

CHROM. 18 539

## HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICAL- LY MODIFIED SILICA

### VI\*. MODIFICATION OF SILICA OF VARIOUS PORE DIAMETERS AND PARTICLE SIZES USING CETYLTRIMETHYLAMMONIUM BROMIDE

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(First received December 20th, 1985; revised manuscript received February 4th, 1986)

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#### SUMMARY

The influence of the pore diameter and the particle size of silica packing materials on the adsorption of cetyltrimethylammonium (CTMA) ions to its surface when used in high-performance liquid chromatography has been investigated. Adsorption isotherms were determined for ten different silica packing materials having mean pore diameters ranging between 4 and 110 nm and particle sizes of 5 or 10  $\mu\text{m}$ . It was found that the adsorbable amount of CTMA per unit area of the silica surface increases with increasing mean pore diameter of the silica. Silica of narrow pore diameter ranges (4–10 nm) are saturated with CTMA at lower concentrations due to steric hindrance.

The particle size of the silica packing material did not exhibit any influence on the amount of CTMA adsorbed per unit area of the silica.

The chromatographic retention of fifteen test solutes was determined over the whole range of CTMA concentrations investigated for each of the ten silica packing materials. The retention data proved that the relationship between the solute retention and the amount of CTMA adsorbed onto the silica is dependent on the particle size of the silica packing material.

The pore diameter of the silica packing material did not influence the chromatographic selectivity, apart from the effects of a partial exclusion of some test solutes on narrow-pore silica materials due to steric hindrance.

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\* For Part V, see ref. 9.

## INTRODUCTION

High-performance liquid chromatographic (HPLC) separations performed on bare silica dynamically modified by the addition of quaternary ammonium compounds to the eluents have been discussed in several publications<sup>1-9</sup>. The modification of silica packing materials of various origin with cetyltrimethylammonium (CTMA) bromide has been investigated<sup>6</sup>, as well as the modification of silica with long-chain or symmetrical quaternary ammonium compounds<sup>7</sup>. Also, the effects of variations in pH, ionic strength and buffer composition of eluents have been elucidated<sup>4,8</sup>. Most recently, the influence of concentration and nature of organic modifier in the eluents was studied<sup>4,9</sup>. The investigations of the dynamic modification approach have been extended to cover silica columns in open-tubular capillary liquid chromatography<sup>10</sup> and silica layers in over-pressurized thin-layer chromatography<sup>11</sup>.

The aim of the present work was to investigate the variations in selectivity and in the amount of CTMA adsorbed onto various silica packing materials having different pore diameters and particle sizes.

## EXPERIMENTAL

### *Apparatus*

The chromatographic testing of the individual systems was performed on a Waters liquid chromatograph consisting of a 6000 A pump, a 710A WISP autoinjector, a 440 UV absorbance detector (operated at 254 nm), a 730 data module and a 720 system controller; or on a liquid chromatograph consisting of a Kontron Model 410 LC pump, a Pye-Unicam LC-UV detector (operated at 254 nm) and a Rheodyne Model 7125 injection valve; or on a liquid chromatograph consisting of an Altex Model 110 LC pump, an Altex LC 254 nm detector and a Rheodyne Model 7120 injection valve. The chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder. Retention data were collected on a Waters 730 data module or on a Hewlett-Packard Model 3353A laboratory data system.

### *Chromatography*

The eluent was methanol-water-0.2 M phosphate buffer (pH 7.5) (50:45:5) containing various concentrations of CTMA bromide. The buffer pH stated is that measured in the undiluted buffer and not in the final eluent. The buffer was prepared from 0.2 mol of potassium dihydrogen phosphate dissolved in 930 ml of water, titrated with 5 M potassium hydroxide to pH 7.5 and finally diluted to 1000 ml. During chromatography, the column was guarded by a silica saturation column situated between the pump and the injection device. The saturation column was 120 × 8 mm I.D. and packed with LiChrorep Si 60, 15-25 μm (Merck, Darmstadt, F.R.G.). The chromatographic system was equilibrated by elution overnight. Following each adsorption experiment, the column was brought to its initial state by elution with methanol-0.1 M nitric acid (50:50) followed by methanol-water (50:50), and finally rinsed with methanol.

