Journal of Chromatography, 285 (1984) 103–114
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,371

ELECTROCHEMICAL DETECTION OF POLYNUCLEAR AROMATIC HYDROCARBONS FOLLOWING REVERSED-PHASE GRADIENT HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING A LARGE-VOLUME WALL-JET DETECTOR

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(First received August 2nd, 1983; revised manuscript received October 18th, 1983)

SUMMARY

Electrochemical detection is rarely used in conjunction with gradient high-performance liquid chromatography. The problem is the changing eluent composition and electrolyte concentration during a gradient run which greatly affects the performance of electrochemical detectors. However, by the use of a large-volume wall-jet detector a means of obviating the problem is afforded. This paper reports on an application to the analysis of polynuclear aromatic hydrocarbons.

INTRODUCTION

Electrochemical detectors are gaining increasing usage in high-performance liquid chromatography (HPLC) for the analysis of a wide range of compounds. Numerous applications have been carried out in conjunction with reversed-phase or ion-exchange chromatography. Recently it was reported[1] that electrochemical detection (ED) can be effectively applied to normal phase HPLC, where use is made of non-polar eluents. Detection in such eluents is made feasible by the addition of a tetraalkylammonium salt (to make the eluent conducting) and of a modified Ag/AgCl reference electrode. In that work, a wall-jet (WJ) electrochemical detector of the Fleet–Little design[2] was employed.

In the design of electrochemical detectors it has been the practice to minimize the geometric cell volume in order to prevent band spreading by convective diffusion. Consequently, various cell designs, which include the WJ, parallel plate and tubular electrode configurations, have sought to limit cell volumes to a few microlitres. However, this reasoning does not apply to the WJ detector for which it was shown[3] that a large-volume cell is, in fact, preferable and does not lead to band spreading.

One of the applications of a large-volume cell WJ detector is in gradient elution HPLC. A significant problem in using ED in conjunction with gradient elution arises as a result of the changing eluent composition. In reversed-phase separations, for example, the eluent composition can vary from aqueous mixtures to one that is totally non-aqueous. This poses two difficulties: first, the eluent dielectric properties change;

0021-9673/84/$03.00

and secondly, the supporting electrolyte must be compatible with the varying eluent composition, in particular, when the eluent is totally non-aqueous.

The large-volume WJ cell affords a means of obviating the effects of changing eluent composition and the problem of electrolyte compatibility in gradient elution HPLC. By way of illustration, this paper reports an application to the gradient reversed-phase separation of polynuclear aromatic hydrocarbons (PAHs). A fluorescence detector was placed in series with the WJ detector for the sake of comparison. Recently, Caudill *et al.*[4] reported the determination of PAHs by reversed-phase HPLC–ED. However, in that work, the elution was isocratic and the electrochemical detector used was a thin-layer type.

EXPERIMENTAL

Electrochemical system

The WJ detector design used by Fleet and Little[2] was actually a constricted wall-jet having a cell volume of less than 100 μl. In contrast, the modified WJ cell used in this work has a capacity of more than 35 ml. The cell body was made of a glass tube fitted onto a PTFE base. The jet nozzle was made to fit through a threaded hole in the base. For most analyses the nozzle was kept 4 mm away from the working electrode. The glass/PTFE cell was positioned below a PARC Model 303 electrode system (Princeton Applied Research Corp., Princeton, NJ, U.S.A.). Use was made of the Model 303 Ag/AgCl reference and platinum counter electrodes without further modification. The working electrode was a 5.5 mm diameter glassy carbon disc (Tokai Mfg, Japan) sealed into a glass housing with epoxy cement. The glassy carbon was joined to a copper lead using silver-loaded epoxy cement; the copper lead was connected to the external working electrode test point of the Model 303, which is the input to the PARC 174 current–voltage converter. Fig. 1 shows a diagram of the modified WJ cell.

The Model 303 electrode system was controlled by a PARC Model 364 polarographic analyser in the d.c. mode. The current output of the 364 was recorded on a Hitachi Model QD15 recorder (Hitachi, Tokyo, Japan).

The glassy carbon electrode was prepared for analysis by polishing to a mirror finish with a fine diamond paste. It was then wiped thoroughly with an ethanol-soaked filter-paper and rinsed in distilled water.

All potentials referred to in this paper are measured with respect to the Ag/AgCl reference electrode.

Fluorescence detection

PAHs show high ultra-violet and fluorescence activity by virtue of their aromatic character. However, fluorescence spectrometry is, by far, the more sensitive and also, the more selective technique.

