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USE OF ELECTROCHEMICAL DETECTION IN NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The application of electrochemical detection to normal-phase liquid chromatography (using non-aqueous eluents of low dielectric constant) holds considerable potential for the determination of a number of important organic compounds. By choosing an appropriate supporting electrolyte and a modified reference electrode, high sensitivity and a large potential working range is attainable. The efficacy of electrochemical detection in normal-phase liquid chromatography is demonstrated in its application to the determination of phenols.

INTRODUCTION

Electrochemical detectors for high-performance liquid chromatography (HPLC) have found important application for compounds which are not easily determined by UV or other detection methods. Most reports on the use of electrochemical detectors have been concerned with aqueous eluents. These eluents consisted either of water having an electrolyte added or acrylonitrile plus the electrolyte¹⁻³.

Such aqueous solvent systems are, however, mainly applicable only for ion-exchange or reversed-phase liquid chromatography; in the case of normal-phase separations where use is made of non-aqueous solvents of relatively low dielectric constant, electrochemical detection has not gained much usage. Presumably, the problem (which may have deterred workers) is that of making the eluent conducting and, attendant with this, obtaining a stable reference potential. However, the application of electrochemical detection to the area of normal-phase chromatography engenders a number of important applications for the determination of compounds that are electroactive but more amenable to this type of separation. Moreover, the voltammetry of organic species in non-aqueous solvent systems leads to tangible benefits in performance: significantly, there could be fewer adsorption problems and increased

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sensitivity and, also, the working potential range (defined by the solvent-electrolyte breakdown potential) can be extended⁴⁻⁷. In this paper we report the use of electrochemical detection in conjunction with normal-phase HPLC for the determination of phenols.

EXPERIMENTAL

Wall jet cell

The wall jet cell was used in this work as the electrochemical detector. The application of the wall jet cell to HPLC detection was first reported by Fleet and Little⁸. This type of detector is easily constructed and, moreover, has a high current sensitivity for a given flow-rate compared to other electrode configurations^{9,10}. For these reasons, the detector has been gaining widespread usage in HPLC. The wall jet cell has also been used for other hydrodynamic voltammetry applications, for example, anodic stripping voltammetry⁹ and kinetic studies. With regard to the latter, the limiting-current equation for the wall jet geometry has been fully defined in terms of various hydrodynamic parameters¹⁰. The wall jet cell can be used for on-line work for which the rotating disc electrode is not amenable.

In this work the body of the wall jet cell as the electrode encasings were fabricated in Kel-F (3M, U.S.A.); thus the cell is resistant to most organic solvents. Otherwise the detector resembled the system described by Fleet and Little⁸. The wall jet cell was controlled by a three-electrode potentiostat controller based on a conventional design¹¹. The current-to-voltage converter of the controller was capable of measuring current in the nano-ampere range.

Reference electrode

The role of the reference electrode is to provide a stable, reproducible potential which would serve as a reference against which the working electrode potential can be measured. To satisfy these requirements ideally, a reversible redox electrode under equilibrium conditions (such as Ag/AgCl in equilibrium with saturated potassium chloride solution) should be used. For HPLC applications the stringent requirements of precise potential control necessary in, for example, cyclic voltammetry studies, can be relaxed and the accurate measurement of current becomes more important. For this reason, it is acceptable to have a quasi-reference electrode which will adopt a steady potential within ± 20 mV¹². For the purpose of HPLC the need is for a simply constructed system which would complement the design of the wall jet cell and, also, be amenable for routine use. It was thus decided to use a silver/silver chloride electrode in conjunction with a salt bridge; a porous ceramic frit afforded contact to the eluent. The role of the salt bridge is to stabilize the junction potential between the saturated potassium chloride solution and the non-aqueous solvent, as well as to minimize cross contamination between solutions. Agar impregnated with saturated potassium chloride solution provided unsatisfactory for this purpose because of crystallisation of potassium chloride in the micro-pores and surface of the ceramic frit resulting in significant potential fluctuations. However, use of a gel of hydroxyethyl cellulose impregnated with saturated potassium chloride solution appeared to work satisfactorily. Fig. 1 shows a schematic diagram of the silver/silver chloride reference electrode. This electrode could be used for several hours without

