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ION-EXCHANGE PHENOMENA AND CONCOMITANT pH SHIFTS ON THE EQUILIBRATION OF REVERSED-PHASE PACKINGS WITH ION-PAIRING REAGENTS*

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SUMMARY

Breakthrough patterns of eluents containing tetrabutylammonium or cetyltrimethylammonium ions (two frequently used ion-pairing agents in high-performance liquid chromatography) have been studied, using reversed-phase columns with octadecyl chains permanently bonded to 10-μm silica particles. The occurrence of pH shifts either before or after the breakthrough of the ion-pairing agents has been demonstrated. An explanation for the breakthrough patterns is offered and evidence for two different binding mechanisms of these agents is presented.

INTRODUCTION

Ion-pair chromatography performed with reversed-phase columns and mobile phases containing ion-pairing agents has been the subject of a number of studies during recent years and has been reviewed by Tomlinson *et al.*[1]. Much attention has been focused on the mechanism underlying this type of chromatography and two models have been proposed: ion-pair formation in the mobile phase or at the interface of the stationary phase and the mobile phase has been proposed by some workers[2–4] and ion exchange of solute ions against the counter ion of the adsorbed reagent by others[5–11]. If the retention behaviour is to be explained by the latter mechanism, a relationship between the amount of reagent adsorbed and the retention volumes of the chromatographed solute ions should exist. Investigations on this relationship were carried out by Terwey-Groen *et al.*[12] and Van de Venne *et al.*[8].

During experiments performed in order to elucidate the retention mechanism of water-soluble corticosteroids, chromatographed with tetrabutylammonium (TBA) containing eluents, the breakthrough patterns of these eluents appeared to be strongly dependent on the history of the columns (*i.e.*, the previous eluents used). In a number of instances breakthrough of the TBA ions occurred in two distinct steps; pH measurements on the collected fractions indicated that these steps corresponded with shifts in the pH of the eluate. Similar observations were made in the breakthrough

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patterns of cetrimide-containing eluents used for studying the retention behaviour of weak acids in this type of chromatographic system.

We therefore decided to study this phenomenon of pH shifts and to investigate the breakthrough patterns with a flow-through detection device.

EXPERIMENTAL

Materials and reagents

Potassium dihydrogen phosphate, potassium bromide, methanol and sodium hydroxide were of analytical-reagent grade and were obtained from Merck (Darmstadt, G.F.R.). Dioctyl sodium sulphosuccinate (DOSS), 85% phosphoric acid ("reinst"), benzoic acid and lithium nitrate were also obtained from Merck. Tetrabutylammonium hydroxide (40%) ("prakt.") and tetrabutylammonium bromide ("puriss.") were purchased from Fluka (Basle, Switzerland). Potassium hydroxide came from EKA (Bohus, Sweden). Cetyltrimethylammonium bromide (cetrimide) was purchased from BDH (Poole, Great Britain) and boric acid ("analyzed" reagent) from Baker (Deventer, The Netherlands). Prednisolone 21-*m*-sulphobenzoate sodium was obtained from Lark (Milan, Italy).

Water was distilled twice from glass after deionization and used immediately.

Tetrabutylammonium dihydrogen phosphate solution was prepared from tetrabutylammonium hydroxide by neutralization with an equimolar amount of phosphoric acid. TBA-containing eluents were prepared by mixing equal weights of filtered methanol and a filtered aqueous solution containing $2 \cdot 10^{-2}$ *M* potassium dihydrogen phosphate and 10^{-2} *M* tetrabutylammonium dihydrogen phosphate (the pH of this aqueous solution was adjusted with potassium hydroxide).

Phosphate buffer eluents were prepared by mixing an equal weight of filtered methanol and a filtered aqueous solution containing $3 \cdot 10^{-2}$ *M* potassium dihydrogen phosphate (the pH of this aqueous solution was adjusted with potassium hydroxide).

The pH values of the eluents used, containing 50% (w/w) of methanol, were measured after calibrating the pH meter against methanol–water (1:1) buffers as described by Bates[13]. The pH meter readouts thus obtained are denoted by pH*. The pH of the aqueous solution used for the preparation of the methanol–water eluent was approximately 1.3 pH units lower than the pH* measured in the eluent itself.

