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PARTIAL ELECTROLYSIS ELECTROCHEMICAL DETECTOR IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

C. BOLLET, P. OLIVA and M. CAUDE

Laboratoire de Chimie Analytique de l'École Supérieure de Physique et de Chimie de Paris, 10 rue Vauquelin, 75231 Paris Cedex 05 (France)

SUMMARY

A partial electrolysis electrochemical detector for high-performance liquid chromatography, using a glassy carbon working electrode, is described and the dead volume, specificity, influence of flow-rate, linearity, reproducibility and sensitivity with aqueous and organic mobile phases are discussed for inorganic and organic solutes.

Applications of the detector in adsorption and partition chromatography are described, as follows: detection of amines and amphetamines (ephedrine, benzphetamine and phenylephrine) and the specific detection of indole in benzene; detection in normal-phase partition chromatography of phenol derivatives (2,4- and 2,3-dimethylphenols) and of active principles commonly measured in pharmaceutical products (propyl *p*-hydroxybenzoate, paracetamol and paroxypropione); adsorption chromatographic separations of a mixture of phenothiazines {(methylamino-3-propyl)-10-chloro-3-phenothiazine, [N-methyl-N-(dimethylamino-3-propyl)amino-3-propyl]-10-chloro-3-phenothiazine and (dimethylamino-3-propyl)-10-chloro-3 phenothiazine N-oxide} and of active principles of pharmaceutical products (butazolidine, amidopyrine and noscapine and its impurities); and reversed-phase chromatographic separations of resorcinol derivatives (methyl-, hexyl-, octyl- and dodecylresorcinol) and of pyrogallol and catechol derivatives (4-*tert.*-butylcatechol and di-*tert.*-butylcatechol).

Finally, gradient elution and reversed-phase partition chromatographic analysis of a new antibiotic, penimocycline, is described.

INTRODUCTION

The lack of a simple, universal detector in high-performance liquid chromatography[1] and the difficulties encountered in coupling a liquid chromatograph with a mass spectrometer have encouraged the development of specific detectors that make use of the physico-chemical properties of the solute. The great sensitivity of electrochemical techniques and their wide range of applications with both inorganic and organic solutes are among the factors that make electrochemical detection a very at-

tractive prospect in liquid chromatography. Several papers on this subject have appeared[2-10] and two detectors are commercially available[11,12].

Certain aspects of continuous-flow analysis and certain requirements peculiar to modern liquid chromatography continue to cause difficulties. Moreover, the applications of electrochemical detectors are at present limited to mobile phases that are solutions of electrolytes, as in ion-exchange chromatography[3-10,13-16], or polar solvents, as in reversed-phase partition chromatography[10,11].

In this paper, we propose a highly sensitive detector based on a solid electrode, which promises to work well in both aqueous and non-aqueous solutions. This detector could, in principle, be used in all types of chromatography, including ion-exchange, adsorption, exclusion, normal-phase and reversed-phase partition chromatography.

Choice of an electrochemical method

There are two general approaches to electrochemical detection in flowing chromatographic effluents, as follows:

(a) to achieve rapid and complete electrolysis of the solute during its passage through the detector; the coulometric response is then directly proportional to the number of moles injected into the chromatograph;

(b) to achieve a micro-electrolysis of a small part of the solute, the current flowing in a micro-electrode is then proportional to the concentration of the solution in the cell at any given time. In this instance, a standardization will be necessary, and this type of detection will be called amperometric (if the proportion of the electrolysed part of the solute is negligible) or semi-amperometric (if the proportion of the electrolysed part is more than 1 %).

The coulometric method has been applied primarily to the detection of inorganic solutes in ion-exchange chromatography[3-6]. It requires excellent contact between the electrode and the solution, which implies the use of porous electrodes, usually made of platinum, that have a high ratio of surface to volume. Unfortunately, the dead volume of such cells is generally so high (often greater than 50 μl) that they cannot be used in rapid liquid chromatography. Moreover, it is virtually impossible to achieve complete electrolysis in a short time, no matter what the nature of the solute may be; this is especially so with organic solutes. Contamination of the porous electrode surface during the long electrolysis time of organic solutes is another problem that can be solved only by frequent cleaning and changes of the working electrode. For these reasons, the coulometric method, which *a priori* is very attractive, cannot be adapted to the needs of modern high-performance liquid chromatography.

However, a coulometric detector with a low dead volume, using glassy carbon electrodes, was recently proposed[17], but its range of coulometric working is limited to very slow flow-rates (less than 30 ml·h^{-1}), which reduces its capabilities in high-performance liquid chromatographic applications.

Development of the detector

We chose to use the classical three-electrode system, which, if used correctly, allows one to control precisely the potential of the working electrode.

Cell geometry. The cell geometry must be chosen very carefully if the cell is to function properly with the wide variety of solvents used in liquid chromatography.

The mobile phases used in ion-exchange chromatography are naturally conductors of electricity, but this is not so for the solvents used in adsorption and partition chromatography. An indifferent electrolyte must be added to non-conducting mobile phases, either before or after their passage through the column. The addition of electrolyte to the influent is preferable to the latter method because it avoids the introduction of a mixing zone between the column outlet and the detectors; however, it is obvious that it can be used only if the added electrolyte does not destroy the chromatographic separations that occur in the column.

Fig. 1 shows the effect of increasing concentrations of lithium perchlorate in the mobile phase (ethyl acetate–methanol, 80:20, v/v) used for separating a mixture of phenothiazines by adsorption chromatography[18]. Up to an electrolyte concentra-
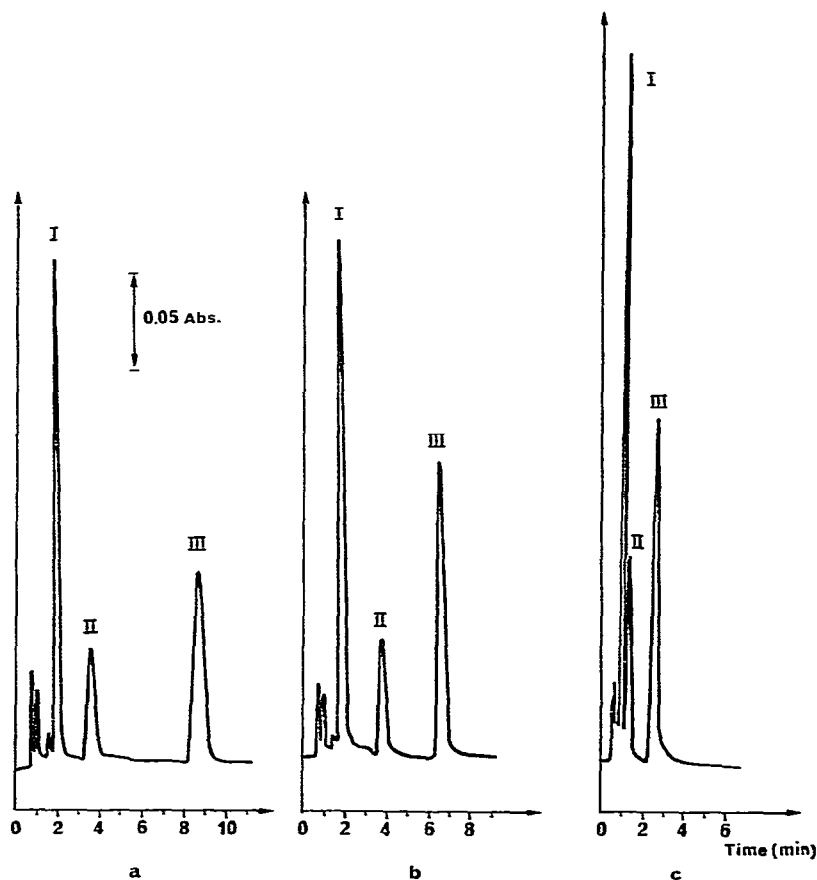

Fig. 1. Separation of a mixture of three phenothiazines by adsorption chromatography. I = (Methylamino-3-propyl)-10-chloro-3-phenothiazine; II = [N-methyl-N-(dimethylamino-3-propyl)amino-3-propyl]-10-chloro-3-phenothiazine; III = (dimethylamino-3-propyl)-10-chloro-3-phenothiazine N-oxide. Column: length 15 cm, I.D. 4.8 mm, packed with Partisil (5 μm). Mobile phase: ethyl acetate–methanol–water–ethylamine (78.2:20:0.6:0.2, v/v). Flow-rate: 180 ml·h^{-1}. Pressure: 100 bar. Injection: 5 μl of a solution in the mobile phase, about 10^{-3} M in each phenothiazine. Detector: UV (254 nm). Concentration of the inert electrolyte (lithium perchlorate in the mobile phase): (a) 0; (b) 0.01; (c) 0.1 M.

tion of 10^{-2} M the selectivity remains virtually unchanged but at higher concentrations (10^{-1} M and above) the selectivity decreases considerably.

