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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICALLY MODIFIED SILICA

III*. MODIFICATION OF SILICA WITH LONG-CHAIN AND SYMMETRICAL QUATERNARY AMMONIUM COMPOUNDS

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SUMMARY

The influence of the nature of quaternary ammonium compounds on retention in high-performance liquid chromatography on dynamically modified silica was investigated. Adsorption isotherms were determined on bare silica (LiChrosorb Si 60) for four alkyltrimethylammonium bromides and two symmetrical tetraalkylammonium bromides, each containing 15-21 carbon atoms. It was found that only the long-chain quaternary ammonium ions are adsorbed on to the silica surface in appreciable amounts and that the affinity increases with increasing number of carbon atoms in the alkyl chain. The maximum amount that can be adsorbed per gram of silica is of the same order of magnitude for each of the four long-chain quaternary ammonium compounds. This amount, however, is reached at lower concentrations in the eluent the longer is the alkyl chain.

The retention of five test compounds was determined over the whole concentration range investigated for each of the modifying agents. The surfactant concentration that causes maximum retention for four of five test compounds coincides with its critical micellar concentration. The retention mechanisms and the influence of the type of the modifying agent on selectivity are discussed, and compared with published results on related experiments on chemically bonded stationary phases.

* For Part II, see ref. 2.

INTRODUCTION

The use of bare silica dynamically modified by adding surfactants such as cetyltrimethylammonium (CTMA) bromide to the eluent in high-performance liquid chromatography (HPLC) has been discussed in recent publications^{1,2}. It was stated that a major advantage offered by the dynamic coating approach over the use of chemically bonded phases is that only slight variations in selectivity for different brands of column material occur.

The aim of this work was to investigate the adsorption of different kinds of quaternary ammonium ions on to the silica surface and their influence on the retention of various test compounds.

EXPERIMENTAL

Apparatus

UV-visible measurements were made on a rebuilt³ Beckman Model DU spectrophotometer.

Breakthrough volumes were measured using a liquid chromatograph consisting of a Gynkotek Model 600 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 ultraviolet 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 Model BD-8 recorder. Retention data were collected on the Waters 730 data module or on a Hewlett-Packard Model 3353 A laboratory data system.

Procedures

Determination of the amounts of quaternary ammonium compounds adsorbed on to the column by the breakthrough method was performed as described previously².

Determination of the surfactants adsorbed on to the column using an elution method was performed by eluting the previously equilibrated column with 100 ml of methanol-0.05 M phosphoric acid (1:1). Surfactant concentrations were measured, after appropriate dilution, using the previously described procedure², or by liquid chromatography using the liquid chromatograph described for the breakthrough experiment, the LiChrosorb Si 60 column and 2.5 mM CTMA in methanol-water-0.2 M phosphate buffer (pH 6.0 or 5.0) (70:25:5) as the eluent. The results were corrected for the dead volume of the column.

The critical micellar concentrations (CMC) were determined by solubilizing 4,4'-diethoxyazobenzene in the eluents. By plotting the absorbance at 375 nm of the saturated solutions against the concentrations of the surfactant two straight lines can be constructed, the intersection of which is equivalent to the CMC.

Chromatography

All experiments were performed on 120×4.6 mm I.D. columns from Knauer (Berlin, G.F.R.), packed by the dilute slurry technique with LiChrosorb Si 60 ($5 \mu\text{m}$) (E. Merck, Darmstadt, G.F.R.). The eluent was methanol-water- $0.2 M$ phosphate buffer (pH 7.5) (50:45:5) with the addition of various kinds and concentrations of quaternary ammonium compounds. During chromatography the column was guarded by a silica pre-column situated between the pump and the injection device. Following each adsorption experiment the column was brought to its initial status by eluting with methanol- $0.05 M$ phosphoric acid (1:1) and finally with methanol.