Cetyltrimethylammonium (CTA) containing eluents were prepared in water–methanol mixtures (final ratio in the eluent = 1:1, w/w) with $5.49 \cdot 10^{-3}$ mol·kg^{-1} (= 0.2%, w/w) of cetrimide and $94.51 \cdot 10^{-3}$ mol·kg^{-1} of potassium bromide (the total bromide concentration is thus 0.1 mol·kg^{-1}); the eluent was buffered with boric acid buffer (0.025 mol·kg^{-1} in the eluent) and adjusted to the desired pH* with sodium hydroxide. Eluents without cetrimide were prepared in the same way; the cetrimide was replaced with an equimolar amount of potassium bromide (resulting in a potassium bromide concentration of 0.1 mol·kg^{-1} in the eluent).

Instrumental

The chromatographic experiments were performed with the following instrumental combination. A 6000A solvent-delivery system was connected to a U6K injection system, modified with a 200-μl sample loop; the two columns used in this study

were a μBondapak C_{18} column (30 cm × 3.9 mm I.D.), particle size 10 μm, and a Radial-Pak A cartridge (10 cm × 8 mm I.D.), in combination with an RCM-100 radial compression module (all from Waters Assoc., Milford, MA, U.S.A.). The packings of both columns were of the reversed-phase type, with octadecyl chains chemically bonded on 10-μm silica particles. Each column was installed between a U6K injection system and a Model 440 differential UV absorbance detector (Waters Assoc.) by means of a Valco rotary six-port 7000 p.s.i.g. valve (Chrompack, Middelburg, The Netherlands). The connection was made in such a way that a bypass could be used to flush the whole system except the column when the solvents were changed. The UV detector was connected to an R401 refractive index detector (Waters Assoc.).

The inlet of a Radiometer (Copenhagen, Denmark) G299A capillary glass pH electrode was connected to the outlet of the refractive index detector. The outlet of the capillary glass electrode was connected to a small conical glass vessel (provided with an outlet for the excess of eluted solvent) in which a Type 373-90 reference electrode (Ingold, Zürich, Switzerland) was placed. The capillary glass electrode and the reference electrode were connected to a Radiometer PHM 64 pH meter. The jacket around the capillary glass electrode was filled with potassium nitrate solution that was electrically connected to the reference electrode in order to avoid instability of the signal of the pH electrode[14].

During the chromatographic experiments the refractive index detector and the columns were kept at 25°C. Flame absorption spectrometry was carried out on a Perkin-Elmer 400 S spectrophotometer (Perkin-Elmer, Norwalk, CT, U.S.A.).

Procedures

The breakthrough pattern of TBA ions was studied in combination with the μBondapak C_{18} column; the breakthrough pattern of CTA ions was investigated with the Radial-Pak A cartridge. These breakthrough patterns were monitored at different pH* values of the eluents, with the UV absorbance detector, the refractive index detector and the pH detector on-line. The UV detection did not give any additional information to the results obtained with the other detectors.

For measuring potassium, TBA and CTA concentrations in the eluate, the pH detector was disconnected and fractions of the eluate were collected according to the response of the refractive index detector. For the determination of potassium the collected fractions were diluted with water to a concentration of 1–2 ppm of potassium and acidified with hydrochloric acid at a final concentration of 0.4 *M*; potassium concentrations were then measured by means of flame absorption spectrometry.

TBA and CTA concentrations were determined by titrating the collected fractions with DOSS in a two-phase system consisting of chloroform and an aqueous buffer of pH 2.8, using methyl yellow as the indicator[15]. The presence of bromide in the eluate was confirmed by the reaction with silver nitrate[16].

Injections of tetrabutylammonium bromide (TBABr), potassium bromide and phosphoric acid, each dissolved in the eluent, were carried out on the μBondapak C_{18} column, after equilibration of the column with a specific mobile phase. The hold-up time on the μBondapak C_{18} column was determined by measuring the retention time of lithium nitrate using the phosphate buffer eluent of pH* 5.8.

