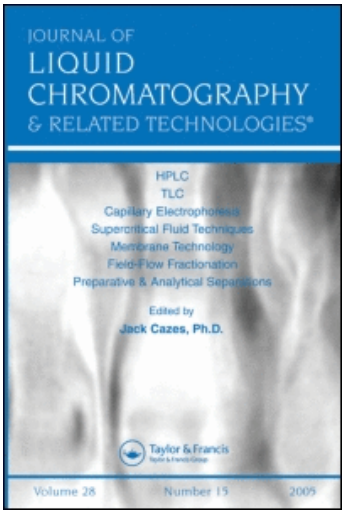


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# Electrokinetic Detection in Reversed Phase High Performance Liquid Chromatography Part II. Quaternary Ammonium Ion-Pairs of Some Volatile Fatty Acids

Wiktor Kemula[a]; Bronisław K. Głód[a]; Włodzimierz Kutner[a]
[a] Institute of Physical Chemistry Polish Academy of Sciences, Warszawa, Poland

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# ELECTROKINETIC DETECTION IN REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PART II. QUATERNARY AMMONIUM ION-PAIRS OF SOME VOLATILE FATTY ACIDS

Wiktor Kemula, Bronisław K. Głód and Włodzimierz Kutner
Institute of Physical Chemistry
Polish Academy of Sciences
Kasprzaka 44/52
01-224 Warszawa
Poland



## ABSTRACT

The conditions of electrokinetic detection were elaborated for tetraethylammonium, $TEA^+$, ion-pairs of volatile fatty acids(acetic, propionic, isobutyric and valeric) in reversed phase high performane liquid chromatography, HPLC. To eliminate the dependence of the retention volume $V_R$, on the concentration of acids found in the first part of this work, $TEA^+$ was added to the non-buffered mobile phase. In the presence of pairing $TEA^+$ ions, $V_R$ appeared to be invariant with the concentration of acids in the sample in a definite concentration range. The detectability of the detector, with a polytetrafluoroethylene, PTFE, capillary as its working unit, was of the order of $10^{-10}$ mole and the reproducibility was 5% (relative standard deviation, R.S.D., for ten consecutive injections). The linear dynamic range extended over two orders of magnitude of the acid concentrations.

## EXPERIMENTAL

The detector and chromatograph construction as well as the chromatographic procedure have been described in part 1 of the present work [1]. As working units of the detector PTFE, borosilicate glass (Jena, GDR) or 1H18N9T stainless-steel open tube capillaries of different diameter and lengh were used.



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The mobile phase solution was prepared using redistilled water and analytical grade methanol (E. Merck, Darmstadt, FRG). Tetraethylammonium perchlorate, $TEACIO_4$, was prepared according to [2] by precipitation of the water insoluble $TEACIO_4$ from the aqueous solution of analytical grade tetraethylammonium bromide, TEABr, (Reahim, USSR) and $HClO_4$ of the same grade (VEB Laborchemie—Apolda, GDR) at 60°C.

## RESULTS AND DISCUSSION

In part 1 of the present work [1] the HPLC separation and electrokinetic detection conditions of volatile fatty acids (acetic, propionic, isobutyric and valeric) were eleborated. The retention volumes of acids appeared, however to be strongly dependent on their concentrations. That is why in the present paper attempts are made to eliminate this effect by separating the acids in the ion-pair reversed phase HPLC mode.

The electrokinetic measurements were carried out with dielectric, i.e. PTFE, borosilicate glass or metal, i.e. stainless-steel, capillaries of different dimensions as working units of the detector. The results, if not stated otherwise, have been obtained with the PTFE capillary (20 x 0.4 mm I.D.).