### *Silica columns*

LiChrosorb and LiChrospher silica packing materials (Merck) of particle size

TABLE I  
PHYSICAL PROPERTIES OF SILICA PACKING MATERIALS INVESTIGATED

<i>Silica packing materials</i>		<i>Particle size</i>	<i>Specific</i>	<i>Specific</i>	<i>Mean pore</i>
<i>No.</i>	<i>Trade name</i>	<i>(<math>\mu\text{m}</math>)</i>	<i>surface area</i>	<i>pore volume</i>	<i>diameter</i>
			<i>(<math>\text{m}^2/\text{g}</math>)</i>	<i>(<math>\text{ml}/\text{g}</math>)</i>	<i>(<math>\text{nm}</math>)</i>
1	LiChrosorb Si 40	5	733	0.7	3.8
2	LiChrospher Si 60	5	513	0.59	4.6
3	LiChrosorb Si 60	5	449	0.64	5.7
4	LiChrosorb Si 60	10	418	0.69	6.6
5	LiChrospher Si 100	5	361	1.12	12.5
6	LiChrosorb Si 100	5	279	1.15	16.5
7	LiChrospher Si 100	10	250	1.29	20.0
8	LiChrospher Si 300	10	199	1.98	40.0
9	LiChrospher Si 500	10	75	1.04	55.0
10	LiChrospher Si 1000	10	37	1.08	110.0

5 or 10  $\mu\text{m}$  having mean pore diameters ranging between 4 and 110 nm were investigated. The range of the materials is given in Table I, together with data on the specific surface area, specific pore volume and mean pore diameter determined by the supplier or by an independent laboratory (Niro Atomizer, Søborg, Denmark). The experiments were performed on 120 or 250  $\times$  4.6 mm I.D. columns from Knauer (Berlin, F.R.G.), packed by the dilute slurry technique.

### *Chemicals*

All reagents were of analytical-reagent grade from Merck and were used as received.

## RESULTS AND DISCUSSION

### *Effects of pore diameter on CTMA adsorption*

The adsorption of CTMA on to the silica was determined using a standard chromatographic system<sup>6</sup>. The columns, packed with the various silica packing materials, were eluted with methanol-water-0.2 *M* potassium phosphate (pH 7.5) (50:45:5) containing concentrations of CTMA bromide ranging between 0 and 50 mM. The upper value was chosen from a previous investigation<sup>7</sup> and was intended to demonstrate the limit of the adsorbable amount of CTMA. The actual amounts adsorbed were determined by the elution method<sup>12</sup>. Fig. 1 shows the CTMA adsorption isotherms for the ten silica packing materials investigated. It appears that the amount of CTMA adsorbed per unit area of the silica surface increases with increasing mean pore diameter. The figure also demonstrates that silica packing materials having small pore diameters are saturated with CTMA at lower concentration levels than the wide-pore materials. This is considered to be due to steric hindrance towards the surfactant molecules in the narrow-pore materials.

As can be seen from Fig. 1, the absorbed amount of CTMA cannot be correlated exactly to the mean pore diameter as such. Some of the packing materials apparently adsorb higher amounts than the general relationship stipulates. However, the level of adsorbable amount of CTMA can be classified according to the shape of