On the Radial-Pak A column the first change in refractive index on injection of a small amount of the eluent to which some methanol had been added was used as an indication of the hold-up time.

RESULTS AND DISCUSSION

The breakthrough patterns on equilibration of the columns with buffer solutions (without TBA or CTA) were found to depend on the history of the columns. If the column had previously been equilibrated with a phosphate buffer eluent of different pH*, the following pattern was observed. Before the hold-up time, the previous eluent is eluted. After the hold-up time, the eluate still exhibits the pH* of this previous eluent. A certain time after the hold-up time, the eluate shows a pH* shift to the pH* of the new eluent. Fig. 1 shows an example of the breakthrough pattern of the phosphate buffer eluent pH* 7.8 after equilibration of the column with the phosphate buffer eluent of pH* 5.8. From these phenomena it was concluded that the columns have some ion-exchange capacity. Apparently, during equilibration with a buffer, part of the residual silanol groups dissociates and the protons are replaced by buffer cations (in these experiments, potassium ions). The equilibration patterns of the columns with TBA- and CTA-containing eluents were also found to depend on the history of the columns.

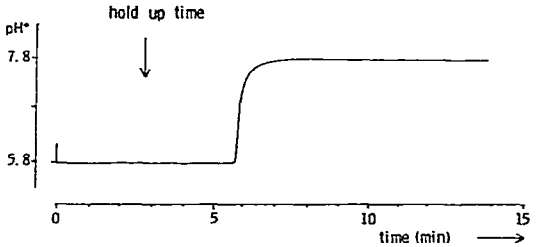

Fig. 1. Breakthrough pattern of the phosphate buffer eluent of pH* 7.8 after equilibration with the phosphate buffer eluent of pH* 5.8. Stationary phase, μBondapak C_{18}; flow-rate, 1.0 ml·min^{-1}.

According to chromatographic theory, the breakthrough volume of a component of the eluent should be the same as its retention volume after injection of a small amount of this component (provided that its distribution isotherm is linear). Injections of TBABr were therefore included in this study and the resulting chromatograms were correlated with the breakthrough patterns found with the TBA-containing eluents. For the interpretation of the chromatograms obtained after injection of TBABr, comparisons with the chromatograms resulting from injection of potassium bromide and phosphoric acid were made. Chromatograms obtained after injection of these compounds and elution with phosphate buffer eluent of pH* 7.3 are shown in Fig. 2. After injection of potassium bromide (Fig. 2a), a peak was observed on the refractive index detector just after the hold-up time. In the eluate corresponding with this peak, the presence of bromide and an increased potassium concentration were demonstrated. Injection of phosphoric acid (Fig. 2b) resulted in two peaks. The first, just after the hold-up time, demonstrated an increased potassium concentration; the second (negative) peak was accompanied by an increased proton concentration, as

