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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICALLY MODIFIED SILICA

II*. MODIFICATION OF VARIOUS SILICA PACKINGS WITH CETYLTRIMETHYLAMMONIUM BROMIDE

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SUMMARY

High-performance liquid chromatography on silica using eluents containing cetyltrimethylammonium (CTMA) bromide was investigated, and adsorption isotherms were determined for two silica packings of different pore diameter and surface area. It was found that about one CTMA ion was adsorbed per square nanometer of the silica surface at pH 7.5 and at a concentration of 6 m*M* CTMA bromide in 50% of methanol.

Fourteen different silica packings were compared using a test mixture. and thirteen were found to exhibit the same selectivity towards the test mixture, which included acids, bases and non-ionic compounds, thus providing a chromatographic system that is largely independent of the origin of the column material.

The retention mechanisms for the five test compounds are discussed.

INTRODUCTION

In the last 5 years several papers (*e.g.*, refs. 1–5) have appeared describing high-performance liquid chromatography (HPLC) on silica that had been dynamically modified by adding different surfactants to the eluent. By this means it seems possible to achieve separations similar to those obtained with chemically bonded octadecyl-

* For Part I, see ref. 5.

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silyl (ODS) silica packings. Although the published works demonstrate the successful application of this technique and to some extent have reported investigations of the amount of surfactant taken up by the column material, none of them incorporated any systematic investigation of the uptake on different silica packings or a clear elucidation of the retention mechanisms for the solute types involved.

This study was carried out using cetyltrimethylammonium (CTMA) bromide as the surfactant with a view to establishing the degree of coverage of the silica surface that can be attained with the new technique compared with covalent bonding. We also investigated whether different brands of dynamically modified silica would constitute chromatographic systems of differing selectivity towards an actual mixture, as is the case for various brands of chemically bonded packings (*e.g.*, refs. 6–8).

EXPERIMENTAL

Apparatus

Breakthrough volumes were measured using a liquid chromatograph consisting of a Gynkotek Model 600 pump and an Optilab Multiref 902 differential refractometer. The detector response and the trace of a 1-ml siphon counter were recorded on a Kipp & Zonen Model BD-8 recorder.

Determination of the CTMA concentrations in the eluates was carried out after ion-pair extraction to dichloromethane at pH 7.5 with naphthalene-2-sulphonate as the counter ion by measuring the UV absorption at 276 nm in the organic phase, using a rebuilt[9] Beckman Model DU spectrophotometer.

Potassium contents were determined with an IL Model 143 flame photometer, utilizing lithium as an internal standard.

pH values were measured by means of a Radiometer Model PHM 64 pH meter.

Testing of the chromatographic properties of the dynamically modified silica was performed on liquid chromatographs consisting of an Altex Model 110 solvent metering pump, a Kontron Model 410 LC pump, a DuPont Model 837 spectrophotometer, a Pye Unicam LC-UV detector and Rheodyne Model 7120 and 7125 injection valves. The chromatograms were recorded on Kipp & Zonen Model BD-8 recorders, and the retention data were collected on a Hewlett-Packard Model 3353A laboratory data system.

Silica columns

Silica packings of particle size 5–7 μm were investigated. The range of materials is given in Table I, together with data on the pore sizes and surface areas provided by the suppliers.

The silica materials were packed into stainless-steel columns (120 × 4.6 mm I.D.) from Knauer (Berlin, G.F.R.) according to a previously described procedure[10]. During all runs the column under study was guarded by the use of a silica precolumn. Following testing, the columns were brought to their initial state by eluting with methanol–0.05 *M* phosphoric acid (1:1) and finally with methanol. The columns were disconnected and emptied, and their silica contents were determined, after drying, by differential weighing.

TABLE I

SILICA PACKINGS INVESTIGATED

Silica packing		*Particle size (μm)*	*Pore diameter (nm)*	*Surface area (m^2/g)*	*Mass (g/column)*
No.	*Trade name*				
1	Chromosorb LC-6	5	12	400	0.735
2	Hypersil	5	10	200	1.094
3	LiChrospher SI 100	5	12	250	0.743
4	LiChrosorb SI 60	5	6	500	0.963
5	LiChrosorb SI 100	5	12	300	0.797
6	Nucleosil 50	5	5	500	1.001
7	Nucleosil 100	5	10	300	0.778
8	Nucleosil 100 V	7	10	430	0.729
9	Partisil	5		400	0.894
10	Polygosil 60	5	6	500	0.882
11	Spherisorb S W	5	8	220	1.285
12	Spherosil XOA 600	5		600	0.932
13	Spherosil XOA 800	5		800	1.311
14	Zorbax SIL	5	7	300	1.250

Chemicals

Naphthalene-2-sulphonic acid was prepared as described by Witt[11]. All other reagents were of analytical-reagent grade from E. Merck (Darmstadt, G.F.R.).

