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SEPARATION AND STREAMING-CURRENT DETECTION OF STRONG ACIDS IN NORMAL-PHASE LIQUID CHROMATOGRAPHY

R. VESPALEC* and M. CIGANKOVÁ
Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Leninova 82, 611 42 Brno (Czechoslovakia)

SUMMARY

The separation and streaming-current detection of strong acids were studied on a model extraction solution of tributyl phosphate which contains, in addition to others, dibutyl and monobutyl phosphates and orthophosphoric acid. All the components of the sample, starting from hydrocarbons and ending with orthophosphoric acid, can be separated within 10 min, provided that silica gel with a large surface area, treated with perchloric acid, is used as a column packing. 2-Propanol–heptane (15:85), saturated to 30% with water, is used as mobile phase. The streaming current can be sensed from a chromatographic column. Phosphoric acids are detected selectively, with a sensitivity of 1–2 orders of magnitude higher than in refractometric detection.

INTRODUCTION

In the last ten years, a few papers have been published on the electrokinetic phenomenon in liquid chromatography[1–7]. This phenomenon was used in reversed-phase systems for detection purposes in the separation of bile[8] and carboxylic[9–11] acids. Capacity factors of weak acids are concentration-dependent[9,11] for mobile phases composed of water and organic polar solvent. This dependence can be avoided by adding an ionized compound, *e.g.* buffer or suitable counter-ion, to mobile phases containing water at a sufficient concentration. Hoewever, this decreases the sensitivity of streaming-current (SC) detection[8,10]. The concentration dependence of the retention of weak acids can also be eliminated by using water-free polar organic solvents as mobile phases[9]. However, such mobile phases show decreased solute retention. In a reversed-phase system, only carboxylic acids with a higher number of carbon atoms in the molecule can be separated by using a non-aqueous polar mobile phase[6,11].

Non-aqueous mobile phases are commonly used in normal-phase systems. Very weak acids, used as acid–base indicators for aqueous (*p*-nitrophenol) and non-aqueous (*o*-nitroaniline) media, can be detected in such systems by measuring the SC. If an acid is present in the stationary phase, even carboxylic acids are eluted in symmetrical zones and are readily detectable[4]. Strong inorganic acids can be washed from silica gel with pure ethanol in the dead-volume (phosphoric acid) or with min-

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TABLE I

DISSOCIATION CONSTANTS OF PHOSPHORIC ACIDS

According to W. D. Kumler and J. J. Eiler, *J. Am. Chem. Soc.*, 65 (1943) 2356.

Constant	*OPA*	*MBP*	*DBP*
pK_1	2.13	1.89	1.72
pK_2	7.21	6.84	–
pK_3	12.32	–	–

imal retention (nitric acid, sulphuric acid). Hydrocarbon mobile phases, containing several percent of butanol and 10 ppm of picric acid, can elute even orthophosphoric acid in a symmetrical zone from silica gel[4]. These facts suggest that in non-aqueous mobile phases the dissociation of hydrogen ions is strongly suppressed. Therefore, it is expedient to study the separation and SC detection of strong acids, which can be detected by common liquid chromatography techniques only with difficulty, in a normal-phase system in greater detail.

A partially hydrolysed extraction solution of tributyl phosphate, which is used, for example, for industrial processing of irradiated fuel[12,13], has been selected for our study as a model, being interesting both theoretically and practically. This extraction solution contains an entirely apolar hydrocarbon solvent, a polar tributyl ester of orthophosphoric acid (tributyl phosphate, TBP), its hydrolytic products dibutylphosphoric acid (dibutyl phosphate, DBP), monobutylphosphoric acid (monobutyl phosphate, MBP), orthophosphoric acid (OPA), butanol and water. There are almost no differences between the first pK_a values of the acids produced by TBP hydrolysis (Table I). The selected model can serve to verify the assumed relationship of SC detection sensitivity and solute dissociation constant[6], to study the effect of the hydrophobicity of a strongly acidic solute on its retention and SC detection, to verify the relationship between the acid retention and SC response value[1], and to investigate the selectivity of the SC detection of compounds of various types.