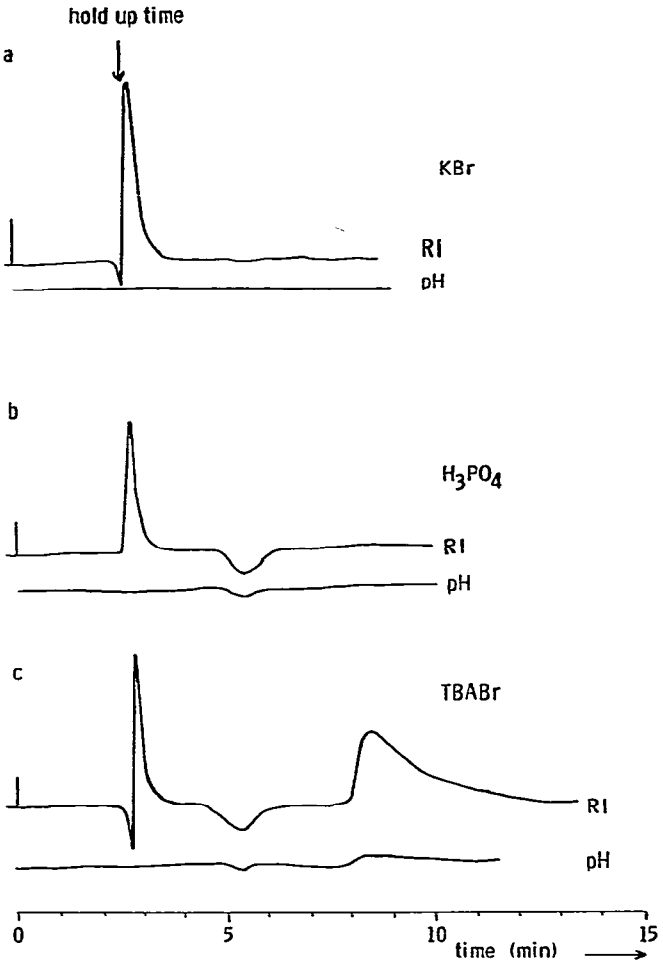

Fig. 2. Chromatograms of 50 μl of (a) a saturated solution of potassium bromide, (b) 20 μg of phosphoric acid and (c) 100 μg of TBABr, all dissolved in the eluent. Chromatographic conditions: μBondapak C_{18}; phosphate buffer eluent of pH* 7.3; flow-rate, 1.0 ml·min^{-1}.

can be seen from the pH detector response. It was therefore concluded that potassium ions are displaced from the stationary phase by protons, as in a cation-exchange system. The liberated potassium ions and the (injected) protons move through the column, each with their own velocity. After injection of TBABr, three peaks were observed with the refractive index detector (Fig. 2c). In the first peak, just after the hold-up, the presence of bromide ions and an increased potassium concentration were demonstrated. The second (negative) peak was similar to the second peak after the phosphoric acid injection (Fig. 2b). Titration of the eluate, corresponding to the third peak, with DOSS confirmed the presence of TBA ions. This last peak was accompanied by a decreased proton concentration. These phenomena can also be explained by cation-exchange processes. TBA is exchanged with potassium ions and protons; these protons in their turn are exchanged with potassium ions. The TBA peak is

eluted, accompanied by a decrease in the proton concentration, for protons will be taken up from the mobile phase by the stationary phase on the release of TBA ions.

At pH* 6.8 the picture is more complicated (Fig. 3), because of the reduction in the retention volume of TBA at the higher proton concentration and the asymmetry of the TBA peak. In the eluate corresponding to the first peak (at 2.8 min) the presence of extra potassium ions and of bromide ions was again demonstrated after injection of different amounts of TBABr. In the eluate of both the second and the third peaks (at amounts of TBA of 150–500 μg) the presence of TBA ions was demonstrated.