The retention volumes of the studied acids in ion-pair reversed phase chromatography appeared to be independent of the concentration of acids in the injected sample in a definite range of concentrations, depending on the concentration of $TEA^+$ in the mobile phase. Thus, the chromatogram obtained under these conditions (Fig. 1) does not differ much from that obtained for acids in the absence of $TEA^+$ (cf. Fig. 1 [1]). Also the dependence of changes of the streaming potential, $\Delta(\Delta\varphi)$, on the mobile phase flow rate, $J$, was analogous to that presented in Fig. 4 of ref. [1]. The change of the sign of chromatographic peak heights (Figs. 1 a and 1 b) is observed as previously for flow rates higher than some critical value, $J^O$. When the mobile phase of the same composition as previously (MeOH + $H_2O$ (10 + 90) v/v) contained additionally $10^{-4}$ *M* $TEACIO_4$ (this mobile phase was used in further experiments), the retention volume is independent of concentration of acids in the injected sample in the acid concentration range $10^{-5}$ —$10^{-3}$ *M* (Fig. 2). Practically up to the acid concentration of $5\times10^{-3}$ *M* the changes of the retention volume are so small that a change of the sequence of chromatographic peaks is not likely. In Fig. 2 the dependence of the retention volume on concentration of acids is shown as determinated directly (dashed line) and by the ion-pair method (solid line) at $10^{-4}$ *M* $TEACIO_4$. That figure shows that after addition of $TEACIO_4$ to the mobile phase at a concentration not exceeding $10^{-4}$ *M* (without a buffer) the retention volume of the studied acids is independent of the acid concentration in a wider range of their concentrations than in the absence of the pairing counter ion (cf. Figs. 1 b, c and 3 b, c in [1]). In Table 1 the influence is presented of $TEA^+$ concentration on the

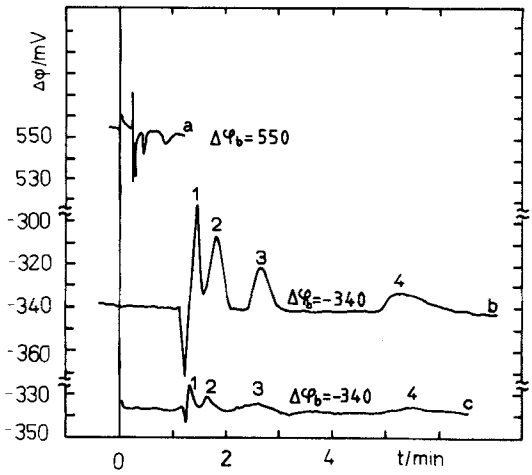


FIGURE 1. HPLC chromatogram of volatile fatty acids separated as ion-pairs on stainless-steel column 150 x 4 mm I.D., LiChrosorb RP-18, 10 $\mu$m, recorded using the electrokinetic detector with PTFE capillary (20 x 0.4 mm I. D.), sample size 5 $\mu$l; mobile phase $10^{-4}$ ***M*** $TEACIO_4$ in MeOH + $H_2O$ (10 + 90) v/v; acids concentrations in ***M***, and flow rates in ml $min^{-1}$: a – $10^{-3}$, 4.2; b – $10^{-3}$, 0.6; c – $10^{-4}$, 0.6. The peaks: 1 – acetic, 2 – propionic, 3 – isobutyric, and 4 – valeric acid.

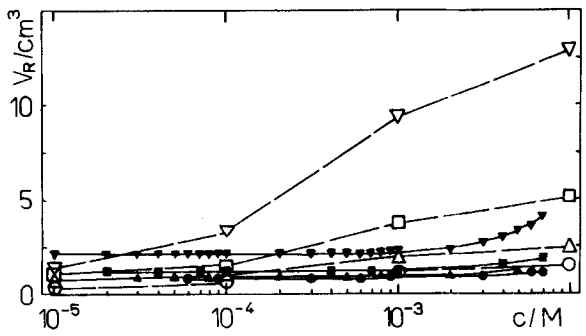


FIGURE 2. Dependence of retention volume on concentration of volatile fatty acids (dashed lines): acetic (○), propionic (△), isobutiric (□), and valeric (▽) and of their ion-pairs (solid lines). Flow rate 0.6 ml $min^{-1}$, other conditions as in Fig. 1.

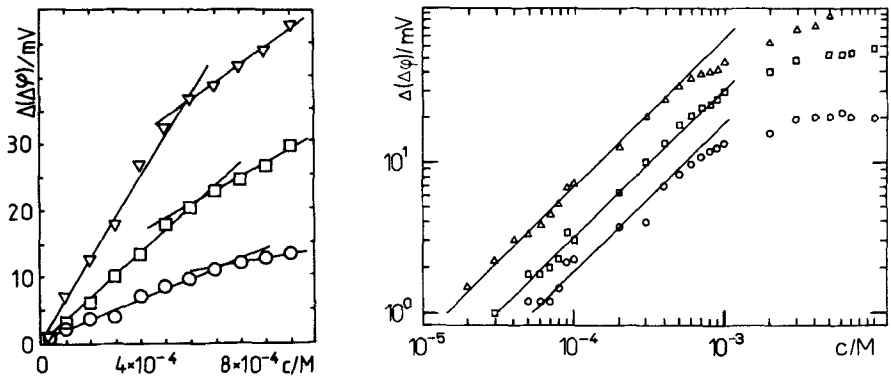


FIGURE 3. Dependence of height (a) and logarithm of height (b) of HPLC chromatographic peaks recorded using the electrokinetic detector with PTFE capillary (20 x 0.4 mm I. D.) on concentration (a) and logarithm of concentration (b) of propionic (Δ), isobutiric (□), and valeric (○), acids separated as ion-pairs in reversed phase HPLC; other conditions as in Fig. 2.