EXPERIMENTAL

A Varian 8500 pulseless pump (Varian, Palo Alto, CA, U.S.A.) and a Knauer Model 2025/50 refractometer (Knauer, Oberursel/Taunus, F.R.G.) with a cell volume of 8 μl, were the main components of the chromatographic apparatus (Fig. 1). The SC, sensed from an electrically screened and isolated column[2], was led to a Vibron Model 33 C electrometric amplifier (Electronic Instruments, Richmond, U.K.). The inlet resistance of the electrometer was 10^8 or 10^9 Ω. The preparation of stainless-steel columns, 200 × 4 mm I.D., is described in ref. 14.

Silica gels from two batches, with a large surface area (Silasorb 600, Lachema, Brno, Czechoslovakia), and mean particle diameters of 7.5 μm (S = 520 m^2/g) and 10 μm (S = 490 m^2/g), were used as stationary phases. After grading, silica gel from both batches was washed with hydrochloric acid and distilled water only. Perfect stability of the bed was reached by washing the packed column with 2-propanol–heptane (15:85) saturated to 80% with water.

The mobile phases always contained analytical-grade *n*-heptane (Reakhim,

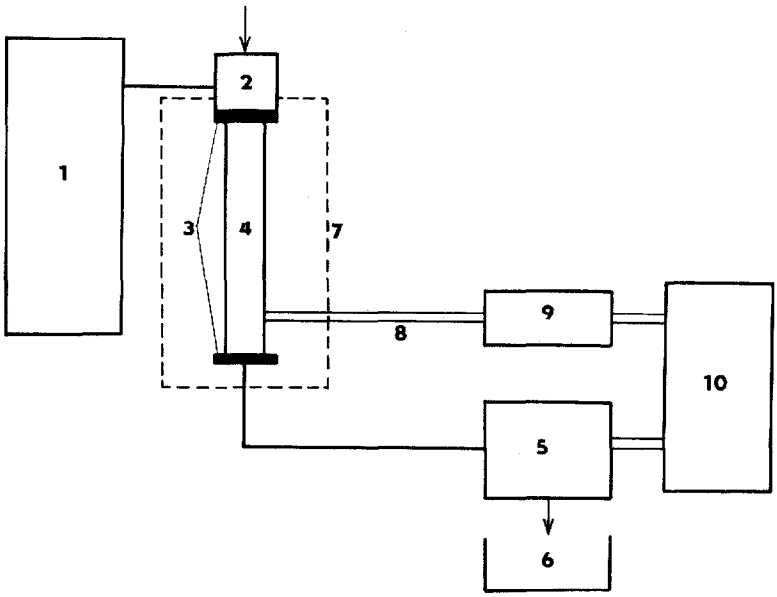

Fig. 1. Diagram of the apparatus. (1) Pulseless pump, (2) injection block, (3) column insulation, (4) chromatographic column, (5) refractometer, (6) mobile-phase waste, (7) column shielding, (8) contact for SC sensing, (9) DC amplifier, (10) dual-pen recorder.

Moscow, U.S.S.R.) with 5–30% analytical-grade 2-propanol (Lachema). Water or analytical-grade perchloric acid (Lachema) at a concentration of 3.2 ppm was added to the heptane–2-propanol mixture. The water content of the mobile phase was controlled within the range of relative saturation of 10–80% by mixing the water-free heptane–2-propanol mixture with the same mixture, saturated with water. All measurements were performed at room temperature. The column was not thermostated. The flow-rate was 1 ml/min.

Squalane (Carlo Erba, Milano, Italy) was selected as a model of the high-boiling hydrocarbon solvent in the extraction solution of TBP. The low-boiling hydrocarbon solvent was modelled with cyclohexane (Lachema), which was also used for the measurement of the column dead-volume. TBP, butanol and OPA were of analytical grade (Lachema). Mixtures of DBP and MBP in the weight ratios 9:1 and 1:1, respectively, were technical products with admixtures of butanol and OPA. Samples were injected with microsyringes.