It seems that, if the electrolyte is to be added to the influent and not to the column effluent, the electrolyte concentration must be kept low. In any event, solvents with a low dissociating power, such as ethyl acetate and methylene chloride, will dissolve only small proportions of indifferent electrolyte such as lithium and tetrabutylammonium perchlorate.

In poorly conducting solutions, it is essential that the distance between the counter electrode and the working electrode be kept as small as possible, to permit the greatest possible electrolysis current. Likewise, precise potential measurement requires that the reference electrode be as close as possible to the other two electrodes.

These requirements led us to use a thin-film electrode system, which had already been studied earlier[19]. The cell is described in detail in the experimental section.

Nature of the electrodes. The choice of electrode material is guided by two requirements: (a) to give the maximum electrode response; (b) to give a stable, non-fluctuating signal.

Fig. 2 shows the variation of detector response as a function of time for injections of $3 \cdot 10^{-7}$ mole of potassium hexacyanoferrate(II) using electrodes of polished platinum and glassy carbon. The carbon electrode gave a response that was greater than that of platinum and perfectly constant with time. Glassy carbon allows one to use a wider range of potential than most other solid electrodes (from -1.3 to $+1.5$ V, relative to the saturated calomel electrode), and most workers report that it behaves very well in non-aqueous solvents.

We therefore chose as our working electrode a disc of glassy carbon, carefully polished with 1-μm diamond paste. For practical reasons (to facilitate the entrance of the solution), the counter electrode was a disc of polished platinum.
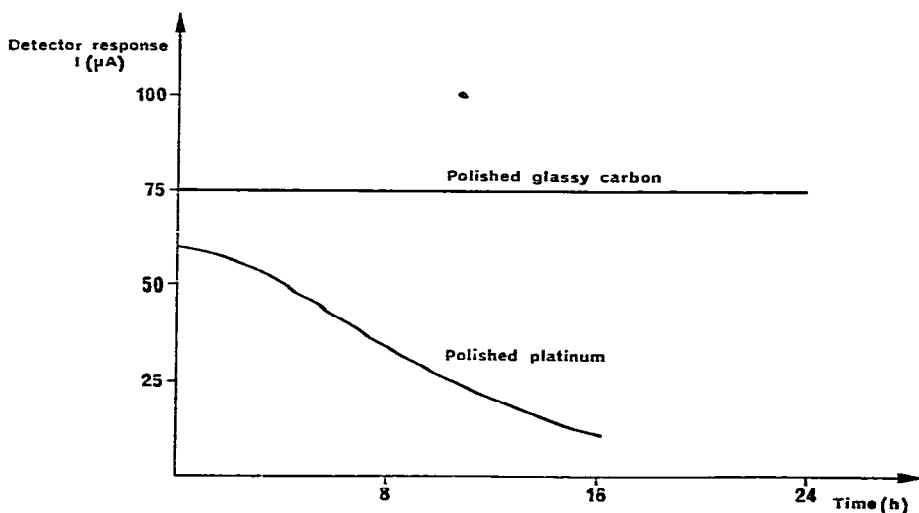

Fig. 2. Variation of the maximum electrolysis current *versus* time for a constant injection of $3 \cdot 10^{-7}$ mole of potassium hexacyanoferrate(II). Column: length 25 cm, I.D. 4.8 mm, packed with glass beads (100–500 μm). Mobile phase: 10^{-2} M potassium chloride solution. Flow-rate: 100 ml·h^{-1}. Applied potential: 700 mV *vs*. S.C.E.

Passivation of the working electrode. Reproducible measurements are essential for quantitative work. Organic compounds often cause the electrode to become passive, which is an important effect that most workers conveniently ignore. Among the possible means of avoiding passivity are the following: mechanical cleaning of the working electrode, which must, in this instance, be easily and rapidly accessible; electrochemical cleaning, by imposing high cathodic or anodic potentials in the form of pulses between measurement of the electrolysis current[11]; and hydrodynamic cleaning, performed by the mobile phase itself; if the cell volume is sufficiently small, linear mobile phase velocities of several hundred centimetres per second can be produced, and these would ensure continuous cleaning[7].

We chose the last method, while retaining the possibility of mechanical cleaning, thanks to a very simple manner of mounting the working electrode.

Compensation for the residual current. Because of the relatively large surface area of the indicator electrode (about 10 mm²), passage of the mobile phase, with its dissolved inert electrolyte, causes a residual current of about 1 μA, which must be compensated for if one is to obtain maximum sensitivity. Fortunately, the current is extremely stable; the fluctuations are less than 1 nA in some instances. Therefore, background compensation can be easily achieved, as described in the experimental section.

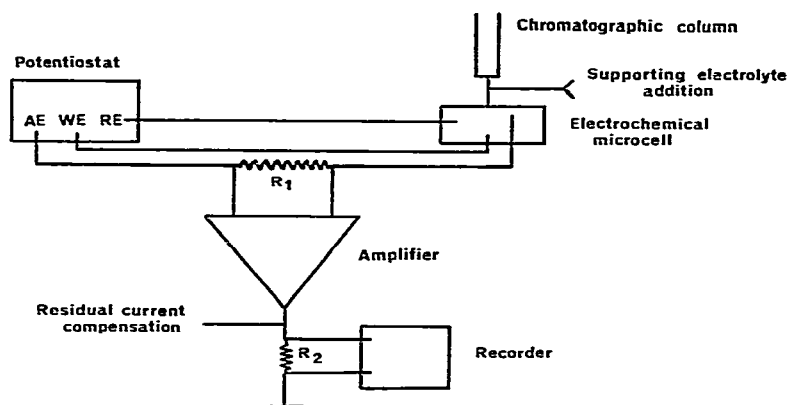Fig. 3 shows the general scheme of the apparatus.

Fig. 3. Principle of the electrochemical detection. WE = working electrode; AE = auxiliary electrode; RE = reference electrode.

EXPERIMENTAL AND RESULTS

Apparatus and materials

Electrochemical cell. The detection cell is shown in Fig. 4. The counter electrode and the working electrode are face to face, separated by a PTFE gasket of adjustable thickness (50–100 μm) that encloses the actual detection chamber.

The arrangement of the three electrodes allows the cell to work satisfactorily with concentrations of inert electrolyte 10^{-2} M and less. The dead volume is 1–10 μl, depending on the thickness of the PTFE gasket.