A fluorescence detector was placed in series between the column and the WJ detector. PTFE tubing (60 cm × 0.55 mm I.D.) connected the two detectors. The fluorescence detector was a Perkin-Elmer Model 60-10S fluorescence spectrophotometer (Perkin-Elmer Corp., CT, U.S.A.) fitted with a Hitachi micro flow cell. It was connected to the column by a 0.5 mm I.D. Swagelok stainless-steel tubing. Based on

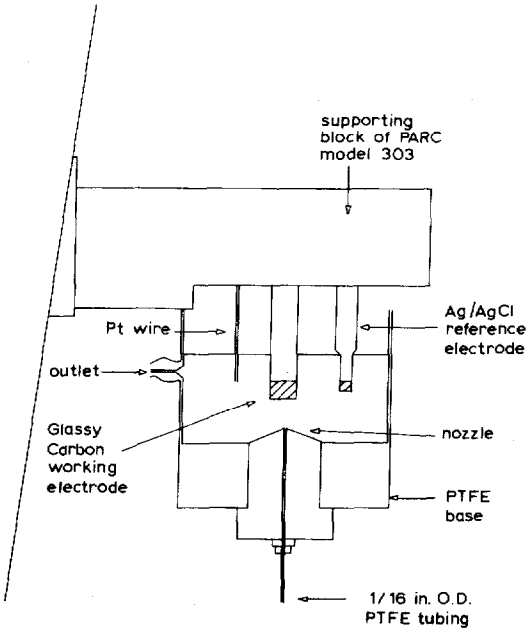


Fig. 1. Schematic diagram of the wall-jet detector.

Ogan's work, the excitation wavelength was set at 305 nm and the emission wavelength at 430 nm.

HPLC system

A Perkin-Elmer Series 4 microprocessor-controlled quaternary solvent delivery system was used. This system has an in-built pulse damper and a solvent chamber for deaerating eluents under pressure (50–60 kPa). Helium gas was used for this purpose. The chromatographic column was a Perkin-Elmer HC/ODS SilX (particle size 5 μm), 0.26 × 25 cm reversed-phase column and the pressures applied were typically 4.2–6.9 MPa. The eluent flow-rate was set at 0.5 ml/min. Samples were injected through a Rheodyne 7125S 6-μl injector valve (Rheodyne, CA, U.S.A.) using a 10-μl syringe.

Chemicals

The eluents were AR grade acetonitrile (E. Merck, Darmstadt, F.R.G.), and triply distilled water with 0.1 *M* potassium nitrate. The PAHs used were fluoranthene, pyrene, dibenz[*a*,*h*]anthracene and benzo[*a*]pyrene (Tokyo Kasei Organic Chemicals, Japan), and 1,2-benzanthracene, benzo[*e*]pyrene and benzo[*g*,*h*,*i*]perylene (Aldrich, WI, U.S.A.). All PAHs were of AR grade and used without further purification. The mixtures of PAHs injected were prepared in acetonitrile. Concentrations varied from 0.06 to 12 m*M*, depending on the fluorescence and electrochemical activity of each compound.

RESULTS AND DISCUSSION

Selection of the gradient program

The analysis of multicomponent PAH mixtures requires separation by reversed-phase HPLC. Gradient techniques are usually employed[5,6]. The gradient program usually has four stages: isocratic elution with an aqueous acetonitrile (or methanol) eluent; gradient elution; isocratic elution with a totally non-aqueous solvent; and finally, a reverse in gradient until the initial starting eluent composition is obtained.

Gradient elution programs previously applied for the separation of PAHs with fluorescence detection (FL) (in particular, Ogan[5]) were found to be unsuitable. The problem arises from the fact that the time between the fluorescence peak and the corresponding electrochemical peak varied from compound to compound. Table I summarizes this variation for the seven PAHs. t(ED − FL) is probably related to the response of the electrochemical detector as well as to the time taken for the sample to travel between the fluorescence and electrochemical detectors. With Ogan's gradient program, fluoranthene and pyrene are found to overlap in the electrochemical trace. The slower response observed for fluoranthene is probably due to adsorption, which causes broadening of the electrochemical trace.

TABLE I

AVERAGED RETENTION TIMES (t_R) AND t (ED − FL) FOR THE SEVEN PAHs STUDIED

t (ED − FL) is the time between the fluorescence retention time and the electrochemical retention time.

