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ELECTROKINETIC DETECTION OF LAURIC, MYRISTIC, STEARIC AND ARACHIDIC ACIDS SEPARATED BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The possibility of electrokinetic detection, by means of our previously described detector, of higher fatty acids (lauric, myristic, stearic and arachidic), separated by reversed-phase high-performance liquid chromatography, is presented. LiChrosorb RP-18 (10 μm) was used as stationary phase and methanol as mobile phase. The sample volume was 5 μl. The working unit of the detector was a poly(tetrafluoroethylene) capillary tube (15 × 0.35 mm I.D.). The results were obtained from measurements on the streaming current, with use of a streaming-current detector (SCD), and on the streaming potential, with use of a streaming-potential detector (SPD). The detectability of the SPD was $1.5 \cdot 10^{-10}$ mole ($3 \cdot 10^{-5}$ *M*) and that of the SCD was $2 \cdot 10^{-9}$ mole ($4 \cdot 10^{-4}$ *M*). Repetitive sample injections resulted in peak heights with relative standard deviations of $< 1\%$ for SPD and 2% for SCD.

INTRODUCTION

The generation of the streaming current and streaming potential during fluid flow through capillary systems has been known since the second half of the nineteenth century[1–5,26]. The application of these effects in detectors for high-performance liquid chromatography (HPLC) dates from only a few years ago. The first such detector for reversed-phase HPLC (RP-HPLC), with measurement of the streaming potential, was constructed by Ando *et al.* in 1967[6]. The same authors also constructed the streaming-current detector (SCD) and applied it in RP-HPLC[7] and in ion-exchange HPLC[8].

The elektrokinetic detection problem was also studied by a research group from Brno[9–14]. The authors described several models of SCD and used them in normal-phase HPLC and in open-tubular capillary liquid chromatography.

SCD was recently used also for assessing the degree of water contamination[15].

In our previous papers[16,17] we described our SPD applied to the detection of volatile fatty acids separated by RP-HPLC and by ion-pair RP-HPLC. In the present paper, SCD and SPD in RP-HPLC and their applications to the detection of higher fatty acids are presented.

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EXPERIMENTAL

In the investigations presented here our detector[16,17], with a poly(tetrafluoroethylene) (PTFE) capillary (15 × 0.35 mm I.D.), was used. The streaming potential (E) and streaming current (I) were measured by using a Z. R. Kasprzak-Unitra, T-219 (Warszaw, Poland) electrometer and were recorded on a Sefram, T-PE (France) recorder. An IChF PAN, T-302 (Warsaw, Poland) high-performance liquid chromatograph equipped with a 5-μl injection valve and a stainless-steel column (150 × 4 mm I.D.) slurry-packed with LiChrosorb RP-18, 10 μm (E. Merck, Darmstadt, F.R.G.) was used. Methanol (E. Merck) and higher fatty acids (P.O.Ch., Gliwice, Poland) were of analytical grade. The mobile phase was degassed prior to chromatographic measurements.

RESULTS AND DISCUSSION

The results for the electrokinetic detection of lauric, myristic, stearic and arachidic acids, separated by use of analytical-grade methanol as a mobile phase, are presented. In such conditions, the inner resistance of the detector calculated from change of measured potential with the change of electrometer resistance, was *ca.* $5 \cdot 10^9\ \Omega$. In Fig. 1, dependence of E and I on volumetric (J) and linear (u) flow-rate of mobile phase is presented. As can be seen in Fig. 1, in the investigated range of flow-rate E and I are proportional to u, which agrees with the Smoluchowski equation[1]. Contrary to the findings in our previous papers[16,17] changes in the sign of E or I with increase of u have not been observed in these experiments. With increase in flow-rate, the chromatographic peaks measured as changes of streaming current (ΔI) (Fig. 2) or of streaming potential (ΔE) (Fig. 3) increased also. A change of sign was observed only in the case of ΔI for $J = 0.7$ cm^3/min. This change is observed for the higher value of J together with the increase in concentration of the acids. This

