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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICAL- LY MODIFIED SILICA

IV*. EFFECTS OF VARIATIONS OF pH, IONIC STRENGTH AND BUFFER COMPOSITION IN ELUENTS CONTAINING CETYLTRIMETHYL- AMMONIUM BROMIDE

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SUMMARY

The influence of pH changes as well as variation of ionic strength and buffer composition on the retention of selected test solutes in high-performance liquid chromatography on dynamically modified silica were investigated. Chromatography was performed on columns of bare silica (LiChrosorb Si 60) using eluents consisting of mixtures of methanol and aqueous phosphate buffers and containing cetyltrimethylammonium (CTMA) bromide.

With increasing pH the amount of CTMA ions adsorbed on to the silica surface rose, thus increasing the retention due to reversed-phase partition.

It was shown that apart from the adsorption of CTMA on to silica, ion exchange plays only a minor role in the retention of cations, except for hydrophobic quaternary ammonium compounds. An increase in the ionic strength of the phosphate buffer caused a decrease in the retention of most ionic test solutes. This effect was shown to be due to interaction with the phosphate ions in the eluent and not to a decrease in the amount of CTMA adsorbed.

The addition of inorganic salts, with anions different from the buffer anion, had no effect on retention apart from ionic strength effects. The addition of alkane-sulphonates was shown to lead to a selective decrease in the retention of anionic solutes.

The nature of the buffer cation was shown greatly to influence retention and selectivity.

* For Part III, see ref. 3.

INTRODUCTION

Dynamic modification of bare silica in liquid chromatography executed by the addition of quaternary ammonium compounds to the eluent has been the topic of several recent investigations¹⁻⁴.

Ion-exchange processes play a major role in the adsorption of the cations on to the silica surface at pH values from 5 to 10, as used in the dynamically modified silica approach. The aim of this work was to investigate (i) the influence of variations of pH, ionic strength and buffer constituents in the eluent on the adsorption of cetyltrimethylammonium (CTMA) bromide on to the silica, and (ii) the influence on the retention of various test solutes.

EXPERIMENTAL

Apparatus

Breakthrough volumes were measured using a liquid chromatograph consisting of a Gynkotek Model 6000 pump and an Optilab Multiref 902 differential refractometer detector. The detector response and the trace of a 1-ml siphon counter were recorded on a Kipp & Zonen Model BD-8 recorder.

Chromatographic testing of the individual systems was performed on a Waters liquid chromatograph consisting of a 6000 A pump, a 710 A WISP autoinjector, a 440 UV absorbance detector (254 nm), a 730 data module and a 720 system controller, or on a liquid chromatograph consisting of an Altex Model 110 solvent metering pump, a Pye-Unicam LC-UV detector (operated at 254 nm) and a Rheodyne Model 7120 injection valve. Chromatograms were recorded on a Kipp & Zonen BD-8 recorder. Retention data were acquired by the Waters 730 data module or by a Hewlett-Packard Model 3353 A laboratory data system.

Procedures

Determination of the amounts of CTMA adsorbed on to the column material by the breakthrough method or by the elution method was performed as described previously^{2,5}.

Chromatography

All experiments were performed on 120 × 4.6 mm I.D. columns from Knauer (Berlin, F.R.G.), packed by the dilute slurry technique with LiChrosorb Si 60 (5 μm) (E. Merck, Darmstadt, F.R.G.). The eluent was methanol-water-phosphate buffer (50:45:5) with the addition of 2.5 mM CTMA bromide. Appropriate phosphate buffers with regard to pH, ionic strength, and nature of cation were used. All pH values stated are those measured in the undiluted buffers and not in the final eluent. The buffers were prepared from potassium dihydrogen phosphate or orthophosphoric acid by titration to the required pH with 5 M potassium hydroxide or the stated amine, respectively, and dilution to a final concentration of 0.2 M. During chromatography the column was guarded by a silica pre-column situated between the pump and the injection device. The chromatographic system was equilibrated by eluting overnight. Following each adsorption experiment the column was brought to its initial status by eluting with methanol-0.05 M orthophosphoric acid (1:1) and finally with methanol.

