Filled tubes as generation elements in electrokinetic detection in liquid chromatography

J. NEČA and R. VESPALEC*

Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Leninova 82, 611 42 Brno (Czechoslovakia)

(First received November 15th, 1989; revised manuscript received April 27th, 1990)

ABSTRACT

A chromatographic column with an electrically conductive wall can be used as an electrokinetic detector even for separations in reversed-phase systems. The high detection sensitivity is comparable to that of electrokinetic detectors placed behind the column. Solutes eluted from the column are detected without additional band broadening. Hence electrokinetic detection using the column as the detector is also promising for microcolumns. The filling increases the detection sensitivity only if the solute responses generated on the tube wall and tube filling are of identical polarities.

INTRODUCTION

Liquid chromatography and streaming current generation involve one common feature, *viz.*, movement of liquid along a solid surface. Hence any chromatographic experiment implicitly provides conditions for streaming current generation. Streaming current generation results from the fact that the streaming liquid carries part of the spatial charge of the electric double layer existing on the boundary between the solid surface (a generating element of the electrokinetic detector) and the flowing liquid[1]. The carried charge can be measured if it is collected by an electrode (sensing element) effected, for instance, by an electrically isolated conductive tube.

The magnitude and polarity of a streaming current depend on the composition of the flowing liquid[1–3]. It is therefore expedient to utilize a streaming current for detection[4]. Measurement of electrokinetic responses to different compounds[2,3] and analyses of bile[5] and carboxylic acids[6–9] and tributyl phosphate hydrolysis products[10] showed that electrokinetic detection of ionizable compounds is sensitive and selective. The properties of electrokinetic detectors behind the column, consisting of empty[6–9] and filled[2,11] capillaries or packs of capillaries[6,7,11], and the properties of the chromatographic columns acting as a detector[11–13] have been studied from different viewpoints[2,6–8,11–14]. However, the influence of the tube wall and its filling on the detection sensitivity and the current generated by the mobile phase stream (background current) were not explained.
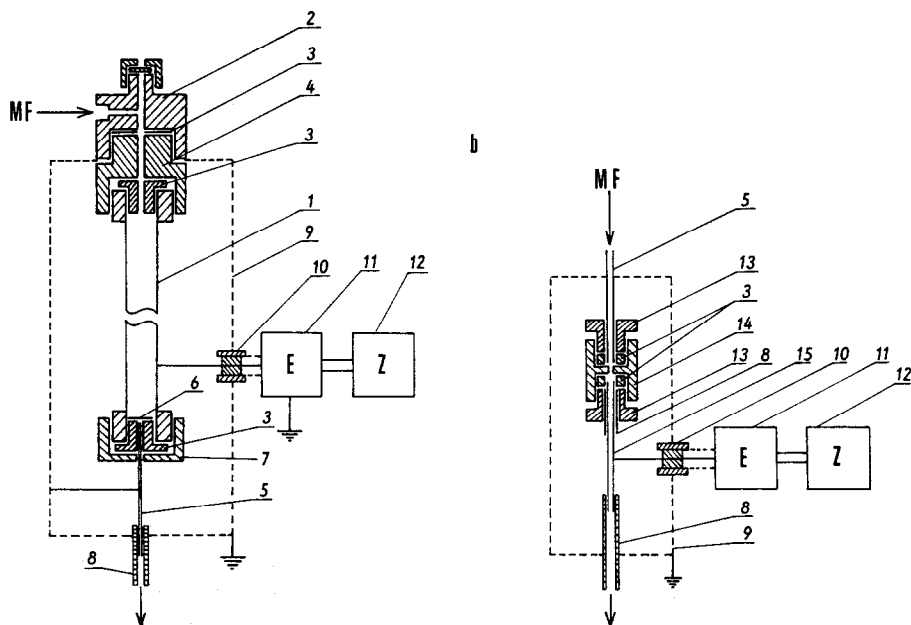
Fig. 1. Scheme of streaming current sensing. (a) From the chromatographic column; (b) from the capillary behind the chromatographic column. 1 = Column; 2 = injection block; 3 = PTFE seal; 4 = isolation element; 5 = stainless-steel capillary (45 × 0.8 mm I.D.); 6 = filter-paper; 7 = end screw joint; 8 = PTFE capillary; 9 = shielding; 10 = connector to electrometer; 11 = electrometer; 12 = recorder; 13 = screw; 14 = stainless-steel couple joint; 15 = stainless-steel capillary (40 × 0.5 mm I.D.).