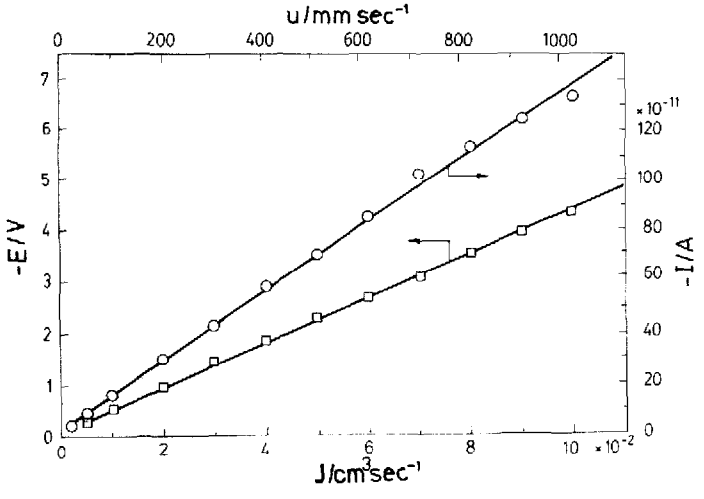

Fig. 1. The dependence of streaming potential (E) and streaming current (I) on volumetric (J) and linear (u) flow-rate of methanol.

effect was encountered because the measured E consists of a few additive component[18]:

$$E = E_T + U_E + U_c + U_{d-h} + U_{d-l}$$

where E_T is the streaming potential, U_E is the electrodes assymetry potential, U_c is the potential difference due to a concentration difference in the absence of pressure difference, and U_{d-h}, and U_{d-l} are the diffusion potentials in the high- and low-pressure points, respectively, of the capillary.

The greatest contributions to E derive from E_T and U_E. The latter value is independent of flow-rate, its small value is deserved even when liquid doesn't flow, while the former is proportional to it and conforms to the Smoluchowski equation[1]. When these potentials have opposite signs in certain values of J, a change of measured E was observed[16,17]. In the same way, changes of sign of ΔE and ΔI with the increase of J may be described. These effects were more noticeable in water because of the greater electrode asymetry potential[16,17].

The dependence of ΔI and of ΔE on the concentration of acids is shown in Fig. 4 and 5, respectively. HPLC chromatograms of the mixture of acids recorded using SCD (a) and SPD (b) are presented in Fig. 6. It can be seen that the linear dynamic range was close to one order of the magnitude of the acids concentration. Because the lauric and myristic acid peaks are superimposed on each other, and the negative peak observed at the beginning of the chromatogram calibration curves for

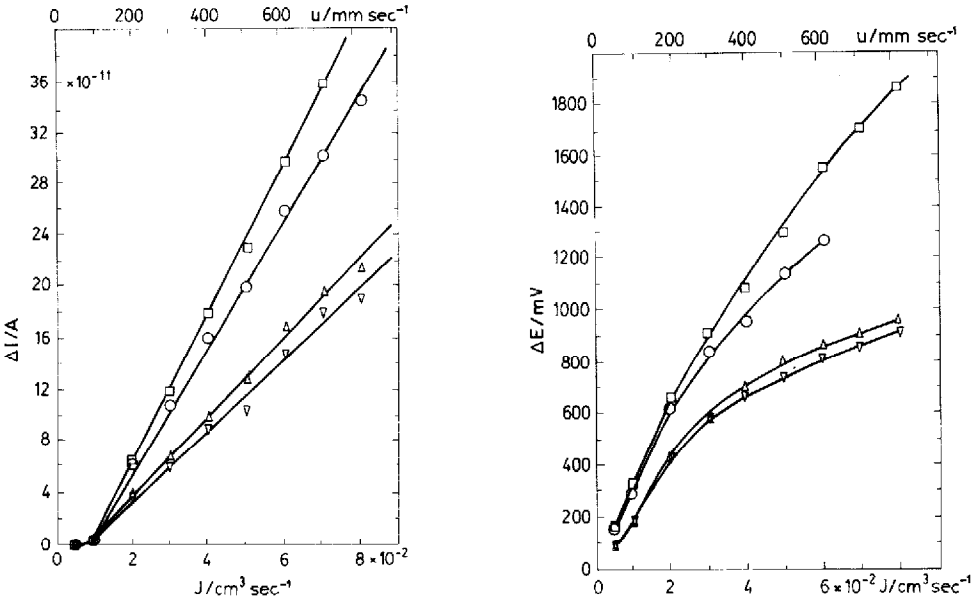

Fig. 2. Dependence of heights of chromatographic peaks measured as changes of streaming current (ΔI) on flow-rate of mobile phase J or u. Analysed acids: ○ = lauric; □ = myristic; △ = stearic; ▽ = arachidic. Elektrokinetic detector with PTFE (15 × 0.35 mm I.D.) capillary. Stainless-steel column (150 × 4 mm I.D.), LiChrosorb RP-18, 10 μm. Sample volume, 5 μl. Mobile phase, methanol.