RESULTS AND DISCUSSION

Differences in the peak shape of injected solutes in refractometric (RI) and SC records were sometimes observed when the effects of the mobile-phase composition on the SC, generated in normal-phase systems[4], were studied. The generation of SC responses of solutes of different types has not yet been studied adequately to explain this experimental fact. The refractometer was therefore used as a universal reference detector to optimize the separation of the sample components. It was also used as a reference detector when the SC records were assessed. The composition of the

TABLE II

DEPENDENCE OF CAPACITY FACTORS ON RELATIVE SATURATION OF MOBILE PHASE WITH WATER

a = Stationary phase: Silasorb, S = 520 m^2/g; b = stationary phase: Silasorb, S = 490 m^2/g.

Solute	*Relative saturation (%)**										
	10	*20*		*30*		*40*		*60*		*80*	
	b	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	a	b
Squalane	−0.11	−0.09	−0.11	−0.12	−0.12	−0.12	−0.13	−0.13	−0.12	−0.12	−0.10
Cyclohexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Propanol	0.08	0.10	0.07	0.09	0.07	0.10	0.09	0.12	0.12	0.22	0.25
TBP	0.32	0.30	0.34	0.32	0.34	0.31	0.33	0.35	0.35	0.38	0.38
DBP	0.45	0.52	0.52	0.65	0.59	0.71	0.80	1.2	0.9	1.2	1.2
Butanol	0.69	0.67	0.73	0.67	0.74	0.71	0.77	0.80	0.87	1.0	1.1
MBP	0.85	0.89	1.3	1.1	1.2	1.3	1.4	2.1	2.0	3.1	3.1
OPA	1.9	2.2	2.4	3.7	3.1	4.5	4.1	6.6	6.8	12.4	14.7
Water	6.6	5.9	6.8	6.5	7.5	–	8.8	12.5	13.5	>17	–

* Expressed in terms of volume parts of 2-propanol–heptane (15:85) saturated with water.

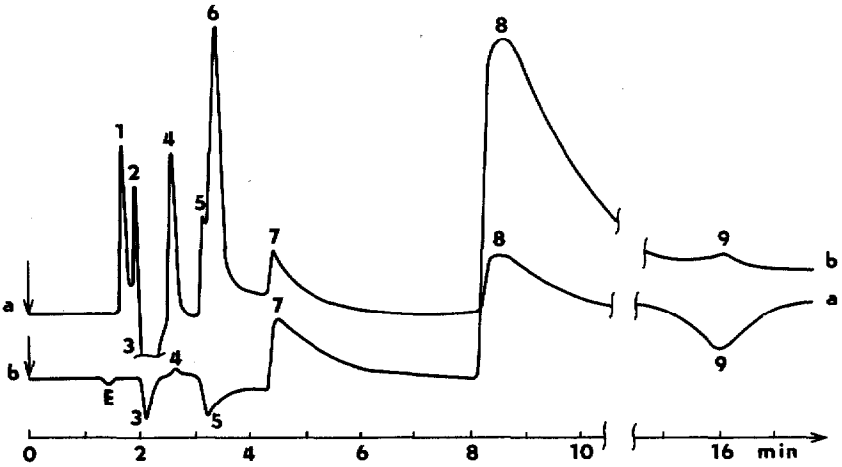

Fig. 2. Separation of the model mixture using a chromatographic system without perchloric acid. Mobile phase: 2-propanol–heptane (15:85) saturated to 30% with water; stationary phase: Silasorb 600, S = 520 m^2/g, d_p = 7.5 μm. a = RI response and b = SC response; basic current: positive, 90 pA. Peak identity: E = exclusion SC response, 1 = squalane, 2 = cyclohexane, 3 = 2-propanol vacancy, 4 = TBP, 5 = DBP, 6 = butanol, 7 = MBP, 8 = OPA, 9 = water.