Chemicals

N,N,N',N'-Tetramethylethylenediamine (TeMED) was obtained from Aldrich-Europe (Beerse, Belgium). Diethanolamine (DEtOHA), diethylamine (DEA), triethanolamine (TEtOHA), and triethylamine (TEA) were obtained from Fluka (Buchs, Switzerland) in analytical-reagent quality. All other reagents were of analytical-reagent grade from E. Merck. All chemicals were used as received from the manufacturers.

RESULTS AND DISCUSSION

Effects of pH

Potassium phosphate buffers of pH between 5 and 10 were used to investigate the influence of pH changes of the eluent on the retention of a number of test solutes. Two series of buffers were used, one with constant phosphate concentration and another with constant ionic strength (Fig. 1). The increasing phosphate concentration with decreasing pH at constant ionic strength seems to have little influence on the retention of the test solutes and on the amount of CTMA adsorbed. At the lowest pH value of 5, however, the amount of CTMA adsorbed is increased relative to the amount adsorbed in the experiment using constant phosphate concentration. The influence of this difference on retention is most pronounced for anionic solutes.

From the figure it is seen that variations in buffer pH may cause considerable changes in selectivity between neutral, cationic and, in particular, anionic species.

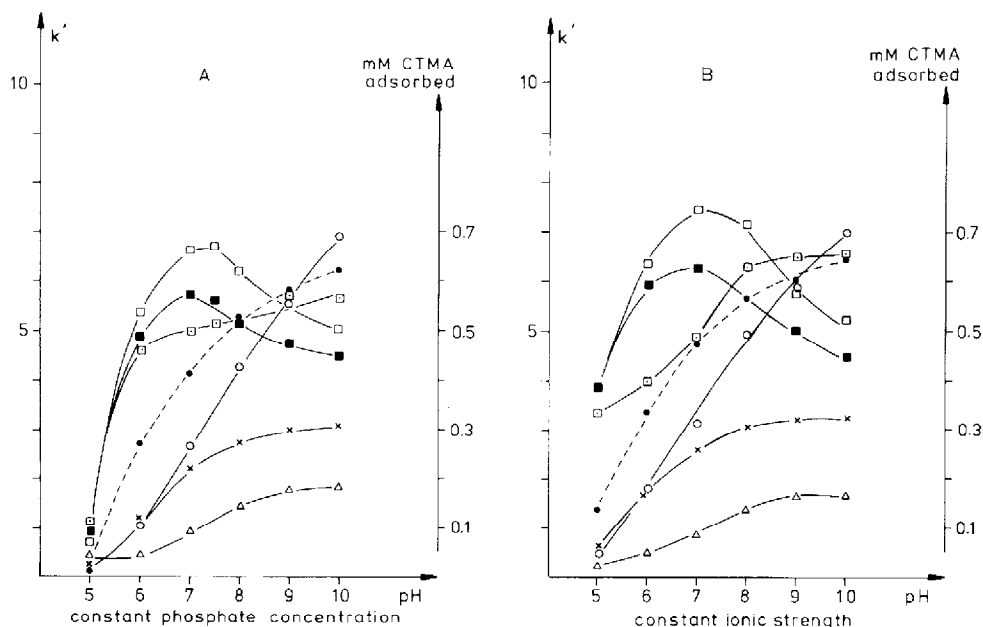


Fig. 1. Relationship between the pH of the buffer in the eluent and the amount of CTMA adsorbed plus retention of various test solutes. Eluent, methanol water potassium phosphate buffer (50:45:5, v/v) with 2.5 mM CTMA added. (A) With constant phosphate concentration. (B) With constant ionic strength. Symbols: ●, CTMA; △, phenylethylamine; ×, benzene; ○, phenol; □, benzoic acid; ■, sorbic acid; ◻, fumaric acid.

It is well known⁶ that silica acts as a cation exchanger at pH values greater than 5. As these experiments are executed using a methanol content of 50% in the eluent which will reduce the cationic effect in general⁷, and as the CTMA ion is a very hydrophobic cation with a great affinity for the cation exchanger, the cationic effect on solutes will be of significance only for very hydrophobic cations.