Compound	*Electrochemical t_R*	*Fluorescence t_R*	*t (ED − FL)*
Fluoranthene	12 min 10 sec	11 min 23 sec	47
Pyrene	13 min 45 sec	13 min 9 sec	36
1,2-Benzanthracene	17 min 40 sec	17 min 8 sec	32
Benzo[*e*]pyrene	19 min 49 sec	19 min 15 sec	34
Benzo[*a*]pyrene	22 min 6 sec	21 min 31 sec	35
Dibenz[*a,h*]anthracene	24 min	23 min 24 sec	36
Benzo[*g,h,i*]perylene	24 min 23 sec	23 min 51 sec	32

The gradient program that was found to be successful is shown in Fig. 2a. Prior to injection of the sample, the eluent composition was held for 20 min at acetonitrile–water (60:40) followed by a further 5 min at the same composition after injection of the sample. This initial elution prior to the actual separation was to allow equilibration of the cell solution and settling of the WJ detector.

Selection of the working potential

The voltammetry of PAHs has been previously studied[7–9]. It is generally accepted that anodic oxidation of these compounds proceeds by way of an electrochemical mechanism leading to nucleophilic substitution at the reactive centre. There have been attempts to correlate fluorescence and electrochemical behaviour and to relate this to carcinogenic properties. Indeed, it has been found that PAHs with marked carcinogenic properties show higher electrochemical activity[8].

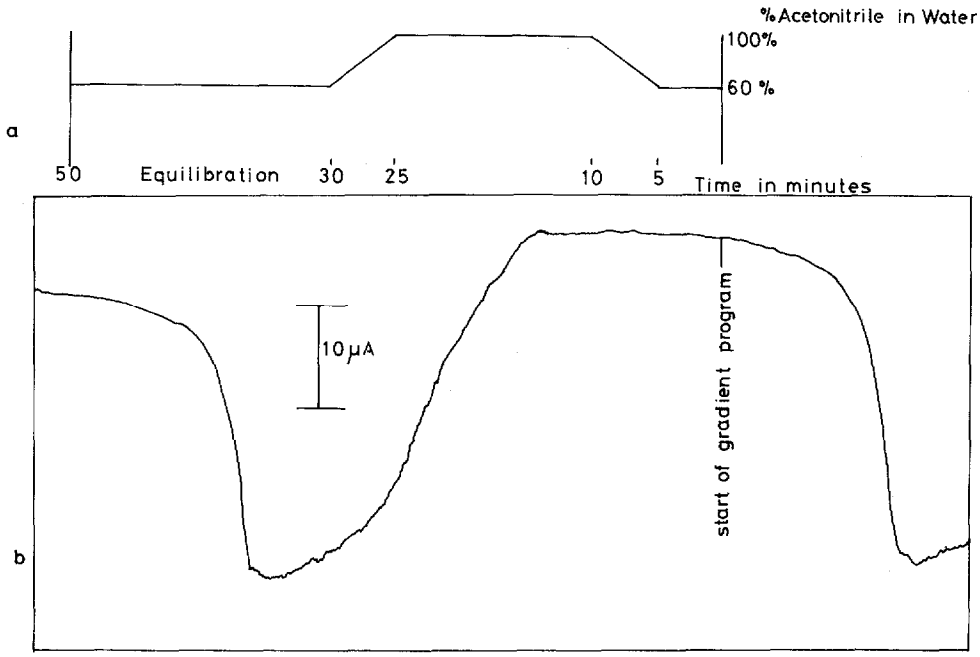


Fig. 2. Variation of the baseline with changing eluent composition during gradient program: a, gradient program; b, electrochemical trace. Wall-jet held at +1.3 V *vs.* Ag/AgCl.

In selecting the optimum potential for the working electrode, individual PAHs were determined by isocratic separation using a acetonitrile–water (80:20) as eluent. The working electrode potential was varied between +1.2 and +1.5 V.

It was found that at higher potentials the peak heights were greater, but upon subsequent injections they decreased dramatically, probably due to adsorption effects. It might be surmised that oxidation at the higher potential leads to a more drastic electrode process, possibly causing the formation of products which are more easily adsorbed on the glassy carbon surface. Whereas +1.4 V is the optimum potential for fluoranthene, benzanthracene and benzo[*a*]pyrene, sufficiently high peaks are obtained at +1.3 V. Therefore a working potential of +1.3 V was selected as a compromise.

As already mentioned, glassy carbon has a variable surface owing to the presence of carbon–oxygen functionalities and to adsorption effects and surface poisoning[10–12]. For a freshly polished electrode, when the potential is applied it takes 10–15 min before a steady state is obtained. This is due to the decay of the double layer charge as well as to the relaxation of surface species to their equilibrium states. The first chromatogram obtained for a freshly polished electrode is usually significantly different from subsequent chromatograms.