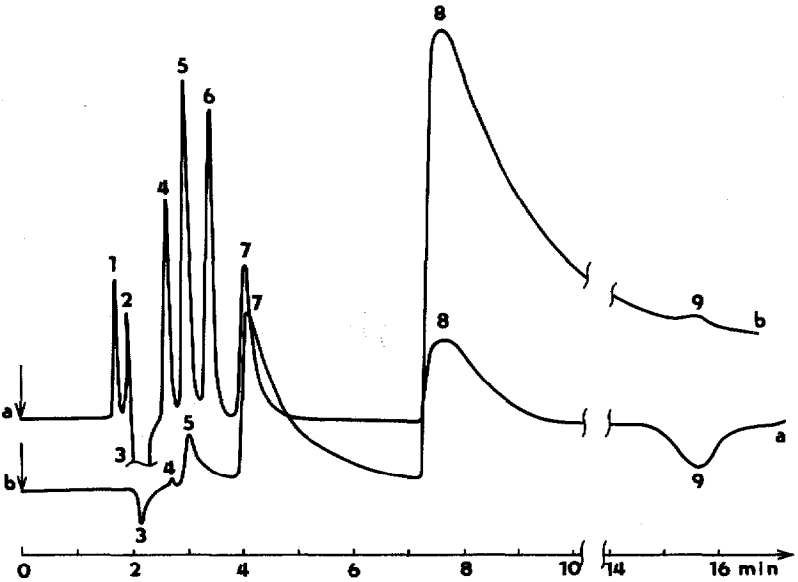

Fig. 3. Separation of the model mixture using a chromatographic system containing perchloric acid in both the mobile and the stationary phase. Mobile phase: 2-propanol–heptane (15:85) saturated to 30% with water, containing 3.2 ppm of perchloric acid; stationary phase: Silasorb 600, S = 490 m^2/g, d_p = 10 μm, equilibrated with the mobile phase. a = RI response and b = SC response; basic current: positive, 100 pA. For peak identity, see Fig. 2.

TABLE III

DEPENDENCE OF CAPACITY FACTORS ON RELATIVE SATURATION OF THE MOBILE PHASE WITH WATER AFTER THE TREATMENT OF SILICA GEL WITH CONCENTRATED PERCHLORIC ACID

Mobile phase: 2-propanol–heptane (15:85); stationary phase: Silasorb, S = 490 m²/g.

Solute	*Relative saturation (%)*						
	10	*20*	*30*	*30**	*40*	*60*	*80*
Squalane	−0.11	−0.11	−0.11	−0.11	−0.11	−0.12	−0.11
Cyclohexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-Propanol	0.06	0.05	0.09	–	0.11	0.14	0.28
TBP	0.31	0.33	0.35	0.36	0.34	0.35	0.38
DBP	0.39	0.45	0.52	0.54	0.62	0.78	1.1
Butanol	0.64	0.69	0.75	0.73	0.78	0.87	1.1
MBP	0.74	0.90	1.1	1.1	1.4	2.1	3.5
OPA	1.8	2.3	3.0	3.1	3.9	7.2	15.6
Water	6.5	6.5	7.1	7.1	8.1	14	–

* The values measured with the mobile phase containing 3.2 ppm of perchloric acid.

chromatographic system was therefore adjusted so as to obtain complete separation of the zones and the greatest possible symmetry of the zones in the refractometric record. Only then the composition of the system was adjusted so as to improve the zone symmetry in the SC record.

Separation

The solubility of the sample components in different organic solvents made it possible to use a hydrocarbon and an alcohol as mobile-phase constituents. Rough adjustment of the retentions of sample components was effected by selecting the alcohol concentration in the mobile phase. However, in the mobile phases consisting of a hydrocarbon and an alcohol, the zones of acidic components were strongly asymmetrical and the separation of DBP, MBP and butanol was impossible. The separation of these components was achieved after water was added to the hydrocarbon–alcohol mixture (Table II). However, the symmetry of acid zones remained poor (Fig. 2). The use of silica gel from another batch did not affect the zone symmetry.

