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AMPEROMETRIC FLOW-THROUGH SOLID ELECTRODE DETECTOR DESIGN: PERFORMANCE AND APPLICATION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE MICRODETERMINATION OF ANTIOXIDANTS IN MONOMERS

A. Ya. LAZARIS*, L. N. BELODED and A. I. KALININ

V. A. Kargin Polymer Chemistry and Technology Research Institute, Dzerzhinsk, Gorky Region, 606006 (U.S.S.R.)

SUMMARY

The development of laboratory-made electrochemical detectors of simple design with a glassy carbon working electrode employed with the conventional type and microbore high-performance liquid chromatographic (HPLC) columns is reported. The dependence of the detector response and performance on the eluent flow-rate, pump pulsation, applied potential, etc., is discussed. The detectors were used for HPLC, flow injection analysis and recording of constant depolarizer flow polarograms.

The application of the detectors to the analysis of hydroquinone, *p*-methoxyphenol and phenothiazine in acrylic monomers with detection limits of 10^{-7}–10^{-8} mg is described.

INTRODUCTION

There is a need to determine residual antioxidants in acrylic monomers because they retard the shelf-life of the polymers. The concentration of antioxidants may be relatively high when, for instance, it is desirable to prevent the formation of peroxides in a monomer during its long-term storage.

High-performance liquid chromatography (HPLC) is the most favoured method owing to its high selectivity and sensitivity. This method permits the analysis of mixtures of antioxidants used for the stabilization of monomer or introduced during technological processes that result in commercial products containing these antioxidants. Previously for the detection of antioxidants by HPLC a UV detector was employed with sensitivity up to 10^{-3}% (w/w)[1-3]. However, a disadvantage of UV detectors is that even the most sophisticated designs suffer from a "refractometric" effect to some extent, *i.e.*, there is a low signal caused by the presence of a substance in the detector which should not absorb light at that wavelength. If we take into account that the monomer-to-inhibitor ratio can reach 10^7–10^6, this would be a considerable disadvantage owing to possible overlapping of the peaks of the monomer or its impurities with the peaks of the antioxidants and also to the diffi-

culties in the separation of the components of the mixture obtained in this instance. From this point of view it would be very convenient to use an electrochemical detector which is extremely sensitive to antioxidants and insensitive to the monomer. High sensitivity and selectivity make this type of detector unique for such an analysis.

Despite the great number of papers on the design and application of electrochemical detectors[4-6], there is a controversy about the optimal design.

In this work an electrochemical detector of simple design was developed, its caracteristics were determined and it was employed for the determination of antioxidants in acrylic monomers.

EXPERIMENTAL

Chromatographic equipment

The chromatographic system with a type a electrochemical detector (Fig. 1) is based on a Tsvet 306 liquid chromatograph (OCBA, U.S.S.R.). The separation column (100 × 6 mm I.D.) is packed with a laboratory-made sorbent prepared from C_{10}-bonded Silasorb-600 (5 μm) silica gel (Chemapol, Prague, Czechoslovakia).

For the chromatographic system with a type b electrochemical detector (Fig. 1) a glass column (100 × 2.7 mm I.D.) was used. The column packed with the same sorbent as in type a, placed in a stainless-steel cartridge. An H-54 liquid delivery system (OCBA) was used for eluent supply (flow-rate 0.2–0.4 ml/min).

Flow injection analysis (FIA) equipment

The eluent was delivered by an MMC-2C pump (Microtechna, Prague, Czechoslovakia) through a six-port switching valve with a 5-μl sampling loop and a PTFE capillary (300 × 0.2 mm I.D.) into the type b detector. Between the pump and the valve there was an additional resistance (a stainless-steel capillary, 200 × 0.25
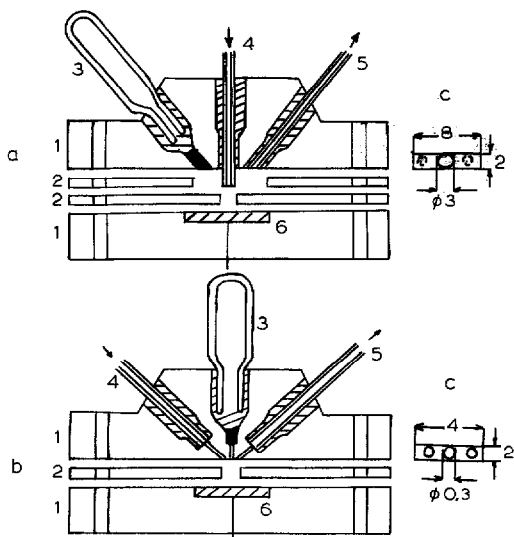