Baseline behaviour

The fluorescence detector is relatively insensitive to changes in eluent composition during the gradient elution. In the case of electrochemical detectors, a high sensitivity can be expected which is clearly dependent on the dielectric properties and

conductance of the eluent as the percentage of acetonitrile, water and electrolyte changes during elution.

In the case of the large-volume cell WJ detector, a stable and reproducible potential can be maintained between the reference and the working electrodes by virtue of the fact that the conductance will be high between these electrodes, even when the jet emerging from the nozzle is completely non-aqueous. This is because of the large volume of the cell which affords a high proportion of water and electrolyte throughout the gradient.

The change in baseline must, then, be related to the variation in potential drop across the thin film of eluent flowing radially over the surface of the electrode (the hydrodynamic boundary layer), rather than to changes in the bulk solution in the WJ cell. Because an aqueous media is always present in the cell, the reference electrode does not need to be modified. In this work a standard Ag/AgCl reference system was used.

Fig. 2b shows the change in the baseline of the WJ detector during two cycles of the gradient. During the initial part of the cycle the baseline is flat. Then, as the concentration of acetonitrile increases, a decrease in the baseline occurs; this becomes more dramatic when the eluent is 100% acetonitrile. A few minutes lag time is seen between the response of the electrochemical detector and the actual gradient output by the solvent delivery system. This arises because of the time taken for solvent to reach the WJ detector and the time lag due to mixing effects.

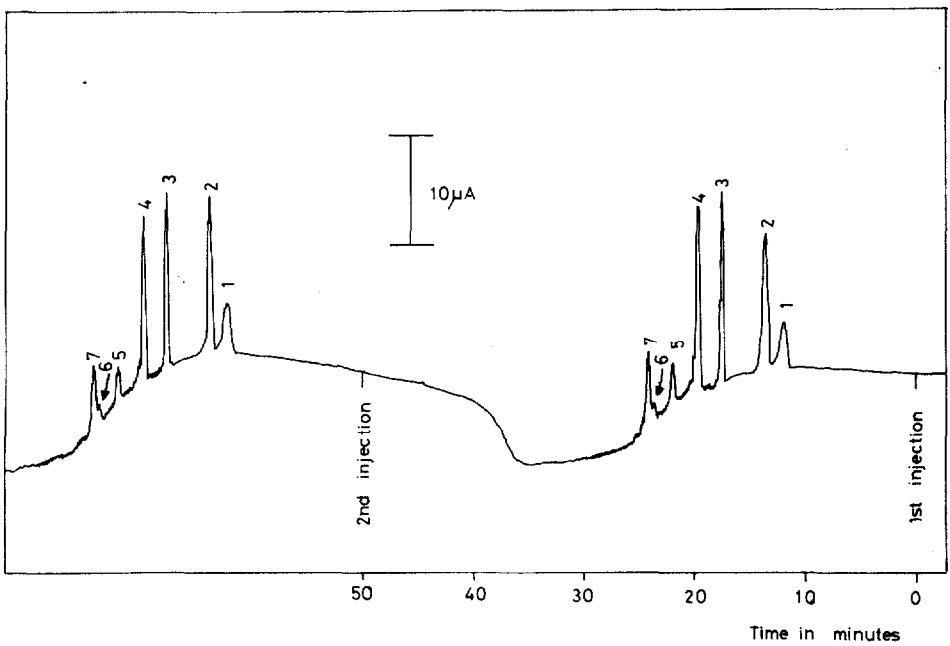


Fig. 3. Successive injections of PAH mixture: 1 = 6.0 m*M* fluoranthene; 2 = 0.9 m*M* pyrene; 3 = 0.97 m*M* 1,2-benzanthracene; 4 = 0.5 m*M* benzo[*e*]pyrene; 5 = 0.09 m*M* benzo[*a*]pyrene; 6 = 0.16 m*M* dibenz[*a,h*]anthracene and 7 = 0.24 m*M* benzo[*g,h,i*]perylene. The wall-jet was held at +1.3 V *vs.* Ag/AgCl.

Comparison of electrochemical and fluorescence detectors

Fig. 3 shows the traces obtained for the WJ detector for successive injections of the seven-component PAH mixture. Fig. 4 shows the corresponding fluorescence traces.

