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## EFFECT OF THE COMPONENTS OF THE CHROMATOGRAPHIC SYSTEM ON THE ELECTROKINETIC STREAMING CURRENT GENERATED IN LIQUID CHROMATOGRAPHY COLUMNS

R. VESPALEC

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

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### SUMMARY

Electrokinetic streaming currents were studied in chromatographic systems comprising two different batches of silica gel and hexane-butanol mobile phases containing water, acetic acid or picric acid. The sign and the magnitude of the basic currents could be stabilized by rinsing the column with the mobile phase. The required volumes,  $10^2$  to  $10^3$  column dead volumes, depended on the history of the solid phase. Variations in the procedure for preparation of the silica gel influenced the basic currents, solutes retentions and responses. Irreversibly sorbed picric acid did not affect solute retention. However, it did change the response ratios in different mobile phases and increased the responses of some solutes to such an extent that they could be detected at concentrations of hundredths of ppm. The electrokinetic effects in liquid chromatographic systems are, therefore, influenced by alterations in the quality of the solid surface which need not be reflected in the mass equilibria.

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### INTRODUCTION

The measurement of electrokinetic streaming currents generated in a chromatographic column on passage of the mobile phase and at the separation of the components of the sample has been described<sup>1-3</sup> for liquid chromatography. However, attempts to apply this technique have drawn attention to certain problems associated with the long-term stability of the basic streaming current and response values.

Variations in the phase equilibria in the surface layer of the solid phase, which are most probably caused by variations in the composition of the mobile phase or by impurities in the injected samples, are manifested as an instability of the streaming current. Experiments have shown that the source of the instabilities may also lie in the differences in the properties of the solid phases caused by variations in their method of manufacture and in their subsequent rinsing with the mobile phase.

In order to examine the effects of these variations, two samples of silica gel, prepared from one technological batch but differing in the final stage of preparation, were used. The properties of the mobile phases were studied by varying the content of water and by adding compounds which exhibit different acidities. Simple organic compounds having different functional groups were used as solutes.

## EXPERIMENTAL

A home-made apparatus was used for the chromatographic measurements. An MC 300 plunger pump (Mikrotechna, N.E., Prague, Czechoslovakia) with a pressure-damping system according to Locke<sup>4</sup> was later replaced with a more suitable pulseless pump (Varian 8500). Streaming currents, from an isolated and electrically screened stainless-steel column<sup>3</sup>, were led to a Vibron Model 33 C electrometric amplifier (Electronic Instrument, Richmond, Great Britain) with an input resistance of  $10^9$  ohms. A Siemens refractometer was used as a reference detector.

The viscosity variant of the filtration technique<sup>5</sup> was used to pack the columns ( $200 \times 4$  mm). Silica gel samples were suspended in cyclohexanol-methanol (95:5, v/v). Petrol-for-medical purposes was used to transfer the packing pressure of 25 MPa. Packed columns were conditioned directly with the mobile phase.

Both Silasorb 500 silica gel samples (Lachema, Brno, Czechoslovakia), with a specific surface area of  $500 \pm 10$  m<sup>2</sup>/g and a particle diameter  $d_p = 10$   $\mu$ m, were from one technological batch. After grading, one sample was treated only with dilute hydrochloric acid (chloride-treated silica gel, "HCl"), and the other was rinsed additionally with phosphate buffer, pH = 7, until the reaction for chloride ions in the eluate was positive (phosphate-treated silica gel, "PO<sub>4</sub><sup>-</sup>"). Both samples were then washed with a small volume of distilled water and dried. The samples supplied by the manufacturer were not treated prior to packing.

The fundamental mobile phase, butanol-hexane (95:5, v/v), was prepared from analytical grade chemicals. Mobile phases with higher water contents were obtained by saturating a portion of the mobile phase with water [a small excess of water above saturation (5-7 drops for 1 l) was ultrasonically stirred into a milk-like suspension which was sedimented freely overnight] and by subsequent mixing with non-saturated mobile phase in the ratio of 4:1 (v/v). The resulting water content was not determined. Acetic acid (HAc) of analytical grade was added to the mobile phases at a concentration of 0.1 % (v/v). picric acid (HPi) at concentrations of 1 and 10 ppm (w/v).

The relative permittivities and the conductivities of the mobile phases were measured by means of a Tesla BM 484 semiautomatic bridge (Tesla, N.E., Brno, Czechoslovakia). A capacitor with a volume of 142  $\mu$ l and air capacitance of 401 pF (stray capacitance 10 pF) was a larger version of the detection cell of the capacitance detector<sup>6</sup>. The input filter of the capacitor was made of stainless steel and PTFE. The relative permittivities of the mobile phases varied from 2.000 to 2.014, and could not be correlated unambiguously with the contents of moderators (water and acids).