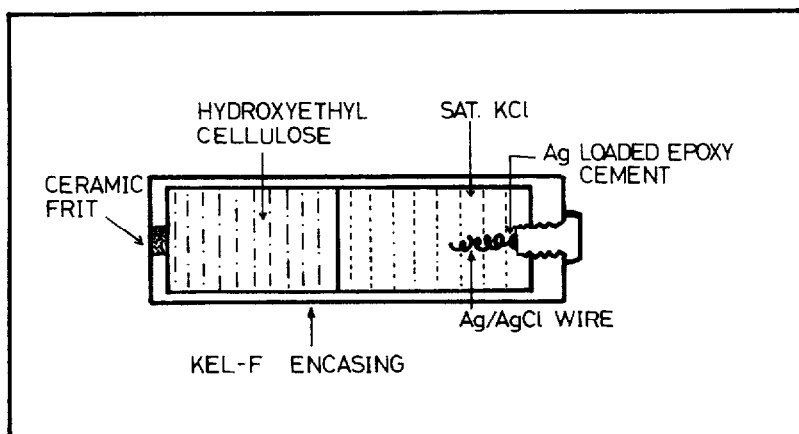


Fig. 1. Schematic diagram of Ag/AgCl reference electrode.

maintenance, apart from periodically refilling the saturated potassium chloride solution to replenish solution lost from the reference compartment into the eluent stream. The resultant contamination of the eluent does not pose a problem as, after striking the wall, the radial flow of the jet, should, in theory, flush such contaminants away from the working electrode.

Working electrode

The choice of working electrode for any continuous monitoring application requires consideration of electrocatalytic activity and surface poisoning. The former has a significant bearing on the sensitivity of the electrochemical detector; the latter most often dictates the reproducibility. The goals of high electrocatalytic activity and low surface poisoning are seemingly contradictory. The most commonly used electrode materials for HPLC detectors are carbon paste and glassy carbon. The former, made popular by Kissinger and co-workers^{13,14}, have been reported to have lower residual currents compared to glassy carbon^{15,16}; they are, however, more difficult to handle and unsuitable for use with non-aqueous solvents and at high flow-rates. Instability at high flow-rates is particularly obvious with the wall jet configuration¹⁷. Noble metal electrodes, such as gold¹⁸, have also been employed. The use of these metals is limited by their high susceptibility to poisoning by trace contaminants, to which glassy carbon appears to have a greater tolerance. However glassy carbon is notorious for its variability in performance, a consequence of the occurrence of carbon-oxygen functionalities on the surface free-valence carbon sites and variations in the surface area¹⁹⁻²². The extent to which functionalities occur depends on the bulk structure: carbons which are more compact have less free-valence carbon atoms at the surface and, of course, the surface area is also less¹⁹. The compactness of glassy carbon depends to a large extent on the manufacturing process, which can vary from one manufacturer to another. Surface groups have been described to serve as mediators in electrode reactions. This mediation can lead to enhancement of electrode reactions; however, it could also lead to increased adsorption problems, as is shown later. Extreme care has to be taken therefore, in the cleaning and potential pretreat-

ment of glassy carbon electrodes to ensure a reproducible surface state. Despite these limitations, glassy carbon appears to be the best choice of working electrode material for use with non-aqueous as well as aqueous solvents. With care in pretreatment and, also, choosing a more compact carbon, the residual current can be kept to acceptable levels even for high-sensitivity current measurements¹⁹.

Eluent

Non-aqueous solvents typically used in HPLC cover a broad range of dielectric constants, from a value of *ca.* 2 for non-polar solvents (benzene, dioxane) to as high as 182 (N-methylformamide). The requirement of solution conductivity precludes the use of solvents having very low dielectric constants such as pure hexane; however solvents of low dielectric constant could be used in mixtures having a small proportion of a solvent of higher dielectric constant: for example, a hexane-ethanol (85:15) mixture in conjunction with 0.01 *M* tetrabutylammonium fluoroborate affords a sufficiently conducting eluent for the purposes of HPLC detection. The hexane-ethanol solvent mixture was used because it is a common eluent for normal-phase separation of polar organic species such as phenols and oestrogen steroids²³, the latter being of particular interest to the authors²⁴. Oestrogen steroids have been successfully determined by electrochemical detection in conjunction with reversed-phase chromatography²⁵.

