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Note

Alternating-current polarographic detection for reversed-phase ion-pair high-performance liquid chromatography of some benzoic acids

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Electrochemical detection has been successfully employed in reversed-phase ion-pair high-performance liquid chromatography (HPLC) in the separation of catecholamines and some other 3,4-dihydroxyphenylamines and carboxylic acids[1–4], with an ionic detergent, octylsulphate[1–3] or anions of strong inorganic acids and of trichloroacetic acid[4] as counter ions. Detection was performed with the aid of a flow-through thin-layer cell with a carbon paste sensor electrode. Because at carbon electrodes the differential capacity of the double layer at the electrode–solution interface cannot be measured reproducibly, only the faradaic response could be monitored in the amperometric mode of operation. Hence the determination was limited to redox substances only. However, using a dropping mercury electrode (DME) as the sensor electrode, non-faradaic signals can also be exploited and thus the presence of electroinactive substances that cause the change in the differential capacity can be detected in the column effluent[5–11]. Alternating-current (a.c.) polarography in both the total current[5–7,10,11] and phase-sensitive[8] modes and also alternating-voltage (a.v.) polarography[9,10] have been used for this purpose and it was possible to determine aromatic hydrocarbons[5–7], alkyl alcohols, alkyl carboxylic and sulphonic acids, different surfactants of the polyethylene glycol monoether type[8], cholanoic acids[9,10] and taurine conjugates of cholanoic acids[11].

In this paper we demonstrate the usefulness of a.c. polarographic detection in which the non-faradaic admittance is recorded for ion-pair HPLC. Benzoic, 4-hydroxybenzoic and acetylsalicylic acids, *i.e.*, compounds that are electroinactive but surface active on the DME, were chosen as model compounds. The separation conditions for these compounds in ion-pair HPLC with UV detection have been given elsewhere[12].

EXPERIMENTAL

Acetonitrile "for chromatography" (E. Merck, Darmstadt, G.F.R.) and doubly distilled water were used for the preparation of mobile phase solutions. Tetrabutylammonium hydroxide (TBAOH) was prepared from tetrabutylammonium iodide (E. Merck) according to a described procedure[12]. All other chemicals were of analytical-reagent grade.

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Chromatographic experiments were performed using a Model 302 HPLC apparatus with a 5-μl injection valve and a type FTPD-101[13,14] flow-through polarographic detector (both made at the Institute of Physical Chemistry, Warsaw, Poland). The column used was a 250 × 4 mm I.D. stainless-steel column slurry packed[15] at 435 kg/m^2 with 10-μm LiChrosorb RP-18 (E. Merck) using tetrachloromethane–dioxan (1:1, v/v) as the suspending liquid. The flow-rate of the mobile phase was 1 ml/min.

A.c. polarographic recording was carried out with the use of a Radelkis OH-105 universal polarograph (Radelkis, Budapest, Hungary), operating at a frequency of 60 Hz and an amplitude of the alternating voltage, $\Delta E_{a.c}$, of 15 mV. The characteristics of the polarographic capillaries measured in the mobile phase solution were as follows in the steady-state a.c. polarographic experiments: m = 5.62 mg/sec, t_1 = 1.6 sec at E = −0.1 V *vs.* Ag/AgCl and h_{Hg} = 60 cm; and in HPLC experiments: m = 3.06 mg/sec, t_1 = 0.81 sec at E = −0.1 V *vs.* Ag/AgCl and h_{Hg} = 55 cm. The potential applied to the DME in the HPLC experiments was E = −0.1 V.

RESULTS AND DISCUSSION

A.c. polarographic detection of non-faradaic signals is possible only if a difference is observed between the curves of the double layer capacity *vs.* potential measured in blank solution and in the presence of a sample substance. Therefore, prior steady-state experiments are necessary for this purpose. For benzoic and 4-hydroxybenzoic acids adsorption studies at the DME only from aqueous solutions have been reported[16,17]. Therefore it was necessary to record a.c. polarographic curves for all of the sample acids in a solution of the composition of the mobile phase for ion-pair reversed-phase HPLC (Fig. 1). The most pronounced differences between