Fig. 1. Electrochemical detector types a and b cells. 1, Covers; 2, spacers; 3, reference electrode; 4, flow inlet; 5, auxiliary electrode outlet capillary; 6, working electrode. (c) Cross-section view (mm).

mm I.D.) and a T-piece with a PTFE capillary damper (300 × 1 mm I.D.) sealed on one side.

Electrochemical equipment

The electrochemical detector is a PU-1 polarographic analyser (Measuring Instrument Factory, Gomel, U.S.S.R.) with a three-electrode circuit. For continuous flow of a depolarizer through the detector an X–Y recorder was used. For recording in the chromatographic and FIA modes LKD-003 recorder (Lenteplopribor, Leningrad, U.S.S.R.) with scale switching starting from 1 mV full-scale was used. In this instance the sensitivity of the recording system was 0.4 nA/cm.

Electrochemical detector construction (Fig. 1)

For both types of detector, one body of the same shape made of Kel-F and having ground-in surfaces is used. In the type a detector the diameter of the holes in the top cover is 1.8 mm and the distance between extreme holes is 7 mm.

The electrochemical detector is of the wall-jet type, with jet radii of 0.5 mm, where the jet is the end of a 0.5 mm I.D. PTFE capillary running directly from the chromatographic column. The distance between capillary end and the working electrode is 0.1 mm. Two polyethylene spacers are used, one 0.15 mm (upper) and the other 0.20 mm (lower). A rectangular channel connecting three electrodes is cut in the top spacer (Fig. 1), and in the lower one there is a round hole for the working electrode.

In the top cover of the type b detector there are three 0.3 mm holes with the extreme ones being at a distance of 4 mm. One 0.2 mm spacer is used in this type of detector.

Glassy carbon working electrodes polished to a mirror-like finish are used in both types of detector. A silver-silver chloride reference electrode filled with 1 M potassium chloride solution is used. A stainless-steel capillary of 0.5 mm I.D. for the eluent waste serves as the auxiliary electrode. The cell resistance was measured with a P 5010 a.c. digital bridge (1000 Hz).

Chemicals

Water was doubly distilled in glass, methanol was purified from oxidizing impurties by treatment with silver oxide and distilled and acetonitrile was rectified on a 0.5 m column. The acetate aqueous buffer was of pH 3.9 (0.56 M acetic acid; 0.1 M sodium acetate), the phosphate aqueous buffer was of pH 5.25 (0.065 M potassium dihydrogen phosphate; 0.0016 M disodium hydrogen phosphate).

RESULTS AND DISCUSSION

Electrochemical detector cell design

The characteristics that have the greatest effect on the detector performance should be considered at the design stage. High sensitivity and a linear response range determine the detector quality. The noise level should be sufficiently low to produce a high sensitivity. Three factors determine the noise level; surface quality of the working electrode[7], ohmic resistance of the cell[4] and pulsation level of the pump[8]. In this design the surface of the working electrode was polished to a mirror-like finish. It is

desirable to have the ohmic resistance of the cell as low as possible, *i.e.*, under a high resistance the cell acts as an antenna and the detector cannot be protected against interferences[9]. To meet this requirement it is necessary to decrease the cell volume in order to reduce the chromatographic zone broadening, which contradicts the first requirement. A lower cell volume is achieved by using a thin spacer, which results in a higher cell resistance. In addition, the reference electrode should be positioned as near to the working electrode as possible to prevent non-compensated cell resistance, which reduces the linear dynamic range[10,11].