Supporting electrolyte

In order to make non-aqueous eluents conducting, a range of tetraalkylammonium salts are available. These salts have gained usage as supporting electrolytes in non-aqueous electrochemical work. In this work use was made of tetrabutylammonium fluoroborate [$(C_4H_9)_4NBF_4$] in the range 0.01–0.05 *M* together with 0.5% of 35% ammonia solution. The fluoroborate can be easily prepared in large quantities by mixing tetrabutylammonium bromide and hydrofluoroboric acid²⁶. For the hexane-ethanol eluent used, 0.01 *M* $(C_4H_9)_4NBF_4$ proved to be the lower concentration limit; below this poor conductivity was observed, manifesting itself in increased noise problems and potential offset. The ammonia solution was added to improve the chromatography; however, this also seemed to enhance the oxidation of the phenols.

HPLC system

The high-pressure pump used for HPLC was a purpose-built system using nitrogen gas pressure applied to a solvent reservoir which consisted of a 2-m coil of 1/4-in. stainless-steel tube. This design ensured a pulse-free delivery of solvent and, also, allowed the solvent to be vacuum-degassed prior to operation.

The chromatographic column used in the present work was a Zorbax 6 × 1/4 in. silica column (particle size, 8 μ m) (DuPont) for measurements using normal-phase separation. Typically, the pressures applied were in the range 300–500 p.s.i. resulting in solvent flow-rates of 0.2–1.0 ml/min. Samples were injected through a septum with a 10- μ l syringe.

RESULTS AND DISCUSSION

Potential range

The potential range of solid electrodes is governed by the solvent-electrolyte breakdown potential. In aqueous solutions and with an 'inert' supporting electrolyte, the limits are set by hydrogen evolution on the cathodic side and oxygen evolution on the anodic side. In practice, however, the anodic and cathodic limits depend on an acceptable background current and this, in turn, depends on the sensitivity required. For example, the potential range of glassy carbon is between -0.8 to $+1.2$ V vs. Ag/AgCl for acidic solutions in the case of nanoampere current measurements. This limitation of potential working range precludes the detection of a large number of compounds. The use of non-aqueous solvents can serve to increase greatly the potential range of glassy carbon. For the hexane-ethanol solvent used in this work, for example, a potential working range of at least $+2$ to -2 V vs. Ag/AgCl can be achieved.

Electrochemistry of phenols

The following studies were carried out with a range of phenols. These compounds are easily oxidized at carbon electrodes, the mechanism depending on the particular solvent system being used and the pH. Two different mechanisms are possible in aqueous solutions: one at low and the other at high pH²⁷. In non-aqueous solvents, phenols are practically unionised and the electrode reaction involves a two-electron transfer. When a base is added the half-wave potential is shifted to a lower value²⁸.

Adsorption of phenols

As the mercury electrode lacks a renewable surface, the adsorption of impurities and products of electrolysis is one of the biggest drawbacks to continuous-monitoring voltammetry. The adsorption of electrolysis products and the formation of polymer films can cause the electrode surface to become passive. The use of non-aqueous solvents affords a means of diminishing (though not obviating) the adsorption problem. One reason for this is the greater solubility of certain organic materials in such solvents, thus increasing the likelihood of the (reverse) desorption process. In other situations, non-aqueous solvents may actually serve to retard polymer film formation²⁹.

Fig. 2 shows the effect of adsorption on glassy carbon working electrodes for successive injections of 3-isopropylphenol. Plots a and b show the change in peak current for glassy carbon with a methanol-water eluent. Plot a is for glassy carbon made by Plessy (Great Britain) while plot b is for glassy carbon made by Tokai Carbon Co. (Japan). In an earlier paper¹⁹ it was shown that the Plessy carbon showed greater surface activity than the Tokai carbon and, as discussed above, this was partially attributed to the greater compactness of the latter. As can be seen in Fig. 2, Plessy glassy carbon shows higher peak currents and, also, increased adsorption of the phenol. Plot c shows the change in peak current for the Plessy glassy carbon using a hexane-ethanol mixture as the eluent. As can be seen, adsorption of the phenols is significantly diminished. This effect could be attributed both to an increased solubility in the organic eluent and to reduction of polymer film formation caused by the interaction of the solvent.