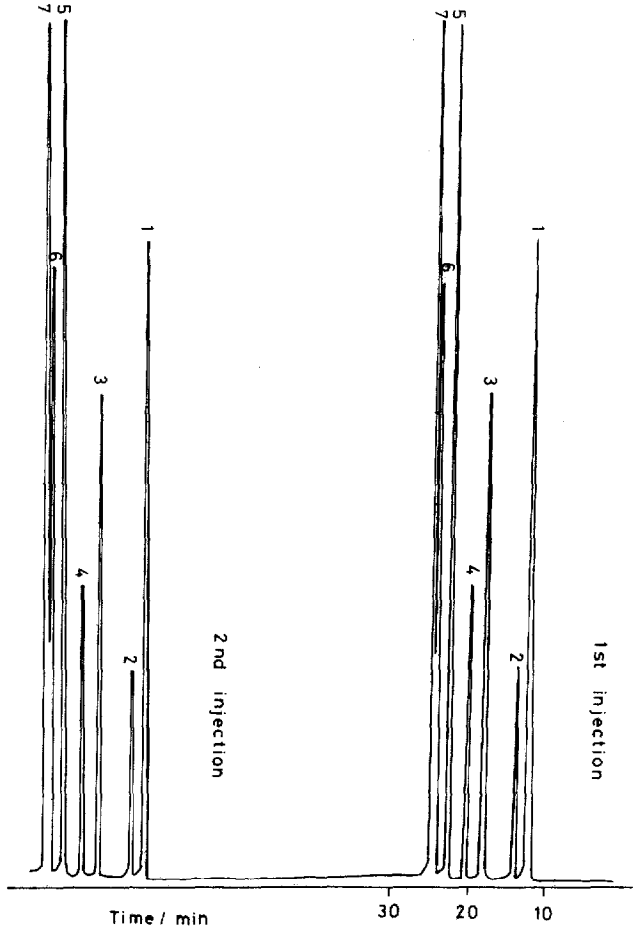
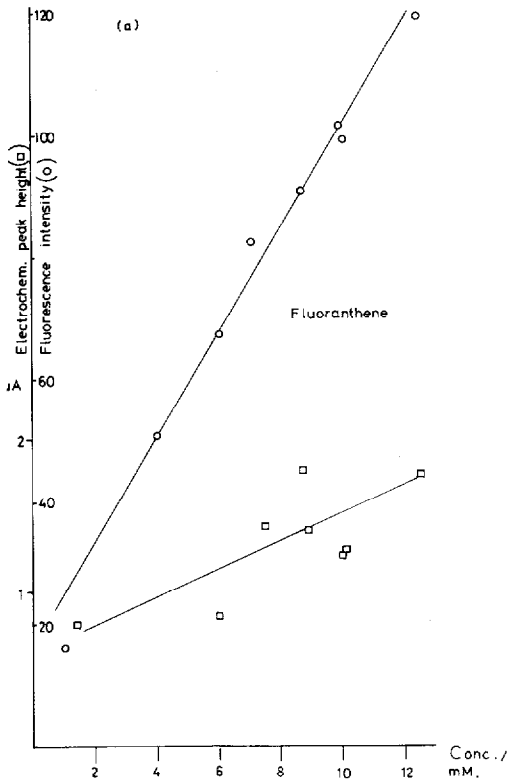
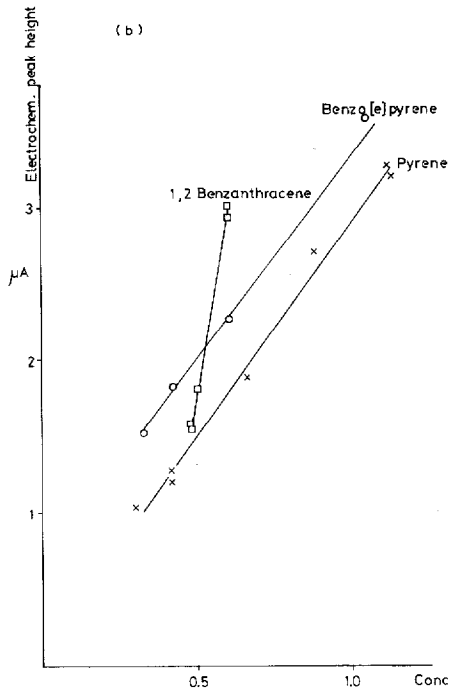


Fig. 4. Fluorescence trace corresponding to Fig. 3. Excitation wavelength = 305 nm and emission wavelength = 430 nm.

After a few injections, a general decrease in peak heights was observed, presumably due to the effect of adsorption. An increase in peak heights evident for fluoranthene and pyrene was only observed in the first three to four analyses. The resolution of the electrochemical trace is comparable to that of the fluorescence trace.

Fig. 5 shows calibration curves for the fluorescence and WJ detectors. The fluorescence and electrochemical activities of the PAHs appear to be correlated. It should be noted that the values were obtained over a period of a few days. This accounts for the larger scatter obtained in the case of the WJ detector, due to changing electrode characteristics.


(a)
Electrochem. peak height(□)
Fluorescence intensity(○)
μA
Fluoranthene
Conc./ mM.