Two detector constructions were designed in order to satisfy these require-ments (Fig. 1). The type a construction is designed for operation with 4–6 mm I.D. columns, *i.e.*, with flow-rates of 1–2 ml/min. The column and the detector are linked by a capillary, serving as a wall-jet design. Two spacers determine the cell shape, the upper spacer having a rectangular channel and the lower spacer a round hole for the working electrode. The auxiliary and reference electrodes are connected to the work-ing electrode through the rectangular channel. The three electrodes of this cell are located near one another but at the same time its resistance does not exceed 4 kΩ*, the geometric volume being 2.5 μm. The type b cell construction is used with 2 mm I.D. columns (flow-rates 0.2–0.4 ml/min) and in the FIA mode. Very narrow channels leading to the working, reference and auxiliary electrodes are concentrated within a 4 mm distance and the cell volume is about 0.8 μm. The resistance of this cells is about 50 kΩ, which is acceptable and both types of cell can be used without screening.

*Evaluation of electrochemical detector performance***

One of the most essential characteristics of the detector performance is the shape of the hydrodynamic polarograms for oxidized substances. Faulty location of the electrodes can result in deformed and prolonged shapes of the polarograms, which increases the value of I_{lim} usually employed for quantitative measurements, and also results in higher noise levels and poorer selectivity. To evaluate the detector perform-ance from this viewpoint it is important to make a correct choice of the sample substance. The most commonly used is $K_4Fe(CN)_6$, but it has been shown[12] that its polarogram on the GC working electrode has poor agreement with that of the the-oretical. Adrenaline used in the continuous flow solution mode, indicates the insta-bility of the polarograms with time[10]. We have found that the best results are ob-tained with phenothiazine solutions. Regardless of method used to obtain the polar-ograms, such as consecutive sample injections (HPLC and FIA) or a continuous flow of substance through the detector, and despite the detector type, the same polarogram shape with an unchanged $E_{1/2}$ value is observed. No electrode fouling was observed after several days of operation. When a polarogram is recorded with a continuous flow of substance through the type b detector, the relationship log $I/(I_{lim} - I)$ *versus* E is plotted, giving $E_{1/2} = 0.43$ V and a slope of 60 mV (least-squares method), which indicates the reversibility of the electrochemical reaction with $n = 1$. The difference in $E_{1/2}$ was only 7 mV (I_{lim} ranged from 3 to 10 μA) for flow-rates of 0.1–2.4 ml/min. The results indicate that the close arrangement of the electrodes selected for both types of detector provides the possibility of measuring and main-

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* Non-flow resistance values are listed for an eluent consisting of methanol (acetonitrile)–buffer.

** All further discussion of detector performance refers to the oxidation mode.

taining the working electrode potential without a high non-compensated resistance, which could disturb main detector characteristics. Hydrodynamic polarograms for phenothiazine, hydroquinone and p-methoxyphenol for the d.c. mode are given in Fig. 2 and demonstrate that by changing the E value one can determine the selectivity for the analysis of the components of a mixture; however, as can be seen from Fig. 3, the selectivity of the differential pulse mode is considerably higher.

Electrochemical detector linear dynamic range

The correct choice of pre-selected detector geometry results in a broad linear response range for both types. A large distance between the electrodes and incorrect potential measurements may reduce the linear range to 10^{11}. From the literature[4,6] it is obvious that the linear response range may reach as high as 10^5-10^6. The detector constructions proposed here are characterized by a linear response range of not less than 10^4 (a wider range to prevent electrode fouling at very high concentrations of depolarizing agents was not checked). Fig. 4 shows an example of the log I = f(log q) relationship (least-squares method), where q is the amount of sample. The correlation coefficient is 0.999 for both types of detector (flow-rate 0.2 ml/min).