Solutes of different origin, mostly of analytical grade, were injected through a septum. Butanol (BuOH), acetic acid (HAc), benzyl cyanide (BC) and 2-bromopyridine (BP) were used without dilution. Lauric acid (HLa), *p*-nitrophenol (NP), 5,5-diethylbarbituric acid (barbital, HB), 5-ethyl-5-phenylbarbituric acid (phenobarbital, HPB) and 1,2-dibromo-1,2-dichloroethyl dimethyl phosphate (Naled, Nal) were injected as solutions in butanol. *o*-Nitroaniline (NA) was dissolved in nitrobenzene (NB).

## RESULTS

*Column equilibration*

At the beginning of the measurements with chloride-treated silica gel, the degree of column adsorbent equilibration with the mobile phases was estimated by the usual manner in chromatography: constancy of the capacity factors of the eluted compounds. In the course of these measurements, variations in the basic streaming current and in the electrokinetic responses were observed, in agreement with experience. In some instances even a change in their sign was noted. This confirms that the usual procedure for the stabilization of sorption properties of a chromatographic system is not sufficient to stabilize the electrokinetic effects generated in them.

At first, rinsing volumes were measured only if a new component of the mobile phase was introduced for the first time or if its concentration was increased. It appeared (Table I) that under the conditions used the consumption of mobile phase never fell below hundreds of column dead volumes. Since the rinsing volumes varied markedly, it is obvious that the volume of the mobile phase passed cannot alone be a criterion for sufficient conditioning of the column from the viewpoint of the electrokinetic phenomena, despite the fact that this volume surpassed by one order of magnitude or more the volumes commonly considered as sufficient for the stabilization of sorption properties of chromatographic systems. From the differences in the required amounts of mobile phase it follows, however, that these volumes can serve as a source of information on phenomena associated with the sorbent equilibration.

In the final washing stage, the basic streaming current always changed very slowly. Therefore, towards the end of the measurements with chloride-treated silica gel and during the whole set of measurements with phosphate-treated silica gel the

TABLE I  
CHARACTERISTICS OF THE INVESTIGATED SYSTEMS

<i>Moderator</i>	<i>Conductivity</i> ( $\mu S$ )	<i>Relaxation</i> <i>time</i> ( <i>sec</i> )	<i>Rinsing volume</i> ( <i>no. of column</i> <i>dead volumes</i> )		<i>Basic current</i> ( <i><math>\mu A</math></i> )	
			<i>HCl</i>	<i>PO<sub>4</sub><sup>3-</sup></i>	<i>HCl</i>	<i>PO<sub>4</sub><sup>3-</sup></i>
—	0.0002	4.0	—	350	—1 to —4	1.1
H <sub>2</sub> O	0.0005	1.6	135	225	2.3 to 6.3	1.9
—	0.0002	4.0	—	—	—2.3	—
—	0.0002	4.0	—	—	0 to —1.3	—
HAc	0.0007	1.1	275	825	51	0.8
HAc*	0.0007	1.1	—	—	31	—
H <sub>2</sub> O, HAc	0.0009	0.9	—	875	6	5.7
H <sub>2</sub> O	0.0005	1.6	—	655	—1.4	12.3
—	0.0002	4.0	—	375	—1.7	2.7
HPi (1)**	0.0011	0.7	600	150	5.3	6.0
H <sub>2</sub> O, HPi (1)	0.0012	0.7	—	400	—	25
HPi (10)	0.0013	0.6	275	900	15.6	15.2
H <sub>2</sub> O, HPi (10)	0.0031	0.3	500	750	27	30

\* Measurement repeated after half a year and further rinsing of the column.

\*\* Figure in parentheses gives the concentration of picric acid in ppm; similarly in Tables III–VII.

column was washed until the basic streaming current remained constant during the passage of at least 70 column dead volumes. After the stabilization in this way, constant responses were always obtained to all the solutes used. Instabilities of the basic streaming current were successfully obviated in all instances except the following two: when phosphate-treated silica gel was equilibrated with mobile phases to which water and acetic acid had been added, and in a next experiment where the acetic acid was omitted and the mobile phase contained an addition of water only. In these two instances the stepwise changes of the streaming current occur after the injection of solutes.

#### *Responses generated by the injection of samples*

In all the present chromatographic systems, positive exclusion responses<sup>7</sup> were recorded upon injection of any sample. A response is considered as positive if it results in an increase in the absolute magnitude of the positive streaming current or a decrease in the absolute magnitude of the negative streaming current.