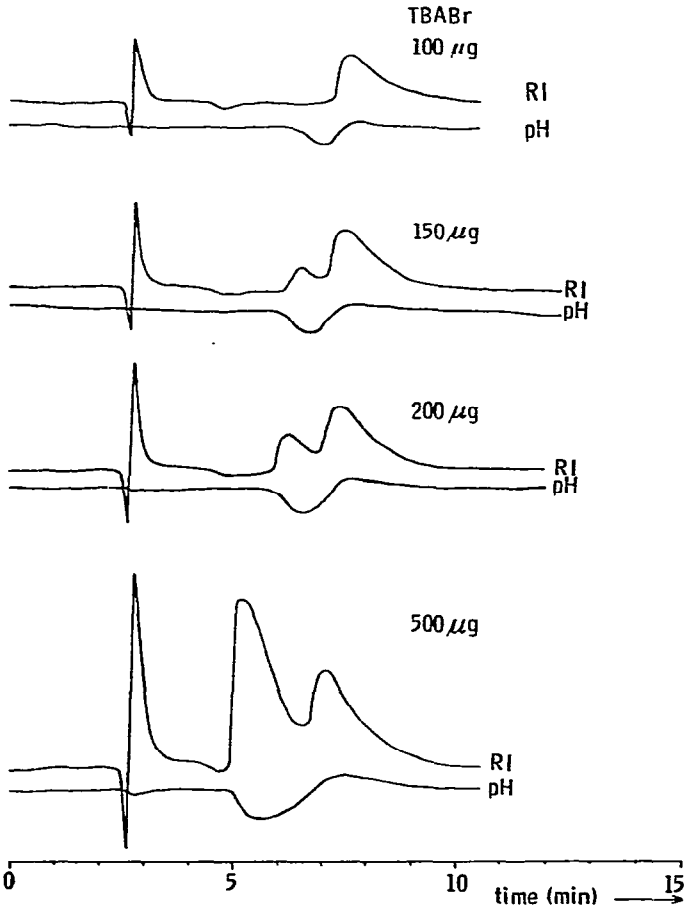

Fig. 3. Chromatograms of 100, 150, 200 and 500 μg of TBABr dissolved in the eluent. Chromatographic conditions: μBondapak C_{18}; phosphate buffer eluent of pH* 6.8; flow-rate, 1.0 ml·min^{-1}.

When 100 μg of TBABr is injected, the retention volume of the TBA zone is still larger than that of the acidic zone. On increasing the amount of TBABr injected, the retention volume of the TBA zone decreases because of the non-linearity of the distribution isotherm. The retention volume of the TBA zone can decrease to such an extent that its front coincides with the acidic zone. As a result, the TBA ions at the

back of the TBA zone move in a mobile phase solution with an increased pH*. The TBA ions at the front of the TBA zone move in a mobile phase solution with a decreased pH*. The alkaline reaction enhances the binding of the TBA ions and the acidic reaction decreases it. The velocity of the TBA ions at the back will therefore be lowered and that of the TBA ions at the front will be increased. Consequently, the TBA zone will split and be detected as a double peak. This splitting of the TBA zone was also observed at pH* values lower than 6.8. Because of the non-linearity of the distribution isotherm of TBA, coincidence of the acidic zone and the TBA zone is also possible at pH* values higher than 6.8, provided that a sufficiently large amount of TBABr is injected. It was established that injection of 1–2 mg TBABr with phosphate buffer of pH* 7.3 resulted in splitting of the TBA zone.

The chromatographic behaviour of injected TBABr explains the differences found in the breakthrough patterns of TBA-containing eluents. During equilibration with TBA-containing eluents protons and potassium ions are liberated continuously.

Fig. 4 shows the breakthrough patterns observed at different pH* values of the TBA-containing eluents after the column was equilibrated with eluents of the same pH* without TBA. Apparently, at this TBA concentration ($5 \cdot 10^{-3}$ *M*) in the eluent, at pH* 7.8 (Fig. 4c) protons and the TBA front move with almost the same velocity.

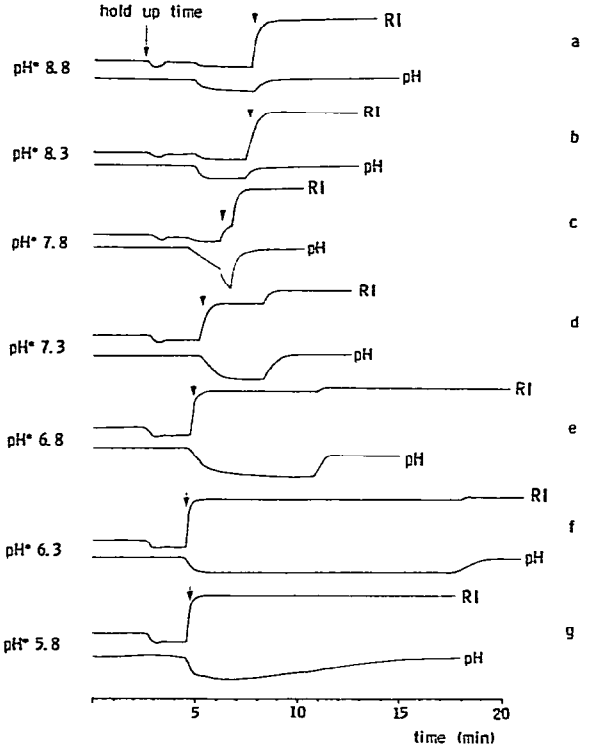

Fig. 4. Breakthrough patterns of the TBA-containing eluents after previous equilibration of the column with phosphate buffer eluents of the corresponding pH*: (a) pH* 8.8; (b) pH* 8.3; (c) pH* 7.8; (d) pH* 7.3; (e) pH* 6.8; (f) pH* 6.3; and (g) pH* 5.8. The arrows denote the breakthrough of TBA ions. Stationary phase, μBondapak C_{18}; flow-rate, 1.0 ml · min^{-1}.