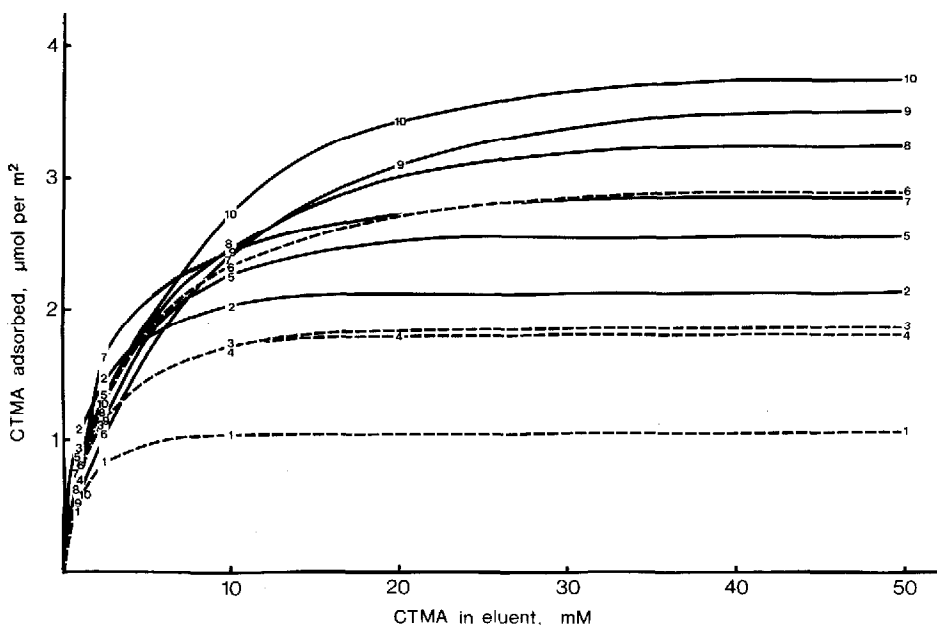


Fig. 1. Adsorption isotherms showing the relationship between the concentration of CTMA in the eluent and the amount of CTMA adsorbed per square meter of the silica surface for ten different silica packing materials. The numbers refer to the type of silica packing material (*cf.* Table I). The packing materials are divided into two groups according to the particle shape: ----, angular; —, spherical. Eluent: methanol-water-0.2 *M* phosphate buffer (pH 7.5) (50:45:5) containing various concentrations of CTMA.

the silica particles. Spherical particles are able to adsorb more CTMA per unit area than those of angular shape. Taking the particle shape into account, the adsorbable amount of CTMA per unit area of silica is clearly related to its mean pore diameter.

The amount of CTMA adsorbed can be correlated to the surface area of the silica as shown previously<sup>6,13</sup>. The correlation for the silica packing materials in the present investigations is illustrated in Fig. 2A, which shows the relationship between the specific surface area of the individual packing materials and the amount of CTMA adsorbed at four different concentrations. The regression lines displayed represent CTMA concentrations of 1 and 2.5 mM in the eluent for the eight silica packing materials having mean pore diameters above 5 nm, and 10 and 50 mM CTMA for the six packing materials having mean pore diameters above 10 nm. Results for some of the narrow-pore materials were discarded in order to fit the data pairs to a straight-line correlation. These results may be explained by the above-mentioned steric hindrance, which is especially distinct for silica packing materials with particles of angular shape (silica packing material Nos. 1, 3 and 4).

The relationship between the mean pore diameter of the silica packing material investigated and the amount of CTMA adsorbed at four different concentrations of CTMA is illustrated in Fig. 2B. The displayed regression lines for eluents containing 1 and 2.5 mM CTMA are almost parallel to the abscissa. The amount of CTMA adsorbed per unit area of the silica surface is thus constant for any pore diameter. The steric hindrance towards CTMA does, however, influence the adsorption of

CTMA at higher concentrations of CTMA (10 and 50 mM), most predominantly for the narrow-pore silica packing materials.

#### *Effects of particle size on CTMA adsorption*

The particle size of the silica packing materials was found not to have any influence on the amounts of CTMA adsorbed or on the maximum absorbable amount of CTMA within the range investigated.

#### *Effects of pore diameter on selectivity*

For testing the individual chromatographic systems, test mixtures of fifteen solutes were chosen to represent non-ionic, acidic and basic compounds. The selectivities, expressed as the separation factor between the individual test substances and benzene, were calculated. Table II contains the results for nine of the test solutes chromatographed with 2.5 mM CTMA in the eluent. The results of silica packing material No. 1 with a mean pore diameter of 3.8 nm are biased by an exclusion effect toward most of the solutes. At the higher end of the pore diameter range, from 40 to 110 nm, minor variations are seen due to small deviations in the generally low retention values. The two dicarboxylic acids, however, exhibit a real shift in selectivity corresponding to the actual pore diameter of the packing material. The shift in selectivity is caused by a size-exclusion effect towards the ion-pair formed between the acids and the CTMA ions. Fumaric acid represents the most pronounced shift in selectivity, and it is also considered to form the most bulky ion-pairs.

#### *Effects of particle size on selectivity*

Fig. 3 shows that the increase in retention of solutes due to increasing amounts of CTMA adsorbed onto the silica is dependent on the particle size of the silica packing material. The regression lines displayed in Fig. 3A and B represent retention data pooled from chromatography of benzene and benzoic acid with eluents containing 1, 2.5 and 10 mM CTMA. The correlations confirm that the retention mechanisms are reversed-phase partition for the non-ionic solute benzene (Fig. 3A), as well as for the anionic benzoate ions forming an ion-pair with CTMA as the counter-ion (Fig. 3B) (*cf.* refs. 2-4). The retention mechanism for basic compounds is more complicated. For 2-phenylethylamine (Fig. 3C), straight lines could only be established for the three CTMA concentrations individually. The variation with particle size was, however, of a similar nature to that for benzene and benzoic acid. The influence of CTMA concentration on the retention of basic solutes is considered to be due to a combination of two different mechanisms. The first is an ion-exchange process taking place at the silanol groups. The second is a reversed-phase partition mechanism occurring in the phase created when CTMA is adsorbed onto the silica surface.