The amounts of the injected samples varied roughly from 0.1 to 3 mg, the injected volumes from 2 to 5  $\mu$ l (Tables V and VI). Since the electrokinetic responses were taken directly from the chromatographic column, the retention data had to be measured in a non-thermostatted column at room temperature. The retentions were characterized by the capacity factors. Butanol served as an unadsorbed compound that provides an electrokinetic signal and was eluted, at the precision of these measurements, within the same volume as the unsaturated hydrocarbons in all the chromatographic systems investigated.

The magnitudes of the electrokinetic responses, measured at a volume flow-rate of 0.9 ml/min, were normalized at a concentration of 10 mg/ml and a volume flow-rate of 1.0 ml/min. The concentrations at the maxima of the eluted zones were calculated for the mean or for the most frequent values of the retentions, assuming that the variations in the shapes of the zones caused by deviations in the retention values or in the composition of the chromatographic system can be neglected to a first approximation. The capacity factors for which the concentrations were calculated are listed in Table II, and the retentions and the responses are summarized in Tables III–VI.

TABLE II

#### INJECTED AMOUNTS OF SOLUTES AND CONCENTRATIONS AT ZONE MAXIMA

<i>Solute</i>	<i>Injected amount (mg)</i>	<i>Concentration at maximum (mg/ml)</i>	<i>Typical retention value, k'</i>
Butanol	1.62	15.3	0
Acetic acid	2.10	3.68	2.3
Lauric acid	0.242	0.708	0.30
Benzyl cyanide	2.02	9.73	0.80
2-Bromopyridine	3.14	5.84	1.5
Nitrobenzene	2.32	18.4	0.24
<i>o</i> -Nitroaniline	0.0966	0.274	1.8
<i>p</i> -Nitrophenol	0.0974	0.301	1.2
Barbital	0.0855	0.119	5.6
Phenobarbital	0.2150	0.323	4.9
Naled	$\approx 0.1$	$\approx 1$	0.20

TABLE III  
CAPACITY FACTORS OF SOLUTES ON SILICA GEL RINSED WITH HCl

Moderator	Basic current ( $\mu A$ )	HAc	HLa	BC	BP	NB	NA	NP	HB	HPB	Nal
-	-1 to -4	3.0	0.47	1.2	1.9	0.25	2.9	2.7	-	7.0	0.30
H <sub>2</sub> O	2.3-6.3	A* 2.9	0.41	0.82	1.2	0.23	2.2	-	-	-	-
-	-2.3	1.8	0.31	0.93	1.6	0.26	1.9	1.2	-	2.7	0.21
-	0-1.3	1.8	0.30	0.86	1.5	0.26	1.9	1.2	-	2.6	0.20
HAc	51	1.3	U**	0.87	1.5	0.25	1.8	1.2	-	2.4	0.21
HAc***	31	A, U	0.23	0.89	1.3	0.25	1.9	1.2	2.7	2.2	0.25
H <sub>2</sub> O, HAc	6.0	2.2	0.42	0.92	1.2	0.26	2.3	2.4	5.4	4.7	0.35
H <sub>2</sub> O	-1.4	2.3	0.46	0.87	1.2	0.29	2.5	2.2	5.7	4.8	0.38
-	-1.7	1.7	0.42	0.84	1.4	0.24	1.8	1.2	2.7	2.7	0.19
HPi (1)	5.3	1.7	0.38	0.82	1.4	0.24	1.8	1.1	3.0	2.4	0.19
HPi (10)	15.6	2.6	0.30	0.82	1.4	0.25	1.7	1.1	2.8	2.8	0.19
H <sub>2</sub> O, HPi (10)	27.0	2.3	0.46	0.90	1.1	0.25	2.2	2.1	5.6	4.9	0.39

\* Anomaly in electrokinetic response.

\*\* The measurement cannot be evaluated.

\*\*\* Measurements repeated after half a year and further rinsing of the column.

## DISCUSSION

### *Equilibration of the system; basic streaming current*

Reference measurements of the relative permittivities and conductivities of the mobile phases showed that the former values were determined by the precision of addition of the butanol. The other components only affected the conductivity (Table I). The relaxation times of the mobile phases were always so long that their effect on the measured electrokinetic streaming currents could be neglected<sup>3</sup>.

Irregular Silasorb sorbents are prepared from silicic acid of high purity. The samples of Silasorb 500 that were used here were not treated hydrothermally, nor did they come into contact with other substances which could chemically modify their surfaces. The HCl and the phosphate buffer were applied under conditions such that there was no reaction with the silica gel surface. Both silica gel samples studied can therefore be considered as relatively pure silicon dioxide, the surface of which should be contaminated only with residues of HCl or of the phosphate buffer and water, which are retained physically.