Electrical components. The potential of the working electrode is controlled by
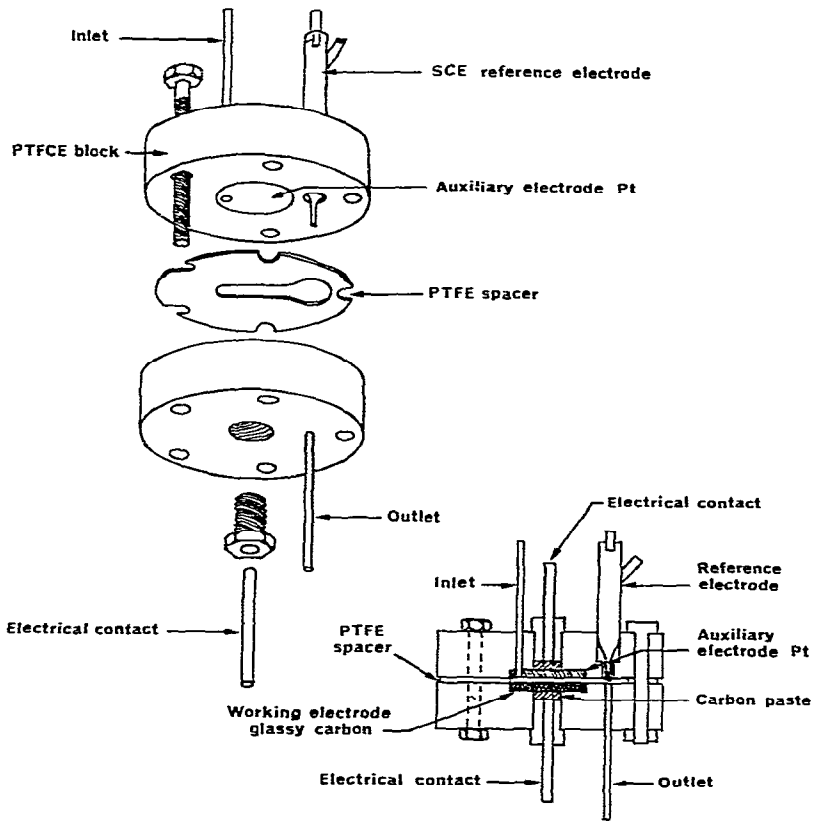
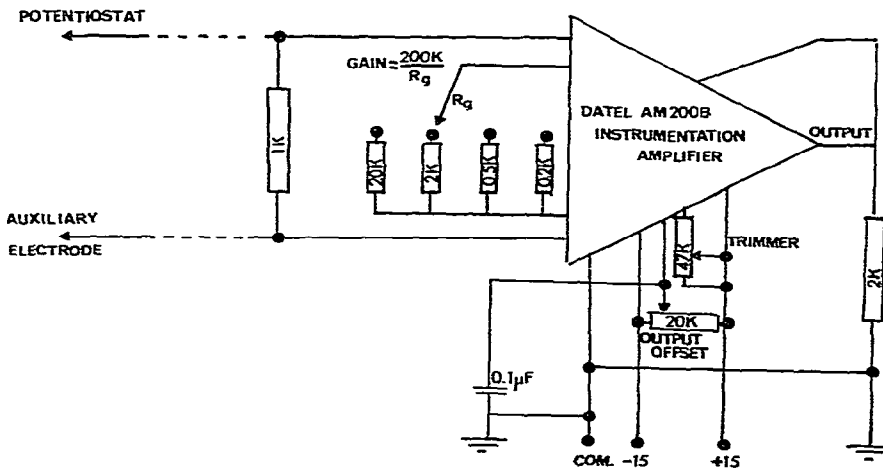Fig. 4. Scheme of the detector electrolysis cell.

Fig. 5. Scheme of the electrical circuit.

a Tacussel potentiostat (PRT-20-2-X) and the current is amplified by a differential amplifier described in Fig. 5.

Chromatographic equipment. The liquid chromatograph was a Varian 8520 (Varian, Palo Alto, Calif., U.S.A.). The spectrophotometer was a Variscan (Varian) working at 254 nm.

Packings. Three stationary phases were used: 5-μm Partisil silica (Whatman, Springfields Mill, Great Britain); Micropak CH (Varian) (porous 10-μm silica bonded with octadecyl groups); and RP 8 (Merck, Darmstadt, G.F.R.) (porous 5-μm silica bonded with octyl groups).

Chemicals. n-Hexane, methylene chloride and isopropanol were Uvasol solvents (Merck). Ethyl acetate was of nanograde quality (Mallinckrodt, Wesel, G.F.R.). Methanol, acetonitrile and aqueous ethylamine were of pro analisi grade (Prolabo, Paris, France). Lithium perchlorate, potassium chloride (Merck) and tetrabutylammonium perchlorate (Eastman-Kodak, Rochester, N.Y., U.S.A.) were of pro analisi grade.

The solutes were obtained from various sources, most of them being of analytical-reagent grade.

Study of the detector performance in aqueous solutions

We tested the performance of the detector by injecting various amounts of potassium hexacyanoferrate(II) into a stainless-steel column, 25 cm × 4.8 mm I.D., packed with glass beads of sizes between 100 and 500 μm. The mobile phase was 0.01 M potassium chloride solution, and injections were made with a syringe through a stop-flow injector.
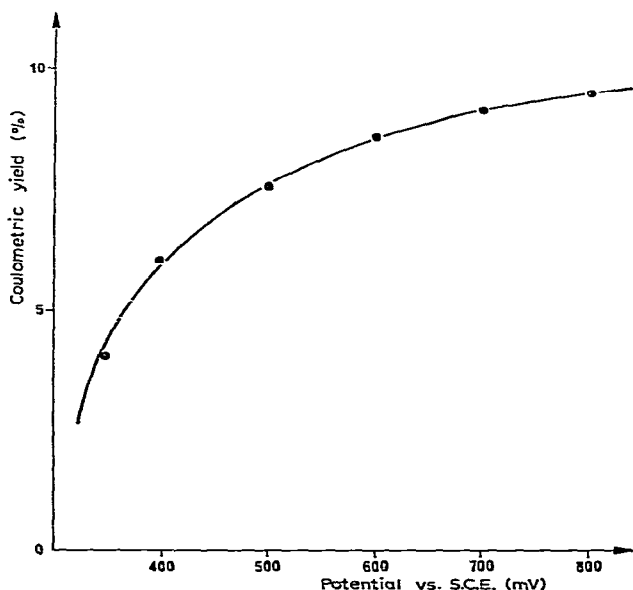

Fig. 6. Variation of coulometric yield *versus* potential applied to the working electrode. Injection: $2 \cdot 10^{-7}$ mole of potassium hexacyanoferrate(II). Other conditions as in Fig. 2.

The term "coulometric yield" used throughout this paper is defined as the percentage of the solute that is actually electrolysed.

Effect of the applied potential on the working electrode. By measuring the detector response for a constant amount of hexacyanoferrate(II) injected, and different applied potentials, we obtained a curve of coulometric yield at constant flow-rate $(100 \text{ ml} \cdot \text{h}^{-1})$, as shown in Fig. 6.

With this curve we could choose the optimum potential, that is, the potential at which the electrode efficiency approaches its limiting value. The coulometric yield is low (about 10%) but, as we shall see later, it is remarkably stable and reproducible. In view of this curve, we chose a working potential of 700 mV relative to the saturated calomel electrode.

It can be seen from Fig. 7 that the form of the peaks is gaussian, except for a slight tailing which arises in the dead volume between the column outlet and the detector.
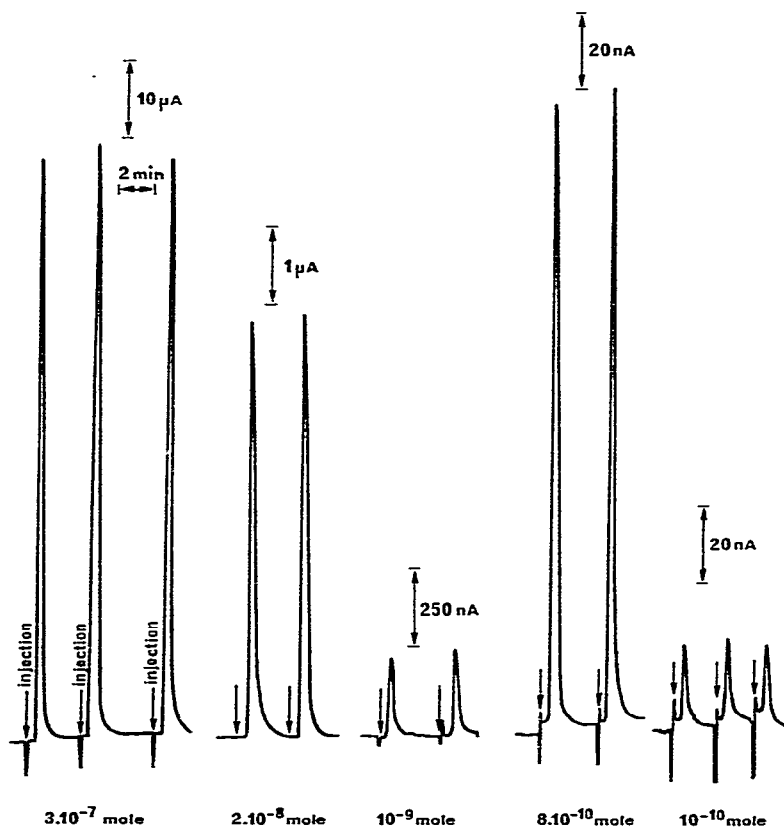

Fig. 7. Elution peaks of potassium hexacyanoferrate(II) for injections between $3 \cdot 10^{-7}$ and 10^{-10} mole. Conditions as in Fig. 2.