The retention of some cationic compounds with an identical hydrophobic moiety has been investigated at different pH values with and without CTMA ions added to the eluent (Fig. 2). Fig. 2A shows the behaviour of the cations in a system without CTMA bromide added. The retention behaviour of the quaternary ammonium compound, which is ionized throughout the pH range, can be taken as an indicator of the true cationic effect. The retention of this solute increases with increasing pH to a certain level, depending of the cation-exchange capacity of the silica. This level is reached when the silica is "fully ionized". When CTMA ions are added to the system (Fig. 2B) the retention of the quaternary test solute decreases to a small constant value at all pH values in accordance with the ion-exchange theory. The hydrophobic part of the molecule is not capable of suppressing the hydrophilic character of the quaternary ammonium group, and thus retention due to the reversed-phase effect is negligible.

Primary, secondary and tertiary amines are chromatographed as cations at low pH. In the system with no CTMA bromide added (Fig. 2A) they show less affinity for the cation exchanger than the quaternary ammonium compound; at increased pH the retention goes through a maximum as the ionization of the amines decreases. The decrease in affinity for the silica at higher pH values is most pronounced for the tertiary amine. This is probably due to its poorer ability to form hydrogen bonds to

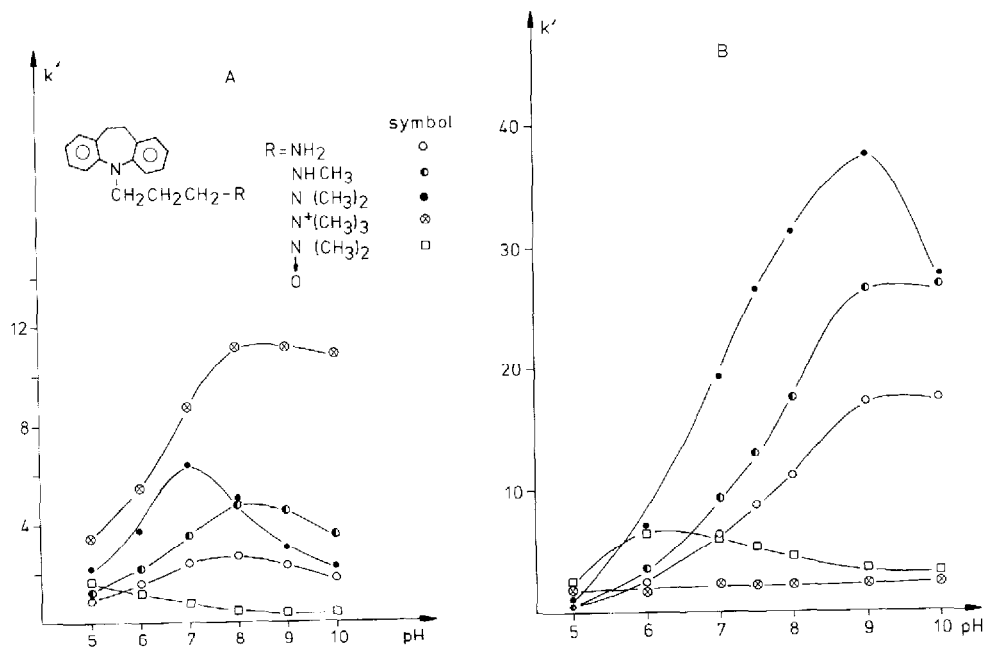


Fig. 2. Relationship between buffer pH and k' values of some cationic solutes. Eluent, methanol-water-0.2 M potassium phosphate (50:45:5). (A) No CTMA added. (B) With 2.5 mM CTMA added.

the silica. When the amines are chromatographed with CTMA ions in the eluent, a hydrophobic interaction with the stationary phase is the main mechanism of retention. The retention of all three amines is increased compared with that in the system with no CTMA present. A decrease in retention appears at higher pH values and is seen only for the tertiary amine. The CTMA adsorbed on to the silica suppresses the hydrogen-bonding effects.

The amine N-oxide is too weak a base to be retained by cation exchange. The N-oxide group forms hydrogen bonds with the silica, and as the pH increases and the silica ionizes these interactions are no longer possible and the retention decreases (Fig. 2A). The hydrophobic part of the molecule can only to a limited extent compensate for the hydrophilicity of the N-oxide group (Fig. 2B).