The concentrations of both water and acetic acid in the stationary phase could be varied reversibly by adjusting the composition of the mobile phase. However, picric acid was retained so strongly that, even after the passage of 2000 ml of the fundamental mobile phase (1000 dead volumes) through a column that had previously been equilibrated with a mobile phase containing 10 ppm of picric acid, no change was observed in the magnitude of the basic streaming current. In the hexane-butanol (95:5) mobile phase the sorption of picric acid can therefore be considered as irreversible.

Rapid elution of picric acid from silica gel can be achieved, for example, by using ethanol. Free inorganic acids, which are strongly ionized in aqueous solutions,

TABLE IV  
ELECTROKINETIC RESPONSES OF SOLUTES ON SILICA GEL RINSED WITH HCl

Values are expressed in pA and normalized at concentrations of 10 mg/ml and flow-rates of 1 ml/min.

Moderator	Basic current (pA)	BuOH	HAc	HLa	BC	BP	NB	NA	NP	HB	HPB	Nal
-	-1 to -4	-2.9	-9.2	-157	-0.57	-0.95	-0.19	-41	26	-	U*	4
H <sub>2</sub> O	2.3-6.3	A**	A -16	10.7	5.4	3.1	4.0	390	-	-	-	-
-	-2.3	-1.7	-26	-115	0.46	1.1	0.61	150	330	-	-38	6.6
-	0-1.3	0.26	-45	-47	2.3	U	2.0	145	330	-	-38	3.3
HAc	51	-1.1	16.8	U	-11.4	-16.5	-8.4	-240	350	-	-47	U
HAc***	31	A	A	-98	-7.3	-12.6	-4.0	-160	53	-120	-34	5.5
H <sub>2</sub> O, HAc	6.0	U	-10.9	-100	-2.8	-10.0	-0.50	-32	86	-47	-17	4.4
H <sub>2</sub> O	-1.4	U	-15	-31	-1.4	-2.7	0.37	49	92	-12	-4.4	3.3
-	-1.7	-1.8	-23	-68	-4.5	-5.2	1.6	41	110	U	45	14.5
HPI (1)	5.3	4.8	-47	-320	8.2	1.6	7.0	280	420	-29	-10	7.7
HPI (10)	15.6	7.5	-48	-370	11	4.3	11	470	620	U	U	14.5
HPI (10), H <sub>2</sub> O	27.0	13	-3.9	-110	9.1	12.8	6.8	490	590	U	21	14.5

\* Response is not detectable.

\*\* Anomaly in electrokinetic response.

\*\*\* Measurement repeated after half a year and further rinsing of the column.

TABLE V  
CAPACITY FACTORS OF SOLUTES ON SILICA GEL RINSED WITH HCl AND PHOSPHATE BUFFER

Moderator	Basic current (pA)	HAc	HLa	BC	BP	NB	NA	NP	HB	HPB	NaI
-	1.1	A* 1.7	0	0.91	1.6	0.26	1.9	1.1	3.4	2.6	0.20
H <sub>2</sub> O	1.9	A 2.6	0.49	A 0.96	A 1.3	0.30	2.4	2.5	6.7	5.7	0.40
HAc	0.8	A 2.6	0.52	1.26	A 0.72	A 0.28	A 2.9	3.1	8.8	7.5	0.40
H <sub>2</sub> O, HAc	5.7	A 2.6	A 0.47	A 0.93	1.2	A 0.29	A 2.3	2.4	6.4	5.5	0.43
H <sub>2</sub> O	12.3	A 3.9	A 0.5	A	A 0.84	A 0.23	A 1.8	2.3	7.7	6.3	0.33
-	2.7	1.9	0	A 0.92	1.5	0.27	1.9	1.4	4.0	3.6	0.24
HPi (I)	6.0	1.8	0	0.86	1.4	0.25	1.9	1.3	3.9	3.0	0.21
H <sub>2</sub> O, HPi (I)	25	2.7	A 0.86	0.83	1.2	A 0.26	2.3	2.4	6.7	5.7	0.41
HPi (10)	15	A 1.5	0.25	0.87	1.5	0.25	1.8	1.1	2.7	2.2	0.22
H <sub>2</sub> O, HPi (10)	30	A 0.43	A 0.45	A 0.94	1.2	0.24	2.2	2.2	5.5	4.5	0.34

\* Anomaly in electrokinetic response.

TABLE VI  
ELECTROKINETIC RESPONSES OF SOLUTES ON SILICA GEL RINSED WITH HCl AND PHOSPHATE BUFFER  
Values expressed as in Table IV.