Effect of flow-rate. The flow-rate of the mobile phase affects the form of the peaks as well as the electrolysis yield.

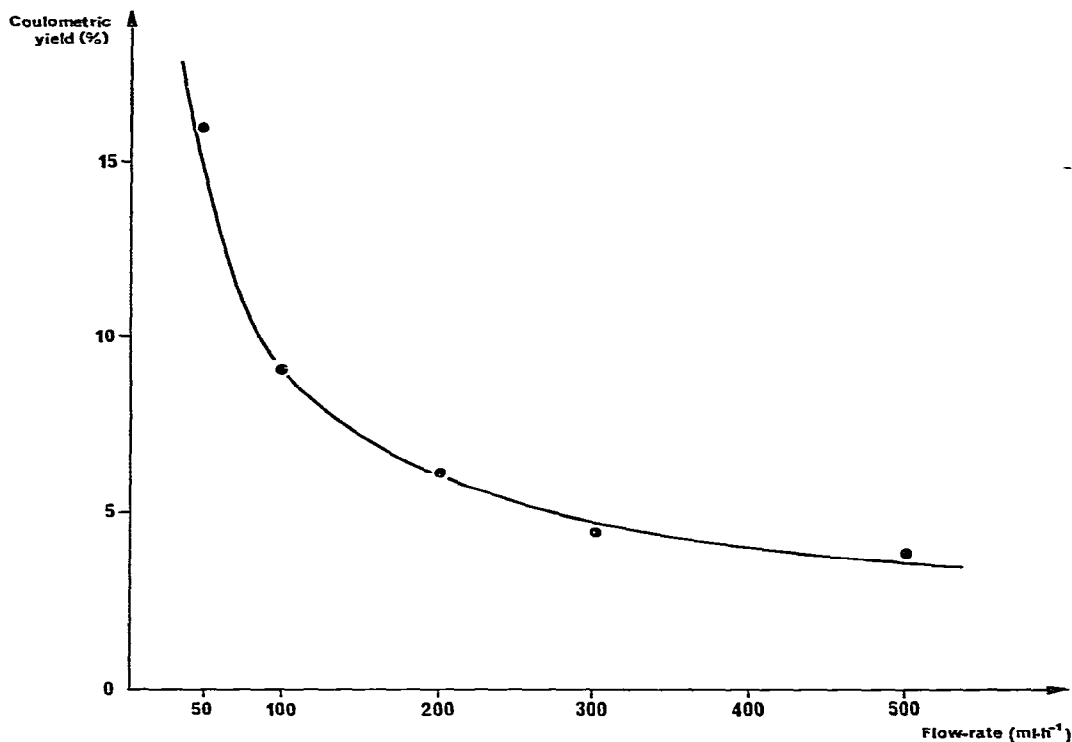*(a) Coulometric yield.* Fig. 8 shows that the coulometric yield decreases as the

Fig. 8. Variation of coulometric yield *versus* mobile phase flow-rate. Conditions as in Fig. 2.

flow-rate increases, being reduced 3-fold when the flow-rate is increased from 50 to 100 ml·h^{-1}.

(b) Peak shape. The maximum electrolysis current increases with the flow-rate of the mobile phase (Fig. 9), which is a common characteristic of electrochemical detectors.

The increasing current helps to compensate for the decrease in efficiency, and provides an advantage over detectors such as the UV and refractometric types, for which the response is inversely proportional to the flow-rate of the mobile phase. Moreover, the gaussian peak shape allows one to use the peak height as a measure of the amount injected.

Response curve of the detector. We made injections of 4 and 8 μl of hexacyanoferrate(II) solutions of different concentrations, representing amounts from 10^{-6} to 10^{-10} mole, with a flow-rate of 100 ml·h^{-1}, commonly used in liquid chromatography.

Fig. 10 shows, on a log–log scale, the relationship between the maximum current and the amount injected. A good linear relationship is obtained, which shows that the coulometric yield does not depend on the amount injected.

Reproducibility. The solutions must be carefully de-gassed if reproducible results are to be obtained. Microscopic air bubbles sticking to the working electrode greatly affect the current. If the solutions are properly de-gassed, however, the reproducibility is excellent. Deviations corresponding to a confidence limit of 95 % are
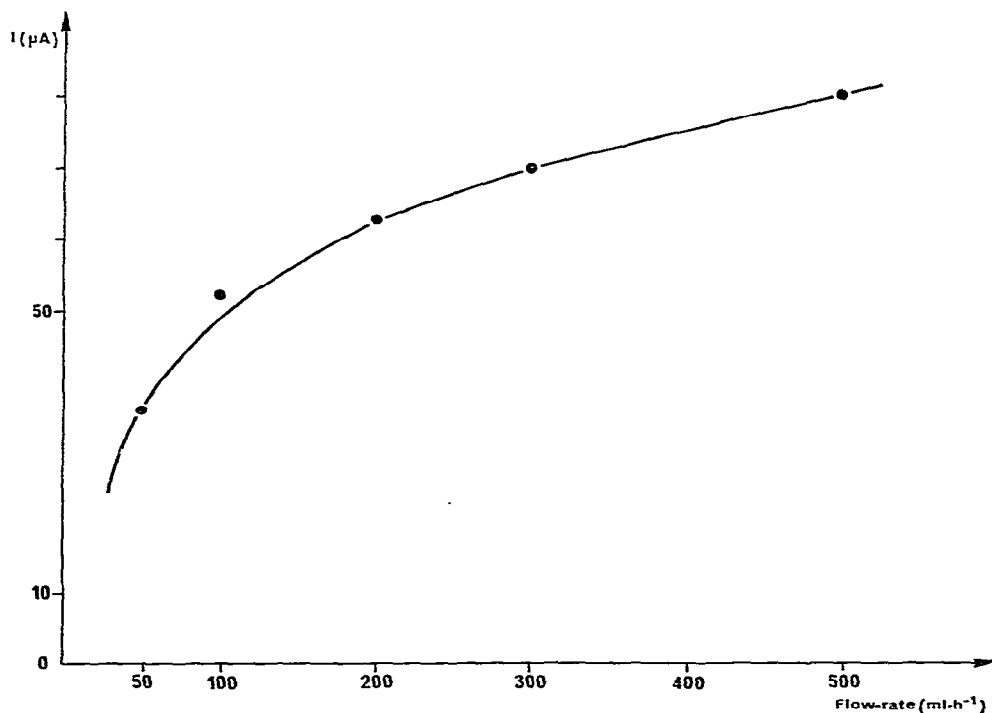
Fig. 9. Variation of maximum peak current *versus* mobile phase flow-rate. Conditions as in Fig. 2.
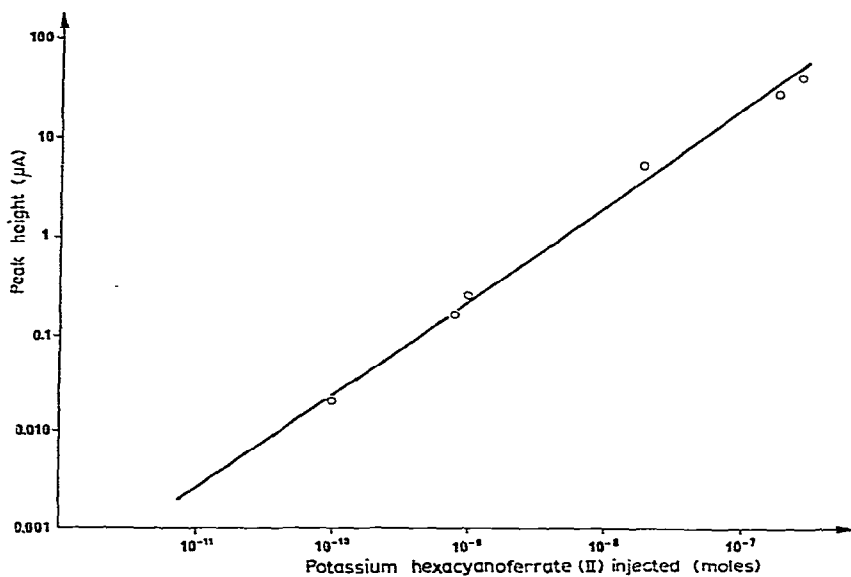