Log Peak Height/nA

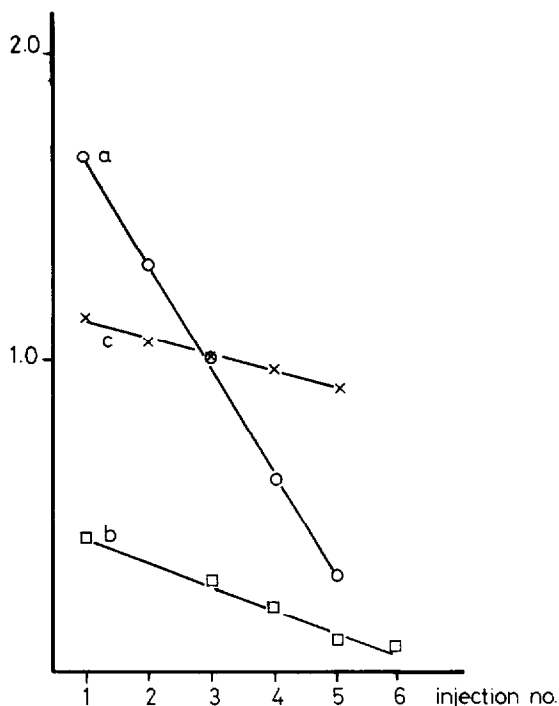


Fig. 2. Adsorption of 3-isopropylphenol on glassy carbon electrodes, $1 \mu\text{g}$ per $5\text{-}\mu\text{l}$ injections: (a) Plessy glassy carbon; eluent, methanol-water (10:90) in $0.1 M$ potassium nitrate; working potential, $+1.0 V$ vs. Ag/AgCl ; (b) Tokai glassy carbon; eluent, methanol-water (10:90) in $0.1 M$ potassium nitrate; working potential, $+1.0 V$ vs. Ag/AgCl ; (c) Plessy glassy carbon; eluent, hexane-ethanol (70:30) $0.05 M$ $(\text{C}_4\text{H}_9)_4\text{NBF}_4$; working potential, $+0.8 V$ vs. Ag/AgCl .

Potential control

The usefulness of detector specificity can be twofold: for identification of species and for the selective determination of species in the presence of others. DiCesare and Ettre³⁰ recently discussed the efficacy of detector specificity but surprisingly omitted mention of the use of electrochemical detectors for this purpose. In fact, electrochemical detection can be made specific by simply altering the working electrode potential. However, there are a few practical problems which require consideration. Electrochemical reactions on solid electrodes are most often irreversible. For example, the oxidation of ferrocyanide is found to be irreversible on glassy carbon electrodes whereas it is reversible on mercury electrodes. Because of their highly irreversible nature, the current-voltage characteristics of solid electrode processes show a gradual rise to the limiting current value. This is seen for the current-voltage curve for Fe^{2+} obtained for the Tokai glassy carbon electrode shown in Fig. 3. In addition, potential offsets could result for solid electrodes as a consequence of surface reactions: for example, oxide formation in the anodic range and hydrogen adsorption in the cathodic side. The adsorption of impurities and products of electrolysis could

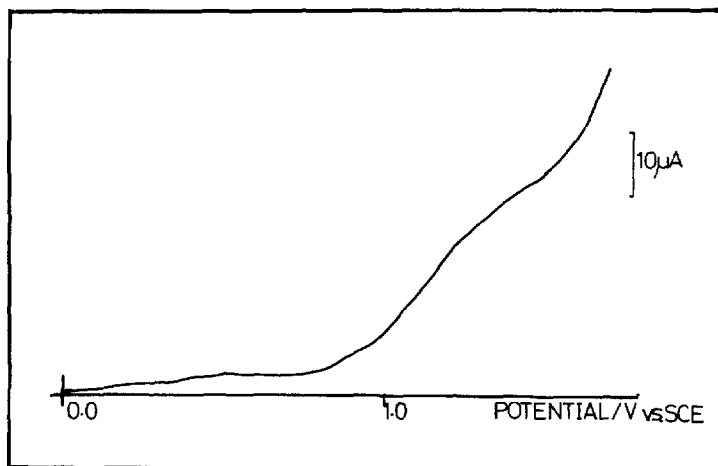


Fig. 3. Current-potential curve for 5 mM Fe^{2+} in sulphuric acid (pH 0.3) for Tokai glassy carbon. Scan rate, 100 mV/sec.

