed by mixing the plasma gas with oxygen[18] and modification of the design of the discharge tube. In addition, this type of plasma can accommodate solutions containing up to 90% (v/v) methanol in water. More powerful and costly He-MIP (500-W) detectors have been also investigated[19,20]. A low power (100-W) He-MIP with a moving wheel sample transport-desolvation interface has also been described[21]. The aqueous solvent is evaporated with a nitrogen flow at elevated temperatures in order to reduce interferences due to the solvent. However, this device is in a preliminary stage and the effect of organic solvents was not reported.

In a recent communication our laboratory reported an alternating current plasma (ACP) detector for gas chromatography (GC)[22]. The self-seeding plasma could tolerate high mass flow-rates of organics without extinguishment. The performance of the ACP detector was comparable with that of the MIP detector for GC. The compatibility with large injected aliquots of organics strongly suggested the possibility of extending its application into HPLC as an inexpensive detector, simple in construction and operation. In this communication the interface and operation of the detector in tandem with HPLC in the reversed-phase mode are reported.

EXPERIMENTAL

Reagents

All the solvents used were HPLC grade. The organomercury compounds, methylmercury chloride (MMC) and ethylmercury chloride (EMC), were purchased from Morton Thiokol (Alfa Products, Danvers, MA, U.S.A.). Stock solutions were prepared by dissolving the appropriate amount of the organomercurial in methanol (J. T. Baker, Phillipsburg, NJ, U.S.A.). Subsequent solutions were prepared by serial dilution of the stock solutions.

Chromatographic equipment

Fig. 1 shows a schematic diagram of the components of the system used in this study. A Spectra-Physics solvent delivery system (Model SP8700, Spectra-Physics, San

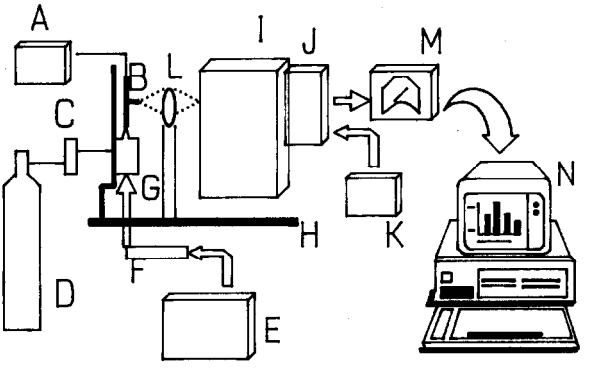

Fig. 1. Schematic diagram of the HPLC–ACP system. A = a.c. power supply; B = discharge tube; C = gas flow controller; D = solvents and solvent delivery system; F = HPLC column; G = nebulizer; H = optical bench; I = monochromator; J = PMT; K = PMT power supply; L = focussing lens; M = picoammeter; N = data acquisition system.

Jose, CA, U.S.A.) delivered the mobile phase to the sample loop injector (Model 7125, Rheodyne, Cotati, CA, U.S.A.) which was connected to a Hypersil ODS column, 100 × 4.6 mm I.D., 5 μm (Hewlett-Packard, Palo Alto, CA, U.S.A.). UV monitoring was performed using a TriDent detector (Perkin-Elmer, Norwalk, CT, U.S.A.).

Spectroscopic equipment

A single-beam McPherson grating monochromator EU700 (McPherson, Acton, MA, U.S.A.) was equipped with a R212 photomultiplier tube (Hamamatsu, Middlesex, NJ, U.S.A.) which was coupled to a voltage power supply (McPherson, Model 7640) and operated at −900 V. An optical bench was equipped with an adjustable optical mount and a 75-mm biconvex quartz lens (Oriel, Stratford, CT, U.S.A.) to focus the plasma into the monochromator with a slit height and width setting of 5 mm and 300 μm, respectively. For spectral background measurements, a slit width of 50 μm was selected. A picoammeter (Model 414s, Keithley Instruments, Cleveland, OH, U.S.A.) monitored the current generated by the photomultiplier tube. A mercury hollow cathode lamp (Perkin-Elmer) was employed to accurately select the analytical emission line (253.7 nm). Data acquisiton was achieved with the Chrom-1AT chromatography data acquisition board controlled by the Lab Calc software (Galactic Industries, Salem, NH, U.S.A.) in conjunction with a Zenith AT compatible microcomputer (Zenith Data Systems, St. Joseph, MI, U.S.A.). The Lab Calc software package provided data smoothing algorithms to reduce random noise and permitted selectable sampling rates. In this investigation a rate of 5 points per second was utilized which is more than adequate to define the peak shape in LC applications where peak widths are typically 15 to 60 s (ref. 13).