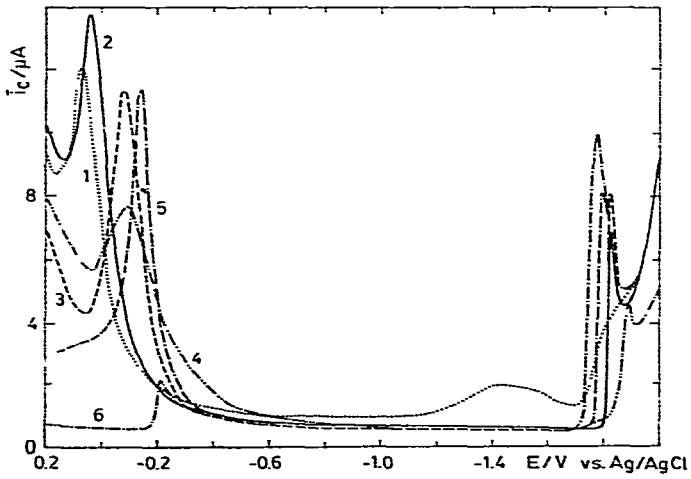

Fig. 1. A.c. polarographic mean capacity current, $\bar{i}_c$, as a function of potential for acetontrile–1/15 M phosphate buffer (pH 6) (20:80, v/v) (1), containing $2 \cdot 10^{-2}$ M TBAOH (2) and $2 \cdot 10^{-2}$ M benzoic acid (3), $2 \cdot 10^{-2}$ M acetylsalicylic acid (4), $5 \cdot 10^{-3}$ M 4-hydroxybenzoic acid (5) or $2 \cdot 10^{-2}$ M 4-hydroxybenzoic acid (6).

the curves of the mean capacity current, $\bar{i}_C$, *vs.* E recorded in the presence and in the absence of the acids are observed at a relatively positive potential range, which is why the potential, $E = -0.1$ V *vs.* Ag/AgCl, was further applied to DME in HPLC experiments (Fig. 2). The $\bar{i}_C$ *vs.* E curve for the blank solution is altered more by 4-hydroxybenzoic acid than by acetylsalicylic acid and least by benzoic acid. The elution of the acids proceeds in the same order (Fig. 2). This order, however, contrasts with the regularity observed, for instance, for homologous alkylbenzenes, for which the stronger the adsorption at the DME the greater is the retention time in a reversed-phase system[18]. In the multi-component system studied here a detailed discussion of the properties of the double layer at the DME is difficult and was not attempted because much more experimental data would be needed.

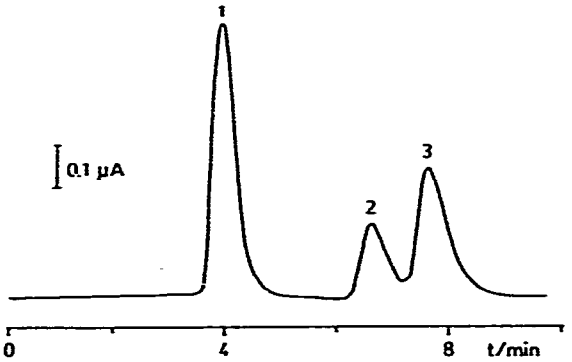

Fig. 2. HPLC results for 125 nmole of each of (1) 4-hydroxybenzoic acid, (2) acetylsalicylic acid, (3) benzoic acid with a.c. polarographic detection. $E = -0.1$ V *vs.* Ag–AgCl, $\Delta E_{a.c.} = 15$ mV; mobile phase, acetonitrile–1/15 M phosphate buffer (pH 6) (20:80, v/v)–0.02 M TBAOH; flow-rate, 1 ml/min; column, 250 × 4 mm I.D., LiChrosorb RP-18 (10 μm); sample size, 5 μl.

It is worth noting that in the mobile phase of pH 2 in the absence of TBAOH, *i.e.*, under conditions relevant to reversed-phase HPLC of the acids[12], alterations of the $\bar{i}_C$ *vs.* E curves by the acids were not large enough for a.c. polarographic detection to be applied, using a chosen potential common for all acids.

Tetrabutylammonium as the counter ion was added to the solution of the mobile phase in the form of TBAOH because other anions were inconvenient either for detection or for separation. For instance, perchlorates, which exhibited negligible adsorption at the DME, interfered strongly in ion-pair separations and peaks 2 and 3 in Fig. 2 were not separated. On the other hand, iodides, which have no effect on ion-pair separations, affected strongly the $\bar{i}_C$ *vs.* E curves and no effect of the acids studied could be measured. The tetrabutylammonium cation is strongly adsorbed at the DME in aqueous solutions at negative potentials[19–21]. It drastically changed the shape of the $\bar{i}_C$ *vs.* E curves in the mixed aqueous–methanol solution of the mobile phase at negative potentials and a sharp peak was observed (curve 2 in Fig. 1). However, at positive potentials the TBA cation caused no characteristic changes in the $\bar{i}_C$ *vs.* E curves.