RESULTS AND DISCUSSION

Adsorption isotherms

A standard chromatographic system chosen from the results presented by Hansen[5] was used. The columns, packed with plain silica, were eluted with methanol–0.2 *M* potassium phosphate (pH 7.5)–water (50:5:45) containing different amounts of CTMA. The adsorption of CTMA to LiChrosorb SI 60 and SI 100 was investigated, these materials having different pore sizes and surface areas (Table I). The amounts of CTMA adsorbed were inferred from the breakthrough volumes[12,13] as indicated by the trace from a refractive index (RI) detector. Fig. 1 shows the RI trace for a stepwise change in the eluent from methanol–water (50:50) to methanol–0.2 *M* potassium phosphate buffer (pH 7.5)–water (50:5:45) and finally to the buffered methanol–water containing 2.5 m*M* of CTMA. Each elution step was continued until equilibrium was established. In order to correlate the shifts in the recorder trace with the composition of the eluate, fractions were collected and their contents of potassium and CTMA and the pH were determined. Fig. 1 shows the results of these determinations together with the RI trace.

When the eluent is changed from methanol–water to buffered methanol–water, the potassium ions break through the column in a stepwise fashion. During the uptake of potassium the pH of the eluate decreases owing to the protons displaced from some of the silanol groups by cation exchange. Similarly, a temporary increase in the concentration of potassium ions in the eluate occurs when the eluent is changed to buffered methanol–water with CTMA added. This is followed by a decrease in pH

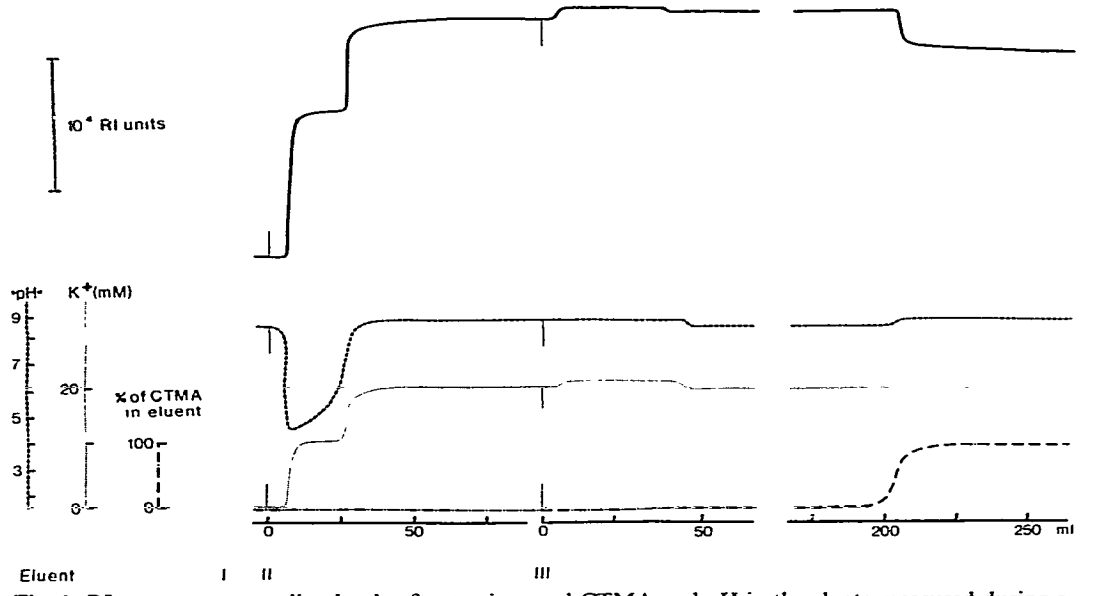


Fig. 1. RI trace, corresponding levels of potassium and CTMA and pH in the eluate measured during a breakthrough experiment. Silica packing: LiChrosorb SI 60. Eluents: I, methanol–water (50:50); II, methanol–0.2 *M* potassium phosphate buffer (pH 7.5)–water (50:5:45); III, as II but with the addition of 2.5 m*M* CTMA bromide. The concentrations of potassium and CTMA and the pH were determined as stated under Experimental.

when the potassium ion concentration has returned to its original value. The reason for this is that CTMA, being a more bulky and hydrophobic ion, has a stronger affinity to the silica than has the potassium ion. An adsorption capacity of 0.52 mmol/g for CTMA in the buffered methanol–water containing 2.5 m*M* CTMA compared with 0.17 mmol/g for potassium ions in the buffered methanol–water ($[K^+]$ = 18 m*M*) was calculated. Thus the CTMA ions first replace potassium ions and then some additional protons on the silica surface.