Discharge tube and interface operation

The discharge tube was constructed from quartz tubing, 6 mm O.D. × 4 mm I.D, and it was similar to that previously reported in our GC study[22]. The discharge tube was attached to an adjustable optical mount (Edmund Scientific, Barrington, NJ, U.S.A.) which was fastened to the optical bench. The plasma was generated across two copper electrodes (1 mm diameter) with an a.c. power supply, operated at 10 000 V, Furnace Ignition Transformer (France, Fairview, TN, U.S.A.). The gap between the electrodes was maintained at 13 mm (ref. 23).

A glass-frit nebulizer was used as the interface between the HPLC column and the ACP and a schematic of the interface is illustrated in Fig. 2. The design closely resembled that which has been used in other studies[24,25]. It consisted of a modified 15-ml Pyrex sintered glass funnel filter, 20 mm in diameter and 4.5–5.0 μm in porosity (Corning Glass, Corning, NY, U.S.A.). A 15 cm length of Flexon high-pressure tubing (0.063 in. O.D. × 0.007 in. I.D.) served as the connecting line from the column eluent to the glass frit. The volume of the chamber was approximately 5 ml. The upper arm of the modified funnel was connected to the bottom end of the PTFE tee union attached to the discharge tube. Helium flow of 6 l/min was introduced into the opposite side of the frit as the nebulizer gas and simultaneously supported the ACP. The drain of the nebulizer was closed to the environment allowing the fine mist produced to be transported toward the plasma. The largest droplets were removed and discarded through the drain to a closed waste collector.

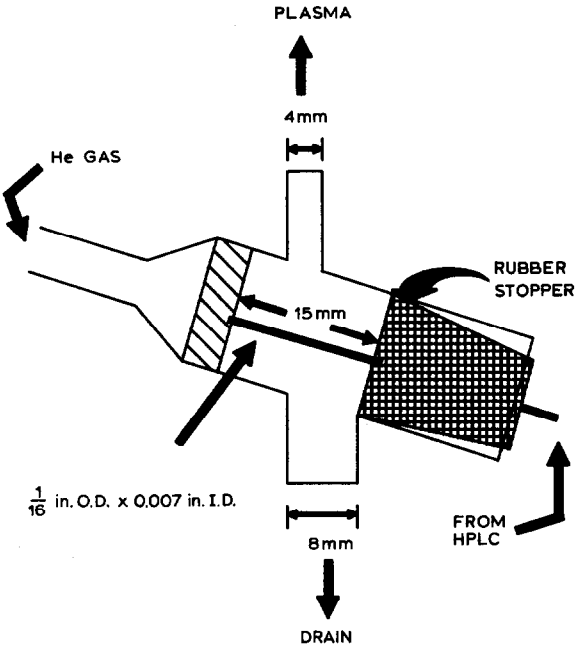

Fig. 2. Schematic of the glass-frit nebulizer.

RESULTS AND DISCUSSION

Interface

The frit nebulizer generates a very fine mist with droplet size distribution smaller than the pneumatic nebulizer[26] and greatly enhances the introduction of organic solvents to the plasma[25]. The nebulizer performed more favorably when the sintered glass disc was positioned in a vertical or angular orientation with respect to the discharge tube, as shown in Fig. 2, as opposed to the preferred horizontal position used with the ICP[24].

The inherently high efficiency of the frit nebulizer[24,25] and the small inner diameter of the transfer tube were responsible for small droplets of the nebulized solution to be deposited as small droplets on the walls of the transfer tube. After several minutes the droplets filled part of the transfer tube presenting disturbances to the plasma and even trapped most of the aerosol at flow-rates greater than 3 l/min. These problems were overcome by maintaining the transfer tube and the connection to the discharge tube at 100°C. Pressures up to 100 p.s.i. can be applied to the nebulizer without producing damage to the sintered glass frit. The ACP response showed a dependence on the gas flow-rate across the frit nebulizer. The maximum response was associated with a He gas flow-rate of 6 l/min delivered at a pressure of 78 p.s.i.