Moderator	Basic current (pA)	BtOH	HAc	HLLa	BC	BP	NB	NA	NP	HB	HPB	Nal
-	1.1	1.4	A* 1.2	A, U**	1.8	0.58	1.4	69	330	U	U	A, U
H <sub>2</sub> O	1.9	A 3.1	A -5.0	-19	A -7.3	A -15	0.36	64	280	-37	-20	2.8
HAc	0.8	A 1.2	A 0.39	2.0	A -0.22	A, U	A, U	A 12	74	U	U	U
H <sub>2</sub> O, HAc	5.7	A 2.6	A -5.1	A 4.8	A 3.1	4.4	A 1.8	A 132	200	-66	-34	3.3
H <sub>2</sub> O	12.3	A, U	A	A 8.0	A, U	A -2.9	A 1.8	A 29	210	-55	-34	U
-	2.7	5.5	-3.1	U	A -2.3	1.2	2.4	160	220	U	U	4.4
HPI (I)	6.0	4.1	-0.91	U	4.9	2.4	3.8	150	160	U	-18	6.6
H <sub>2</sub> O, HPI (I)	25	3.9	0.91	A -4.8	5.3	6.4	2.5	120	140	-61	-10	3.3
HPI (10)	15	13	A 4.7	-43	18	5.3	14	630	850	-56	-31	6.6
H <sub>2</sub> O, HPI (10)	30	8.0	A	A -55	A 8.0	14	6.3	340	360	U	U	2.8

\* Anomaly in electrokinetic response.

\*\* Response is not detectable.

TABLE VII  
INFLUENCE OF PRE-ADSORPTION OF PICRIC ACID ON PHOSPHATE-TREATED SILICA GEL AND OF MOBILE PHASE MODERATORS ON RETENTION AND ELECTROKINETIC RESPONSE  
Electrokinetic responses expressed as in Table IV.

Composition, stationary/mobile phase	Basic current (pA)	Nitrobenzene		o-Nitroaniline		p-Nitrophenol		Benzyl cyanide		2-Bromopyridine	
		k	pA	k	pA	k	pA	k	pA	k	pA
-/-	1.1	0.26	1.4	1.9	69	1.1	330	0.91	1.6	1.6	0.58
HPI/-	1.8	0.25	24	1.8	1160	1.1	2800	0.85	24	1.5	9.4
HPI/HPI	15	0.25	14	1.8	630	1.1	850	0.87	18	1.5	5.3
HPI/HPI, H <sub>2</sub> O	30	0.24	6.3	2.2	340	2.2	3.60	0.94	8.0	1.2	14



can also be eluted with ethanol. They are eluted in the dead volume ( $\text{H}_3\text{PO}_4$ ) or have negligible retentions ( $\text{HClO}_4$ ,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ). The sodium or potassium salts of phosphoric, boric or hydrofluoric acids could, however, not be eluted from silica gel with pure alcohol<sup>8</sup>. It follows that the ionization of free acids is strongly suppressed in alcoholic media.

With a mobile phase containing water, phosphoric acid and 10 ppm of picric acid it was possible to observe the elution of  $\text{H}_3\text{PO}_4$  from the phosphate-treated silica gel by measuring the electrokinetic streaming current and even by refractometry.

It follows from the above experiments that a certain ability to elute free inorganic acids from silica gel can be ascribed to all the mobile phases used. This, together with previous knowledge of the origin of electrical double-layers on the surface of silicon dioxide, makes it possible to describe qualitatively some variations in the magnitude or in the sign of the charge of the electrical double-layer on the surfaces of the packings tested.

The conditions in the part of the liquid that is in direct contact with the solid phase are crucial insofar as the surface charge is concerned. With a multicomponent mobile phase containing a large amount of hydrocarbon and with silica gel as adsorbent, the liquid which is contiguous with the solid phase comprises the adsorbed layer of polar components; this may also contain compounds remaining on the surface from earlier treatments. It follows from the work of Scott and Kucera<sup>9</sup> that in all these systems a compact sorbed layer of butanol was formed on the surface of the solid phase. In the systems containing water or acids the butanol layer was enriched by these components<sup>10</sup>.

The surface charge of adsorbents of the oxide type, determined by the concentrations of  $\text{H}^+$  and  $\text{OH}^-$ , is dependent on the pH in aqueous media. The sign changes on passing through the isoelectric point<sup>11</sup>. pH values of 1.3–3.7 or 3–5 are observed<sup>12,13</sup> for the isoelectric point of silicon dioxide in water. In alcoholic media,  $\text{SiO}_2$  gains surface charge by the same mechanism as in water<sup>14</sup>. Alcohols, however, possess greater electronegativity than water<sup>14</sup> and therefore the isoelectric point is expected to lie at higher pH values. This means that, at the same pH value, silica gel gains positive surface charge (or increases it) on passing from an aqueous to an alcoholic medium. When substances capable of ionizing via the elimination of a proton or hydroxyl group are present on the silica gel surface or in the adsorbed layer, it is necessary that these ionisation processes and their course in semi-aqueous or alcoholic media should be considered.