acids detectability, $W$, and on the maximum concentration of acids, $c^{max}$, at which the changes of $V_R$ are so small that a change in the elution sequence of acids could not occur. As it is seen, the increase of $TEA^+$ concentration in the mobile phase causes a decrease of detectability but also a shift of the acid concentration range, in which $V_R$ is independent of the concentration of acids, towards higher concentrations. When the concentration of $TEA^+$ increases by one order of magnitude, the peak heights decrease several times. At the concentration of $TEA^+$ equal to $10^{-4}$ $M$ the peak heights are about ten times smaller than in the absence of $TEA^+$. Fig. 3 shows the dependence of the peak heights (3 a) and the logarithm of the peak heights (3 b) on concentration (3 a) and the logarithm of concentration of acids (3 b) determined as ion-pairs. From this figure it is seen that the linear dynamic range extends to at least one order of magnitude, i.e. $2\times10^{-5}-7\times10^{-4}$ $M$ or $2\times10^{-5}-1\times10^{-3}$ $M$ for the cases presented in Fig. 3 a nd 3 b, respectively. The slope of all curves in Fig. 3 b, $d\log[\Delta(\Delta\varphi)]/d\log c$, is the same and equal to about unity. Within the linear sections of the calibration curves the response of the detector may be described by the known formula

$$\Delta(\Delta\varphi) = \Delta(\Delta\varphi)^{O} + k\,c^{n},$$

where: $\Delta(\Delta\varphi)^{O}$ is the residual signal of the changes of streaming potential, $k$ the sensitivity and $n$ the detector response index.
Then for $\Delta(\Delta\varphi) \gg \Delta(\Delta\varphi)^{O}$ we have

$$\log\Delta(\Delta\varphi) = \log k + n\log c.$$

TABLE 1

Dependence of the Detectability of Propionic Acid, $W_{C2H5COOH}$, and Its Maximum Concentration $c^{max}_{C2H5COOH}$, at which the Changes of $V_R$ are so Small That the Change in the Sequence of Peaks Could not Occur, on $TEA^+$ Concentration.

| $c_{TEACIO_4}$ [M] | $W_{C_2H_5COOH}$ [M] | $c^{max}_{C_2H_5COOH}$ [M] |
|---|---|---|
| 0 | $5x10^{-7}$ | $5x10^{-5}$ |
| $10^{-6}$ | $(2-5)x10^{-7}$ | $8x10^{-5}$ |
| $10^{-5}$ | $(2-5)x10^{-6}$ | $5x10^{-4}$ |
| $10^{-4}$ | $(2-5)x10^{-5}$ | $5x10^{-3}$ |
| $10^{-3}$ | $5x10^{-4}$ | ——— |
| $10^{-2}$ | $5x10^{-4}$ | ——— |

For the studied acids in the range of almost two orders of magnitudes of concentration $n = 1$, and the sensitivity of the detector for all acids is in the limits of (2–8)x $x10^{-4}$ for concentrations of acids smaller than $7x10^{-4}$ M, and for higher concentration it is about half that value. In ion-pair HPLC of acids the reproducibility is better than 5% (R.S.D. for ten consecutive injections), like in direct HPLC determination of acids. With the increase of flow rate in the ion-pair chromatography method for $J < 3.4$ ml $min^{-1}$ a decrease of the peak heights was observed. For $J = 3.4$ ml $min^{-1}$ the peaks disapear altogether in the chromatogram. For $J > 3.4$ ml $min^{-1}$ the peaks change their sign to negative and their absolute value increase.

In Fig. 4 the dependence of HPLC chromatographic peak heights of propionic acid of different concentration ($1x10^{-4}$, $5x10^{-4}$, $10x10^{-4}$ M) on the flow rate is presented. Analogous curves were obtained for other acids. As it is seen, the value of the flow rate at which the relationship $\Delta(\Delta\varphi) = f(J)$ passes the zero point, $J^0$, is independent of the acid concentration. For the PTFE capillary (20 x 0.35 mm I.D.) this value is 1.65 ml $min^{-1}$. It should be noted that at higher flow rates, in this case higher than 4.8 ml $min^{-1}$, we observe like in the direct method a decrease of the absolute value of peak heights (i.e. at $J = 4.8$ ml $min^{-1}$ the relationship $\Delta(\Delta\varphi) = f(J)$ has a minimum).