Fig. 3. Dependence of heights of chromatographic peaks measured as changes in streaming potential (ΔE) on flow-rate J or u. Other conditions as in Fig. 2.

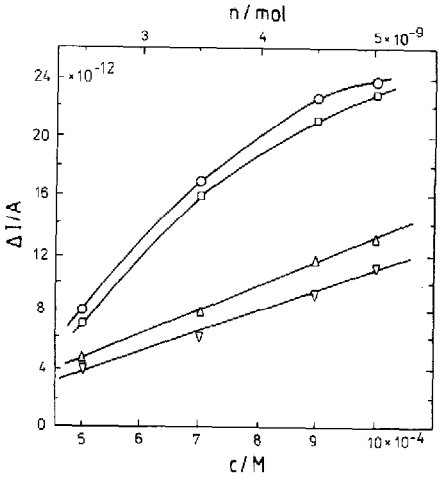

Fig. 4. Dependence of ΔI on concentration of analysed acids (c) or number of their moles in samples (n). Flow-rate, 10^{-2} cm³/sec. Other conditions as in Fig. 2.

these acids are not linear, the height of the lauric acid peak was lower than that of the peak for myristic acid. The same effects were observed for volatile fatty acids[16,17].

The detectability of acids using SCD was equal to $2 \cdot 10^{-9}$ mole ($4 \cdot 10^{-4}$ M) and SPD $1.5 \cdot 10^{-10}$ mole ($3 \cdot 10^{-5}$ M), and were independent of flow-rate. The better detectability of SPD is caused by the fact that it reacts not only like SCD on changes in permittivity, electrokinetic potential and viscosity, but also on changes in conductivity, which may be very high[16,17]. It was observed that, as in the detection of volatile fatty acids[16,17], the retention of acids depends on their concentration. It appeared that these changes (Fig. 7) are so small that changes in the sequence of acids were not observed on the chromatogram. This may be explained by the as-

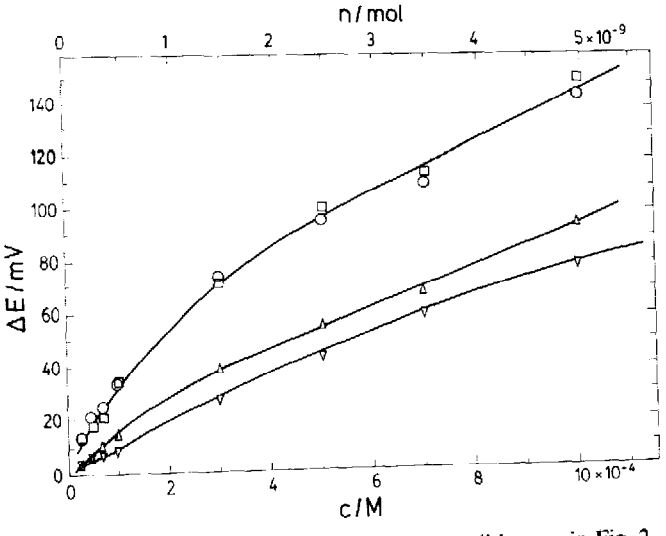

Fig. 5. Dependence of ΔE on c and n. Other conditions as in Fig. 2.

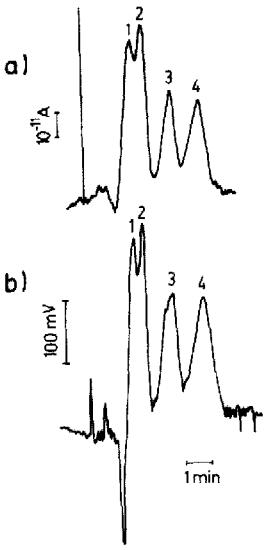

Fig. 6. HPLC chromatograms of higher fatty acids 1 = lauric; 2 = myristic; 3 = stearic; 4 = arachidic of concentration 10^{-3} *M* recorded by SCD (a) and SPD (b). Flow-rate; $2 \cdot 10^{-2}$ cm^3/sec. Other conditions as in Fig. 2.

sumption that, in the chromatographic processes, only undissociated forms of fatty acids are retained on the stationary phase. So their dissociated form may exist only in the mobile phase. From the well-known dependences of the retention time (t_R) on the capacity coefficient k' and of k' on the partition coefficient[19] and the Ostwald dilution law[20] we obtained:

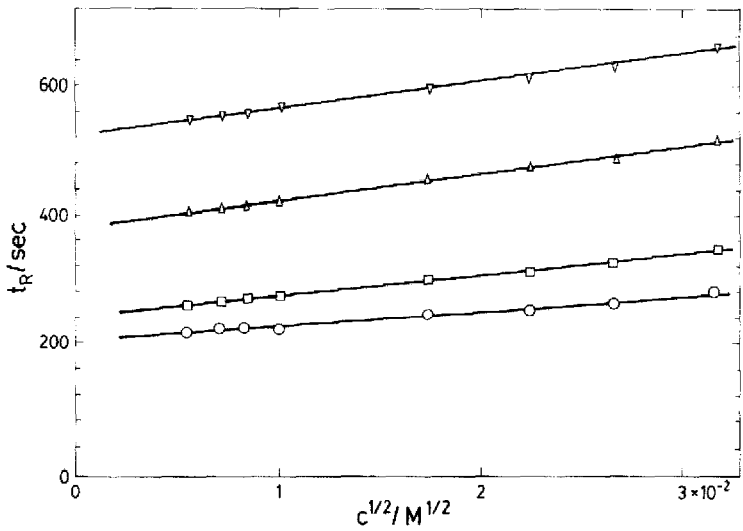

Fig. 7. Dependence of retention time (t_R) of higher fatty acids on square root of their concentration $c^{1/2}$. Flow-rate, 10^{-2} cm^3/sec. Other conditions as in Fig. 2.

$$k' = \frac{1 - \alpha}{\alpha} \cdot \frac{A}{V}$$

and then

$$t_R = \frac{L}{u}\left[1 + \frac{A}{V}\left(\frac{c^{1/2}}{K_a^{1/2}} - 1\right)\right]$$

where L is the length of the chromatographic column, α is the degree of dissociation, A is the surface area of the stationary phase, V is the volume of the mobile phase, and K_a is the acids dissociation constant.

This equation shows that the t_R of weak acids are proportional to the square root of their concentration:

$$t_R = \text{const. (1)} + \text{const. (2)} \cdot c^{1/2}$$

It agrees with the experimental measured dependences t_R on concentrations of higher fatty acids presented in Fig. 7. Ten-fold repetitive injections yield $10^{-2}M$ myristic acid peak heights with relative standard deviations (R.S.D.) of $< 1\%$ for SPD and 2% for SCD. It is caused by the lower stability of the baseline current in comparison with the baseline potential. The so-called day-to-day reproducibility was *ca.* 6%. This was due to irreversible changes of the inner surface of the capillary[16,17] and by changes in retention times of the acids.

At the start of a set of measurements it takes some time before a stationary state is reached. The time necessary to establish the baseline E and I after change of J was of the order of 30 and 5 min, respectively. The sample injection yields on the chromatograms spikes (4 sec for SPD and 1 sec for SCD) generated by the disturbance of flow-rate by the movement of the injector. They play the role of markers. Because the reological relaxation time[21] and the electrical one[22] calculated from the Debye equation are equal to $4 \cdot 10^{-3}$ sec and $2 \cdot 10^{-3}$ sec, respectively, these effects may be caused only by the settlement of adsorption equilibrium in the capillary.

The mobile phase was degassed prior to the chromatographic measurements. Along the chromatographic column a large pressure gradient exists. This can cause gas to evolve in the form of bubbles on its end, which subsequently greatly disturb electrical measurements. The injection of pure mobile phase results in two peaks on the chromatogram, which disappear after degassing.

CONCLUSIONS

It was shown that, with the electrokinetic detector with a single PTFE capillary, it is possible to detect the higher fatty acids in RP-HPLC. SPD is characterised by higher linear dynamic range, better detectability ($1.5 \cdot 10^{-10}$ mole) and reproducibility (R.S.D. = 1%) than SCD ($2 \cdot 10^{-9}$ mole and 2%, respectively).

The electrokinetic detector may work only with certain chromatographic setups. It may by used in RP-HPLC especially when the mobile phase is pure solvent without any electrolyte. It cannot be used with mobile phases of high conductivity

because a short-circuit effect occurs[16,17]. The results obtained with the electrokinetic detector are similar, in respect of the application, to those obtained with the conductivity and capacitance detectors[23–25].

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