The Helmholtz–Smoluchovsky equation describing the generation of a streaming current, I_s, has the general form

$$I_s = A\zeta P \tag{1}$$

for both empty[1] and filled[15–17] tubes. Constant A depends on the geometry of the generation element and the composition of the streaming liquid; ζ is the zeta potential and P is the pressure gradient on the generation element. The high additional pressure drop on an electrokinetic detector placed behind the column, necessary for a high detection sensitivity, may be completely eliminated if the chromatographic column with an electrically conductive wall acts as an electrokinetic detector[11–13].

The flow dependence of the background current generated in the column was found to be non-linear at the beginning in normal-phase systems[11]. The contradiction between the experiment and eqn. 1 was explained by the idea of back-discharging of the charge carried by the liquid flowing out of the column[11]. A consequence of this idea was the assumption that the chromatographic column can act as an electrokinetic detector only if mobile phases with conductivities of 10^{-9} $\Omega^{-1}m^{-1}$ or lower are used[12]. However, that assumption has never been confirmed experimentally.

This work was aimed at establishing whether the column with an electrically conductive wall can be used as a detector in reversed-phase systems where the mobile

phase conductivities substantially exceed the limit of 10^{-9} $\Omega^{-1}m^{-1}$. Further, the sensitivity of detection using the column as a detector in reversed-phase systems was tested and the effects of the column wall and the column filling on both background current and solute responses were studied.

EXPERIMENTAL

An HPP 4001 syringe pump (Laboratory Instruments, Prague, Czechoslovakia) served as a pulseless source of the mobile phase flow. Stainless-steel columns with beds of 100 × 2 mm, 150 × 2 mm, 100 × 4 mm and 100 × 6 mm were closed at the outlets with filter-paper and sealed with Teflon (Fig. 1a). The columns were isolated from the injection block with a nylon spacer. Streaming currents were measured with a Vibron Model 33 C electrometer (Electronic Instruments, Richmond, U.K.). A Model 427 current amplifier (Keithley, U.S.A.) was used for model analyses. A reference detector (a refractometer or UV photometer) with an 8-μl cell was connected behind the column. Solutes were injected with a Hamilton (Bonaduz, Switzerland) Model 1801 microsyringe.

The currents generated in the chromatographic column were sensed from the column wall (Fig. 1a) or from the outlet capillary 5 (45 × 0.8 mm I.D.). On sensing from the chromatographic column both the whole chromatograph and the outlet capillary 5 were earthed. On sensing from the isolated capillary 5 the column was earthed.

To sense the streaming current generated in the capillaries, the electrokinetic detector (Fig. 1b) was connected behind the column. A 40 × 0.5 mm I.D. stainless-steel capillary (15), electrically isolated and screened, served as both generation and sensing element. If the generation capillary was filled with a sorbent, its outlet was closed with a cotton-wool wick. The capillary leading out from the column (capillary 5 in Fig. 1a) was earthed. The sensing resistances of the meters (10^5 Ω for acetone and 10^4 Ω for aqueous mobile phases) were chosen so that the conditions for the measurements of current could be achieved[8,18].

Irregular Silasorb 300 silica gel (Lachema, Brno, Czechoslovakia) with a mean particle diameter d_p = 12.4 μm and specific surface area S = 347 m^2/g, and the same material modified with octadecyl groups (Silasorb C$_{18}$), were used as stationary phases. Prior to use the packed columns were washed with 40 ml of distilled water, 100 ml of 20 mM nitric acid (flow-rate 0.2 ml/min), 25 ml of distilled water and 40 ml of ethanol. Analytical-reagent grade solvents used for the preparation of the mobile phases were not purified. Solutes from different sources were of analytical-reagent grade.