From the investigations described it can be concluded that cation exchange plays only a minor role in the retention of solutes on dynamically modified silica.

Effects of ionic strength

The influence of ionic strength on retention and thus on selectivity was investigated at pH 7.5 by varying the buffer concentration in the eluent.

From Fig. 3 it is seen that the amount of CTMA adsorbed on to the silica is not influenced by the change in the potassium ion concentration in the eluent. This is due to the much higher affinity of CTMA for the cation exchanger. As shown

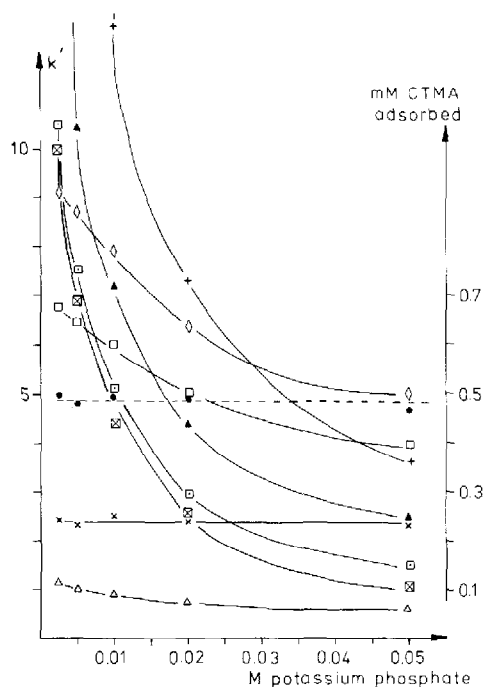


Fig. 3. Relationship between buffer concentration, amount of CTMA adsorbed and retention of various solutes. Eluent, methanol-water-0.2 M potassium phosphate, pH 7.5 (50:(50 - x):x, x = from 1.25 to 25) with 2.5 mM CTMA added. Symbols: +, phthalic acid; ▲, terephthalic acid; ◇, *p*-toluenesulphonic acid; ⊠, maleic acid; other symbols as in Fig. 1.

TABLE I
EFFECTS OF ADDITION OF VARIOUS ANIONS TO THE ELUENT ON THE RETENTION (k') OF VARIOUS TEST SOLUTES IN DYNAMICALLY MODIFIED SILICA

Eluent, methanol water 0.2M potassium phosphate (500:487.5:12.5) containing 2.5 mM CTMA bromide. Column temperature, 39.6°C. t_0 = Void volume in minutes.