Plasma stability

The presence of organic solvents in the ACP increases the noise level of the

detector; therefore, the high-frequency random noise was digitally removed by smoothing the data once collected, improving signal to noise ratio. Since methanol and acetonitrile are the most commonly used organic modifiers in reversed-phase HPLC, the characteristics of the plasma were further studied with the binary mixtures of these solvents with water. The color of the plasma (pink-purple with the introduction of pure water) changed to a bluish green color when aqueous solutions of the organic solvents were introduced. This color change, resulting from CN molecular emission, became more intense with increasing concentration of the organic solvent. However, if increased oxygen levels are present in the plasma, such as is the case when aqueous solutions of methanol are introduced, decreasing cyanogen formation occurs and the more stable CO molecular species are formed instead resulting in decreased intensity of the bluish-green color[27]. The CN molecular species formation results from nitrogen impurities in the He gas, when acetonitrile is not used as the organic modifier. The ACP did not extinguish when pure methanol was nebulized into the plasma at a rate of 1 ml/min for a period of 30 min and the recorded baseline (noise) did not show any irregular perturbation. However, the introduction of aqueous mobile phase containing 10% acetonitrile did extinguish the plasma after 5 min. In addition, with acetonitrile entering the plasma in place of methanol, the formation of carbon species increases with a concurrent decrease in the amount of oxygen-containing species and results in the accumulation of combustion deposits in the vicinity of the plasma. Carbon deposition with acetonitrile was also observed on the walls of the discharge tube above the plasma plume but with methanol very little residue was noted in this vicinity.

Organomercury detection

The analytical emission line used throughout this study was the Hg(I) 253.7-nm line. In order to observe the influence of the mobile phase in the vicinity of the analytical line, a profile of the plasma emission was generated. No interference is observed at the wavelength corresponding to Hg(I) line.

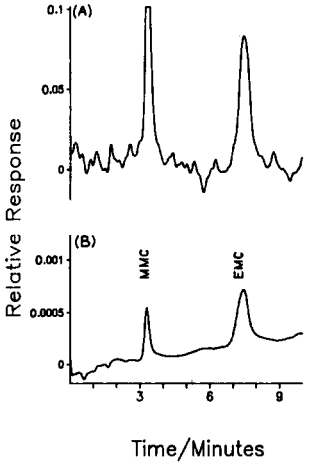

Fig. 3. Chromatograms of MMC (669 ng as Hg) and EMC (505 ng as Hg) with (A) ACP detection and (B) UV detection; mobile phase, 0.01% 2-mercaptoethanol in methanol–water (22:78); column, Hypersil ODS 5 (100 mm × 4.6 mm I.D.); flow-rate, 1.0 ml/min; injection volume, 25 μl; wavelength, 253,7 nm.

The HPLC–ACP chromatograms of MMC and EMC were monitored with a fixed-wavelength UV detector (254 nm) operated at its maximum output sensitivity. In Fig. 3 parallel chromatograms of MMC and EMC which clearly display the improved sensitivity of the ACP detector are presented.

Linearity and detection limits

Calibration plots for MMC and EMC were prepared by injecting six repetitive injections of known amounts into the HPLC–ACP system linearity of over three orders of magnitude for both probe solutes in the range studied was observed. Correlation coefficients of the log–log plots were 0.999 and 0.998 for MMC and EMC, respectively. Detection limits (D) were calculated based on the integrated baseline noise[6,28] and according to eqn. 1

$$D = 3\sigma/m \tag{1}$$

where σ refers to the standard deviation of the noise and m is the sensitivity. The sensitivity is defined as the slope of the calibration curve[29] multiplied by the peak width at 0.607 height of the analyte peak[30] to account for the capacity factor, k'. The detection limits for MMC and EMC, expressed in terms of elemental Hg, were calculated to be 4.5 ng/s and 2.2 ng/s, respectively. The actual values observed corresponded to 25 μl injection of 2.8 ng/μl MMC (2.2 ng/μl or 70 ng as Hg) and 4.0 ng/μl EMC (3.0 ng/μl or 70 ng as Hg). The repeatability of a standard solution at twice the detection limit was under 10% relative standard deviation ($n = 5$) for both MMC and EMC. Detection limits for similar compounds analyzed by HPLC interfaced with ICP by means of a conventional nebulizer have been reported to be 232 and 302 ng/μl as Hg for MMC and EMC, respectively; improved results were also reported but the cold vapor generation technique for mercury was used after the analytical column[31].