RESULTS AND DISCUSSION

In order that the column may serve as a source of streaming current, the bed outlet must be closed with electrically non-conductive material and the metallic outlet capillary must be isolated from the column wall (Fig. 1a). Except for model analyses, the injected solutes were chosen so that their symmetric zones may be eluted in the dead volume or in its vicinity. The electrokinetic responses of such solutes could be quantified only by the changes in the background current, ΔI_s, in the zone maxima.

        The streaming current will reach the maximum if the time of contact between the liquid and the bypassed surface is at least ten times greater than the relaxation time of the liquid[12,19]. The relaxation time of the acetone used was $\tau = 1.9 \cdot 10^{-5}$ s and those of aqueous mobile phases were even shorter[20]. The time the liquid spent in the empty capillary (45 × 0.5 mm I.D.) was 0.1 s at a flow-rate of 5 ml/min. Hence all the capillaries used either as generation or sensing elements guaranteed the maximum charge density in the flowing liquid and its perfect discharge[12,19].

Column as a detector
        The possibility of sensing the streaming current generated in a metallic column through which a relatively conductive mobile phase flows was shown by experiments with stainless-steel columns of dimensions 100 × 2 mm, 100 × 4 mm and 100 × 6 mm I.D. filled with Silasorb 300, through which acetone flowed. The streaming current was sensed from the column wall (Fig. 1a). After multiplying streaming currents by a correction factor of $4/d_c^2$, where d_c is the column diameter in millimetres, the flow-rate dependences of the background currents obtained for columns of varying diameter agreed (Fig. 2).
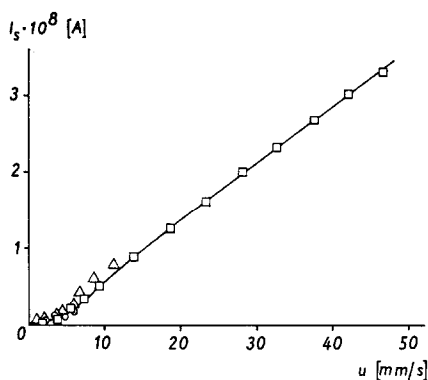

Fig. 2. Dependence of the background current on the linear velocity of the mobile phase in the column standardized to the column of I.D. 2 mm. Stationary phase, Silasorb 300; mobile phase, acetone. Columns: 100 × 6 mm, 100 × 4 mm and 100 × 2 mm I.D.

        The streaming current, I_s, is the product of the volumetric flow-rate of the liquid, F and the mean charge density in the streaming liquid, $\bar{q}(u)$:

$$I_s = F\bar{q}(u) \tag{2}$$

The mean charge density at a certain linear velocity of the liquid, u, did not depend on the column diameter and, starting from a linear velocity of 12 mm/s, it did not change at all with the linear velocity. The flow-rate dependence of the background current had the same course as the dependence measured with a hydrocarbon mobile phase[1] whose conductivity was lower by six orders of magnitude than that of the acetone used. This suggests that the initial non-linearity of the background current flow-rate dependence is obviously not associated with the conductivity of the flowing liquid and thus not

even with its relaxation time[12,19]. The reason may rather be the dependence of $\bar{q}(u)$ on the linear velocity of the liquid.

The independence of the streaming current of the time of the liquid transport from the column outlet into the capillary 5 withdrawing the effluent (Fig. 1a) was verified by changing the distance of the capillary from the bed (Table I). The acetone–water (1:1) mobile phase had a specific conductivity of $3.6 \cdot 10^{-5} \, \Omega^{-1} m^{-1}$. The streaming current did not change even if the outlet capillary 5 was removed from the Teflon sealing and the effluent dripped freely out of the column. These results agree with the observations of Hurd and Hackerman[18].