(b)
Electrochem. peak height
μA
Benzo[e]pyrene
Pyrene
1,2 Benzanthracene
Conc

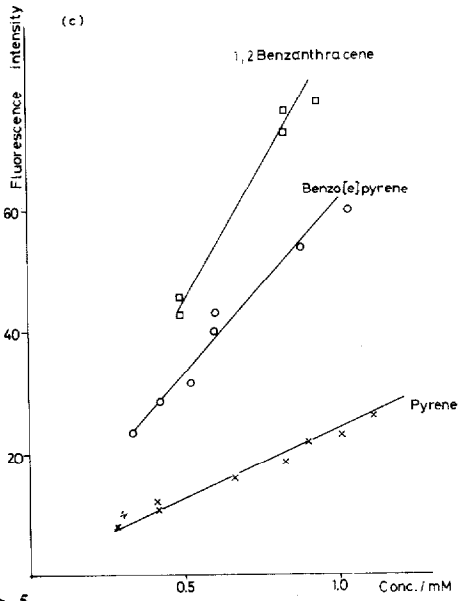

(c)
Fluorescence intensity
1,2 Benzanthracene
Benzo[e]pyrene
Pyrene
Conc./ mM

Fig. 5.

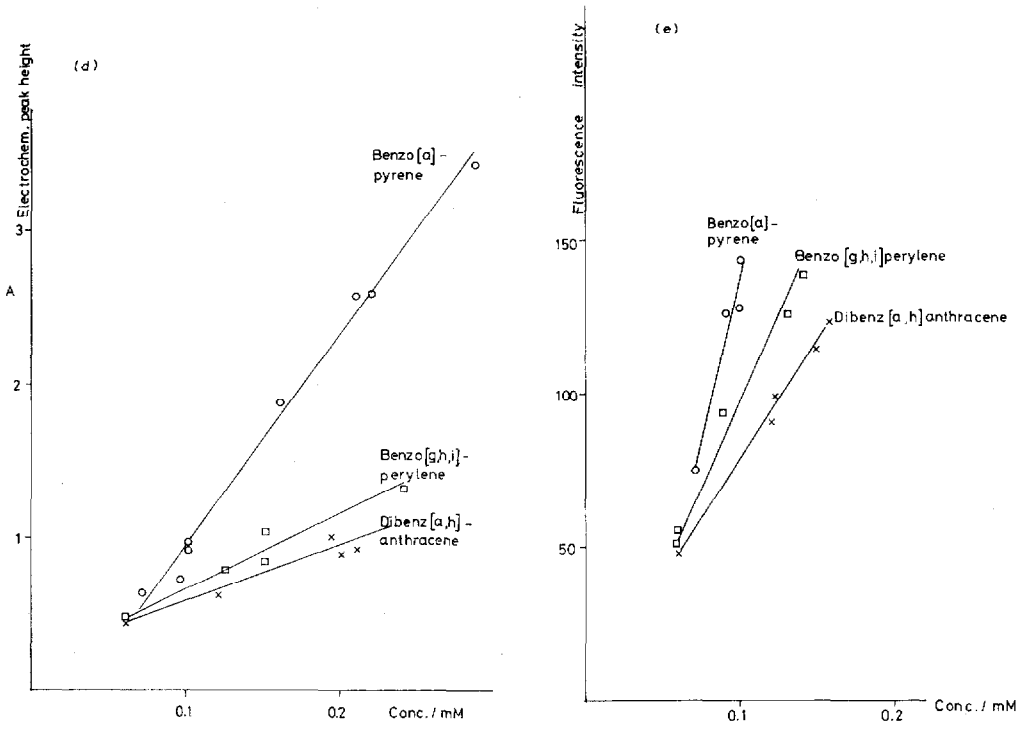


Fig. 5. Plots of peak heights against concentration for fluoranthene (a), pyrene, 1,2-benzanthracene and benzo[*e*]pyrene (b, c) and benzo[*a*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene (d, e). The wall-jet was held at +1.3 V *vs.* Ag/AgCl and the sensitivity at 10 μA. Fluorescence detection: excitation wavelength = 305 nm, emission wavelength = 430 nm; sensitivity, 0.1.

Hydrodynamic considerations

As already mentioned, hitherto, the popularly held belief was that the cell volume of an electrochemical detector should be minimized to obviate band spreading. This may be the case for thin-layer cells or tubular systems, where the flow of eluent —as it emerges from the inlet— is parallel to the surface of the electrode. For the wall-jet configuration, this is not a limitation.