With the increase of the PTFE capillary diameter, of 15 mm length, the peak heights decrease (Fig. 5). At the same time the stability of the baseline potential is improved. Changes in the capillary diameter do not influence the relative reproducibility of the detector. However, for capillaries of smaller diameters longer time was required for the baseline potential to stabilize. With the increase of the capillary length (Fig.

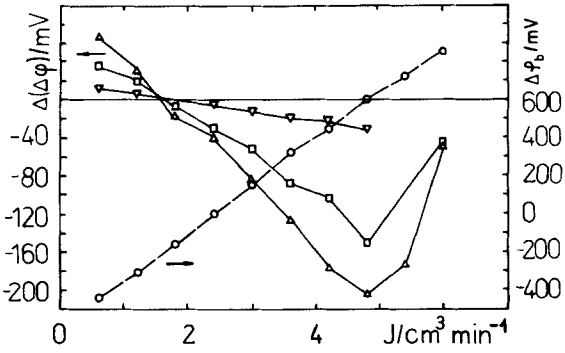


FIGURE 4. Plot of chromatographic peak height, $\Delta(\Delta\varphi)$, against flow rate, $J$, for 0.1 (▽), 0.5 (□), and 1 m$M$ (△) propionic acid, dashed line—the baseline potential; other conditions as in Fig. 2.



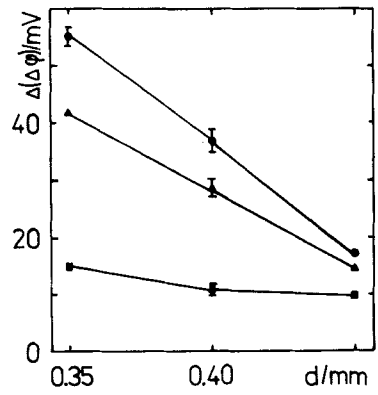


FIGURE 5. Plot of chromatographic peak height, $\Delta(\Delta\varphi)$, *vs* inner diameter, $d$, of PTFE capillary 15 mm long for 1 m$M$ propionic (○), isobutric (△), valeric (□) acids; other conditions as in Fig. 2.

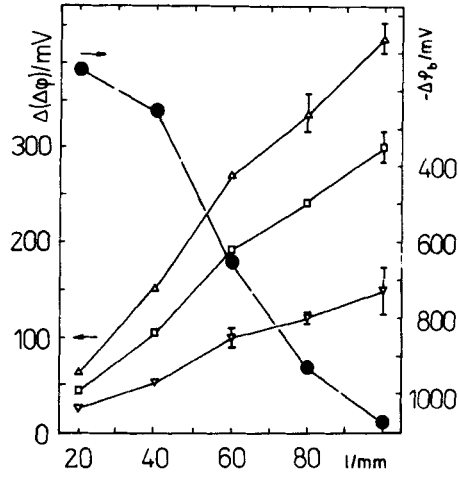


FIGURE 6. Plot of chromatographic peak height, $\Delta(\Delta\varphi)$, *vs* length, *l*, of PTFE capillary (0.2 mm I.D.) for 1 m*M* propionic (Δ), isobutric (□), valeric (∇) acids; dashed line—the baseline potential; other conditions as in Fig. 2.



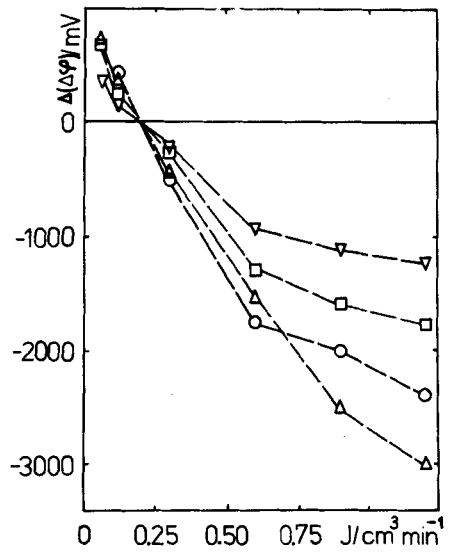


**FIGURE 7. Dependence of height of HPLC chromatographic peak,** $\Delta(\Delta\varphi)$, on flow rate, *J*, for 1 m*M* acetic (○), propionic (Δ), isobutyric (□), valeric (∇) acids. The detector working unit—borosilicate glass capillary (20 x 0.13 mm I.D.) other conditions as in Fig. 2.