At equilibrium the chromatographic system then consists of the silica surface, partly covered with CTMA, probably held to the silanol groups by ion–ion interactions. This leaves the long carbon chain to point into the mobile phase in the same manner as for corresponding covalently bonded ODS groups. Contrary to the findings of Ghaemi and Wall[2], it was found that rinsing with methanol–0.05 *M* phosphoric acid (1:1) brings the silica column back to the initial state.

When the amount of CTMA adsorbed per gram of support is plotted against the concentration of CTMA in the eluent, convex adsorption isotherms result (Fig. 2A). Using the amount of CTMA adsorbed per unit area of the support instead, the isotherms for the two packings intersect as shown in Fig. 2B. At low concentrations the silica with the larger surface area, LiChrosorb SI 60, adsorbs more CTMA per unit area than does LiChrosorb SI 100. At higher concentrations of CTMA this situation is reversed. This may be attributed to the differences in pore size, a smaller pore size leading to a higher degree of steric hindrance at the pores as increasing amounts of CTMA are adsorbed.

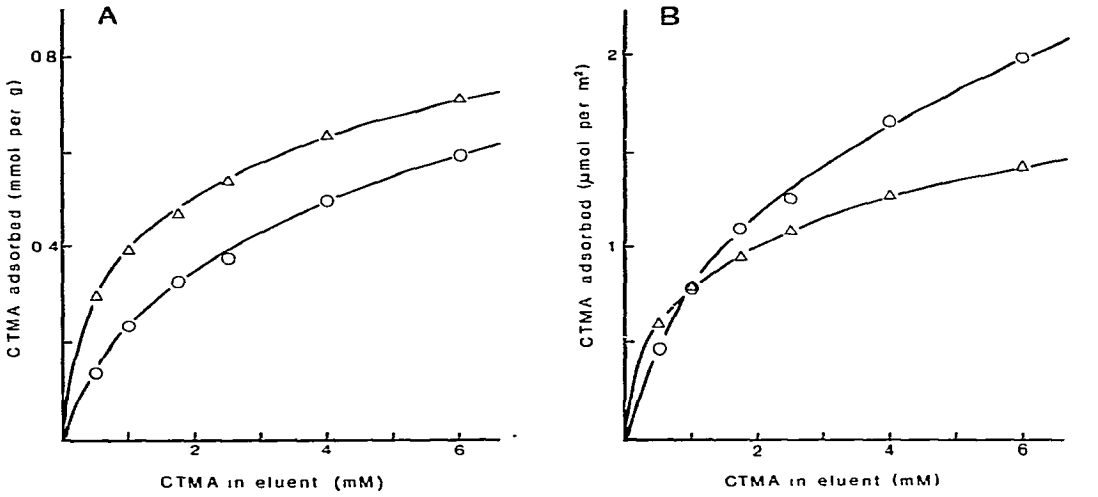


Fig. 2. Relationships between the concentration of CTMA in the eluent and the amount of CTMA adsorbed per gram silica (A) or per square metre of silica surface area (B). Silica packings: △, LiChrosorb SI 60; ○, LiChrosorb SI 100. Eluents: methanol–0.2 *M* potassium phosphate buffer (pH 7.5)–water (50:5:45) with the stated concentrations of CTMA.

Surface coverage

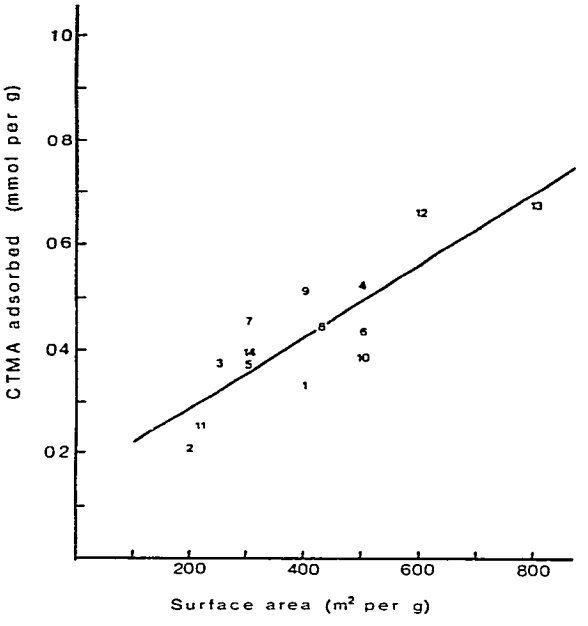
The number of CTMA ions adsorbed at a CTMA concentration of 6 m*M* in the eluent is 0.8 and 1.1/nm^2 for LiChrosorb SI 60 and SI 100, respectively, calculated from the results in Fig. 2B. This is in good agreement with the results found by others[2], taking the pH and the CTMA concentration into consideration. These amounts are comparable to those reported for the most highly covered chemically bonded ODS silicas[14].