The wall-jet defines the flow of a jet of fluid which impinges perpendicularly onto a wall and flows radially over its surface. It is hydrodynamically well defined and a complete solution relating the limiting current to the various hydrodynamic parameters has been derived by Yamada and Matsuda[13] and Gunasingham and Fleet[3]. The equation for the limiting current is

$$i_{lim} = 1.38nFCD^{2/3}\nu^{-5/12}V^{3/4}R^{3/4}a^{-1/2} \quad (1)$$

where n is the number of electrons, C is the concentration, D is the diffusion coefficient, ν is the kinematic viscosity, V is the volume flow-rate, R the electrode radius and a the inlet diameter.

Eqn. 1 shows that the limiting current of the wall-jet electrode is dependent on the flow-rate to the power 3/4. This contrasts with the situation for tubular and

parallel plate electrodes where the dependency is to the powers 1/3 and 1/2 respectively. Eqn. 1 does not show any dependency on inlet-electrode separation. It is assumed that the jet is laminar and remains intact as it emerges from the inlet. However, it has been found that eqn. 1 holds only when the inlet-electrode separation is greater than a certain empirically derived value. This arises because of the interference of the inlet body within the hydrodynamic boundary layer which results in a reduction of momentum transfer (and hence a reduction of the limiting current)[3].

The boundary-layer thickness, δ_{bl}, for the wall-jet has been derived by Gunasingham and Fleet[3]:

$$\delta_{bl} = 5.8\,\pi^{3/4}\nu^{3/4}V^{-3/4}R^{5/4}a^{1/2} \qquad (2)$$

For $\nu = 10^{-2}$ cm^2/sec, $V = 0.5$ ml/min, $a = 0.3$ mm and $R = 0.27$ cm, δ_{bl} is about 5.4 mm. Thus, for the best response, the inlet-electrode separation should be greater than the boundary-layer thickness.

Varying the inlet-electrode separation has little effect on band spreading, in agreement with the assumption that the effective cell volume will be of the order of the hydrodynamic boundary layer. The jet, therefore, remains intact regardless of the inlet-electrode separation. This has been found to be so for separations greater than 10 mm. Fig. 6 shows chromatograms obtained from the WJ detector at inlet-electrode separations of 1 and 4 mm; there is negligible band spreading.

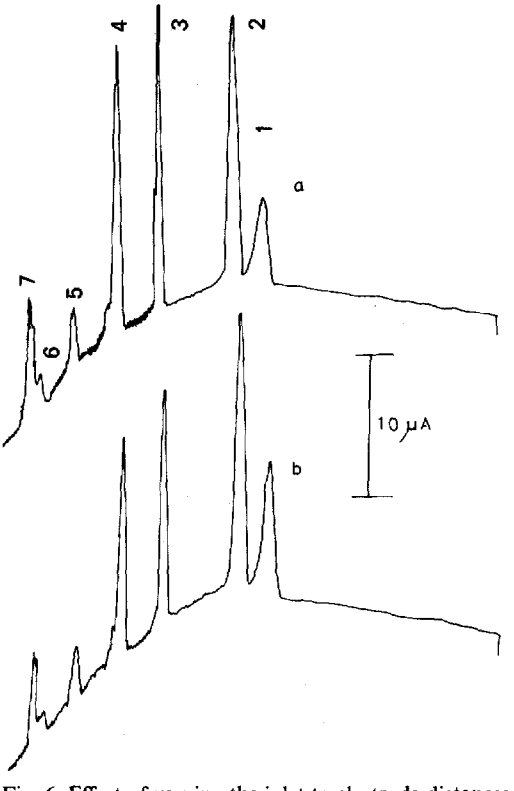


Fig. 6. Effect of varying the inlet to electrode distances on peak resolution: a, 4-mm separation; b, 1-mm separation. Peaks as for Fig. 4. Wall-jet held at +1.3 V *vs.* Ag/AgCl.

While band spreading is negligible in the laminar free jet itself, it occurs to a greater extent within the boundary layer. Eqn. 2 shows that the boundary-layer thickness increases rapidly from the centre of the working electrode. In order to minimize band spreading the electrode area should be small. There is, consequently, a trade-off with sensitivity. Eqn. 2 also shows that the boundary-layer thickness decreases with increasing volume flow-rate, and increases with viscosity and inlet diameter.

Effect of electrode polishing

When the glassy carbon electrode was polished between analyses it was found that the peaks for pyrene and fluoranthene become less intense. In the case of the other PAHs, electrode polishing either results in an increase in peak height (as for benzo[*e*]pyrene and dibenz[*a*,*h*]anthracene) or has no effect (benzo[*g*,*h*,*i*]perylene, benzo[*a*]pyrene and 1,2-dibenzanthracene). Fig. 7 shows the effect of polishing.