Fig. 10. Detector response curve for potassium hexacyanoferrate(II). Conditions as in Fig. 2.

only 1–2 % for amounts between 10^{-7} and 10^{-10} mole. This precision is comparable with that obtained with conventional liquid chromatographic detectors, and it can be attributed to the great stability of the residual current, the coulometric yield and the flow-rate of the mobile phase.

In conclusion, the properties of this detector seem to be suitable for its use in high-performance liquid chromatography.

Study of the detector in organic media

The possibilities of electrochemical detection go far beyond the range of aqueous solutions and ion-exchange chromatography, to which its use has generally been restricted. We shall show in the next section that it is feasible to perform electrochemical detection in such solvents as alcohols, acetonitrile, methylene chloride and even mixtures of hexane with methylene chloride and 2-propanol that have been made conducting by the addition of lithium perchlorate or tetrabutylammonium perchlorate.

Examples will be given to show the linearity of response, reproducibility and sensitivity in such instances.

Linear range. Fig. 11 shows response curves obtained for injections of pyrogallol and diphenylamine in a column packed with glass beads. The mobile phase is 10^{-2} *M* acetonitrile in lithium perchlorate. In both instances the response is very close to linear over the range 10^{-11}–10^{-6} mole. As in aqueous solutions, the coulometric yield is very nearly constant, regardless of the amount injected.

Reproducibility. Tests of the reproducibility of the behaviour of these two solutes gave results very like those found with inorganic solutes in aqueous solutions.
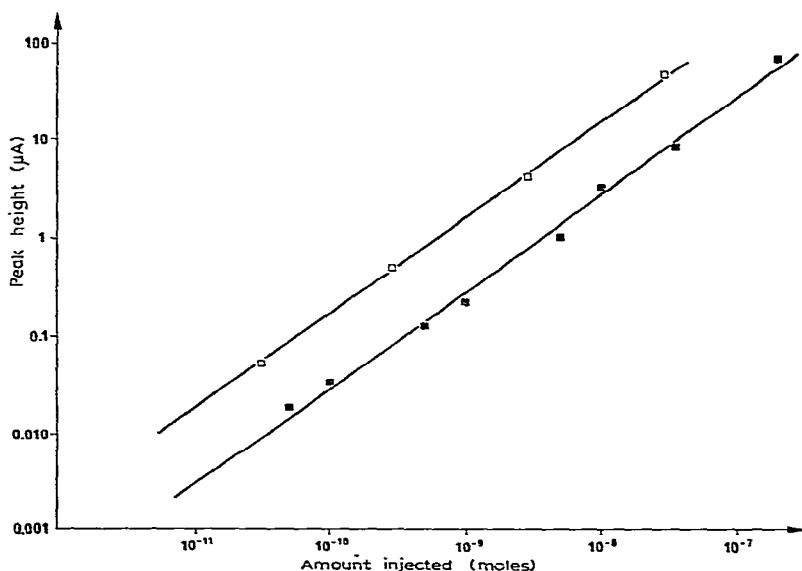

Fig. 11. Detector response curve for pyrogallol (■) and diphenylamine (□). Column: length 15 cm, I.D. 4.8 mm, packed with glass beads (100–500 μm). Mobile phase: acetonitrile + LiClO₄ (0.02 *M*). Flow-rate: 100 ml·h⁻¹. Applied potential: 1 V *vs.* S.C.E.

TABLE I

DETECTABILITY OF THE ELECTROCHEMICAL DETECTOR FOR VARIOUS ORGANIC SOLUTES

Organic compounds	Minimum detectable amount (moles injected)
Aromatic amines	10^{-14}
Indoles	10^{-14}
Carbazoles	10^{-14}
Quinolines	10^{-10}
Pyridines	Difficult to detect (10^{-7})
Phenols	10^{-14}
Thiophenes	Difficult to detect (10^{-7}–10^{-10})
Amphetamines	10^{-10}–10^{-14}

There was no evidence of any passivation of the electrode under the conditions we used.

Minimum detection limits. The minimum detectable amounts depend on the nature of the substance, but range between 10^{-10} and 10^{-14} mole injected for most solutes that can be detected electrochemically. Table I summarizes the values obtained with different types of compounds. It can be seen that the limits are very low for phenols and aromatic amines (about 10^{-14} mole), which suggests interesting possibilities for biological studies, such as the measurement of certain steroids in
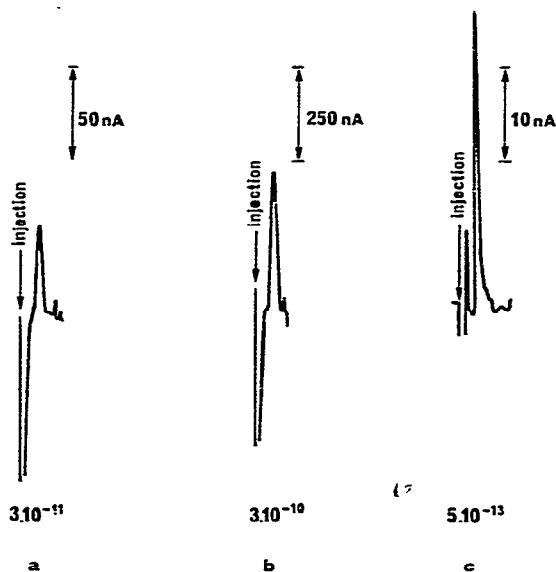

Fig. 12. Electrochemical detection of various organic solutes. Column: length 15 cm, I.D. 4.8 mm, packed with Partisil (5 μm). (a) Injection of $3 \cdot 10^{-11}$ mole of diphenylamine. Mobile phase: acetonitrile + LiClO$_4$ (0.02 M). Flow-rate: 100 ml·h^{-1}. Applied potential: 1 V vs. S.C.E. (b) Injection of $3 \cdot 10^{-10}$ mole of indole. Conditions as in (a). (c) Injection of $5 \cdot 10^{-13}$ mole of o-toluidine. Mobile phase: acetonitrile + LiClO$_4$ (0.1 M). Flow-rate: 100 ml·h^{-1}. Applied potential: 1.5 V vs. S.C.E.

physiological fluids, as well as the active ingredients of many medicines. Furthermore, most of the components of petroleum that contain oxygen, sulphur or nitrogen can be determined by electrochemical methods, which offer the great advantage of being selective in presence of compounds of aromatic character. Fig. 12 shows examples of detection by electrochemical means.