From the specific surface areas of the 14 different silica packings in Table I it is possible to correlate the amount of CTMA adsorbed to the surface area of the silica, as shown in Fig. 3. The number of silanol groups per unit area may vary for silica packings of different origin but values between 4 and 5/nm^2 are usual[14]. The data in Fig. 3 correspond to an adsorption of 0.5–1.0 CTMA ion/nm^2 at a CTMA concentration of 2.5 m*M* in the eluent.

Selectivity

The 14 columns packed with silica from different suppliers were tested by chromatographing a mixture of five substances chosen to represent acidic, basic and non-ionic compounds. Fig. 4 shows a representative chromatogram of a test mixture consisting of benzoic acid, phenol, phenethylamine, pyridine and benzene.

In Fig. 5 the capacity factors (k') for benzene as a function of the amount of CTMA adsorbed on the individual columns are given. It appears that the k' values increase linearly with increasing amounts of CTMA adsorbed, and corresponding linearities were demonstrated for the other four test substances. The amount of CTMA adsorbed, however, is not an independent variable but is strictly controlled by the accessible surface area of the individual columns and can be taken as a measure of that area.



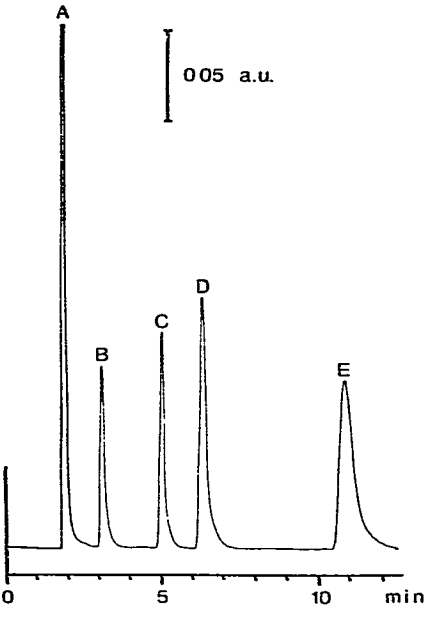


Fig. 3. Relationship between the specific surface area of the individual silica packings and the amounts of CTMA adsorbed. Regression line: $y = 0.007x + 0.16$. The numbers refer to the brand of silica (*cf.*, Table I).

Fig. 4. Chromatogram of test mixture. Column: LiChrosorb SI 60 (120 × 4.6 mm I.D.). Eluent: methanol–0.2 *M* phosphate buffer (pH 7.5)–water (50:5:45) with 2.5 m*M* CTMA. Flow-rate: 1 ml/min. Detection wavelength: 254 nm. A, Pyridine, 2.5 μg ($k' = 0.3$); B, phenethylamine, 25 μg ($k' = 1.2$); C, benzene, 30 μg ($k' = 2.5$); D, phenol, 10 μg ($k' = 3.4$); E, benzoic acid, 10 μg ($k' = 6.4$).

The selectivities of the silica packings tested, defined as their ability to separate each of the test substances from benzene in terms of separation factors, are given in Table II. Only one column material (Spherosil XOA 800) differs substantially from the others, which are remarkably alike.

Retention mechanisms

Fig. 6 presents the results of the chromatography of the test mixture using different concentrations of CTMA in the eluent. The non-ionic, hydrophobic compound benzene seems to be chromatographed according to a purely reversed-phase mechanism, its retention being proportional to the amount of CTMA adsorbed to the silica when calculated from the adsorption isotherm measurements. Benzoic acid is fully ionized at the pH of the eluent; the benzoate ions can form ion pairs with CTMA which are retained by a reversed-phase mechanism, thus explaining the strong retention at high CTMA concentrations. Phenol is only slightly ionized at this pH and it may therefore be chromatographed partly as an ion pair and partly as the free phenol by a reversed-phase mechanism. The k' value of phenethylamine decreases with increasing CTMA concentration, which may be explained by retention being due to a cation-exchange mechanism. The retention of pyridine, which is unionized at pH 7.5, remains unaffected by addition of CTMA to the eluent.