Dependence of electrochemical response on flow-rate

Many workers have attempted to use electrochemical hydrodynamic equations to describe the behaviour of electrochemical detectors[4-6,13], but obtained contradictory results. Only recently was it demonstrated[14,15] that with a small thickness of the cell (0.05-0.2 mm) there is no point in differentiating between thin-layer and wall-jet
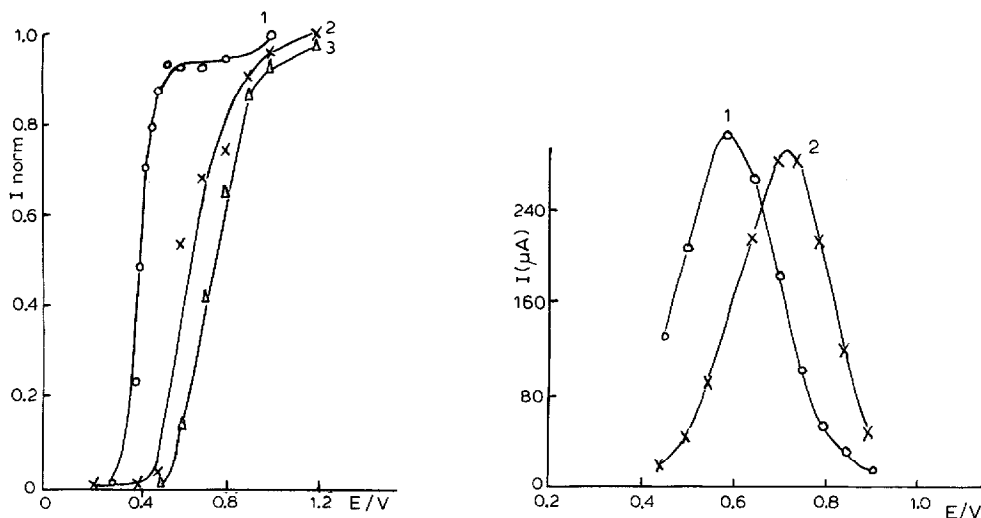

Fig. 2. D.c. hydrodynamic polarograms. 1, Phenothiazine [FIA mode, type b detector, acetonitrile–acetate buffer (1:1), flow-rate 0.40 ml/min]; 2, hydroquinone; 3, p-methoxyphenol [HPLC mode, type a detector, 100 × 6 mm I.D. C_{10}-bonded column, methanol–phosphate buffer (1:1), flow-rate 1.5 ml/min].

Fig. 3. Differential pulse hydrodynamic polarograms. 1, Hydroquinone; 2, p-methoxyphenol. HPLC mode, type a detector, 100 × 6 mm I.D. C_{10}-bonded column, methanol–phosphate buffer (1:1), flow-rate 1.5 ml/min, E = 0.45 V, amplitude 100 mV, frequency 2 Hz.
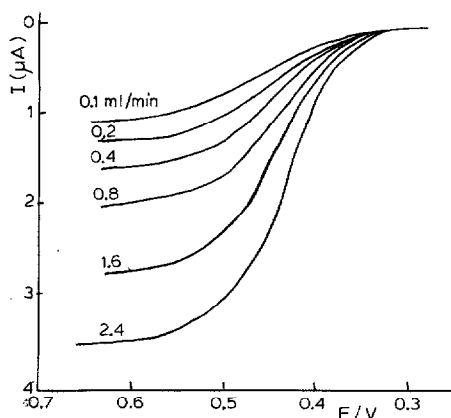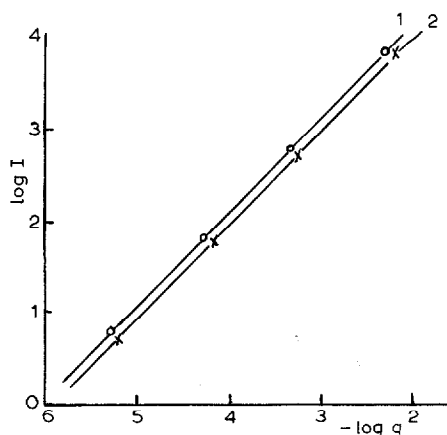
Fig. 4. Calibration graphs. 1, Hydroquinone; 2, p-methoxyphenol. HPLC mode, type a detector, 100 × 6 mm I.D. C_{10}-bonded column, methanol–phosphate buffer (1:1), flow-rate 1.5 ml/min, $E = 0.8$ V.

Fig. 5. Constant-flow polarograms for phenothiazine: $10^{-5}\%$ (w/w) acetonitrile–acetate buffer (1:1) solution. Type b detector. Scan rate 2 mV/s.

cells; wall-jet dynamic specific features are not realized. Both cell types are well defined by the equation

$$I = kC_0\bar{u}^{1/3}$$

where k includes terms relating to the number of electrons, the Faraday constant, diffusion coefficient, kinematic viscosity of the solution and the cell dimentions, C_0 is the depolarizer concentration and $\bar{u}$ is the average eluent volume flow-rate.