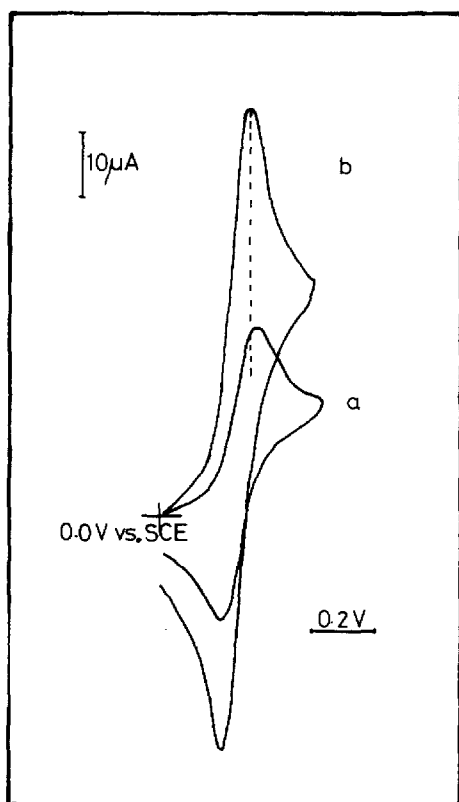


Fig. 4. Effect of potential pretreatment of platinum electrode on cyclic voltammogram of ferrocyanide: (a) Pt cleaned with concentrated nitric acid; (b) after electrode pretreatment (electrode held at +1.5 V vs standard calomel electrode for 30 sec followed by 1 min at +0.4 V. Scan-rate, 20 mV/sec.

also contribute potential offsets. The effect of changing the surface characteristics of solid electrodes on the current-voltage profiles of solid electrodes is illustrated in Fig. 4 which shows the effect of potential pretreatment of a platinum electrode on the cyclic voltammogram of ferrocyanide. The enhanced reversibility is presumably due to formation of surface oxides which serve as bridging bonds for electron transfer. The effect of surface oxidation and reduction on the cyclic voltammogram of *p*-phenylenediamine and nitrobenzene on glassy carbon are similarly illustrative of the effect of changing surface characteristics³¹. In HPLC detection it would be desirable to set the working potential at the limiting-current part of the current-potential waveform. However, as shown by the above examples, the susceptibility to potential offsets of several millivolts due to changing surface characteristics obviates efforts at precise potential control. In the case of non-aqueous solvents, at least trace quantities of water would be present even in high-grade eluents; consequently similar effects could result, although to a considerably lesser extent. Thus, successful application of potential control for the purpose of selectivity requires an empirical approach to determining the appropriate potential setting that would afford a reproducible and sensitive current response.

Fig. 5 shows the efficacy of potential selection for the determination of a mixture of phenols. By varying the potential controls by 100–200 mV, a number of the phenols can be selectively determined. The specificity for the phenols for a given potential setting was found to be reproducible even though peak heights diminished for successive injections, presumably because of adsorption effects.

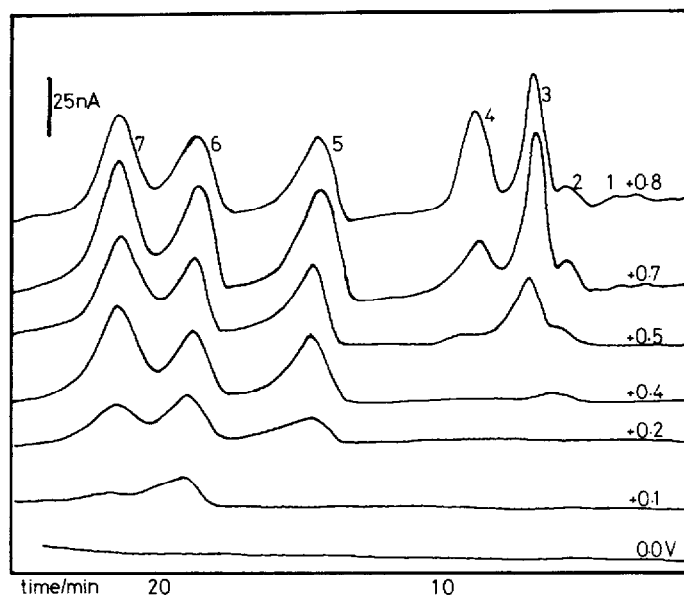


Fig. 5. Application of potential selection for the determination of phenols. Eluent, hexane-ethanol (70:30) in 0.05 M $(C_4H_9)_4NBF_4$, 0.5% ammonia added; Plessy glassy carbon. Peaks: 1 = impurity; 2 = 2,6 diisopropylphenol; 3 = 3-isopropylphenol; 4 = phenol; 5 = *o*-aminophenol; 6 = *p*-aminophenol; 7 = *p*-phenylenediamine. Potentials vs. Ag/AgCl reference.