The influence of particle size on retention was not accompanied by any other effects on the chromatographic properties as seen in Fig. 4, when it is taken into consideration that 5- and 10- $\mu\text{m}$  silica packing materials are packed in columns of 120- and 250-mm lengths, respectively.

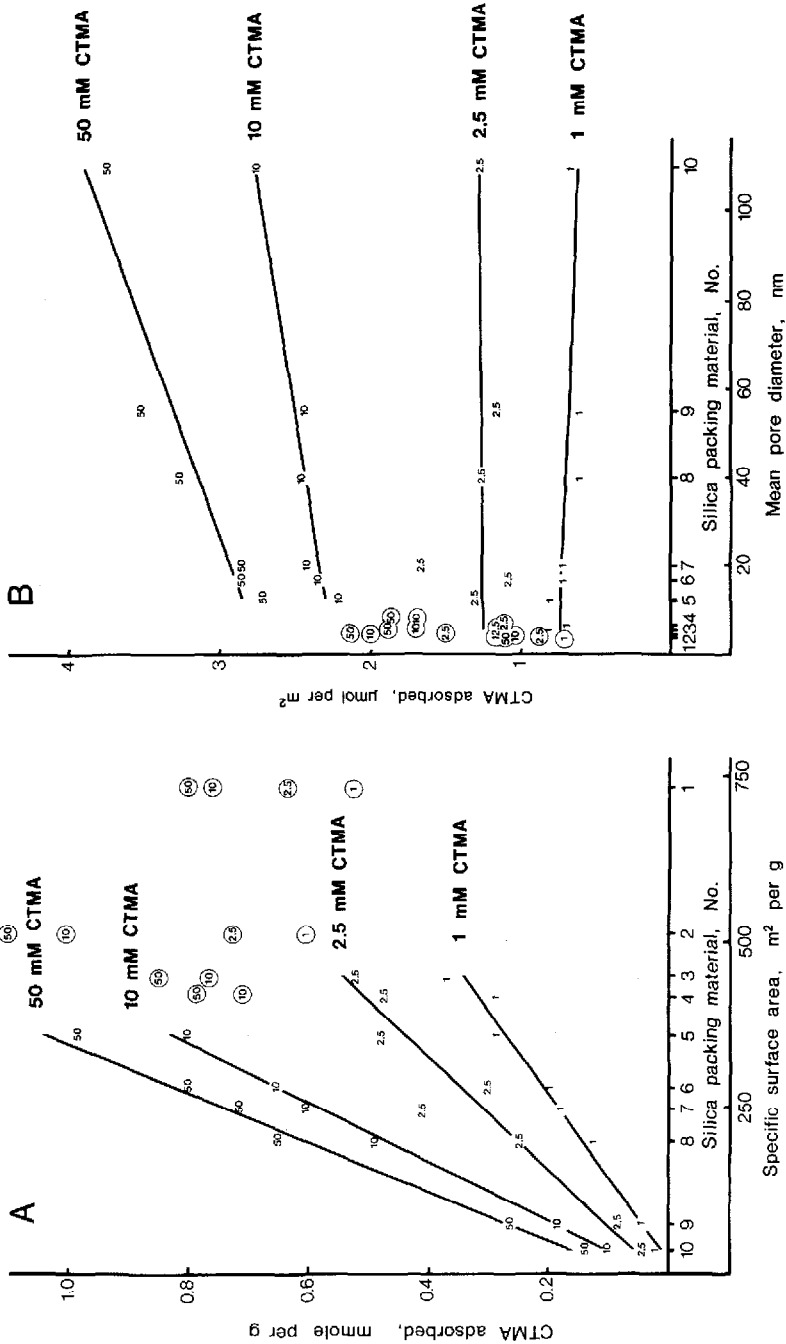


Fig. 2. Regression lines showing the amount of CTMA adsorbed per gram of silica as a function of the specific surface area (A), or per square metre of silica surface area as a function of the mean pore diameter (B) of the individual silica packing materials for four different concentrations of CTMA. The points encircled are not included in the calculation of the regression lines. The numbers refer to the type of silica packing material (cf. Table I). Eluents as in Fig. 1, containing the stated concentrations of CTMA.