The surface of chloride-treated silica gel in a butanol medium gained a negative charge (Table I). The gradual decrease in the magnitude of this charge with increasing volume of the non-aqueous mobile phase passed can be explained by gradual washing out of HCl. On passage of a mobile phase enriched with water, the content of water in the sorbed phase increased. The establishment of the positive surface charge can be ascribed to dissociation of HCl and to the associated decrease in pH below the isoelectric point. The reason for the establishment of the negative charge on the surface of silicon dioxide in a butanol medium in the presence of HCl is not clear.

In the medium containing relatively electronegative acetic acid, silica gel gains a considerable positive charge. If water is added to the system, the content of acetic acid in the sorbed phase decreases with the simultaneous increase in the water content. Both these processes lead to reduction of the positive surface charge. The

increase in the concentration of hydrogen ions caused by the dissociation of acetic acid in the presence of water is, bearing in mind the strength of acetic acid and pH of the isoelectric point for silica gel, obviously negligible.

Picric acid is known to ionize even in non-aqueous media. Thus its presence can so reduce the pH of the butanol phase that the silica gel surface gains a positive electric charge. On increasing the acid concentration, this charge increases. In the presence of water, the picric acid ionization increases, which appears as an even greater positive electric charge on the gel surface.

The systems containing phosphate-treated silica gel behave differently. On injection of samples, composite responses are often observed (Tables V and VI) similar to composite signals generated almost simultaneously by different sources<sup>15</sup>. Such responses cannot be ascribed to either the mobile phase or the manner of sensing the electrokinetic signals, which was selected so as to eliminate the generation of responses caused by the interaction of the effluent with the sensing element<sup>15</sup>. They must therefore be caused by the packing of the column. The variations in the magnitude of the basic streaming current after the injection of samples, observed, for example, in the system containing the mobile phase enriched with water and acetic acid, can also be ascribed to the sorbent. Rinsing of the silica gel with a small volume of distilled water after the application of phosphate buffer, used in the final stage of the preparation of the sample, does not ensure the quantitative removal of the phosphate.

Dobiáš<sup>16</sup> found that, with incompletely soluble solid phases of ionic character ( $\text{BaSO}_4$ ,  $\text{CaF}_2$ ), the potential-determining ions are the grid ions. The solubility of salts is very limited in alcoholic media. Moreover, the amount of the salt that can be transferred into the layer of the sorbed liquid is limited also by the volume of this liquid. It can therefore be assumed that solid residues of the buffer on the silica gel surface behave in the same way as an incompletely soluble ionic phase and may be an independent source of the streaming current.

A characteristic of the phosphate-treated silica gel is that it is positively charged in all the systems. From the increase in the positive charge after the addition of water to the mobile phase, which was always observed, it can be concluded that acidic components from the preparation, capable of ionizing easily, are present on the surface of the sample. The addition of acetic acid, which reduced the amount of water in the sorbed phase and suppressed, by its higher electronegativity, the ionization of these components, reduced the positive electric charge on the gel surface.

The addition of picric acid had the same effect as in the systems with chloride-treated silica gel. The similarity in behaviour of this compound and in the magnitudes of the basic streaming currents found in comparable systems suggest that of all the moderators tested picric acid possesses the strongest ability to affect the electric relationships at the phase interface. It can therefore be recommended for standardization of electrokinetic phenomena in comparable chromatographic systems or, at least, for the suppression of differences between them.

#### *Retentions and responses of solutes*

Model solutes were mostly selected to represent compounds of different types. It is seen from Tables III–VI that the differences in the mobile phase composition and in the history of the solid phase affect the values of both retention and electrokinetic

response. Furthermore, the retentions and electrokinetic responses of different solutes are influenced by the variations in the properties of the chromatographic phases in different ways.

Regardless of the treatment of the silica gel, the addition of water to the mobile phase had a significant effect on the retentions of hydrophilic solutes or of compounds having markedly hydrophilic groups (acetic and lauric acids, barbiturates, *p*-nitrophenol), the retentions increasing by more than 100%. The retentions of *o*-nitroaniline, 2-bromopyridine, benzyl cyanide and naled changed by 20–60%, but there was almost no effect on the retention of nitrobenzene. In the majority of cases, the mode of silica gel treatment was reflected in the absolute values of the retentions. The relative changes in retention caused by the variations in water content in the mobile phase were, however, practically independent of the treatment.