TABLE I

DEPENDENCE OF BACKGROUND CURRENT, I_s, SENSED FROM THE COLUMN ON THE DISTANCE, d, OF OUTLET CAPILLARY 5 (FIG. 1) FROM THE COLUMN

Column, 100×2 mm I.D.; sorbent, Silasorb 300; mobile phase, acetone–water (1:1); flow-rate, 1 ml/min.

d (mm)	$I_s \cdot 10^8$ (A)
0.5	-1.1
3	-1.0
5	-1.1

The dependence of the background current, I_s, and solute responses, ΔI_s, on the composition of the separation system is shown in Tables II and III. The differences in Table II can be ascribed unambiguously to modification of the silica gel with octadecyl groups, as Silasorb C_{18} was prepared from Silasorb 300 used in this work. When sensing streaming currents from the outlet capillary, the same phenomenon as during measurements with a hydrocarbon–alcohol[11,13] mobile phase was observed, i.e., the polarities of both the background currents and solute responses reversed, but their absolute values did not change. In electrokinetic and refractometric detection both the retention volumes of the solutes and the peak shapes agreed.

The sensitivity of the detection of retained ionizing solutes in sensing streaming currents from the metallic column was tested for nitrophenols (Fig. 3) and carboxylic acids (Fig. 4). The calculated detection limits (Table IV) generated responses twice as high as the noise. The response was calculated from the measurements in which the

TABLE II

BACKGROUND CURRENTS, I_s, AND SOLUTE RESPONSES, ΔI_s, ON SILASORB 300 AND SILASORB C_{18}

Column, 100×4 mm I.D.; mobile phase, acetone; flow-rate, 0.5 ml/min.

Sorbent	$I_s \cdot 10^8$ (A)	$\Delta I_s \cdot 10^8$ (A)		
		Ethanol	p-Nitrophenol	2,3-Dihydroxynaphthalene
Silasorb 300	$+0.2$	$+0.2$	$+0.4$	$+0.3$
Silasorb C_{18}	$+0.5$	-1.5	-2.0	-2.0

TABLE III

INFLUENCE OF THE MOBILE PHASE COMPOSITION ON BACKGROUND CURRENT, I_s, AND SOLUTE RESPONSES, ΔI_s, GENERATED ON SILASORB C_{18}

For experimental details, see Table II.

Mobile phase	ε_r^a	κ^b $(\Omega^{-1}m^{-1})$	$I_s \cdot 10^8$ (A)	$\Delta I_s \cdot 10^8$ $(A)^c$				
				EtOH	ONA	PNA	PNP	2,3-DHN
Acetone	23.0	$1.1 \cdot 10^{-5}$	+0.5	−11.5	− 1.8	− 1.85	− 2.0	− 2.0
Acetone–methanol (90:10)	24.9	$3.9 \cdot 10^{-5}$	+1.6	− 1.9	− 1.6	− 1.6	− 1.6	− 1.2
Methanol	34.4	$4.4 \cdot 10^{-5}$	−6.2	+22.3	+10.2	+ 5.6	+ 3.2	+ 2.5
Acetonitrile	39.5	$6.4 \cdot 10^{-5}$	+1.3	−28.0	−25.0	−25.0	−32.0	−22.5
Acetone–water (70:30)	45.0	$1.5 \cdot 10^{-4}$	−0.5	− 0.5	0.0	− 0.1	− 1.3	− 0.7

^{a} Relative permittivity.

^{b} Electrical conductivity.