Chemicals

4,4'-Diethoxyazobenzene was prepared by coupling diazotized 4-ethoxyaniline to phenol followed by ethylation using diethyl sulphate; the crude product was recrystallized from methanol-water. Stearyltrimethylammonium (STMA) bromide was prepared from stearylamine by dimethylation with formaldehyde-formic acid⁴ and subsequent methylation of the tertiary amine with methyl bromide⁵. Dodecyltrimethylammonium (DTMA) bromide and tetradecyltrimethylammonium (TTMA) bromide were obtained from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical-reagent grade from E. Merck.

RESULTS AND DISCUSSION

Adsorption isotherms

The adsorption of four alkyltrimethylammonium compounds, DTMA, TTMA, CTMA, and STMA, and of two symmetrical tetraalkylammonium compounds, tetrabutylammonium (TBA) bromide and tetrapentylammonium (TPA) bromide, was investigated at concentrations ranging from 0 to 50 mM . The upper value represents an increase relative to that previously investigated² for CTMA, in order to establish for each component the limit of the amount adsorbed. Measurements of the amounts adsorbed at high concentrations of the surfactant-type compounds were not possible by means of the previously described breakthrough technique. At these concentrations it was found that the buffer capacity of the eluent was insufficient to maintain a constant pH, as protons on the surface of the silica were exchanged for surfactant ions. This gave rise to a decrease in the pH, which at low concentrations of surfactant ions was so small (0.2 pH units for 2.5 mM CTMA^2) that it caused no problems. At higher concentrations larger shifts were measured (2.5 pH units for 25 mM CTMA); thus the ionization of the silanol groups is affected considerably. The ability to adsorb surfactant ions is temporarily decreased, giving rise to a partial breakthrough. The early breakthrough was observed from *ca.* 10 mM for the four surfactants, and the amounts adsorbed at concentrations above 10 mM were determined by the elution method.

Fig. 1 shows the six adsorption isotherms. It appears that the structure of the quaternary ammonium compounds rather than the number of carbon atoms present is of vital importance for the amount adsorbed. The two symmetrical compounds TBA and TPA are adsorbed to only a minor extent, and only a small difference between the two compounds, which differ by four carbon atoms, is seen. For the alkyltrimethylammonium compounds at lower concentrations the affinity to silica

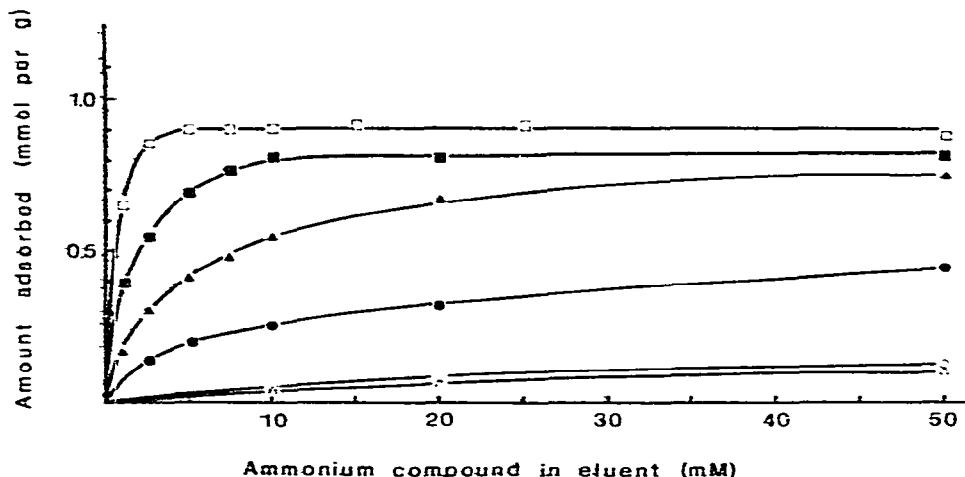


Fig. 1. Relationship between the concentration of quaternary ammonium compound and the amounts adsorbed per gram of silica. O, TBA; Δ , TPA; \bullet , DTMA; \blacktriangle , TTMA; \blacksquare , CTMA; \square , STMA. Eluent: methanol-water-0.2 M phosphate buffer (pH 7.5) (50:45:5) plus quaternary ammonium compounds at the indicated concentrations.