The influence of acetic acid depended significantly on the treatment. In the chromatographic systems with chloride-treated silica gel, acetic acid affected the retentions of only two solutes: acetic and lauric acids. In the systems with phosphate-treated silica gel, the addition of acetic acid caused multiple changes in the retentions of some compounds. The magnitude and the character of these changes also depended on the presence of water. Picric acid had a significant effect on the retentions only of acetic and lauric acids, this being more marked in the systems with phosphate-treated silica gel.

The differences in the retentions caused by the differences in the treatment of the solid phase are surprisingly large, amounting to  $\pm 80\%$  for some solutes in comparable systems (*i.e.*, in systems with the same mobile phases). Despite this they usually do not play an important role in practice. Providing solutes of the same or related type are separated, the retention values can be adjusted by varying the mobile phase composition. The differences in retention which are sometimes found on silica gels of different type or on products of different origin are usually explained in terms of discrepancies between the declared and actual specific surface areas, differences in the structure and size of pores or variations in the degree of surface hydration. The results discussed above and those in ref. 8 show, however, that another possible reason may be differences in the mode of preparation of the silica gel.

The variability of the electrokinetic responses and their dependence on both components of the chromatographic system is greater than in the case of retention. It is useful to interpret in terms of electrokinetic responses the variations in the streaming currents caused by a reversible short-term change in the mobile phase composition<sup>3</sup>. Constancy of the basic streaming current can be considered as evidence of the stability of the relationships at the phase interface between consecutive injections of quantitatively eluted solutes. Two factors determine the value of the electrokinetic response: the relationships at the liquid–solid phase interface, and the short-term changes in these relationships caused by variations in the mobile phase composition (*i.e.*, by the solute concentration pulse).

The relationships at the phase interface (mass and charge balance equilibria) that are established in the course of the column equilibration evidently depend on the ability of the mobile phase to elute from the surface of the solid phase the components or their reaction products that were captured earlier, and on the ability of the solid phase to bind to its surface the components of the mobile phase. These equilibration processes and their dependence on the history of the solid phase and on the mobile

phase composition can be deduced from the rinsing volumes and their variations (Table I). The influence of the quality of the solute on the electrokinetic response is obvious (see, for example, Tables III and IV), and the dependence of the response on the concentration of the solute, derived theoretically, was also verified experimentally<sup>2</sup>.

Šlais and Krejčí<sup>2</sup> derived the following relationship for the value of the electrokinetic response

$$\frac{dI_{\text{str}}}{dc_i} = \frac{\kappa}{D} \cdot \frac{(\varphi_i - \varphi_0)}{c_{si}^0} \cdot K_d F_m \quad (1)$$

where  $I_{\text{str}}$  is the streaming current (the measured electrokinetic response),  $c_i$  is the concentration of solute  $i$  in the mobile phase,  $\kappa$  is the conductivity of the mobile phase,  $D$  is the molar diffusivity of the charge,  $\varphi_i$  is the potential of the outer plane of the Stern layer comprising a monolayer of the molecules of compound  $i$ ,  $\varphi_0$  is the corresponding potential when the surface is not covered by compound  $i$ ,  $K_d$  is the distribution constant of compound  $i$  and  $F$  is the volume flow-rate of the mobile phase. The mobile phase is thus characterized by the term  $F_m \kappa/D$ , the stationary phase and the solute by the factor  $(\varphi_i - \varphi_0) K_d/c_{si}^0$ , where  $c_{si}^0$  is the maximum interface concentration of component  $i$ . For a selected chromatographic system (and for the given solute) the right-hand side of the equation is constant.

It follows from this equation that if a column is rinsed consecutively with two mobile phases, the respective basic streaming currents and responses to any solute will be reproducible. However, after washing the column with a mobile phase containing picric acid, the values that were measured initially could not be reproduced on replacing this phase with the fundamental mobile phase.

It is seen from Table VII that the introduction of picric acid into the stationary phase does not measurably affect the mass equilibria of the solutes listed, expressed in terms of the capacity factors. (The changes that were observed are within the limits of experimental error.) There is essentially no difference between the mechanism of generation of the basic streaming current and that of the solute response. The differences in the magnitudes of the basic streaming currents in systems with the same mobile phases (lines 1 and 2 in Table VII) can therefore be explained, in terms of eqn. 1, by differences in  $\varphi_0$  only, which is equivalent to the changes in quality of the surface of the solid phase. Thus, from electrokinetic phenomena in chromatographic systems, not only electrical relationships at the phase interface but also changes in the quality of the solid phase surface, which may not be revealed by means of adsorption measurements, can be evaluated.

