

CHROM. 15,751

Note

Determination of some polyaromatic compounds by reversed-phase liquid chromatography with electrochemical detection

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(Received February 4th, 1983)

Synthetic fuels derived from sources such as coal and oil shale are being increasingly analyzed for components that may present certain health hazards. While the multicomponent separation and identification methods combined with extensive fractionation schemes^{1–6} are important to fully characterize these materials, simple measurements are also needed for the selected compounds that possess mutagenicity and carcinogenicity. Polycyclic aromatic primary amines (APAHs) were found among the potent mutagens contained in petroleum substitutes^{4–8}.

A key feature of liquid chromatography (LC) with electrochemical detection (EICD) is the selectivity that can be afforded by the judicious choice of the applied potential. In this communication, we show that amperometric detection can be employed to selectively determine APAHs in the presence of other polycyclic aromatic compounds (PACs). This selectivity may, therefore, be of great utility for routine determinations. The electrochemical oxidation of PACs in solvents such as acetonitrile has been investigated^{9,10}. In general, APAHs are oxidized at less positive potentials than polycyclic aromatic hydrocarbons (PAHs). Polycyclic aromatic nitrogen heterocycles (PANHs) are more difficult to oxidize than either of the above classes.

APAHs have been previously determined by strong cation-exchange LC–EICD¹¹. However, recent advances in column technology and cell design provide considerable improvement in this analysis scheme. The use of glassy carbon electrodes and inert materials throughout the amperometric cell permit a wide range of solvents to be employed. While separation of PACs can be achieved by reversed-phase LC with acetonitrile as the mobile phase, electrochemical detection is still possible. Since non-aqueous LC–EICD is an unexplored area, we have characterized the performance of the detector in this application.

EXPERIMENTAL

Reagents

Standard solutions of anthracene (Gold Label; Aldrich, Milwaukee, WI, U.S.A.) and 2-aminoanthracene (Aldrich) were prepared immediately before chromatographic analysis in acetonitrile (HPLC Grade, Fisher, Cincinnati, OH, U.S.A.)–water (doubly distilled) (90:10) which contained sodium perchlorate (0.1 *M* or 0.01 *M*). The 2-aminoanthracene was sublimed (200°C) prior to use. Standard solutions of

anthracene, 2-aminoanthracene and acridine ($1 \cdot 10^{-3} M$, Sigma, St. Louis, MO, U.S.A.) were prepared immediately before cyclic voltammetric characterization in acetonitrile which contained sodium perchlorate (0.1 M).

Apparatus

LC system consisted of a constant-flow reciprocating pump (Model 396 mini pump; Milton Roy, Riviera Beach, FL, U.S.A.), a loop injector (50 μ l, Model 7010; Rheodyne, Berkeley, CA, U.S.A.) and a reversed-phase column (Chromanetics Spherisorb, ODS-2, 25 cm \times 5 mm I.D., 5 μ m particle size; PJ Cobert Assoc., St. Louis, MO, U.S.A.). A stainless-steel tube (0.1 m \times 5.0 mm I.D.) between the pump and the loop injector served as a pulse dampener. The mobile phase, which contained sodium perchlorate (0.1 M or 0.01 M) in acetonitrile–water (90:10) was recycled by pumping the effluent into the buffer reservoir. The mobile phase reservoir was sealed to prevent solvent evaporation. The flow-rate was 1.2 ml/min.

Amperometric detection was accomplished with a working electrode of glassy carbon (Tokai GC-20; Atomergic, New York, NY, U.S.A.). The glassy carbon was polished to a mirror finish with the use of 600 grit sandpaper followed by polishing successively on a felt cloth with 5 μ m, 0.3 μ m, and 0.05 μ m alumina (Fisher, Cincinnati, OH, U.S.A.). The auxiliary electrode, which is also the upper portion of the cell, was made of stainless steel as described previously¹². A pre-cut spacer (51 μ m thick, Tefzel; DuPont, Wilmington, DE, U.S.A.) was laminated (20 min at 300°C) onto the auxiliary electrode. The lamination of the Tefzel spacer was necessary to prevent the leakage that occurred between the auxiliary electrode and spacer when PTFE or polyethylene spacers were employed. The entire assembly was then clamped together with 4 bolts. The working electrode area was 0.46 cm² and the cell volume was 2.3 μ l. The reference electrode in these studies was a silver/silver perchlorate (saturated) electrode in acetonitrile–water (90:10). The reference electrode potential was checked daily *versus* a saturated calomel electrode and was found to be 0.40 V more positive. Voltammetric studies employed a PARC Model 174A (Princeton, NJ, U.S.A.) and a disk-shaped glassy carbon electrode (area 0.058 cm²).

RESULTS AND DISCUSSION

The cyclic voltammograms of 2-aminoanthracene, anthracene and acridine in acetonitrile with sodium perchlorate (0.1 M) as supporting electrolyte at a glassy carbon electrode gave oxidation peak potentials +0.16 V, +0.82 V and +1.17 V, respectively. These results are similar to those obtained at a rotating platinum electrode in acetonitrile^{9,10}. Analysis of the relative peak potential differences indicates that LC–EICD should be able to determine 2-aminoanthracene in the presence of anthracene and acridine. However, as indicated in the introduction, a detector which is physically stable (*i.e.* will not degrade, dissolve, etc.) in acetonitrile, was needed. To achieve this requirement an amperometric detector was constructed in which the mobile phase contacts only stainless steel, Tefzel, glassy carbon, and the reference electrode.