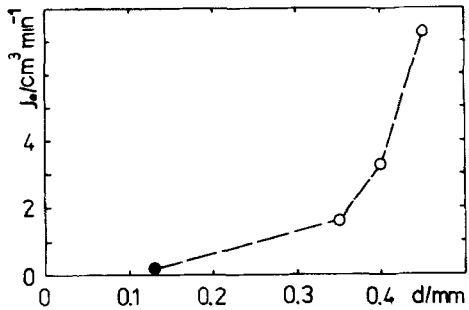


FIGURE 8. Dependence of flow rate, $J^O$, corresponding to the zero point of the relationship $\Delta(\Delta\varphi)$ on $J$, on diameter, $d$, of dielectric capillary 20 mm long; PTFE (●), borosilicate glass (O); other conditions as in Fig. 2.



6) the peaks heights increased as well but at the same time the baseline potential became less stable and shifted towards more negative values.

Substitution of the PTFE capillary (20 x 0.4 mm I.D.) for the borosilicate glass one (20 x 0.13 mm I.D.) led to a fortyfold increase of the peak heights (Fig. 7). However, the baseline potential was less stable, what was the reason why the relative reproducibility was only about 5% (R.S.D. for ten consecutive injections). Hence, the general conclusion can be made that the application of a capillary of a smaller inner diameter leads to the increase of the absolute value of the baseline potential (this was why the use of the borosilicate glass capillary at $J > 1.2$ ml min$^{-1}$ was impossible) and of the peak heights, and also makes $\Delta$ ($\nabla$6) more sensitive to changes of $J$. For the glass capillary $J^O = 0.21$ ml min$^{-1}$. Fig. 8 shows the dependence of $J^O$ on the diameter of the capillary made of a dielectric. As it is seen, $J^O$ increases with the increase of the capillary diameter.

The chromatographic peaks obtained using a stainless-steel capillary (40 x 0.2 mm I.D.) were about twice smaller than these obtained when using the PTFE one (20 x 0.4 mm I.D.); when a stainless-steel capillary (20 x 0.2 mm I.D.) was used the peaks were hardly distinquishable from noise. The reproducibility for the stainless-steel capillary (40 x 0.2 mm I.D.) was better than 30% (R.S.D. for ten consecutive injections). The nature of the recorded chromatograms was unaffected irrespective of whether streaming potential was measured against earth at the working (3) or at draining (6) capillary (see Fig. 1 in [1]). No change of sign was observed at the dependence of peak heights on the flow rate in the accessible range of $J$, i.e. from 0.06 to 6.0 ml min$^{-1}$ for the stainless-steel capillary (40 x 0.2 mm I.D.). Moreover, in contrast to the working capillary made of a dielectric, only a minor increase of heights of the chromatographic peaks with the increase of flow rate was observed. This is why the stainless-steel capillary seems to be more useful for analytical purposes.

## GENERAL DESCUSSION AND CONCLUSIONS

The electrokinetic detector is specific for ionic and universal for nonionic substances [3]. This may be explained by the S m o l u c h o w s k i equation [4 – 6], which may be expressed in following forms

$$\Delta\varphi = q\,\delta\,\Delta p/\eta\kappa = \epsilon\epsilon_0\Delta p/4\pi\eta\kappa = \epsilon\epsilon_0\zeta Iv/2\pi\kappa\delta,$$

were:

- $q$ – surface density of charge, C $m^{-2}$,
- $\delta$ – thickness of the mobile part of the electric double layer, m,
- $\Delta p$ – pressure difference at capillary ends, Pa,
- $\eta$ – dynamic viscosity coefficient, P,
- $\kappa$ – specific conductivity, $\Omega^{-1}$ $m^{-1}$,
- $\epsilon$ – dielectric constant, n. dim.,
- $\epsilon_0$ – dielectric constant of vacuum, $8.9\times10^{-12}$ F $m^{-1}$,
- $\zeta$ – electrokinetic potential, V,
- $v$ – flow velocity of fluid, m $s^{-1}$,
- $r$ – radius of capillary, m.