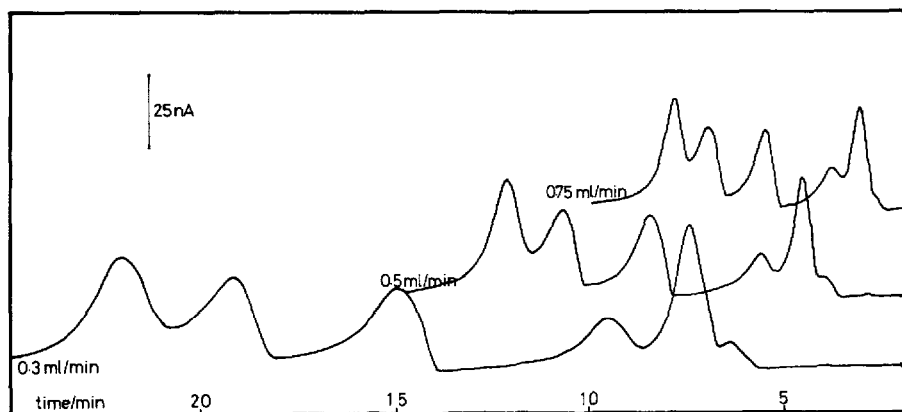


Fig. 6. Effect of flow-rate on chromatogram of phenol mixture. Eluent, hexane-ethanol (70:30) in 0.05 M $(B_4H_9)_4NBF_4$, 0.5% ammonia added. Plessy glassy carbon; working potential, +0.7 V vs. Ag/AgCl.

Flow-rate dependency

The dependence of the current on the flow-rate is described by the Yamada-Matsuda equation¹⁰. This equation shows that the limiting current is dependent on the volume flow-rate to the power 3/4 when the jet is in the laminar flow regime. Fig. 6 shows the effect of flow-rate on the peak profiles of the phenol mixture. As would be expected, retention times are significantly decreased and the peak profiles nar-

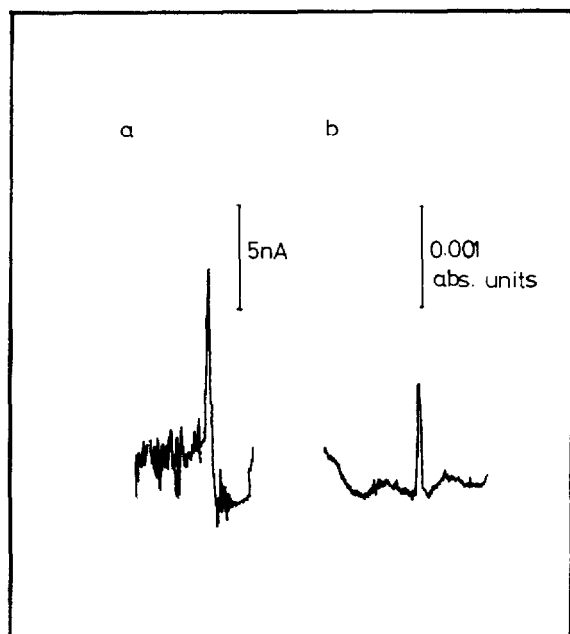


Fig. 7. Comparative response of wall jet cell (a) and UV (b) detector for the determination of 10 ng per 5 μ l injection of 3-isopropylphenol. Eluent, hexane-ethanol-ammonia (73:27:0.5) in 0.05 M $(C_4H_9)_4NBF_4$. Wall jet cell; Plessy glassy carbon held at +1.2 V vs. Ag/AgCl. UV detector set at 280 nm.

rowed. However, the expected increase in peak height with increase in flow-rate was not apparent. This can be attributed to the effect of adsorption of the phenols.

Sensitivity

Fig. 7 shows the comparative response for isopropylphenol for the wall jet cell and UV detector placed in series. The UV detector was set at its optimum wavelength for this compound. The trace for the wall jet cell was obtained without filtering of the potentiostat output; use of a 500-Hz low-pass filter serves to enhance the signal-to-noise characteristics of this trace considerably, such that a higher sensitivity than the UV detector is feasible. The high signal-to-noise ratio attainable with non-aqueous solvents is indicative of the stability of the reference electrode and the conductivity of the eluent.