The employment of this equation based on the assumption of stationarity of the depolarizer flow in the pulse injection mode (HPLC and PIA) is not correct, because a narrow concentration profile appears, having an approximately Gaussian shape. Each of the above modes should be considered individually; therefore, whilst studying the relationship between eluent flow-rate and I_{lim} for the type b detector we recorded first the hydrodynamic polarograms at different continuous flow-rates of phenothiazine solution (Fig. 5). The polarograms show good reproducibility and, as was previously noted, there is almost no variation of $E_{1/2}$ with flow-rate. Fig. 6 shows the relationship between I_{lim} and flow-rate. It can be seen that I_{lim} increases abruptly with increase in flow-rate. Plotting of this relationship for log $I_{\text{lim}} = f(\log \bar{u})$ by the least-squares method ($r = 0.997$) gives a slope of 0.36 ± 0.04, which corresponds closely to the theoretical value of 1/3 for stationary flow. This method of recording hydrodynamic polarograms at different depolarizer flow-rates through the detector is very informative for both cell and depolarizer evaluation.

A detector operating in the pulse injection mode can be considered as a concentration detector. It can easily be shown with all necessary approximations that the peak height, i.e., current, should be independent of flow-rate, whereas the peak width and the peak area, or the response in coulombs, undergo reduction with increase in flow-rate.

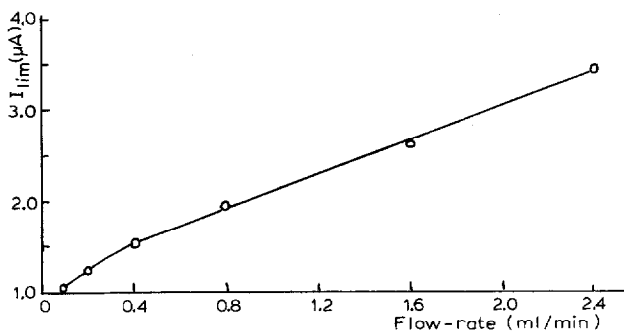An experimental relationship between the peak height and eluent flow-rate for

Fig. 6. Relationship between I_{lim} and flow-rate (see Fig. 5).

different electrochemical detector constructions has been given[13]. As a rule, the peak height increases slightly with increasing flow-rate. It has been demonstrated[15] that the scatter is fairly high, but the value of m in the equation $I = f(u^m)$ approaches zero (0.05), $i.e.$, there is a very weak relationship between peak height and flow-rate. In Fig. 7 the relationship between peak height and flow-rate for a type b detector for the FIA mode is shown. The upper points were obtained for the pure electrode and the others for states of electrode fouling (1-week experiment). The upper curves demonstrate a tendency for some growth, but the range of values does not exceed 25%. The peak heights for the most fouled electrode do not depend on flow-rate.

The curves for the increase in peak height have three regions. For low flow-rates (0.1–0.4 ml/min) and flow-rates higher than 0.8 ml/min the peak height does not depend on the flow-rate, whereas in the range 0.4–0.7 ml/min there is a transition region. The same tendency has been found previously[13], but all efforts to explain the phenomenon have been unsuccessful. One can only accept that the behaviour of the electrochemical detector is similar to that of the conventional concentration detector and the dependence of peak height on flow-rate is low.

Fig. 8 shows the dependence of the electrochemical yield for phenothiazine on
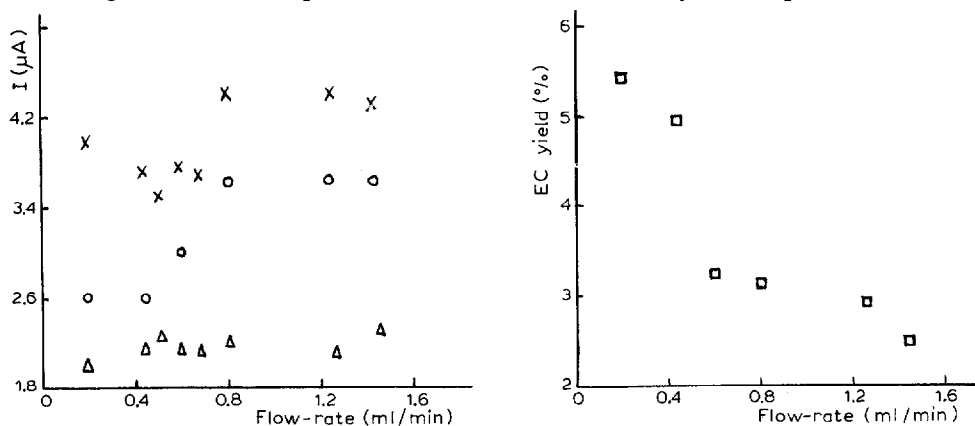