increases with increasing carbon number of the alkyl chain. The maximum amount adsorbable, however, seems to be of the same order of magnitude for STMA, CTMA and TTMA, indicating that the limitation is due mainly to steric reasons. The maximum is *ca.* 0.9 mmol/g, corresponding to *ca.* 2 $\mu\text{mol}/\text{m}^2$ (calculated from the surface area per gram of silica as stated by the supplier). For DTMA only *ca.* 0.45 mmol/g is adsorbed at a concentration of 50 mM but the curve is still rising. The degree of coverage of the silica surface attainable is comparable to that achieved by derivatizing with long-chain alkylsilanes⁶ and no indication of the formation of a double layer or a multi-layer is found.

Retention mechanism

For the chromatographic testing of the individual systems a test mixture containing pyridine, phenethylamine, benzene, phenol and benzoic acid was used. Figs. 2a-d show the capacity factors (k') for four of the test substances as functions of the concentration of the individual quaternary ammonium compounds. The retention of the fifth compound, pyridine, was largely unaffected by the type and concentration of modifying agent, as was previously found² for CTMA.

When significant amounts of an alkyltrimethylammonium compound are adsorbed on to the silica surface, neutral compounds such as benzene are chromatographed according to a reversed-phase partition mechanism, acidic compounds such as benzoic acid by reversed-phase ion-pair partition and phenol by an intermediate mechanism as shown earlier for CTMA². As was found in the adsorption experiments, only a monolayer of surfactant seems to be present. This supports the statement that the retention of acidic solutes is due to ion-pair partition and not ion exchange as has been reported to take place in chromatography with surfactants on chemically bonded phases⁷. The previously reported results with CTMA were found for concentrations ranging from 0 to 6 mM, whereas the experiments underlying Fig.