Solute	Inorganic salts				<i>n</i> -Alkanesulphonates											
	Phosphate, pH 7.5				Cl ⁻	Br ⁻	NO ₃ ⁻	SO ₄ ²⁻	Hexane	Octane, 0.02 M	Decane, 0.02 M					
	0.01 M	0.02 M	0.04 M	0.08 M												
Benzene	1.94	2.18	21.9	1.89	1.89	1.96	1.88	2.01	1.75	1.87	2.05	2.18	2.23	2.38	2.45	3.21
Toluene	3.84	4.15	4.08	3.57	3.47	3.47	3.68	3.57	-	-	3.87	3.46	4.34	4.67	5.84	5.74
β -Methylnaphthalene	13.7	15.0	15.2	13.6	13.3	13.3	13.9	13.7	-	-	14.9	15.0	16.7	17.7	23.0	21.9
Phenanthrene	28.9	31.4	31.4	28.8	27.9	30.0	29.2	29.2	-	-	31.8	32.8	35.2	36.8	50.8	47.2
Phenol	2.19	2.52	2.42	1.97	1.98	1.99	2.05	2.05	1.90	1.93	2.21	2.38	2.30	2.34	2.39	2.83
Phenethylamine	1.13	1.08	1.00	0.95	0.85	1.00	0.88	0.88	1.05	0.89	0.93	0.91	1.06	1.10	2.60	3.78
Tyramine	0.93	0.75	0.67	0.64	0.55	0.67	0.55	0.55	0.71	0.59	0.61	0.57	0.67	0.65	1.49	2.54
Tryptamine	2.90	2.62	2.33	2.20	1.84	2.27	1.90	1.90	2.33	1.98	2.13	2.04	2.36	2.69	5.66	10.8
Imipramine (IP)	22.1	22.0	20.5	20.9	19.6	21.2	19.8	19.8	20.7	20.1	21.2	21.1	21.5	21.1	27.1	41.7
Desmethyl-IP	13.4	12.4	11.1	10.8	9.2	11.1	9.4	9.4	11.1	9.7	10.5	10.7	11.6	11.9	21.8	51.2
Didesmethyl-IP	7.86	7.55	7.03	6.04	5.5	6.4	5.7	5.7	6.5	5.8	6.4	6.5	7.13	7.58	13.5	29.5
N-Methyl-IP	3.00	2.49	2.53	2.38	2.28	2.46	2.35	2.35	2.61	2.42	2.41	2.42	2.75	2.79	7.74	22.0
IP-N-Oxide	4.00	3.80	3.90	4.22	4.45	4.31	4.54	4.54	4.23	4.49	4.27	4.31	4.20	3.89	4.54	5.05
Benzenesulphonic acid	3.16	2.52	2.13	-	2.17	2.36	2.15	2.15	2.29	2.01	2.33	2.09	1.83	1.19	0.41	0.22
Benzoic acid	4.26	3.54	3.12	3.51	3.67	3.44	3.36	3.36	3.30	-	3.33	2.97	2.40	1.86	0.65	0.49
Phthalic acid	13.3	5.81	3.72	6.39	4.37	5.92	3.71	3.71	5.72	3.70	5.52	3.93	4.20	2.08	0.47	0.23
Isophthalic acid	13.0	6.64	4.65	7.45	5.61	7.03	4.84	4.84	6.74	4.78	6.38	4.55	4.40	2.30	0.58	0.00
Terephthalic acid	6.21	3.31	2.24	3.81	2.70	3.56	2.31	2.31	3.44	2.30	2.85	1.95	2.14	1.05	0.22	0.00
1,3,5-Benzenetricarboxylic acid	109.5	22.9	10.5	27.9	12.9	24.9	9.73	9.73	23.8	10.5	16.4	7.90	9.5	3.55	2.48	0.00
Sorbic acid	3.91	3.25	2.73	3.20	3.15	3.10	2.99	2.99	3.01	2.80	3.14	2.72	2.25	1.61	0.58	0.43
Maleic acid	4.50	2.21	1.57	2.38	1.57	2.22	1.34	1.34	2.18	1.33	1.83	1.28	1.49	0.66	0.07	0.00
Fumaric acid	4.83	2.49	1.60	2.85	1.92	2.62	1.63	1.63	2.55	1.63	2.01	1.34	1.56	0.69	0.07	0.00
t_0 (water)	1.04	1.02	1.02	1.04	1.04	1.04	1.03	1.03	1.05	1.04	1.03	1.02	1.00	1.00	0.92	0.82

previously⁴ the retention of benzene is dependent only on the amount of CTMA adsorbed, and hence the retention of benzene is constant.

The decrease in retention of the cationic compound phenethylamine must be due to interactions within the mobile phase, as the cation-exchange and reversed-phase mechanisms do not seem to be affected.

A decrease in retention with increasing ionic strength is also seen for the various anionic compounds, *i.e.* carboxylic and sulphonic acids. The effect is more pronounced for the divalent than for the monovalent acids. Kraak and Huber⁸ have previously shown that an increase in the ionic strength has a stronger effect on retention in ion-pair partition chromatography on divalent sulphonic acids than on monovalent ones.

The effect of the addition of different inorganic anions as potassium salts to the eluent on the retention of a number of test solutes was also investigated (Table I). The neutral salts are added in great excess of the CTMA (2.5 mM) and the buffer concentration (2.5 mM). As the retention of the neutral solutes benzene, toluene, β -methylnaphthalene and phenanthrene are hardly altered (within $\pm 5\%$), the amount of CTMA adsorbed on to the silica can be considered to be constant (*cf.* ref. 4). The retention of other solutes, which are also primarily retained by a reversed-phase mechanism (*e.g.* amines and phenols) show correspondingly small changes. The retention of anionic compounds, however, is greatly influenced.