Finally, as an example of the power of this technique, Fig. 13 shows that the electrochemical detection of three amphetamines is much more sensitive than absorptiometric detection at 254 nm. The minimum detectable amounts of ephedrine and benzamphetamine are 50 times smaller, and that of phenylephrine is 200 times smaller.
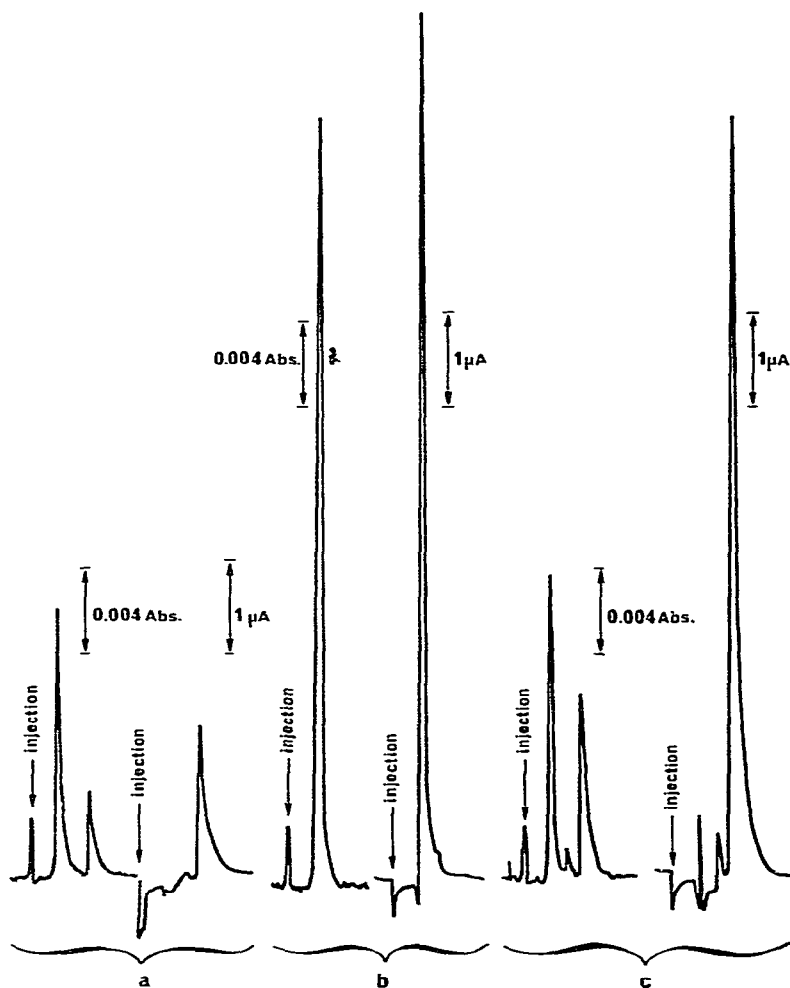

Fig. 13. Comparison between electrochemical detection and UV detection at 254 nm for amphetamines. Column: length 15 cm, I.D. 4.8 mm, packed with Partisil (8 μm). Mobile phase: methanol–2 M aqueous ammonia–1 M aqueous ammonium nitrate (90:6.6:3.4, v/v). Flow-rate: 50 ml·h^{-1}. Applied potential: 1 V vs. S.C.E. (a) Injection of $3 \cdot 10^{-8}$ mole of ephedrine; (b) injection of $2 \cdot 10^{-8}$ mole of benzphetamine; (c) injection of $2.5 \cdot 10^{-8}$ mole of phenylephrine.

Examples of applications

We shall illustrate the performance of the detector by a few applications in adsorption and partition chromatography, after which we shall show how it can be used in reversed-phase gradient elution. Most of the chromatograms were recorded with an ultraviolet absorption detector at 254 nm, which was mounted ahead of the electrochemical detector and in series with it.

Adsorption chromatography. As we have already noted, in adsorption chromatography one can add an indifferent electrolyte to the solvent, at concentrations of 10^{-2} M or less, without affecting the chromatographic separation. We shall now give a few examples of this procedure.

Fig. 14 shows the separation of a mixture of phenothiazines by adsorption chromatography[18], after making the solvent 10^{-2} M in lithium perchlorate. Even though the electrochemical detector was placed downstream from the ultraviolet
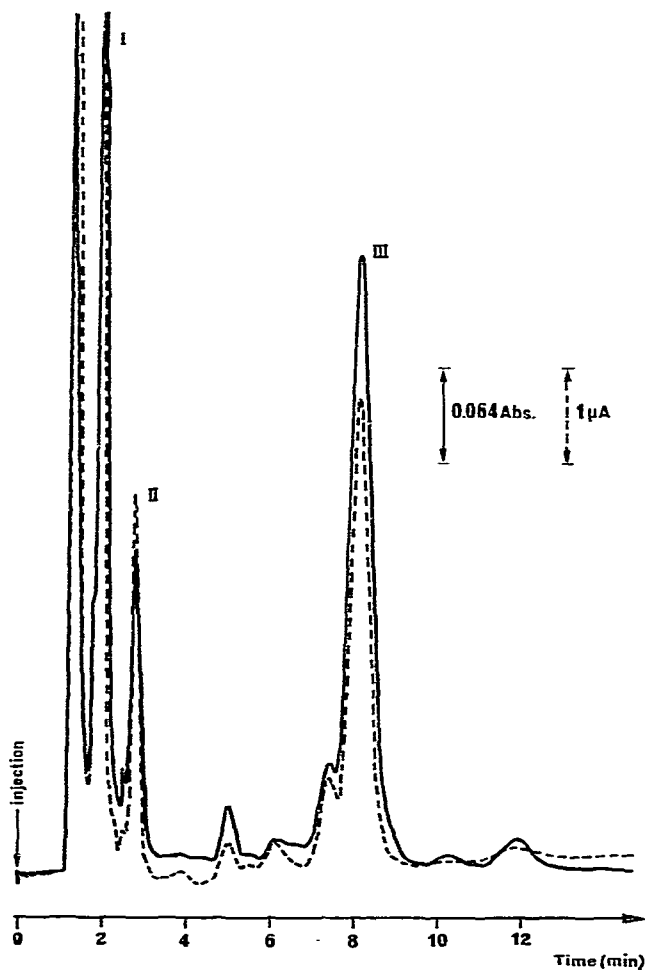

Fig. 14. Separation of a mixture of phenothiazines by adsorption chromatography. Conditions as in Fig. 1 (b). Applied potential: 1.25 V *vs.* S.C.E. Only peaks I, II and III have been identified.

detector, it showed no decrease in resolution, which would have occurred if the dead volume of the detector had been significant. In this instance there is little difference in sensitivity between the two detectors; however, it is worth noting that electrochemical detection can be used with solvents of ordinary grade and low cost, whereas ultraviolet detection at 254 nm usually requires spectroscopic-grade solvents that are much more expensive. Another point is that solvents such as benzene and acetone can be used with electrochemical detection, and that thin-layer chromatography performed with these solvents can easily be adapted to column chromatography.

The selectivity and sensitivity of the electrochemical detector can also be exploited for the measurement of trace constituents in media that absorb strongly in the UV region. Thus, Fig. 15 shows a chromatogram obtained by injecting 3 μl of benzene containing indole at a concentration 10^{-3} M (*ca.* 100 ppm). Electrochemical detection permits one to determine the indole without having to separate it from the benzene.