After sample injection the value of $\epsilon$, $\kappa$, $\zeta$, and $\eta$ of the mobile phase change. In dilute solutions the change of $\epsilon$ and $\eta$ are very small. If, in injected sample solution, an ionic substance is present, $\kappa$ and $\zeta$ also change markedly, due to the change of the thickness of the electric double layer. For instance, for water of very high purity at 20° C $\eta$ = 1.002 cP and $\kappa = 10^{-8}-10^{-7}\,\Omega^{-1}$ $cm^{-1}$, wheres for 20% acetic acid at the same temperature $\eta$ = 1.41 cP and $\kappa$ = 1.61 $\Omega^{-1}$ $cm^{-1}$ [7].

The electrokinetic detector appeared to be very sensitive even to minor contaminations of the surface of the inner capillary wall. If the surface was "poisoned" with irreversibly adsorbed substances (e.g. quaternary ammonium ions, or higher fatty acids of high concentration) two or even three peaks were observed. To avoid this effect the detector had to be washed with 20 or even 200 $cm^3$ of the mobile phase which was pumped through it [8].

In the presented detector design the working capillary was connected directly to the chromatographic column. Because of the very small dead volume of the detector of *ca.* 2 $\mu$l the detector seems to be particularly suited for liquid capillary chromatography [9, 10].

The detector model described here was used for measuring the potential. Therefore it is particularly useful for reversed phase liquid chromatography in which the electrokinetic conductivity of the mobile phase is usually higher than $10^{-7}-10^{-6}$ $\Omega^{-1}$ $cm^{-1}$.

The construction of the presented model of the electrokinetic detector is very simple. The detector may be made from materials available in any laboratory. The peak heights measured are almost invariant with temperature. The detector is cheap and easy to handle. However, its reproducibilty might be the object of improvement. The detector can be used only to the limited number of separated systems. It is less sensitive to non-polar substances and it is difficult to use when the mobile phase contains buffers or substances which adsorb specifically on the inner surface of the capillary wall. It cannot be used in LC with gradient elution or flow rate. The mobile phase must be degassed before use because any bubble of gas when entering the detector cuts the electric circuit and as a result a peak is formed on a chromatogram.

As it was shown, better detectability and reproducibility were obtained with the dielectric capillary than with the metallic one. The detectability for acids determined with the use of the PTFE capillary (20 x 0.4 mm I.D.) was of the order of $10^{-12}$ mole, and for non-ionic substances (e.g. ketones) of the order of $10^{-10}$ mole [11]. The reproducibilty for this capillary was better than 5% (R.S.D. for ten consecutive injections). The linear dynamic range of the detector extended to more than one order of magnitude of concentration. Nearly the same linear dynamic range of fatty acids was reported for the electrokinetic detector in which the streaming current was measured [3] and for the UV (210 nm) detector [12]. But the detectability of the former one was only $5\times10^{-9}-1\times10^{-8}$ g [3], and the lower limit of the linear dynamic range of the latter was $2.5\times10^{-7}$ mole [12].

The presented detector model reveals a much smaller dependence of its baseline on the flow rate as compared with the detector with which streaming current was measured [3]. With the increase of $J$ by 0.1 ml $min^{-1}$ in the range of 0.6–1.8 ml $min^{-1}$, the baseline potential increased by only 35 mV for the mobile phase of the composition MeOH + $H_2O$ (10 + 90) v/v or of 2.5 mV for $10^{-4}$ *M* $TEACIO_4$ in MeOH + $H_2O$ (10 + 90) v/v, respectively.

It has been shown that even at concentrations of the quaternary ammonium cations without buffers as small as $10^{-5}-10^{-4}$ *M* the determination of volatile fatty acids was possible in the ion-pair chromatography system. The addition of quaternary ammonium pairing cations to the mobile phase made the retention volume independent of acid concentration in the injected sample in a definite range of concentrations. However, in this case the reproducibilty of the detector was poorer (e.g. $10^{-10}$ mole for $c_{TEA^+} = 10^{-4}$ *M*).

For capillaries made of a dielectric a pronounced dependence was observed of chromatographic peak heights on the flow rate. The value of $J^O$ was independent of the concentrations of acids, but it was the higher the greater was the diameter of the capillary used. The longer was the capillary or the smaller was its diameter the higher were the peaks observed, but the relative reproducibility for all of them was unaffected.

The reproducibility and detectability of the detector equipped with the stainless-steel capillary was in fact poorer than that of the detector with the PTFE ca-

pillary and equaled about 30% and $2.5 \times 10^{-10}$ *M*, respectively; however, the heights of peaks in the former were less dependent on the flow rate, what might be advantegeous in analytical practice.

## ACKNOWLEDGMENTS

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