CONCLUSION

This paper shows that electrochemical detection can be applied to the determination of compounds which are electroactive but which are more suitable for separation by normal-phase HPLC. The main requirement for this type of work is the use of an appropriate supporting electrolyte and a suitable reference electrode: tetrabutylammonium fluoroborate served the purposes of the former and an Ag/AgCl system in conjunction with a salt bridge was found to be satisfactory for the latter. Performance benefits were also found in the use of non-aqueous solvents: in particular, diminishing of adsorption problems and enhanced working potential ranges. Comparison with UV detection shows higher sensitivity for the wall jet cell for the determination of phenols. Based on these results we have to disagree with the view of Kissinger¹³ that electrochemical detection has little application to normal-phase HPLC.

REFERENCES

- 1 C. B. Ranger, *Anal. Chem.*, 53 (1981) 20A.
- 2 A. Pryde and M. T. Gilbert, *Applications of High Pressure Liquid Chromatography*, Chapman and Hall, London, 1979, p. 43.
- 3 R. E. Shoup and G. S. Mayer, *Anal. Chem.*, 54 (1982) 1164.
- 4 J. C. Schaur and D. E. Smith, *Anal. Chem.*, 54 (1982) 1589.
- 5 J. C. Schaur and D. E. Smith, *J. Electroanal. Chem.*, 100 (1979) 145.
- 6 A. L. Woodson and D. E. Smith, *Anal. Chem.*, 42 (1970) 242.
- 7 J. Boadoz-Lambling and C. Cauquis, in H. W. Nurnberg (Editor), *Electroanalytical Chemistry*, Wiley, Chichester, 1974, p. 335.
- 8 B. Fleet and C. J. Little, *J. Chromatogr. Sci.*, 12 (1974) 747.
- 9 B. Fleet, H. Gunasingham, T. A. Berger, G. de Damia and C. J. Little, *Physicochemical Hydrodynamics*, Advance Publications, London, 1977, vol. 1, p. 273.
- 10 H. Yamada and J. Matsuda, *J. Electroanal. Chem.*, 44 (1973) 189.
- 11 T. A. Berger, *Ph.D. Thesis*, London University, London, 1975.
- 12 D. T. Sawyer and J. L. Roberts, *Experimental Electrochemistry for Chemists*, Wiley, Chichester, 1974, p. 50.
- 13 P. T. Kissinger, *Anal. Chem.*, 49 (1977) 447A.
- 14 P. T. Kissinger, C. Refshauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
- 15 K. Štulík and V. Pacáková, *J. Electroanal. Chem.*, 129 (1981) 1.
- 16 K. Štulík, V. Pacáková and B. Stárková, *J. Chromatogr.*, 213 (1981) 41.
- 17 K. Štulík and V. Pacáková, *J. Chromatogr.*, 208 (1981) 269.

- 18 F. Kreuzig and J. Frank, *J. Chromatogr.*, 218 (1981) 615.
- 19 H. Gunasingham and B. Fleet, *Analyst (London)*, 10 (1982) 896.
- 20 R. E. Panzer and P. J. Elving, *Electrochim. Acta.*, 20 (1975) 635.
- 21 W. E. van der Linden and J. W. Dieker, *Anal. Chim. Acta.*, 119 (1980) 1.
- 22 D. Laser and M. Ariel, *J. Electroanal. Chem.*, 52 (1974) 291.
- 23 Jian-Tsyh Lin and E. Heftmann, *J. Chromatogr.*, 212 (1981) 239.
- 24 H. Gunasingham and B. Fleet, submitted for publication.
- 25 W. R. Prescott, B. K. Boyd and J. F. Seaton, *J. Chromatogr.*, 234 (1982) 513.
- 26 H. O. House, E. Feng and N. P. Peet, *J. Org. Chem.*, 36 (1973) 2371.
- 27 F. J. Vermillion and I. A. Pearl, *J. Electrochem. Soc.*, 111 (1964) 1392.
- 28 S. D. Ross, M. Finklestein and E. J. Rudd, *Anodic Oxidation*, Academic Press, New York, 1975, p. 271.
- 29 P. T. Kissinger, *Interface*, 20 (1977) 2.
- 30 J. L. DiCesare and L. S. Ettre, *J. Chromatogr.*, 251 (1982) 1.
- 31 B. Fleet and H. Gunasingham, in preparation.