TABLE II  
SEPARATION FACTORS OF TEST SUBSTANCES FROM BENZENE  
Eluent: methanol-water-0.2 M phosphate buffer (pH 7.5) (50:45:5) containing 2.5 mM CTMA bromide.

<i>Silica packing materials</i>		<i>Test substances</i>									
No.	Trade name	Pyri- dine	2-Phenyl- ethyl- amine	Hydro- corti- sone	Phenol	Maleic acid	Fuma- ric acid	Beta- metha- sone	Ben- zoic acid	Imi- pra- mine	
1	LiChrosorb Si 40-5	0.12	0.68	0.87	1.56	1.29	1.29	1.92	2.46	15.65	
2	LiChrospher Si 60-5	0.11	0.49	1.00	1.47	1.70	1.84	2.08	2.62	12.32	
3	LiChrosorb Si 60-5	0.12	0.47	1.09	1.39	1.83	2.00	2.17	2.52	11.83	
4	LiChrosorb Si 60-10	0.11	0.46	1.07	1.35	1.72	1.92	2.09	2.34	11.17	
5	LiChrospher Si 100-5	0.18	0.60	1.13	1.23	1.82	2.06	1.98	2.22	11.51	
6	LiChrosorb Si 100-5	0.16	0.53	1.18	1.22	1.89	2.15	2.10	2.32	10.72	
7	LiChrospher Si 100-10	0.15	0.51	1.19	1.20	2.13	2.46	2.08	2.38	10.52	
8	LiChrospher Si 300-10	0.16	0.56	1.20	1.16	1.98	2.25	2.05	2.21	10.82	
9	LiChrospher Si 500-10	0.28	0.66	1.29	1.14	2.00	2.34	2.00	1.91	11.09	
10	LiChrospher Si 1000-10	0.25	0.60	1.29	1.12	2.06	2.43	2.02	2.06	10.64	