At pH* 8.8 and 8.3 (Fig. 4a and b) the protons move faster than the TBA front. The TBA ions will be exchanged against protons and potassium ions and the protons in their turn against potassium ions. The patterns found at these two pH* values can be explained as follows. Until the hold-up time (2.7 min), the previous eluent will be eluted. After this hold-up time, a potassium-enriched zone (compared with the TBA-containing eluent) is eluted. The end of this potassium-enriched zone is the starting point of an acidic zone in the eluate; the pH* shifts to a lower value and the potassium concentration shifts back to that of the eluent. After a certain time, the TBA breaks through and the pH* shifts to that of the eluent. At pH* 7.3 and at lower pH* values (Fig. 4d–g) the protons move slower than the TBA zone. Consequently, the TBA ions will move through the column in a zone with lower pH* than the pH* of the eluent itself. At the front of this zone potassium ions will be exchanged against TBA ions and protons. The breakthrough patterns in Fig. 4d–g can now be explained as follows. Until the hold-up time the previous eluent is eluted. After the hold-up time, eluent in which the TBA ions are replaced by potassium ions is eluted. After a certain time the pH* will shift back to that of the eluent and the TBA concentration does the same. At pH* 5.8 this shift back to the pH* value of the eluent occurs gradually (Fig. 4g).

These observations on the breakthrough patterns are in good agreement with the observations concerning the injections. At pH* 8.8 and 8.3 (Fig. 4a and b), the time at which the pH* shifts to a lower value is almost the same as the retention time observed for the second peak after the injection of a small amount of phosphoric acid in these eluents without TBA. It was also found at pH* 7.3 and 6.8 (Fig. 4d and e), that the shift in pH* back to the pH* value of the eluent occurs at the retention time found for the acidic zone after injection of TBABr in TBA-containing eluents (results not shown).

If during the equilibration with TBA-containing eluents TBA ions and protons are exchanged against potassium ions, the potassium concentration in the first part of the eluate (*i.e.*, directly after the hold-up time) is expected to be increased. Fractions of that part of the eluate were collected and the potassium concentration was measured. The increases in the potassium concentration in that part of the eluate compared with that of the eluent itself are reported in Table I. The data indicate that

TABLE I

INCREASE IN POTASSIUM CONCENTRATION IN THE ELUATE, COLLECTED DIRECTLY AFTER THE HOLD-UP TIME, OF TBA-CONTAINING ELUENTS OF DIFFERENT pH*

pH of eluent*	*Increase in potassium concentration* ($mmol \cdot l^{-1}$)
5.8	2.2
6.3	2.2
6.8	2.8
7.3	3.0
7.8	3.0
8.3	3.4
8.8	3.8

the potassium concentration of the eluate collected directly after the hold-up time is significantly increased. If ion exchange were the only binding mechanism of TBA ions, an increase of approximately $5 \cdot 10^{-3}$ *M* should have been found, as this is the TBA concentration in all of the TBA-containing eluents. The values in Table I are significantly lower than this value of $5 \cdot 10^{-3}$ *M*, which indicates that at least one other binding mechanism also plays a role in the binding of TBA ions on the column packing material. These other binding mechanisms are based on the binding of TBA ions together with anions. These anions have to be phosphate ions as these are the only anions available. The binding of TBA ions and phosphate ions occurs either as ion pairs or in a double layer in which the TBA ions are bound by hydrophobic bonding to the octadecyl surface, their charge being compensated by phosphate ions. Recent investigations indicate that the double-layer model is probably correct[10,17,18].