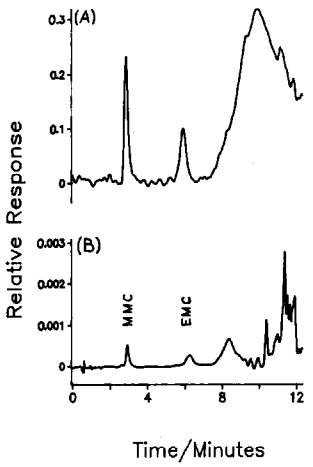

Fig. 4. Chromatograms of a water sample taken from a local river spiked with MMC and EMC, with (A) ACP detection and (B) UV detection: mobile phase, 0.01% 2-mercaptoethanol in methanol–water (22:78), after 5 min methanol was increased to 100%; column, Hypersil ODS 5 (100 mm × 4.6 mm I.D.); flow-rate, 1.0 ml/min; injection volume, 25 μl; wavelength, 253.7 nm.

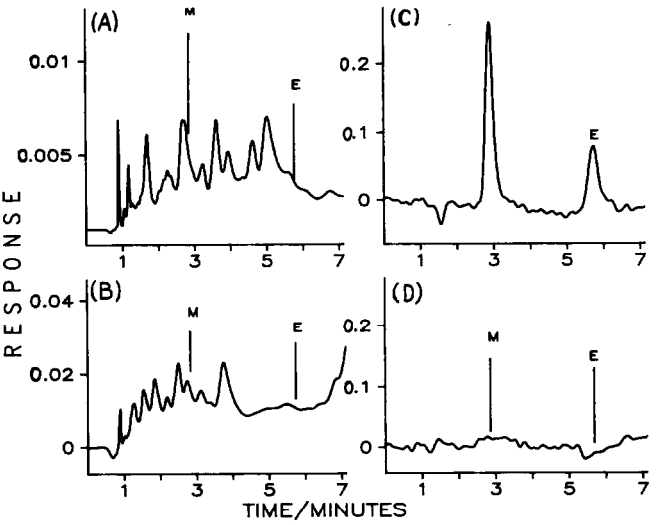

Fig. 5. Chromatograms of (A) diluted gasoline spiked with MMC and EMC by UV detection, (B) unspiked gasoline with UV detection, (C) ACP detection of the spiked gasoline and (D) unspiked gasoline detected by ACP; M and E points out the elution time of MMC and EMC, respectively. Conditions as in Fig. 4.

Selectivity

In order to demonstrate the selectivity of the ACP, known, amounts of MMC and EMC were spiked to samples of different matrix composition, namely, a sample of local river water and gasoline. Chromatograms of each spiked sample in Figs. 4 and 5 were monitored in parallel by UV detection at 254 nm and the ACP. The samples were spiked in such a manner that a 25-μl injection corresponded to 840 ng (669 ng Hg) and 668 ng (505 ng Hg) of MMC and EMC, respectively. In order to elute all strongly retained components from the column, the amount of methanol in the mobile phase was increased after 5 min from injection (from 22% to 100%). In Fig. 5 the selectiviy of the ACP detector is further demonstrated with chromatograms of gasoline: UV detection of (A) gasoline spiked with MMC and EMC and (B) unspiked gasoline, and ACP detection of (C) spiked and (D) unspiked gasoline. The rapid increase of methanol in the mobile phase (after 5 min of injection) also yielded a higher plasma background emission when the methanol reached the plasma as a "plug" of solvent. After approximately 4 min the baseline restabilized but at an elevated level. Moreover, the baseline stability of the ACP detector in HPLC was not affected by injection of a complex matrix whereas in our GC studies we found baseline instability to be problematic at the beginning of chromatograms associated with high organic solvent concentrations in the plasma[22].

CONCLUSION

The ACP detector described here offers a number of attractive features as an element-selective detector for the reversed-phase mode of HPLC. The detector is inexpensive, simple in design and can accept pure methanol with no adverse

performance. The ACP offers considerable potential for the determination of organomercurials by HPLC, especially where complex sample matrix may be a difficulty with other types of detectors. The nebulizer used as the interface contributed to the development of the ACP detector for reversed-phase HPLC. Further improvements in detector design including the use of other nebulizing systems should provide even improved performance of the ACP. One such area is the use of discharge tubes of smaller inner diameters to further reduce the overall dead volume of the system.

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