Fig. 7. Relationship between I and flow-rate, FIA mode. Type b detector, acetonitrile–acetate buffer (1:1), 5 μl of 10^{-2}% (w/w) solution. $E = 1.0$ V.

Fig. 8. Relationship between electrochemical yield and flow-rate (see Fig. 7).
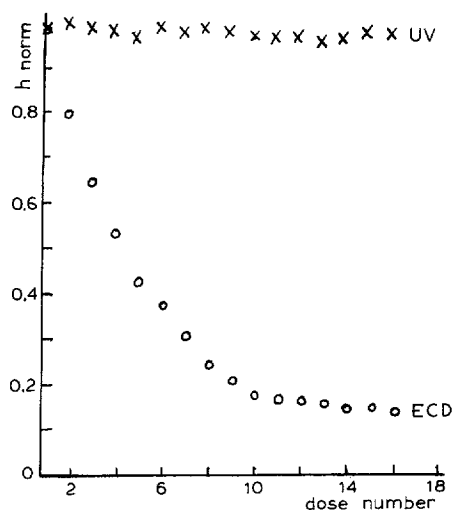
Fig. 9. Electrochemical and UV detector responses (normalized peak height) for repeated diphenylolpropane sampling. Type a electrochemical detector, 100×6 mm I.D. C_{10}-bonded column, acetonitrile-acetate buffer (1:1), flow-rate 1.5 ml/min, $E = 1.2$ V. Each sample contained 0.01 mg.

flow-rate. As could be predicted, the yield decreases from 5 to 2% with increasing flow-rate, *i.e.*, to a common value for amperometric detectors[4,5].

Detector noise

The residual current for both detectors is $5 \cdot 10^{-7}$–10^{-8} A and can be compensated for in the process of measurement. The detector sensitivity is determined by the noise level, which is 0.5 nA for type a ($E = +1.0$ V, flow-rate 1.5 ml/min) and 0.2 nA for type b ($E = 1.0$ V, flow-rate 0.2 ml/min). The noise level falls with a reduction in E and data reported here are dependent on conditions used for antioxidant analysis (detection limits calculated on the basis of these data are given below). The observed noise is typical of low-frequency noise and depends on the pump pulsations. It can be assumed that the employment of a pulsless delivery system would have allowed the noise level to be reduced considerably.

Electrochemical detector signal repeatability and reproducibility

It is believed that a newly polished GC electrode is more sensitive than when it is stabilized[10]. The detectors developed here also showed a 10–15% initial sensitivity increase, which is higher than its subsequent stabilized value. A 1-week test run after signal stabilization gave a relative standard deviation (repeatability) of 1.5–3% during a 1-day chromatographic analysis of hydroquinone, p-methoxyphenol and phenothiazine and the same reproducibility on each subsequent day.