^{c} EtOH = Ethanol; ONA = o-nitroaniline; PNA = p-nitroaniline; PNP = p-nitrophenol; 2,3-DHN = 2,3-dihydroxynaphthalene.
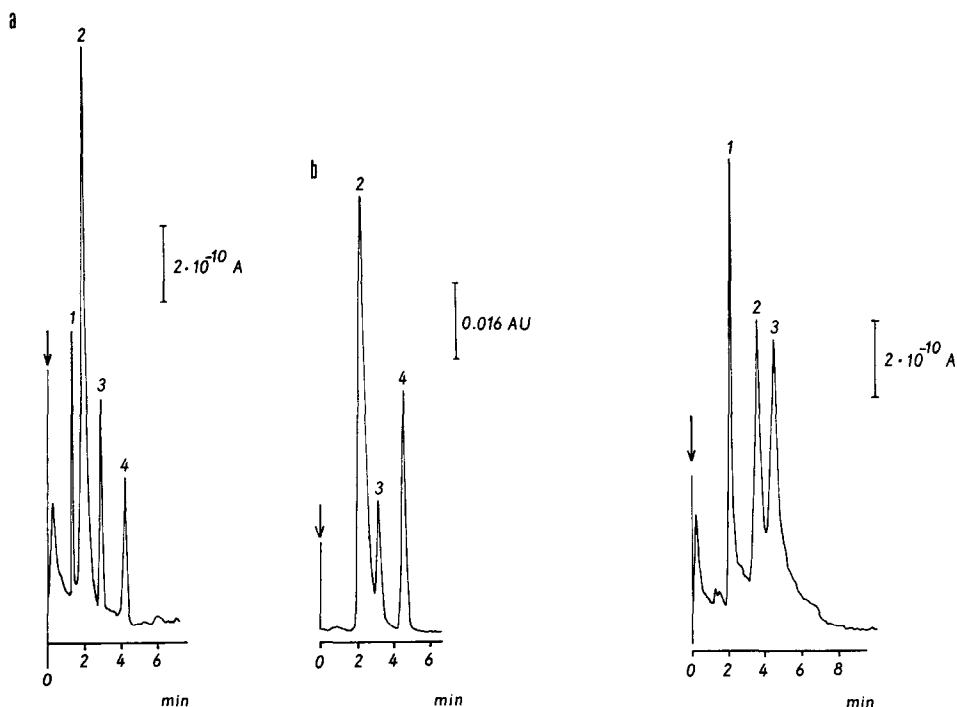

Fig. 3. Chromatogram of a mixture of nitrophenols. Detection: (a) sensing of streaming currents for the column; (b) UV (254 nm). Column, 150 × 2 mm I.D.; stationary phase, Silasorb C_{18}; mobile phase, methanol–water (4:6); flow-rate, 0.2 ml/min; amount of solutes injected, $5 \cdot 10^{-9}$ mol. Noise level (peak to peak): $2.2 \cdot 10^{-11}$ A. Peaks: 1 = methanol; 2 = 2,5-dinitrophenol; 3 = p-nitrophenol; 4 = o-nitrophenol.

Fig. 4. Chromatogram of a mixture of carboxylic acids. Mobile phase, methanol; amount of solutes injected, $2 \cdot 10^{-9}$ mol; other conditions as in Fig. 3. Noise level (peak to peak): $3.4 \cdot 10^{-11}$ A. Peaks: 1 = caprylic acid; 2 = palmitic acid; 3 = stearic acid.

TABLE IV

CAPACITY FACTORS, k', AND DETECTION LIMITS OF RETAINED IONIZING SOLUTES CALCULATED FROM CHROMATOGRAMS IN FIGS. 3 AND 4

Solute	k'	Detection limit $(mol \cdot 10^{10})$
2,5-Dinitrophenol	0.7	2.7
p-Nitrophenol	3.7	6.7
o-Nitrophenol	7.3	8.8
Caprylic acid	0.8	1.2
Palimitic acid	2.0	1.8
Stearic acid	2.7	1.9

detection sensitivity had not been optimized for individual solutes. In spite of that, the values obtained were comparable to the best results obtained with the electrokinetic detector behind the column[2]. The better resolution of nitrophenols with the electrokinetic than with the photometric detection (Fig. 3) is due to the fact that the eluted zones are detected without extra column broadening. Electrokinetic detection is therefore a promising technique for microcolumns. However, with decreasing column diameter the importance of the wall effect for both background current and solute responses increases.