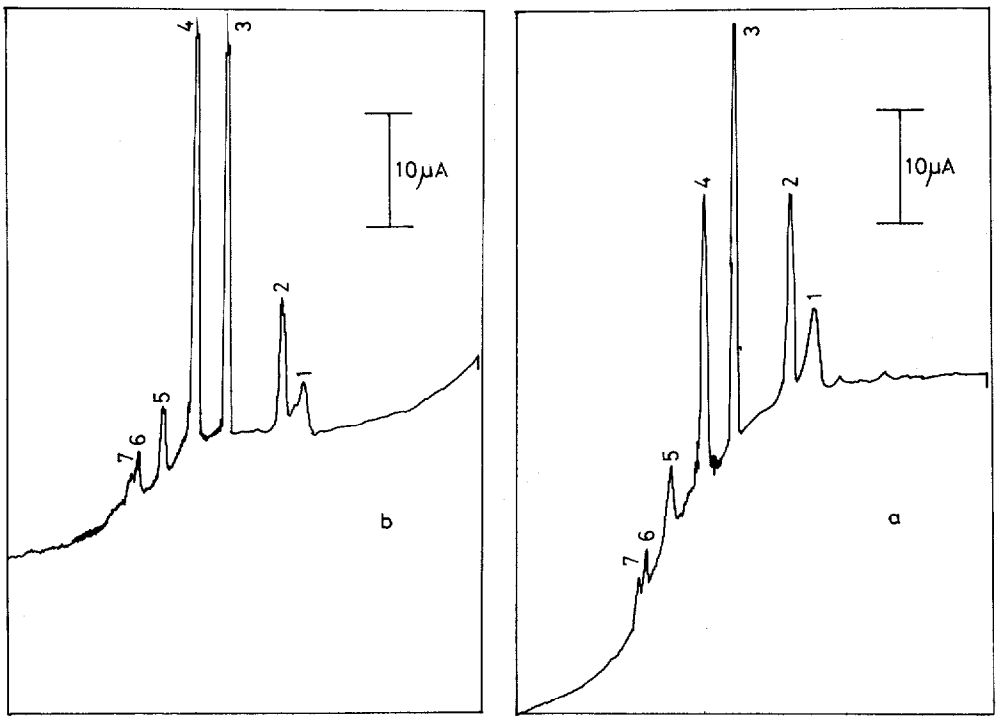


Fig. 7. Electrochemical trace for electrode used for several analyses (a) and freshly polished electrode (b). Peaks: 1 = 7.05 m*M* fluoranthene; 2 = 0.41 m*M* pyrene; 3 = 0.6 m*M* 1,2-benzanthracene; 4 = 0.6 m*M* benzo[*e*]pyrene; 5 = 0.1 m*M* benzo[*a*]pyrene; 6 = 0.12 m*M* dibenzanthracene and 7 = 0.06 m*M* benzo[*g*,*h*,*i*]perylene.

CONCLUSIONS

One of the advantages of the large-volume WJ cell is that it obviates the need to make the non-aqueous component of the eluent conducting; electrolyte need only be added to the aqueous fraction. Even when the eluent is completely non-aqueous

(as is the case for a major part of the gradient separation of the PAHs) there will be sufficient water and electrolyte present in the cell to maintain a conducting medium. Additional benefits arise because of the large volume of the cell: first, the flow of the jet is not disturbed as in the case of the Fleet–Little design[3]; secondly, the reference electrode does not have to be modified; thirdly, during the actual gradient elution, the effect of changing eluent composition is damped; and finally, the cell construction is greatly simplified. The use of a large-volume WJ cell does not lead to band spreading, as might be expected in the case of thin-layer cells. This is because the effective volume of the WJ detector is only of the order of the hydrodynamic boundary layer (adjacent to the electrode surface) and not the geometric cell volume.

By the use of a large-volume WJ cell, it has been shown that gradient elution techniques can be used in conjunction with electrochemical detection. This greatly extends the application of this mode of detection.

The sloping baseline behaviour could easily be compensated for by means of background subtraction. Work remains to be done in characterizing this baseline behaviour, and in the variation in peak response for the different PAHs.

Although this work shows that fluorescence detection is more sensitive than electrochemical detection, the latter does offer the possibility of selective detection of the seven carcinogenic PAHs (investigated in this work) in the presence of the other PAHs which show significantly less electrochemical activity.

ACKNOWLEDGEMENT

The award of a Research Grant and a Research Scholarship to B.T.T. is gratefully acknowledged.

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