Working electrode fouling

We were unable to determine hydroquinone, p-methoxyphenol and diphenylolpropane simultaneously with the type a detector. The relative sensitivity for diphenylolpropane was much lower than that for the other compounds. Repeated sampling of diphenylolpropane with parallel recording using electrochemical and UV detectors
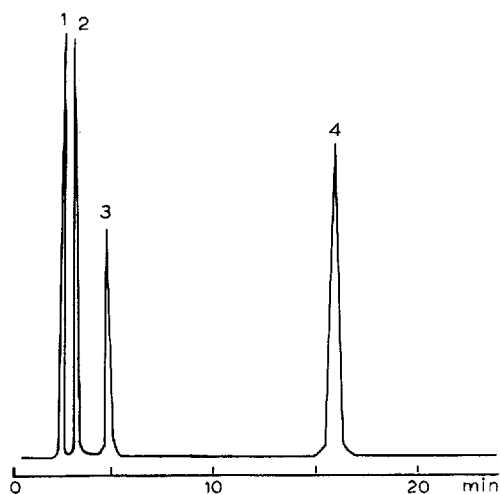
Fig. 10. Chromatogram obtained during the separation of (1) hydroquinone, (2) p-methoxyphenol, (3) diphenylolpropane and (4) phenothiazine. Type b detector, 100 × 2.4 mm I.D. C_{10}-bonded column, acetonitrile–acetate buffer (1:1), flow-rate 0.2 ml/min, E = 1.0 V, 1.4 μl, $4 \cdot 10^{-2}$% (w/w) of each component, 1 μA full-scale.

was performed. The results are shown in Fig. 9 and demonstrate an abrupt loss of electrochemical detector sensitivity. For the type b detector the electrode fouling was much less pronounced, which apparently can be explained by the abrasive effect of the eluent solution in the narrow channel at a rather high linear velocity.

Analytical application

    Both types of detector were used in the HPLC analysis of hydroquinone, p-methoxyphenol, phenothiazine and diphenylolpropane impurities in acrylic monomers. Reversed-phase HPLC separation was not subject to any difficulties (Fig. 10). We could expect two kinds of problems: (a) low eluent solubility for most monomers, which could cause sampling disturbances, as it was necessary to introduce 1–5 mg of monomers for the analysis; (b) possible detector response for such large amounts of monomer.

    The solubility of most monomers in water is lower than 1.5–2%; in acetonitrile–acetate buffer eluent the solubility is twice as high. To prevent the formation of two layers at the column inlet, the monomer sample was diluted with acetonitrile (1:1). The elution volume of monomers is not higher than 0.3–0.5 ml (6 mm I.D. column, UV recording), so the average monomer concentration in the eluted zone is below the solubility, and it is safe to say that a homogeneous mixture is entering the detector.

    Fortunately, the second assumption also was not true, *i.e.*, both detector constructions proved to be insensitive to the monomers and did not record a false signal even at a sensitivity of 0.8 nA/cm. The detectors responded only to the presence of the oxidized substances. The absence of these two undesirable effects allowed the high-sensitivity analysis of the antioxidants. Because the electrochemical detector can be regarded as a concentration detector, the main chromatographic parameter de-

termining the sensitivity of analysis is the maximum peak concentration. Two column types, of 6 mm and 2.7 mm I.D., were employed for the analysis. As the increase in the maximum peak concentration is proportional to the square of the column diameter, one would expect a 5-fold sensitivity increase for the 2.7-mm I.D. column. For the 6-mm I.D. column the limit of detection of all the antioxidants considered was $5 \cdot 10^{-7}$–$7 \cdot 10^{-7}$ mg (three times the noise level). Hence, on introducing a 1-mg sample, antioxidant concentrations at the $5 \cdot 10^{-5}\%$ (w/w) level can be determined; however based on our experience we can say that a 10-μl sample of monomer solution in acetonitrile (1:1) does not overload the column, and therefore a sensitivity of $1 \cdot 10^{-5}\%$ (w/w) may be achieved. Under these conditions we determined methyl, butyl and octyl methacrylate and butyl and octyl acrylate monomers. Propylene glycol monomethacrylate, ethylene glycol monomethacrylate and methoxydiethylene glycol methacrylate were determined on the 2.7-mm I.D. column. As expected, the detection limit dropped to $8 \cdot 10^{-8}$–$2 \cdot 10^{-7}$ mg (hydroquinone).

However, in some instances the monomers influenced the detector response; during the determination of triethylene glycol dimethacrylate, the detection limit for hydroquinone was $1 \cdot 10^{-6}$ mg, whereas during the determination of polyethylene glycol 600 dimethacrylate on the 2.7-mm I.D. column was $1 \cdot 10^{-7}$ mg, *i.e.*, worse than for the other monomers. The reason for this phenomenon is not understood but the sensitivity obtained is adequate for any high-purity monomer analysis.

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