The existence of at least two binding mechanisms was confirmed by stripping experiments. It was found that part of the TBA ions bound by the column could be stripped from the column with a methanol–water mixture. The remainder of the TBA ions could only be stripped with a buffer. Stripping with methanol–water will disturb the double layer by removing the phosphate ions. The TBA ions bound by ion exchange can be eluted only when cations are available in the stripping eluent. These TBA ions can therefore not be eluted efficiently with a methanol–water mixture. Table II gives the amounts of TBA ions bound to the column (as calculated from the breakthrough patterns) and the amounts of TBA ions stripped with methanol–water (1:1, w/w) and the phosphate buffer eluent of pH* 5.8.

TABLE II

AMOUNTS OF TBA BOUND TO THE COLUMN AFTER EQUILIBRATION WITH TBA-CONTAINING ELUENTS OF DIFFERENT pH* CALCULATED FROM THE BREAKTHROUGH VOLUMES AND THE AMOUNTS OF TBA WHICH CAN BE STRIPPED FROM THE COLUMN BY THE SUBSEQUENT ELUTION WITH METHANOL–WATER (1:1, w/w) AND WITH PHOSPHATE BUFFER ELUENT OF pH* 5.8 AND THE CAPACITY FACTOR (*k'*) FOR PREDNISOLONE 21-*m*-SULPHOBENZOATE SODIUM IN THE TBA-CONTAINING ELUENTS

*pH**	*TBA bound (μmol)*	*TBA stripped with methanol–water (μmol)*	*TBA stripped with eluent of pH* 5.8 (μmol)*	*k'*
5.8	8.5*	4.8	3.9	3.8
6.3	9.0*	5.8	5.5	4.2
6.8	10.5*	3.2	4.9	3.9
7.3	13.5*	2.7	15.1	3.4
7.8	21.0	1.0	14.8	3.3
8.3	24.0	0.6	22.5	3.0
8.8	28.5	0.9	24.9	2.6

* These figures apply to a condition in which the final equilibrium has not yet been fully established.

The data in Table I indicate that with increasing pH* the binding of TBA ions by ion exchange increases compared with the binding by other mechanism(s). The data in Table II indicate that the absolute amount of TBA ions bound by ion exchange increases with increasing pH*. This is in accordance with the expectation that

the silanol groups will show an increasing tendency to dissociate with increasing pH*. The data in Table II also indicate a decrease in the amount of TBA ions bound by a mechanism other than ion exchange with increasing pH*. This apparent decrease is stronger than might be expected from Table I. No fully satisfactory explanation can be offered for this discrepancy at this stage. However, the data in Tables I and II clearly indicate the existence of at least two binding mechanisms for the TBA ions. They also strongly suggest that ion exchange accounts for the major part of the reagent bound to the column packing material.

Comparable breakthrough patterns were found for CTA-containing eluents with the Radial-Pak A columns. The radial compression can compensate for the reduction in the volume of the packing on dissolution of the silica matrix during chromatography. These columns therefore remain free of voids and channels. This makes it possible to use eluents of higher pH than is permitted with the usual narrow-bore stainless-steel columns, without significant losses in column efficiency. In this study eluents with pH* values up to 11 were used.

The breakthrough patterns of CTA were studied at pH* 7, 9 and 11. At pH* 7 and 9, comparable results were obtained to those with TBA-containing eluents below pH* 7.8 (discussed above). After pumping through 150–200 ml of eluent, containing 0.2% (w/w) of cetrimide, the breakthrough volume was reached, as indicated by the response of the refractive index detector. This was confirmed by titration of the eluate fractions with DOSS.

At this moment the pH* shifted to a lower value. Because of the optimal buffer capacity of the boric acid buffer near pH* 9, the shift at this pH* could only be demonstrated with a non-buffered eluent. These large differences in volume before the pH* shifts back, observed in repeated experiments, are probably caused by the difficulty of removing all cetrimide from the stationary phase before a new experiment was started. It is assumed that some of the silanol groups can bind the CTA ions very tightly. If during the stripping process a smaller amount of CTA ions has been removed from the stationary phase, fewer protons will have the opportunity to be exchanged against CTA ions in the next experiment, and consequently the volume needed for the shift back in pH* will be smaller.