To suppress the asymmetry of the acid zones, perchloric acid was applied. The symmetry of the acid zones in the refractometric record improve substantially (Fig. 3) after column equilibration with a mobile phase containing 3.2 ppm of perchloric acid. Acid retentions decreased simultaneously (Table III). The greatest relative decrease in capacity factor was found for DBP (19%); the capacity factor for MBP decreased by 13%. The least decrease (11%) was measured for the OPA zone. Better acid zone symmetry and decrease in acid retention favourably influenced the resolution of DBP, MBP and butanol (Fig. 3). The shape of zones and the retention of TBP, butanol and water did not change on adding traces of perchloric acid to the mobile phase. If the column, equilibrated with the mobile phase containing perchloric acid, was washed with 250 ml of ethanol (125 dead-volumes), both retention and

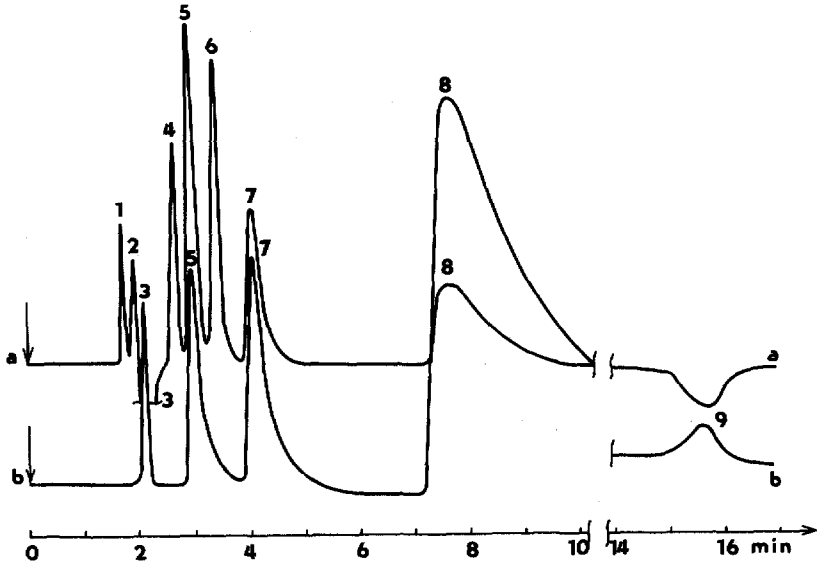

Fig. 4. Separation of the model mixture using a chromatographic system containing perchloric acid in the stationary phase only. Mobile phase: 2-propanol–heptane (15:85) saturated to 30% with water; stationary phase: Silasorb 600, S = 490 m²/g, d_p = 10 μm, treated with concentrated perchloric acid. a = RI response and b = SC response; basic current: positive, 140 pA. For peak identity, see Fig. 2.

zone shape observed prior to the addition of perchloric acid to the mobile phase were reproduced.

If concentrated perchloric acid (32%) was injected into a column washed with the mobile phase without perchloric acid several times, the same retentions were measured (Table III) as in the elution of the solute with a mobile phase that contained perchloric acid. In the refractometric detection, the shapes of acid zones were also identical in both instances (Figs. 3 and 4). Perchloric acid, retained in the stationary phase, could be washed out with ethanol, even when the column had been treated with the concentrated acid. Perchloric acid was also being washed very slowly from the stationary phase with the mobile phase containing 15% of 2-propanol. The loss of perchloric acid from the stationary phase appeared as an increase in the capacity ratio of acids and as a worsening of the symmetry of their zones. The injection of the concentrated acid once a day sufficed to maintain perchloric acid in the stationary phase at a sufficient concentration.

Complete separation of all the sample components can thus be accomplished if a ternary mobile phase, composed of a hydrocarbon, alcohol and water, is used and silica gel with a large surface area is equilibrated with it. Zone asymmetry of acidic solutes can always be suppressed in the refractometric record if a sufficiently strong acid is added to the chromatographic system. Squalane is separated from cyclohexane due to a molecular-sieve effect. The retention volume (1.25 ml) of the exclusion SC response, recorded for the chromatographic system that does not contain perchloric acid (Fig. 2) is equal to the exclusion volume of the column[3,15]. The pore volume of the column packing[15] equals 0.78 ml. From these data and from the formal negative capacity factor of squalane (0.12), it follows that 36% of the sta-

tionary-phase pore volume that is accessible to cyclohexane is inaccessible to squalane.