Fig. 16 illustrates the use of the detector for the control of pharmaceutical products. It can be seen that in the injection of 2 μl of a solution containing 3 mg·ml^{-1} of noscapine, with the mobile phase ethyl acetate–methanol (80:20, v/v), two uniden-
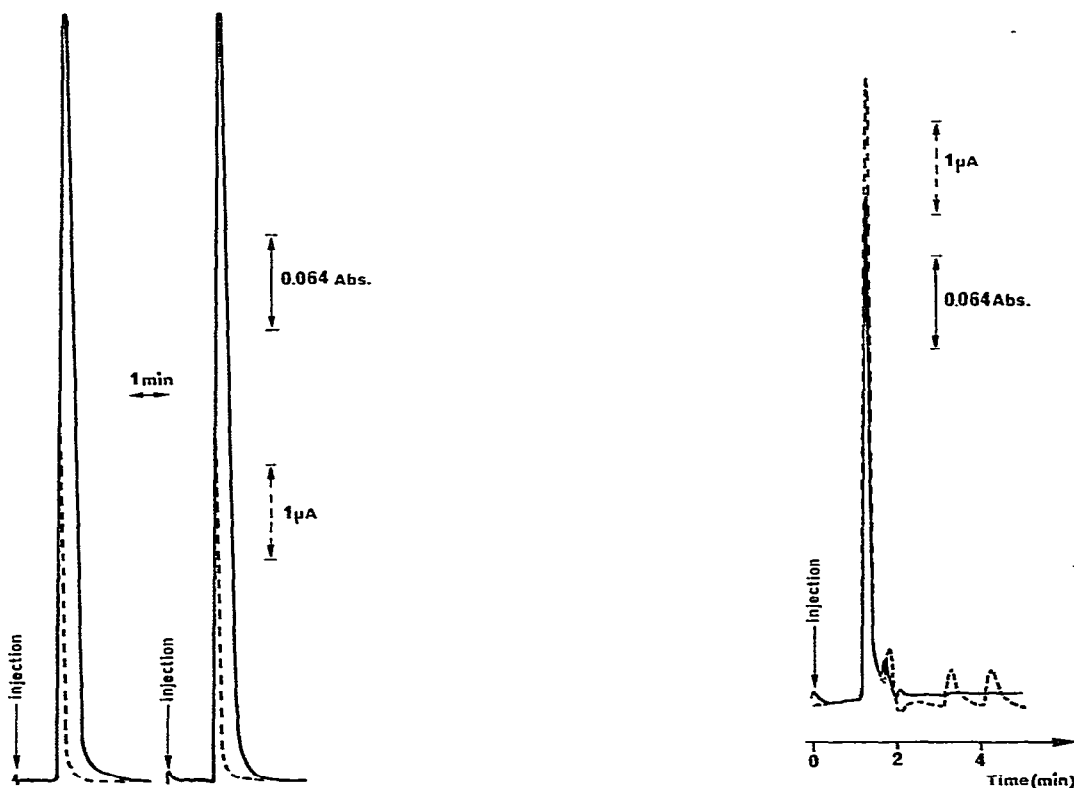

Fig. 15. Selective detection of indole (0.01 %) in benzene solution. Injection: 3 μl. Conditions as in Fig. 1 (b) except flow-rate = 100 ml·h^{-1} and pressure = 80 bar. Applied potential: 1 V *vs.* S.C.E.

Fig. 16. Chromatogram obtained from the injection of 2 μl of a 7.5·10^{-3} M noscapine solution. Conditions as in Fig. 15.

tified impurities appear that cannot be detected by ultraviolet absorption at 254 nm. This application shows the complementary character of ultraviolet and electrochemical detection. We consider that it would be advantageous to use a certain number of detectors in series, each being more or less specific, rather than to try to develop a universal detector, which would be complicated and costly and would require columns of high resolution for complex mixtures.

Fig. 17 shows the separation of two active principles, amidopyridine and butazolidine, commonly measured in pharmaceutical products[20]. The electrochemical detector is much more sensitive for amidopyridine than is the ultraviolet detector.

Partition chromatography. (a) Reversed-phase. Reversed-phase partition chromatography lends itself particularly well to the use of the electrochemical detector, because the solvents commonly used are mixtures of water with methanol or acetonitrile,
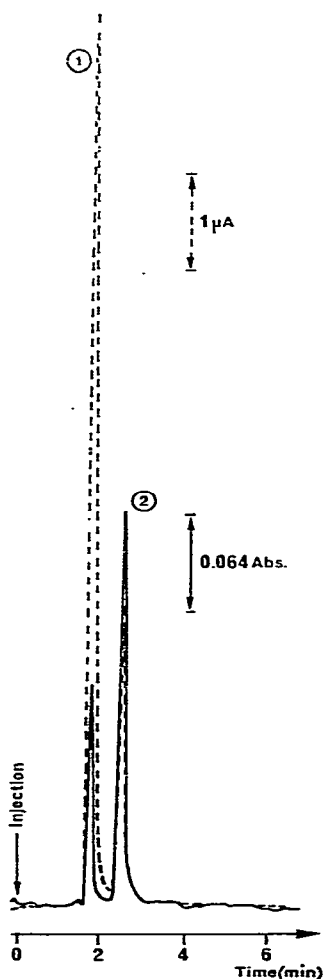

Fig. 17. Separation of two active principles associated in an antirheumatismal medicine. Injection: 5 μl of a solution 10^{-3} M in (1) butazolidine and (2) amidopyrine. Conditions as in Fig. 1 (b) except flow-rate = 80 ml·h^{-1} and pressure = 50 bar. Applied potential: 1 V *vs.* S.C.E.

and they can easily be made conducting by adding an inert electrolyte such as lithium perchlorate. Figs. 18 and 19 show the separation of phenolic compounds as examples. The inert electrolyte concentrations are only $5 \cdot 10^{-3}$ M.
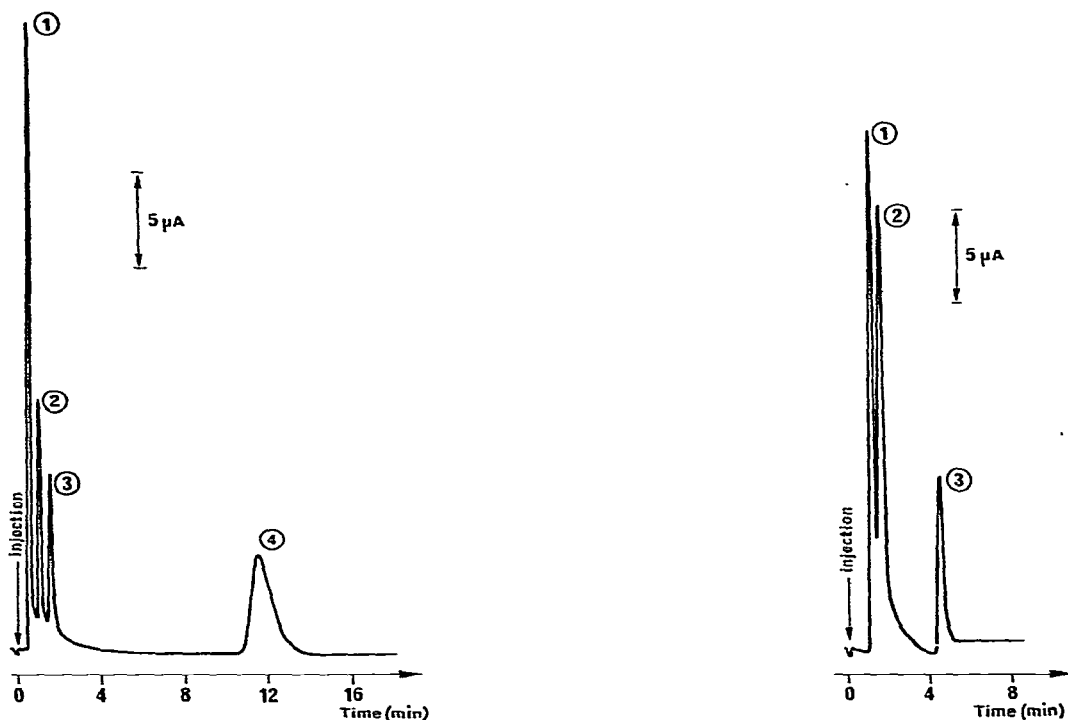

Fig. 18. Reversed-phase separation of resorcinol derivatives. Injection: 3.5 μl of a solution containing (1) methyl-, (2) hexyl-, (3) octyl- and (4) dodecylresorcinol, each at 0.01 M concentration. Column: Micropak CH (Varian), length 25 cm, I.D. 2.8 mm. Applied potential: 1 V vs. S.C.E. Mobile phase: acetonitrile–water (65:35, v/v) + 0.005 M lithium perchlorate. Flow-rate: 60 ml·h^{-1}. Pressure: 80 bar.