The overall effects seem to be caused by the increase in ionic strength, similar to what is seen when the buffer concentration is increased. The nature of the inorganic anions had no specific effects on the retention, when differences in ionic strengths are taken into account.

The addition of alkanesulphonates to the mobile phase has a more complex effect (Table I). The retention of solutes retained by a reversed-phase mechanism increases slightly with increasing amount of alkanesulphonate. Generally the retention of these solutes also increases with increasing chain length of the alkanesulphonate, but for the aromatic hydrocarbons a slight decrease is seen when using C₁₀-sulphonate, possibly due to micelle formation in the eluent (*cf.* ref. 3). The increase in retention can be explained by formation of a thicker layer of stationary phase, confirmed by the decrease in the void volume of the column.

The addition of alkanesulphonates causes a decrease in the retention of anionic solutes, indicating competitive ion-pair formation with CTMA ions. Furthermore the alkanesulphonates in the eluent will form ion pairs with the cationic solutes and hence cause a large increase in the retention of the cationic solutes.

The effects described above may be used to cause a selective decrease in the retention of anionic solutes.

Influence of changes in buffer cations

In reversed-phase ion-pair chromatography using chemically bonded phases it has been shown^{9,10} that the nature of the cation of a buffer is of vital importance for the chromatographic system with respect to resolution and efficiency.

The effect of amine phosphate buffers on retention and selectivity in chromatography on dynamically modified silica has been investigated using buffers of constant pH 7.5 and with constant phosphate concentration, as these parameters are of greater importance than the cation concentration.

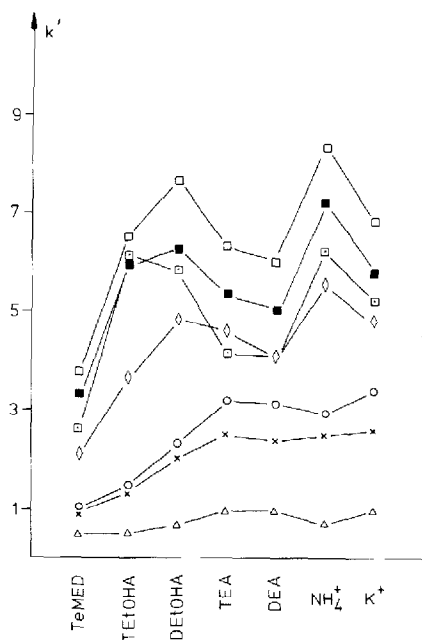


Fig. 4. Relationship between the nature of the buffer cation and the retention of various solutes. Eluent, methanol water-0.2 *M* phosphate buffer, pH 7.5 (50:45:5) with 2.5 *mM* CTMA added. Symbols: ◇, maleic acid; all other symbols as in Fig. 1.

Fig. 4 depicts how the nature of the buffer cation influences the amount of CTMA adsorbed on to the silica as indicated by the retention of benzene (*cf.* ref. 4). The retention of neutral and cationic solutes increases with increasing amount of CTMA adsorbed on to the silica.

The retention of the anionic solutes using various amine phosphate buffers is rather unpredictable and, in fact, the retention of fumaric acid and maleic acid is reversed when using triethylammonium phosphate buffer relative to their retentions in the other systems.

Again the effect on the retention of various solutes is assumed to be due to interactions primarily within the mobile phase. The solute anions, CTMA ions, buffer cations and phosphate ions all interact with one another and the mutual equilibria determine the resulting retention.

CONCLUSION

It has been demonstrated that the pH and ionic strength, as well as the buffer constituents, greatly influence the retention and selectivity of solutes chromatographed on dynamically modified silica. The degree of retention due to the reversed-phase mechanism is dependent on the amount of CTMA adsorbed and thus on the pH, whereas ionic strength variations are of little importance for this mechanism. The ion-exchange mechanism has been shown to be of minor importance apart from the adsorption of CTMA on to the silica.

Ion-pair formation and other ionic interactions within the mobile phase play

important roles in the dynamic modification approach, and changes in pH and in the nature and concentration of the buffer ions therefore have large effects on the retention of solutes.

The investigations also demonstrated that alkanesulphonates possess a selective strong elution power for anionic solutes brought about by competitive ion-pair formation.

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