The remaining solutes are separated owing to the dissolution mechanism, since the equilibration of silica gel with the stationary phase used gives rise to a liquid–liquid separation system[16]. The elution order of TBP and its hydrolytic products is in accord with the increase in hydrophilicity of these sample components. The elution order DBP < butanol < MBP remains unchanged, even in the separation system without perchloric acid. It follows that the acidity of solutes and their dissociation in the stationary phase associated with it do not influence retention very much. However, it causes marked tailing of chromatographic zones, which can be suppressed by the presence of a strong acid in the stationary phase (ion-suppression effect)[17].

Streaming-current detection

An SC was generated on washing the column with each of the mobile phases used. Both the polarity and the magnitude of the basic current markedly changed with the mobile-phase composition. The dependence of the basic current on the 2-propanol contents in the mobile phase can serve as a typical example (Table IV). The mobile-phase volume required for the stabilization of the basic current after the change in the mobile-phase composition or after the injection of phosphoric acids varied, depending on the mobile-phase composition. The higher the alcohol content in the mobile phase (and thus also its eluting power), the more rapid was the column equilibration.

The exclusion SC response, generated after sample injection[3,15], was greatest for the 2-propanol–heptane mixtures to which neither water nor perchloric acid was added. As the 2-propanol concentration in the mobile phase increased, the exclusion response rapidly decreased. The exclusion response was also suppressed by an increase in water content or by the presence of perchloric acid in the chromatographic system, or by a combination of both effects (Figs. 2 and 3). The above results confirm the previous observations[3,4,15] that the exclusion SC response is generated only in a chromatographic system with minimal concentrations of ionizable compounds (*e.g.* strong acids) or solvents capable of supporting ionization (*e.g.* alcohols, water). Hence, it can be said that the generation of the exclusion SC response is associated with a limited capability of the chromatographic system to restore, sufficiently fast, the electrical equilibrium between the stationary and the mobile phases that was disturbed by the sample injection or some other effect.

The mobile-phase composition also influenced the sign, magnitude and time

TABLE IV

DEPENDENCE OF BASIC CURRENT, I_B, ON THE 2-PROPANOL CONCENTRATION IN THE MOBILE PHASE

Current sign: negative; stationary phase: Silasorb 600, S = 520 m²/g, d_p = 7.5 μl; flow-rate: 1 ml/min.

	2-Propanol (%)					
	5	*10*	*15*	*20*	*25*	*30*
I_B (pA)	0.7	2.8	28	140	700	2160

course of the responses of injected compounds of different types. The SC response of the compounds modelling hydrocarbon solvents (squalane, cyclohexane) and the SC response of butanol were observed in none of the mobile phases. The SC response of TBP, if registered at all, and the SC response of water always agreed, in elution volumes and shape, with refractometric response. The elution volume of the zone maximum was always in accord with the refractometer response as far as the SC responses of phosphoric acids are concerned. The tailing of the acid zone, however, depended markedly on the mobile-phase composition and, as a rule, differed from the shape of the same zone in the refractometric record (Figs. 2–4).

In elution by mobile phases without perchloric acid, the zones of phosphoric acids in the SC record were less symmetrical than in the refractometric record. The zone asymmetry always increased in the following order: DBP < MBP < OPA. With lower alcohol and water contents in the mobile phase, elution of phosphoric acids in the SC record was often so slow that it appeared as a change in the baseline (in the basic current) after injection (Fig. 2). However, the amount of acid trapped by the stationary phase in this way from a single injection was so small that it could not be determined quantitatively by aassessing the refractometer record.