Fig. 19. Reversed-phase separation of catechol derivatives. Injection: 1 μl of a solution containing (1) pyrogallol, (2) 4-$tert$.-butylcatechol and (3) di-$tert$.-butylcatechol each at 0.1 M concentration. Column: Micropak CH (Varian), length 25 cm, I.D. 2.8 mm. Mobile phase: acetonitrile–water (56:44, v/v) + 0.005 M lithium perchlorate. Flow-rate: 80 ml·h^{-1}. Pressure: 110 bar. Applied potential: 1 V vs. S.C.E.

(b) *Normal-phase.* No normal-phase separations are presented here, but some examples are given of the detection of several compounds dissolved in mobile phases usually used in this type of chromatography, namely hexane–dichloromethane and hexane–2-propanol mixtures. The compounds were injected into a 25 cm × 4.8 mm I.D. column packed with glass beads (Fig. 20).

Completely non-polar mobile phases, such as pure hexane, cannot be used with the electrochemical detector unless they are mixed with a more polar solvent, such as 2-propanol, at the outlet of the chromatographic column.

Gradient elution. Gradient elution is now used routinely in liquid chromato-
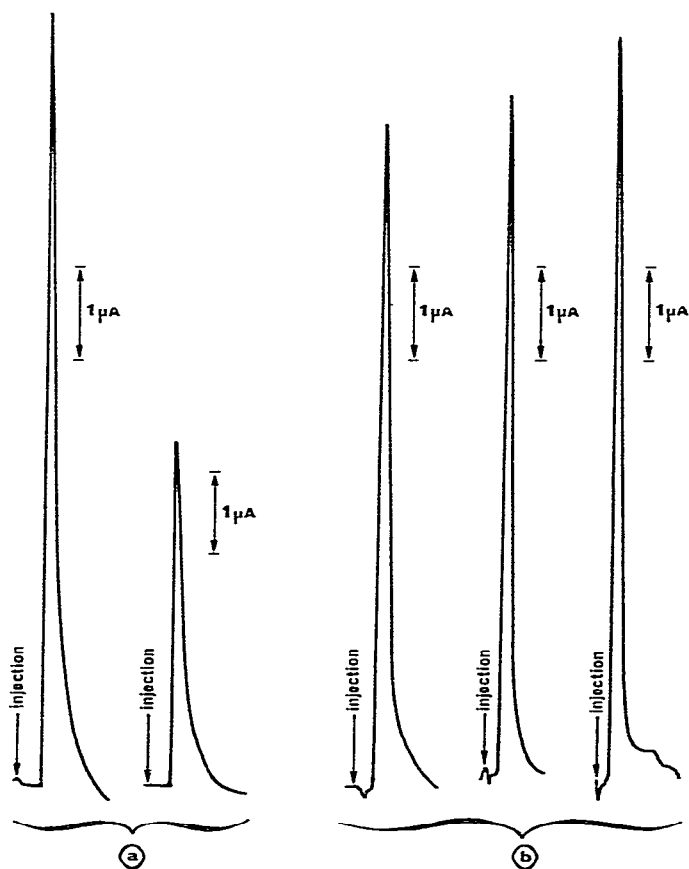
Fig. 20. Detection of various organic solutes. Column: length 15 cm, I.D. 4.8 mm, packed with glass beads (100–500 μm). Applied potential: 1.5 V *vs.* S.C.E. (a) Injection of 3 μl of a 10^{-3} M solution of 2,4- and 2,3-dimethylphenol. Mobile phase: hexane–methylene chloride (20:80, v/v) + 0.01 M tetrabutylammonium perchlorate. Flow-rate: 100 ml·h^{-1}. (b) Injection of 3 μl of a 10^{-3} M solution of propyl *p*-hydroxybenzoate, paracetamol and paroxypropione. Mobile phase: hexane–2-propanol (75:25, v/v) + 0.01 M lithium perchlorate. Flow-rate: 100 ml·h^{-1}.

graphy. Without gradient elution it would be impossible to resolve, in any reasonable time, very complex mixtures that have a wide range of polarities.

Fig. 21 shows the use of the electrochemical detector with a water–methanol gradient and reversed-phase partition chromatography for the analysis of a mixture of a new antibiotic, penimocycline[21]. In this instance the mobile phase is naturally conducting, because it contains a sodium phosphate buffer, $3.6 \cdot 10^{-2}$ M. We found that the baseline drift was small, and no more than one would find with an ultraviolet detector at 254 nm.

CONCLUSION

The electrochemical detector described here can be used, owing to its special cell geometry, in a variety of solvents, both aqueous and non-aqueous. The very close
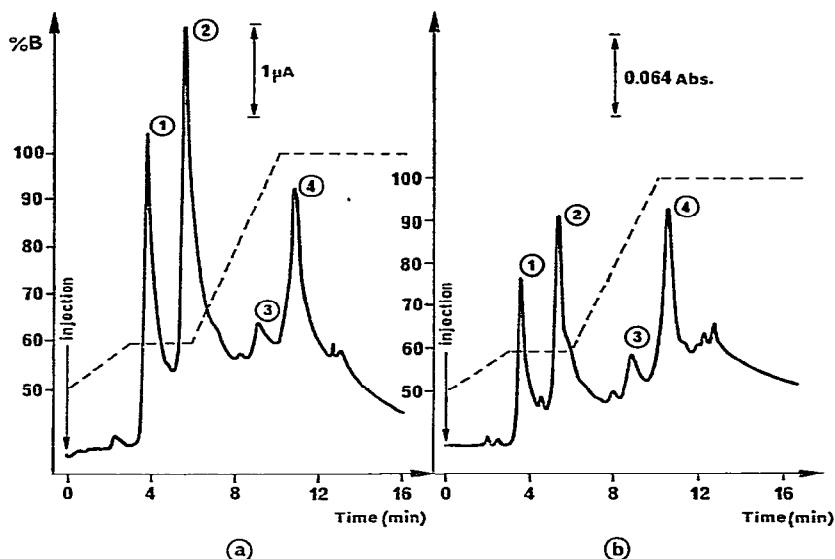
Fig. 21. Chromatogram obtained from a 4-μl injection of penimocycline solution in dimethyl sulph-oxide (10 mg·ml^{-1}). Column: length 20 cm, I.D. 4.8 mm, packed with RP 8 (5 μm) (Merck). Mobile phase: gradient elution with water–methanol mixture + 0.036 M phosphate buffer at pH 7, with methanol content varying from 36 to 60% (v/v). Gradient curve is represented by dashed lines. A Pump: water–methanol (90:10, v/v). B Pump: water–methanol (40:60, v/v). Flow-rate: 80 ml·h^{-1}. Pressure: 150–200 bar. (a) Electrochemical detection. Applied potential: 1 V vs. S.C.E. (b) UV detection. Peaks: 1 = 4-epi-tetracycline; 2 = tetracycline; 3 = 4-epi-penimocycline; 4 = penimocycline.

proximity of the working electrode to the counter electrode reduces Ohm's law power losses in poorly conducting, weakly polar mobile phases, and the rapid flow-rate of the liquid through a thin-film cell assures continuous hydrodynamic cleaning of the electrode surfaces, which, in turn, reduces considerably the passivation encountered with other cell geometries.

Therefore, it is possible to use this type of detector without changing the composition of the mobile phase, by adding a very low concentration (5·10^{-5} to 10^{-2} M) of inert electrolyte which does not affect the resolution of separations.

Moreover, completely non-polar eluents can be used if a solution of an inert electrolyte in a more polar solvent such as 2-propanol is added at the outlet of the column.

The various examples of separations given show that the detector is especially well adapted to the analysis of complex mixtures and for the determination of trace amounts in high-performance liquid chromatography. Moreover, the high sensitivity, moderate cost and ease of operation of this detector are advantages which should assure it of a special place in the field, so far largely undeveloped, of specific liquid chromatographic detectors.

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