A difference with the TBA experiments is the fact that many more CTA ions are held up by the column. This difference cannot be explained by the use of different types of columns[19]. The amount of cetrimide bonded to the column material was calculated to be 824 μmol at pH* 7, 934 μmol at pH* 9 and 1044 μmol at pH* 11. This phenomenon of increased breakthrough volumes at increased pH* values was also observed in the TBA experiments (Fig. 4). This might be explained again by assuming an increased dissociation of the silanol groups at higher pH* values so more binding places are available for quaternary nitrogen compounds. At pH* 11 the breakthrough pattern of CTA was comparable to those of the TBA experiments above pH* 7.8. After the hold-up time the pH* gradually shifted to a lower pH* value. At the moment of the breakthrough of the CTA ions the pH* shifted back to the pH* of the eluent.

The results indicate that CTA ions, as TBA ions, are partly bound by an ion-exchange mechanism. Analogous to the TBA experiments, the potassium concentration was determined in the eluate of the CTA-containing eluents collected directly after the hold-up time. A small but not significant increase in the potassium concen-

tration was measured. This suggests that the contribution to the binding of CTA ions by ion exchange is comparatively small. A major part of the CTA ions will be bound by another, presumably hydrophobic, interaction with the stationary phase.

The retentions of a number of solutes were determined using both the TBA- and CTA-containing eluents.

In Tables II and III, two examples from the solutes studied are given (both solutes may be regarded as completely ionized over the pH range studied). It appears that the increase in the total amount of reagent that is bound to the column is accompanied by a decrease in the retention of anionic solutes. The same tendency was found for all of the anionic solutes studied. This clearly indicates that a correlation between the total amount of the reagent bound to the column and the retention of the anionic solutes does not make sense.

TABLE III

CAPACITY FACTOR (k') OF BENZOIC ACID IN CTA-CONTAINING ELUENTS OF DIFFERENT pH*

*pH**	*Total amount of CTA bound (μmol)*	k'
6.8	824	8.4
9.0	934	4.4
11.0	1044	3.1

When, however, the retention of prednisolone 21-*m*-sulphobenzoate sodium is compared with the amount of reagent bound to the column by a mechanism other than ion exchange, a positive correlation is found. When making this comparison, it should be kept in mind that there is some uncertainty about the amounts bound at higher pH values, and that this compound is partially retained by at least one other mechanism, as it also exhibits some retention when phosphate buffer eluents without TBA ions are used.

The data show that dissociation of silanol groups plays a role over a wide pH range. The explanation for this phenomenon probably lies in the fact that the pK_a values of silanol groups change progressively with the degree of neutralization of the packing material[20].

CONCLUSION

The existence of at least two binding mechanisms for TBA ions and CTA ions is of importance for the interpretation of the chromatographic behaviour of anionic solutes. The first binding mechanism is based on an ion-exchange process, in which TBA ions and CTA ions are exchanged against protons and potassium ions bound to residual silanol groups. The second binding mechanism is assumed to be based on the hydrophobic bonding of the TBA ions and the CTA ions to the stationary phase, their charges being compensated by a double layer of anions in the mobile phase. The exchange of anionic solutes against anions from the double layer could explain the

retention of anionic solutes. In this concept, the retention of anionic solutes must be correlated with the amount of TBA ions and CTA ions bound to the stationary phase by this second mechanism only, not with the total amount of adsorbed TBA and CTA. However, the amount bound by hydrophobic bonding could not be determined with sufficient accuracy with the techniques used in this study to make such correlations meaningful.

Finally, there is a practical implication of the results of our investigations. Our studies show that in a number of instances the moment of breakthrough of the ion-pairing reagent precedes the moment that equilibrium is reached. With TBA-containing eluents the column is comparatively quickly equilibrated; elution with 25 ml will usually be sufficient. When using CTA-containing eluents, the volumes needed for equilibration of the column are much larger; more than 1 l of eluent may then be needed.

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