The introduction perchloric acid into the chromatographic system eliminated the changes in the basic current after the injection of phosphoric acids. The increase in the concentration of strong acid in the hydrocarbon–alcohol mobile phase containing water, raises the basic current more rapidly than do the responses of acidic solutes[4]. A low concentration of perchloric acid in the mobile phase (3.2 ppm) was therefroe used. This concentration of acid was sufficient to improve the symmetry of the zones of acids in the refractometric record (Figs. 2 and 3). In the SC record, the polarity of DBP response changed, MBP and OPA responses increased slightly, and the change in the basic current after injection of the phosphoric acids was avoided. After elution of the OPA zone maximum, the basic current was established very slowly (Fig. 3).

The optimal results of SC detection of very weak acids in the normal-phase system were obtained if strong acid was present in the stationary and not in the mobile phase[4]. SC detection of phosphoric acids was therefore tested after column treatment by injection of concentrated perchloric acid. After this silica gel treatment, the TBP response was not registered, but the responses of MBP and DBP further increased, and the symmetry of the zones of phosphoric acids improved markedly. After OPA elution, the basic current reached an almost constant value before elution of the water zone (Fig. 4). This chromatographic system, being the best from the viewpoint of zone shape in the SC record, was also the best from the viewpoint of the sensitivity of SC detection of phosphoric acids (Table V). However, the phosphoric acid zones remained less symmetrical than in RI detection. The injection of varying weight amounts of the phosphoric acids confirmed that the zone height was directly proportional to the amount injected.

The zone shapes of the phosphoric acids differed in the RI and SC records for all the mobile phases used. These differences were most obvious in the RI and SC chromatograms obtained in the system without perchloric acid (Fig. 2). The injected weight amounts of all samples were so small that the refractometer operated in the linear range. It follows that, at least after elution of the maxima of the phosphoric acid zones, the response in SC detection of descending zone boundaries was not

TABLE V

MINIMUM DETECTABLE CONCENTRATIONS (IN g/ml) OF THE SOLUTES IN THE EFFLUENT

a = Stationary phase treated with concentrated perchloric acid, mobile phase: 2-propanol–heptane (15:85) saturated to 30% with water; b = stationary phase equilibrated with the mobile phase given above to which 3.2 ppm of perchloric acid were added.

Solute	*RI*		*SC*	
	a	*b*	*a*	*b*
TBD	$2 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	–	–
DBP	$2 \cdot 10^{-6}$	$2 \cdot 10^{-6}$	$1 \cdot 10^{-7}$	$6 \cdot 10^{-7}$
Butanol	$3 \cdot 10^{-6}$	$3 \cdot 10^{-6}$	–	–
MBP	$4 \cdot 10^{-6}$	$4 \cdot 10^{-6}$	$2 \cdot 10^{-7}$	$3 \cdot 10^{-7}$
OPA	$1 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$2 \cdot 10^{-8}$	$2 \cdot 10^{-8}$
Water	$4 \cdot 10^{-6}$	$5 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$3 \cdot 10^{-6}$

directly proportional to the solute concentrations in the mobile phase.

In view of the theories of retention of ionizing compounds[17] and SC generation[1,7,18], one can say that the distortion of the zones of phosphoric acids in the SC record is associated with the dissociation of these acids in the stationary phase. The origin of the responses of phosphoric acids in the systems studied can be described by the following model.

In the mobile phase, which is substantially a mixture of a hydrocarbon with an alcohol, the dissociation of phosphoric acids is neglectable. However, in the stagnant liquid on the surface of the solid phase, which, in comparison with the mobile phase, is substantially enriched with alcohol and water, phosphoric acids dissociate. Released hydroxonium cations protonize the least acidic silanol groups on the silica gel surface[19], thereby increasing the surface positive charges. The anions of phosphoric acids remain in the stagnant part of the electric double-layer. The leak of anion from the stagnant part of the double-layer is supported by the hydrophobicity of the anion. Hence, the ability of phosphoric acid to change the basic current after the injection increases in the sequence DBP < MBP < OPA.