Wall effect
    Let I_1 be the background current influenced by the cylindrical tube wall of radius a and I_2 the streaming current generated by the tube filling. According to eqn. 2, it holds that

$$I_1 = F_1 \bar{q}_1 \qquad (3)$$

$$I_2 = F_2 \bar{q}_2 \qquad (4)$$

The volumetric flow-rate of the liquid carrying the spatial charge of the double layer on the tube wall, F_1, is

$$F_1 = 2\pi a \kappa^{-1} u \qquad (5)$$

where u is the linear liquid velocity; the double-layer thickness, κ^{-1}, depends only on the properties of the flowing liquid[19].
    It follows from the Guy–Chapman theory of the electric double layer[1] that the mean charge density in the liquid is directly proportional to the zeta potential. Therefore,

$$\bar{q}_1 = A_0 \zeta_1 \qquad (6)$$

where ζ_1 is the zeta potential of the tube wall. The proportionality constant, A_0, depends on the properties of the liquid.

The flow-rate of the liquid carrying the spatial charge of the double layer of the filling not affected by the wall is

$$F_2 = \pi(a - \kappa^{-1})^2 A_2 u \tag{7}$$

where A_2 is the ratio of the cross-section of the liquid in which exists the double layer on the particles of the filling to the total bed cross-section, and depends on the bed geometry and κ^{-1}. It holds for the mean charge density, $\bar{q}_2$, in the liquid fraction, F_2, in analogy with eqn. 7 that

$$\bar{q}_2 = A_0 \zeta_2 \tag{8}$$

where ζ_2 is the zeta potential of the filling particles.

The relative contribution of the current affected by the wall, I_1, to the total current, $I_1 + I_2$, is

$$\beta = I_1/(I_1 + I_2) \tag{9}$$

Substituting into eqn. 9 from eqns. 3–8 and assuming $a \gg \kappa^{-1}$ we can calculate the minimum radius of the filled tube, a_{min}, in which wall effect does not exceed β:

$$a_{min} = 2\kappa^{-1} \frac{[1 - \beta(1 - A_2\zeta_2/\zeta_1)]}{\beta A_2|\zeta_2/\zeta_1|} \tag{10}$$

In order to determine a_{min}, let us assume a cylindrical tube filled with spherical particles of $d_p = 12\,\mu m$, with acetone as mobile phase ($\kappa^{-1} = 0.24\,\mu m$[20]), and assume also that $|\zeta_2/\zeta_1| \approx 1$. For such a system $A_2 = 4 \cdot 10^{-2}$. In this instance the minimum tube diameter having a negligible wall effect ($\beta = 0.01$) is $2a \approx 2.4$ mm.

Analogous considerations concerning the solute responses is not possible, as the generation of solute responses has not yet been sufficiently investigated. The influence of the tube wall and its packing was therefore studied experimentally. The electrokinetic detector behing the column (100 × 4 mm I.D.) with a stainless-steel capillary (45 × 0.5 mm I.D.) acting as a generation element (Fig. 1b), was used. The reference background current and solute responses were sensed from the column used.

In order that identical properties of the silica gel surface in the column and in the capillary may be guaranteed, in contrast to preceding experiments, both the filled column and the capillary were washed only with the mobile phase.

In all the experimental arrangements (Table V) the background current and the responses of methanol and ethanol injected into the empty column were below the sensitivity limit of the meter, $1 \cdot 10^{-9}$ A. Hence the responses of solutes measured from the filled column were generated in the bed of sorbent only. In accordance with refs. 11 and 13, the absolute values of the responses sensed from the column wall or from the outlet capillary 5 (Fig. 1a) were identical.

At a sufficiently high linear velocity of the liquid, with $\bar{q}(u)$ being constant, the streaming current generated by a given volumetric flow-rate, F does not depend on the bed cross-section (eqn. 2, Fig. 2). Therefore, the responses of methanol and ethanol generated on a silica gel bed in the column and the filled capillary of the detector

TABLE V

INFLUENCE OF GENERATING AND SENSING ELEMENTS ON THE MAGNITUDE AND POLARITY OF STREAMING CURRENT RESPONSES, ΔI_s

Mobile phase, acetone; flow-rate, 0.5 ml/min.