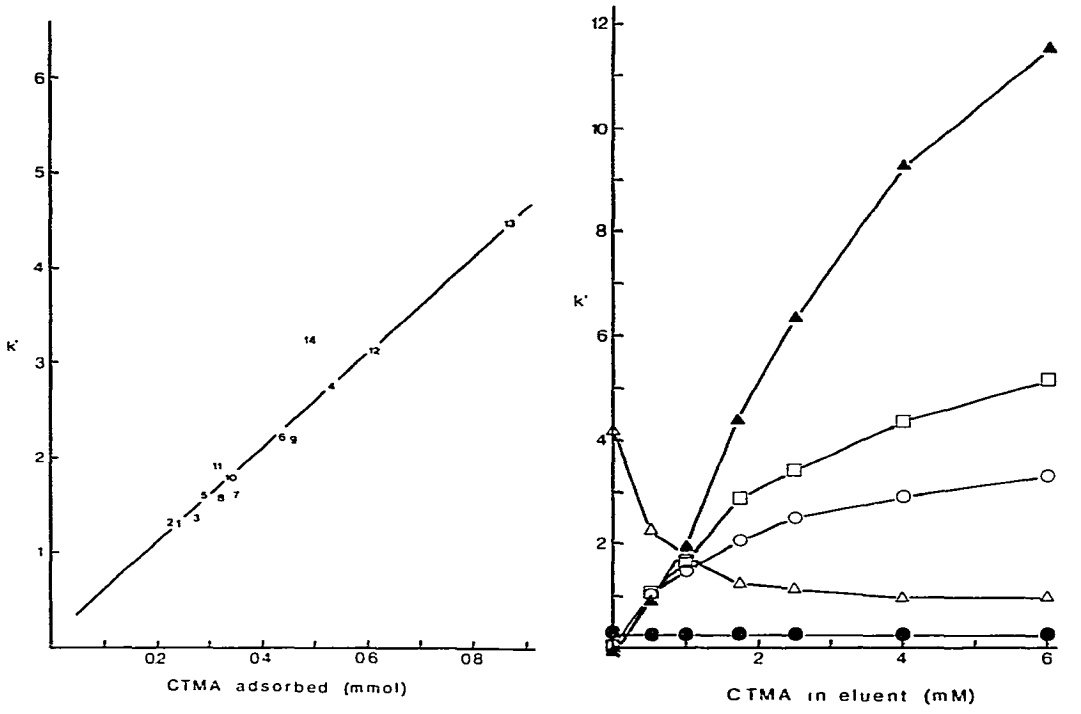


Fig. 5. Relationship between the amount of CTMA adsorbed on the individual silica packings and k' for benzene. Regression line: $y = 5.04x + 0.08$. The numbers refer to the brand of silica (*cf.*, Table I).

Fig. 6. Relationship between the concentration of CTMA in the eluent and k' for the five components of the test mixture. Column: LiChrosorb SI 60 (120 × 4.6 mm I.D.). Eluents: as in Fig. 2. ●, Pyridine; △, phenethylamine; ○, benzene; □, phenol; ▲, benzoic acid.

TABLE II

SEPARATION FACTORS BETWEEN BENZENE AND OTHER TEST SUBSTANCES MEASURED ON 14 DIFFERENT SILICA COLUMNS

*Column No.**	*Separation factor*			
	Pyridine	*Phenethylamine*	*Phenol*	*Benzoic acid*
1	0.13	0.48	1.27	2.64
2	0.12	0.46	1.33	2.64
3	0.14	0.51	1.32	2.64
4	0.12	0.45	1.38	2.67
5	0.15	0.52	1.29	2.55
6	0.11	0.52	1.38	2.63
7	0.15	0.55	1.30	2.50
8	0.13	0.54	1.30	2.59
9	0.12	0.49	1.38	2.70
10	0.12	0.46	1.34	2.63
11	0.12	0.46	1.38	2.52
12	0.12	0.50	1.41	2.65
13	0.18	1.17	1.55	2.26
14	0.10	0.43	1.51	2.58

* See Table I.

CONCLUSION

The adsorption isotherms for CTMA in chromatography on dynamically modified silica have been determined and it is shown that only part of the silanol groups on the silica surface are covered. The amount of CTMA adsorbed per gram for a fixed eluent composition is dependent on the specific surface area of the silica packing.

Thirteen different silica packings exhibited the same selectivity towards a test mixture. Thus it is demonstrated that using dynamically modified silica it is possible to standardize an HPLC system without the restrictions relating to the origin of the column material which usually apply for HPLC systems based on chemically bonded ODS-silica.

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