Phosphate-treated silica gel when equilibrated by a mobile phase containing 10 ppm of picric acid can, with respect to the adsorption strength of picric acid, be considered as a solid phase of constant quality in all the cases listed in Table VII. The conductivities of butanol-hexane, butanol-hexane + 10 ppm of picric acid and butanol-hexane + 10 ppm of picric acid 80% saturated with water increase approximately in the ratio 1:7:15 (Table I). However, the responses of none of the solutes listed in Table VII follows this increase. On the contrary, with the exception of 2-bromopyridine, the measured responses decrease with increasing conductivity of the mobile phase. At the same time, the ratios of solute responses in different mobile phases vary.

The discrepancy between the response values found and the values expected

from eqn. 1 cannot be explained by the changes in  $\varphi_0$ . As defined, the value of  $\varphi_1$  is only dependent on the charge of the sorbed particles of the solute and on their maximum surface density. The values of the retentions,  $K_d$ , and diffusivities,  $D$ , remained constant<sup>3</sup>. Sorption competition of polar components of the mobile phase (water, picric acid) or of their ions, which would be concentration dependent only, could not change the response ratios of the various pairs of solutes in different mobile phases so markedly. It is therefore necessary to consider the specific effects of minor polar components of the mobile phase on the ionization of solutes in non-aqueous media.

The response data in Table VII are also interesting from a practical standpoint. They show that by a suitable modification of the relationships at the phase interface and in the mobile phase not only the sensitivity but also the selectivity of the electrokinetic detection can be affected by orders of magnitude. Moreover, that the modification need not be achieved only by a change in the chemical composition of the stationary phase or its surface<sup>2</sup>, but also by the addition of compounds that can, depending on the selection of mobile phase, be reversibly retained. The data also suggest that picric acid is a suitable modifying compound, and that it is advantageous to work with a mobile phase containing the lowest possible amount of highly polar components.

In this context, the effect observed with phosphate-treated silica gel and the fundamental mobile phase is worthy of note. Upon injection of naled the responses recorded exceeded by at least three orders of magnitude those obtained upon its injection into other chromatographic systems. Even though it was not possible to differentiate unambiguously the increase in the basic current from the response itself, this observation suggests that some other, and perhaps more efficient, way of controlling the sensitivity or selectivity of the response can be found than that which follows from, for example, eqn. 1 or by use of picric acid.

## CONCLUSIONS

This work has shown that electrokinetic phenomena in a chromatographic system can be stabilized by passing a sufficiently large amount of the mobile phase. The volumes required were always at least an order of magnitude greater than those usually considered to be sufficient to stabilize the retention properties. In the systems tested, these volumes never decreased below hundreds of column dead volumes. The history of the solid phase, *i.e.*, the mode of its preparation, the individual operations associated with application of the sorbent and their sequence have a significant influence on the volume of the mobile phase required for stabilizing the electrokinetic phenomena.

However, the stability thus obtained does not ensure that the initial state can be reproduced precisely after executing any cycle of column operations. The experiments with picric acid show that the reasons for this lack of reproducibility should be found in the limited ability of the mobile phase to establish equilibria among all the components in both chromatographic phases. The standardization of electrokinetic phenomena in chromatographic systems thus remains an unsolved problem.

The variations in the streaming current, observed during the equilibration of silica gel samples with various mobile phases, have been explained qualitatively.

Although the explanation involves some assumptions that could not be verified in the present work, it is consistent for both silica gel samples and can be used to describe even the systems with picric acid.

The washing of silica gel with phosphate buffer led, in chromatographic systems with the same mobile phase, to variations in solute retentions of up to  $\pm 80\%$ . Together with previous results<sup>8</sup>, this shows that even small differences in the silica gel preparation can result in considerable variations in the sorption selectivity of the final product, even when the specific surface area, pore volume and degree of surface hydration do not change.

When interpreting the responses, in addition to the quality of the surface of the solid phase, it is necessary to consider also the interactions of the solutes with the components of the mobile phase during the formation of ions in the solution and at the phase interface. The content of highly polar components, which ionize or can affect the ionization of the solute, has a significant influence on the resulting electrokinetic phenomena. Both these effects complicate the standardization of the electrokinetic responses and their application to detection purposes.

On the other hand, these dependences provide information on the quality of the solid phase surface and probably also the ionization phenomena in non-aqueous media that cannot be obtained by sorption measurements. The dependences of the electrokinetic phenomena on the quality (purity) of the solid phase surface and on the changes in the mobile phase composition, which are negligible from the viewpoint of retention, can also be used for detection purposes.

By the addition of picric acid, and apparently also of some other compounds, the sensitivity of the detection can be increased substantially and also its selectivity can be improved. For instance, for some tested solutes the increase in the response was so high that at the minimum detectable change in the current,  $1 \cdot 10^{-14}$  A, which is easily accessible, these compounds could be detected in concentrations of hundredths of ppm.

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