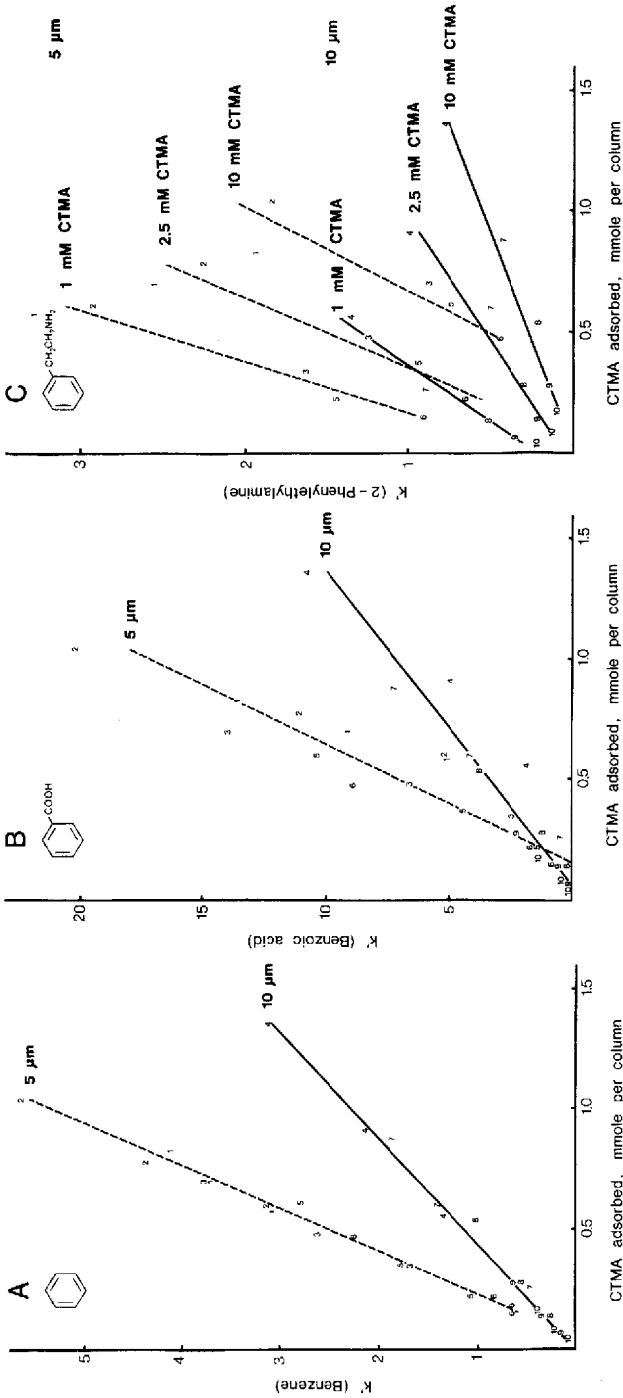


Fig. 3. Regression lines showing the retention of benzene (A), benzoic acid (B) and 2-phenylethylamine (C) as a function of the amount of CTMA adsorbed onto the individual silica classified according to particle size, 5 or 10  $\mu\text{m}$ . The numbers refer to the type of silica (cf. Table I). Eluents refer to the type of silica (A, B) or individually (C).



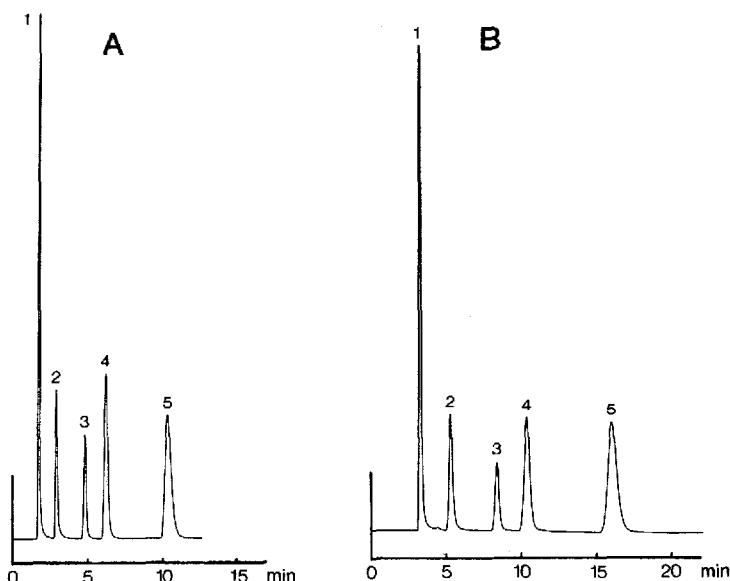


Fig. 4. Chromatograms of five test solutes performed on silica columns of 5 and 10  $\mu\text{m}$  particle size: LiChrosorb Si 60, 5  $\mu\text{m}$ , 120  $\times$  4.6 mm I.D. (A) and LiChrosorb Si 60, 10  $\mu\text{m}$ , 250  $\times$  4.6 mm I.D. (B). Eluent: methanol-water-phosphate buffer (pH 7.5) (50:45:5) containing 2.5 mM CTMA bromide. Detection: 254 nm. Test solutes: 1 = pyridine, 2 = 2-phenylethylamine, 3 = benzene, 4 = phenol, 5 = benzoic acid.

## CONCLUSION

Determination of CTMA adsorption isotherms for various silica packing materials has shown that the amount of CTMA adsorbed per unit area of the silica surface increases with increasing mean pore diameter of the silica packing material. Narrow-pore silica packing materials are saturated with CTMA at lower CTMA concentrations than wide-pore materials, due to steric hindrance. The results confirm that the adsorption of CTMA per mass unit of silica is controlled by the specific surface area of the silica packing material.

The particle size did not influence the amount of CTMA adsorbed. Chromatographic testing, however, showed that the slope of the linear relationship between retention and amount of CTMA adsorbed is dependent on the particle size of the silica packing material. Thus, the surface area accessible to the test solutes seems to be substantially different for 5- and 10- $\mu\text{m}$  material, and the difference is not reflected in the specific surface areas actually measured.

The findings of the present work emphasize that column packings for dynamically modified silica need standardization only as regards the specific surface area, a certain minimum pore diameter and the particle size.

## ACKNOWLEDGEMENTS

F. Eisenbeiss (Merck) is thanked for providing some of the silica packing materials investigated and the data for their specific surface areas, specific pore volume

and mean pore diameter. V. Bødstrup and E. Johansen (Niro Atomizer) are acknowledged for the determination of the actual specific pore volume and mean pore diameter. This work was supported by the Danish Medical Research Council, Grant No. 12-0649.

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