Fig. 3 shows (a) calibration graphs of the peak surface area against the number of moles injected and (b) the logarithm of the peak surface area against the logarithm

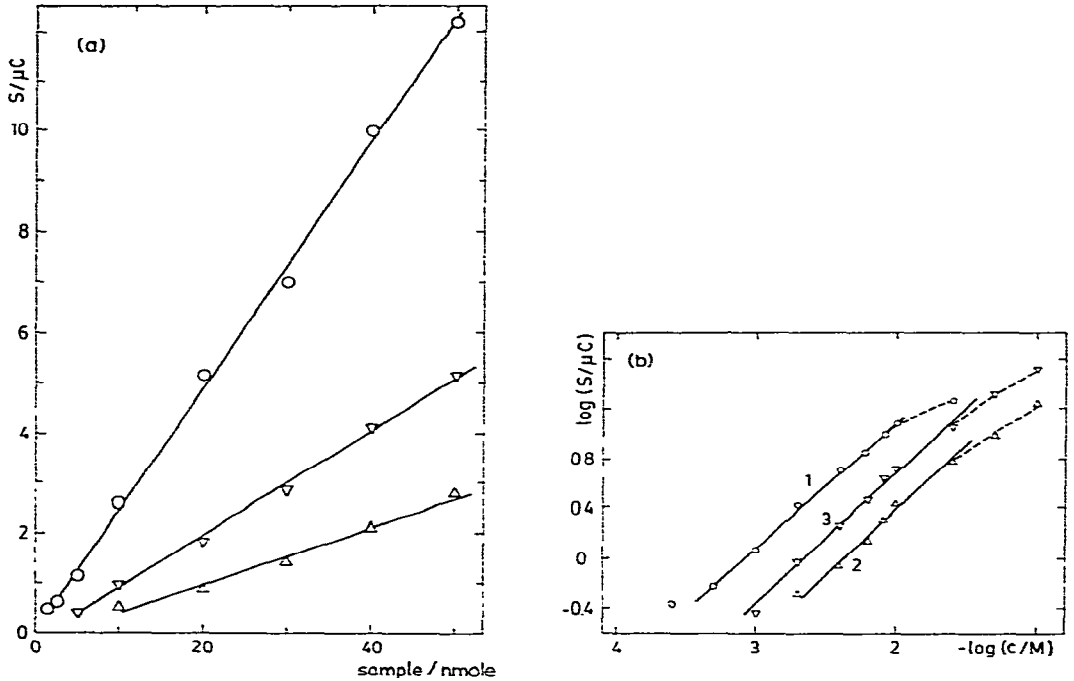

Fig. 3. Dependence of (a) peak surface area on amount of sample injected and (b) logarithm of peak surface area on logarithm of concentration, for (1) 4-hydroxybenzoic acid, (2) acetylsalicylic acid and (3) benzoic acid. Separation conditions as in Fig. 2.

of concentration over a wider concentration range. The response index of the detector, calculated for all acids from the slope of the curves in Fig. 3b, was close to unity (the mean value of the correlation coefficient was 0.997) over a concentration range of at least one order of magnitude. At higher concentrations deviations from linearity were observed (broken lines in Fig. 3b) owing to the non-linearity of the adsorption isotherms of acids at the DME. For concentrations of 4-hydroxybenzoic acid exceeding $4 \cdot 10^{-2}$ *M* the HPLC peak was split into two. This may be explained by the observed strong dependence of the adsorption peak on the $\bar{\tau}_C$ *vs.* *E* curve on the concentration of 4-hydroxybenzoic acid *i.e.* with an increase in the concentration of 4-hydroxybenzoic acid the adsorption peak decreases and shifts towards more negative potentials. At high concentrations the shape of the $\bar{\tau}_C$ *vs.* *E* curve is changed and a decrease in $\bar{\tau}_C$ is observed at positive potentials (curve 6 in Fig. 1). This may indicate phase transition of the adsorbate at the DME. Because of this complicated adsorption effect, careful potential control is essential for detection in HPLC experiments.

The sensitivities calculated from the intercept in Fig. 3a or the slope in Fig. 3b of the straight lines were 1240, 280 and 480 μC/mole for 4-hydroxybenzoic, acetylsalicylic and benzoic acid, respectively. The linear dynamic range extends for at least one order of magnitude and the detectability of 4-hydroxybenzoic acid is 0.625 nmole per 5-μl injection at a signal-to-noise ratio of 2.

In conclusion, it has been demonstrated that under carefully chosen conditions a.c. polarographic detection based on the changes in the double layer differential capacity is possible even in such a complicated system as reversed-phase ion-pair HPLC.

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