Generating element	Sensing element	$\Delta I_s \cdot 10^8$ (A)	
		Methanol[a]	Ethanol[a]
Column 100 × 4 mm I.D., + Silasorb 300	Column 100 × 4 mm I.D., + Silasorb 300	−2.0	+1.9
Column 100 × 4 mm I.D., + Silasorb 300	Stainless-steel outlet capillary, 40 × 0.8 mm I.D.	−1.9	−1.8
Detector stainless-steel capillary, 40 × 0.5 mm I.D.	Detector stainless-steel capillary, 40 × 0.5 mm I.D.	−0.4	−0.4
As above + Silasorb 300	As above + Silasorb 300	+1.6	+1.4

[a] Volume injected: 10 µl.

behind the column had to be identical. Hence the additivity applies to the solute response affected by the tube wall and its filling in the same way as to background currents. The tube filling evidently increases the detector sensitivity only if the solute responses generated on the tube wall and on the tube filling have the same polarities.

The minimum diameter of the packed tube through which acetone flows and in which the wall effect on the background current is negligible is 2.4 mm. In the filled tube, 0.5 mm in diameter, one sixth of the total charge was generated on the tube wall by the zone passing through the detector with the use of the same mobile phase. With a tube diameter of 4 mm the influence of the wall could not be proved (Table V). It can therefore be judged that with the use of the same mobile phase the minimum tube diameter whose wall does not influence the solute response is comparable to the minimum tube diameter in which the influence of the wall on the background current is negligible. If responses are sensed from the columns behaving as columns of infinite diameter[21], the solute does not come into contact with the tube wall. In such a column the solute responses are not affected at all by the wall.

REFERENCES

1 R. J. Hunter, Zeta Potential in Colloid Chemistry, Academic Press, London, 1981.
2 S. Terabe, K. Yamamoto and T. Ando, Can. J. Chem., 59 (1981) 1531.
3 R. Vespalec, J. Chromatogr., 210 (1981) 11.
4 N. Ando, Y. Tanizaki and F. Hasegawa, U.S. Pat., 3 352 643 (1967).
5 S. Terabe, K. Yamamoto and T. Ando, J. Chromatogr., 239 (1982) 515.
6 W. Kemula, B. K. Glod and W. Kutner, J. Liq. Chromatogr., 6 (1983) 1823.
7 W. Kemula, B. K. Glod and W. Kutner, J. Liq. Chromatogr., 6 (1983) 1837.
8 B. K. Glod and W. Kemula, J. Chromatogr., 321 (1985) 433.
9 B. K. Glod and W. Kemula, J. Chromatogr., 366 (1986) 39.
10 R. Vespalec and M. Cigánková, J. Chromatogr., 364 (1986) 233.
11 K. Šlais, Thesis, Brno, 1978.
12 K. Šlais and M. Krejčí, J. Chromatogr., 148 (1978) 99.
13 M. Krejčí, K. Šlais and K. Tesařík, J. Chromatogr., 149 (1978) 645.

14 M. Krejčí, D. Kouřilová and R. Vespalec, *J. Chromatogr.*, 219 (1981) 61.
15 H.-S. Jacobasch, G. Bauböck and J. Schurz, *Colloid Polym. Sci.*, 263 (1985) 3.
16 S. M. Neale, *Trans. Faraday Soc.*, 42 (1946) 473.
17 L. Dresner and K. A. Kraus, *J. Phys. Chem.*, 67 (1964) 990.
18 R. M. Hurd and N. Hackerman, *J. Electrochem. Soc.*, 102 (1955) 594.
19 J. Gavis and I. Koszman, *J. Colloid Sci.*, 16 (1961) 375.
20 J. Neča, unpublished results.
21 J. J. De Stefano and H. C. Beachell, *J. Chromatogr. Sci.*, 8 (1970) 434.