Fig. 1A shows the chromatogram of 2-aminoanthracene (5 pmol) at +0.4 V in the presence of anthracene (5 pmol). The mobile phase was acetonitrile–water (90:10) with 0.1 M sodium perchlorate as the supporting electrolyte. The working electrode

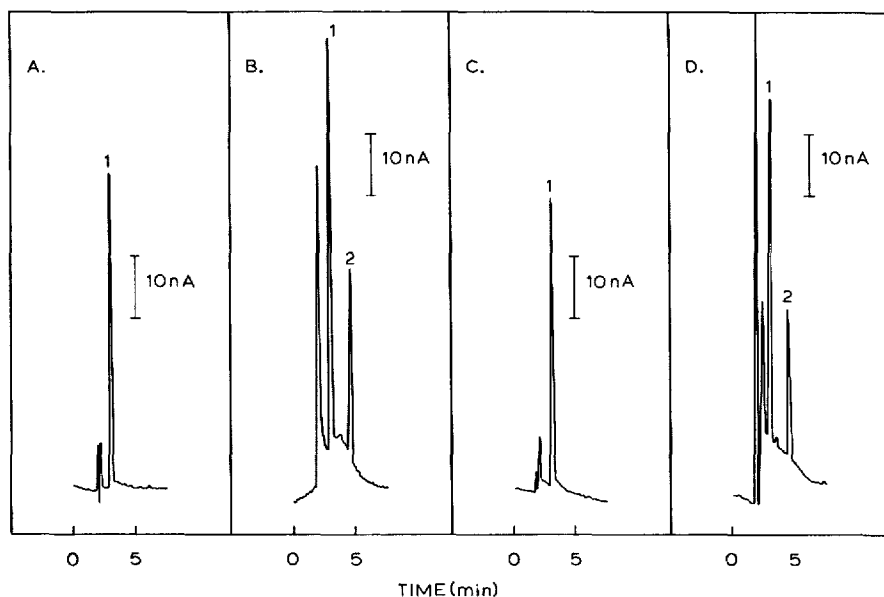


Fig. 1. Chromatograms of standard solutions of 2-aminoanthracene (5 pmol) and anthracene (5 pmol) with acetonitrile–water (90:10) mobile phase. (A) Working electrode potential, $E_{app} = +0.4$ V with 0.1 M sodium perchlorate as supporting electrolyte; (B) $E_{app} = +0.8$ V with 0.1 M sodium perchlorate as supporting electrolyte; (C) $E_{app} = +0.4$ V with 0.01 M sodium perchlorate as supporting electrolyte; (D) $E_{app} = +0.8$ V with 0.01 M sodium perchlorate as supporting electrolyte. Peaks: 1 = 2-aminoanthracene; 2 = anthracene.

potential was then altered to +0.8 V and the simultaneous detection of 2-aminoanthracene and anthracene was accomplished (Fig. 1B). The signal-to-noise ratios are reported in Table I and these compare favorably with those observed with this detector with aqueous LC–EICD¹³. Thus, LC–EICD with non-aqueous solvents does not compromise the high sensitivity associated with aqueous LC–EICD. For 2-aminoanthracene, the calculated minimum detection limit (assuming a signal-to-noise ratio of 2) was less than 50 fmoles.

For the analysis of the non-polar PACs and for the longevity of the reversed-

TABLE I

SIGNAL-TO-NOISE RATIOS OF 2-AMINOANTHRACENE AND ANTHRACENE

E_{app}	Sodium perchlorate concentration*	2-Aminoanthracene (5 pmol)	Anthracene (5 pmol)
+0.4 V**	0.1 M	226	
	0.01 M	419	
+0.8 V**	0.1 M	99.2	45.2
	0.01 M	68.2	30.6

* Supporting electrolyte concentration in acetonitrile-water (90:10).

** Versus Ag/AgClO₄ (saturated) reference electrode.

phase column, it may be advantageous to use relatively low concentrations of supporting electrolyte. As seen in Fig. 1C and D, chromatograms obtained with 0.01 *M* sodium perchlorate in the mobile phase are essentially identical to those with 0.1 *M* sodium perchlorate. The signal-to-noise ratios are also given in Table I for the low sodium perchlorate concentration and are virtually the same as those obtained with 0.1 *M* sodium perchlorate. The residual currents, however, increased from 57 nA to 81 nA at +0.4 V and from 560 nA to 700 nA at +0.8 V. An increase of this nature may arise because the lower supporting electrolyte concentration increases the solution resistance.

This work clearly indicates that non-aqueous LC-EICD at carbon electrodes may be a viable technique for the selective analysis of PACs. As shown, the method possesses the selectivity (detection of 2-aminoanthracene in the presence of anthracene), sensitivity (subpicomole) and low cost (recycling of buffer) that are associated with aqueous LC-EICD. For the analysis of very complex samples (coal tars and oil shale), the technique may provide valuable qualitative information in sample screening efforts (e.g., APAHs in the presence of PAHs). For fractionated samples, the technique may provide supportive information for identification purposes. If more component resolution becomes a necessity, a miniaturized version of this detector¹⁴ could be used in conjunction with high-efficiency capillary LC¹⁵. Further, while gradient elution is problematic with electrochemical detection (due mainly to residual current shift), it has been shown that quantitative results on the picomole level can be obtained with small (<10%) gradients¹⁶. Larger gradients may also be possible; however, it is expected that there will be a decrease in detection limits.

ACKNOWLEDGEMENTS

This research was supported by the National Science Foundation (CHE-81-21422 to R.M.W.) and in part by the Department of Energy (DE-AC02-81ER60007 to M.V.N.). R.M.W. is an Alfred P. Sloan Fellow and the recipient of a Research Career Development Award from N.I.H. (PHS K04 NS 356). Discussions with T. Wozniak are appreciated.

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