If a very strong hydrophilic acid is added to the stationary phase, it suppresses the dissociation of the phosphoric acids. The decrease in the dissociation of phosphoric acids appears as a decrease in their capacity factors down to the value corresponding to the distribution of uncharged molecules[17] and as the elimination of the retention dependence on concentration[11]. The elimination of the concentration dependence of retention will show itself in the normal-phase system as the disappearance of the tailing of the zones of these acids in the refractometric record (Fig. 3). The anions of strong acids can also substitute for the anions of phosphoric acids in the stagnant part of the electric double-layer. At a sufficiently high concentration, strong acids will suppress the dissociation of phosphoric acids entirely and substitute for them completely, also in the protonation of silanols. The competition of very strong acids manifests itself in an improved agreement between the shapes of zones of the phosphoric acids in the RI can SC records.

It can be seen from Tables I, III and V that the sensitivity of SC detection of

phosphoric acids increases with retention the sequence DBP < MBP < OPA, despite the fact that their first dissociation constants decrease from DBP to OPA (Table I). This finding is in contrast with the assumption made by Terabe *et al.*[6] that for weak acids under reversed-phase conditions the magnitude of the dissociation constants of solutes will determine the sensitivity of SC detection. The finding would instead justify the theoretical conclusion by Šlais and Krejčí[1] that the sensitivity of SC detection of retained solutes will increase in proportion to their distribution constants. However, it follows from Figs. 2–4 and from the results of ref. 4 that in the given chromatographic system a simple direct proportion between the solute response and its distribution constant (retention), which was derived in ref. 1, is not generally valid either. Undoubtedly, SC detection sensitivity will depend on solute retention and the ability of a solute to produce ions. However, the changes in the sensitivity of the detection of phosphoric acids with the composition of the chromatographic system and previous results[4,6,8–11] suggest that, in addition to the above two, some other factors will still appear. Some of them, *e.g.* the magnitude of the basic current[4,6], the total concentration of ions in the mobile phase[8–10], and the concentration of substances that can either ionize or support ionization[4], have already been reported in the literature.

CONCLUSIONS

All the compounds occurring in a hydrolysed extraction solution of TBP can be separated in a normal-phase system and, by using SC detection, the acidic hydrolysis products DBP, MBP and OPA can be detected selectively. The SC can be sensed directly from the chromatographic column. The retention of zones in SC detection is identical with the retention found by RI detection. The magnitude of the SC response of sample components in the zone maxima is directly proportional to the amount injected. However, after elution of the zone maximum, the magnitude of the SC response of the acids does not correspond to their concentration in the effluent. The shape of zones in the SC record differ from that obtained by refractometric detection. Zone tailing is greater in SC detection. The analysis must therefore be evaluated quantitatively from zone heights. The physical reason for the disagreement between the zone shapes of phosphoric acids in RI and SC records in inherent in the different mechanisms of generation of the two responses.

The sensitivity of detection of the various acids depends on the composition of the chromatographic system. It increases with retention of the components in a given chromatographic system. From the viewpoint of the magnitudes of the retentions, the shapes of zones in the refractometric and SC records, and from the viewpoint of the sensitivity of SC detection, the following separation system is optimal. A mixture of a hydrocarbon with an alcohol, partly saturated with water, serves as the mobile phase. It is expedient that silica gel with a large surface area, which was treated with a strong acid prior to injection, be selected as the stationary phase. The silica gel, which had been washed only with the solutions of acids and with water during its production and treated with concentrated perchloric acid after having been packed into the column, proved to be acceptable. If the specific surface area of silica gel is 500 m^2/g, a mixture of 2-propanol with a hydrocarbon (15:85), saturated to 30% with water, is suitable as the mobile phase. Hydrocarbon solvents TBP, DBP,

MBP and OPA can be separated in this chromatographic system on a 200 × 4 mm I.D. column at a flow-rate of 1 ml/min within 10 min. Water is eluted within about 16 min. The separated components can be detected refractometrically in the eluent at a sensitivity in the ppm range. SC detection of acidic components in the sample is more sensitive by 1–2 orders of magnitude.

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