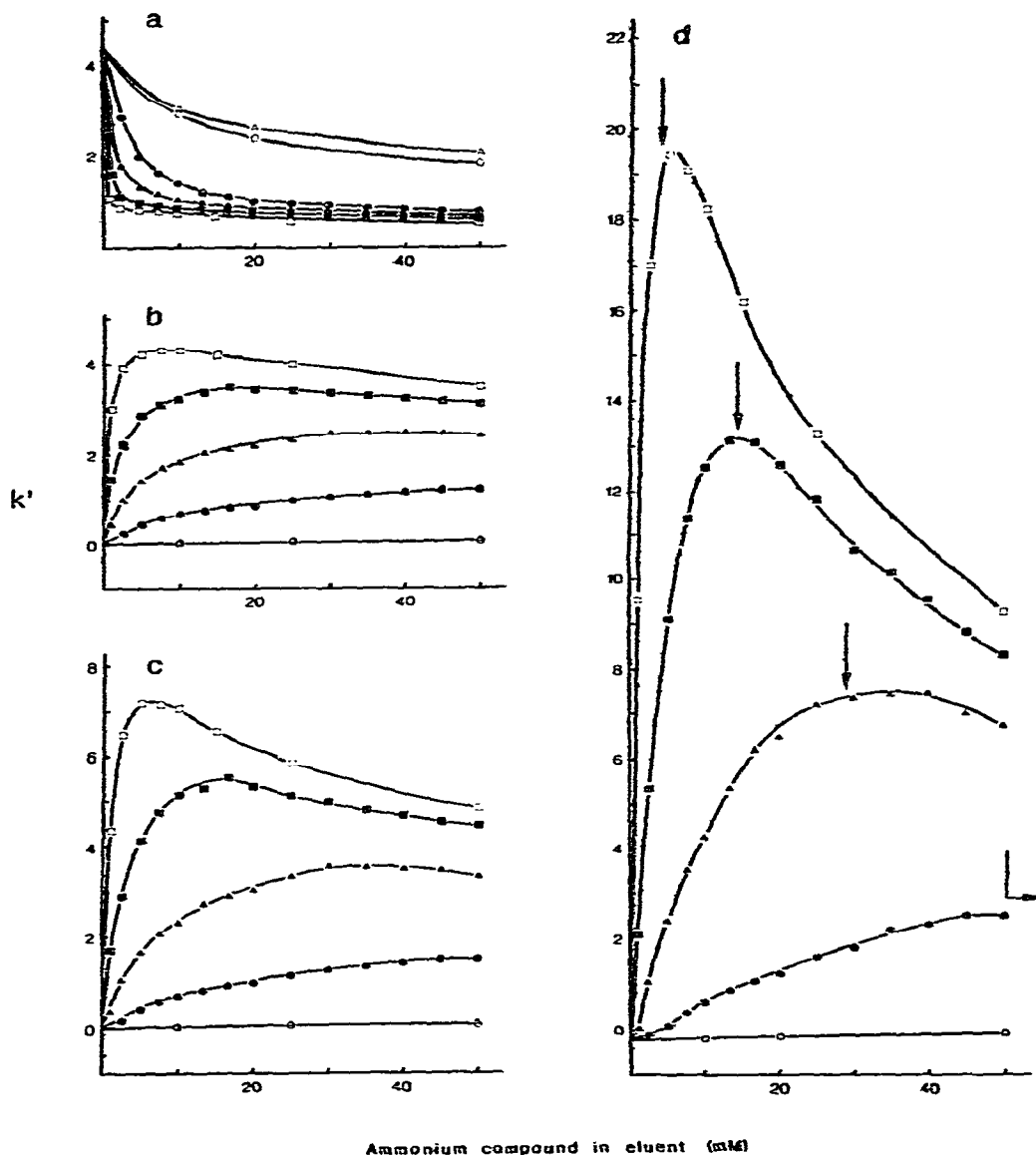


Fig. 2. Relationship between the concentration of quaternary ammonium compound in the eluent and k' for four of the test substances: (a) phenethylamine; (b) benzene; (c) phenol; (d) benzoic acid. Symbols and eluent as in Fig. 1. The arrows in (d) indicate the estimated critical micellar concentrations (see Experimental).

2 were extended to 50 mM. At higher concentrations, for all four surfactants the retention of the neutral and acidic solutes exhibit a distinct maximum. In chromatography on chemically bonded phases it has recently been shown^{8,9} that the retention of acidic solutes is influenced similarly by both symmetrical and surfactant-type quaternary ammonium compounds. Although it has been claimed that micelle forma-

tion (which is possible for the surfactant-type compounds only) had no influence, other workers^{10,11} have found that for alkyl sulphates the maximum retentions of various solutes coincide with the CMC values. In the work presented here (*cf.*, Fig. 2) the maximum retentions of both acidic and neutral solutes show a high degree of coincidence with the CMC values found for the four surfactants (57, 27, 16 and 3.3 mm, for DTMA, TTMA, CTMA and STMA, respectively), indicating that micelle formation apparently increases the hydrophobicity of the eluent.

The two symmetrical compounds, TBA and TPA, affect the retention of the neutral and acidic compounds to only a minor extent. This is in accordance with the above-mentioned fact that only minor amounts of the said compounds are adsorbed on to the silica surface and thus reversed-phase partition is not possible. A certain decrease in the retention of phenethylamine is seen, which indicates that the symmetrical quaternary ammonium compounds do influence the ion-exchange process of the basic solute but to a much smaller extent than do the surfactant-type compounds.

We investigated whether any effect on the retention could be observed on adding a symmetrical pairing ion to an eluent containing a surfactant-type ion of the same charge. Table I shows the retention of benzene, phenethylamine, phenol and

TABLE I

INFLUENCE OF THE ADDITION OF TBA ON RETENTION

Eluent: 2.5 mM CTMA in methanol-water-0.2 M phosphate buffer (pH 7.5) (50:45:5) plus TBA at the stated concentrations.

Solute	Concentration of TBA (mM)				Decrease in k' from 0 to 20 mM (%)
	0	5	10	20	
Phenethylamine	0.68	0.67	0.66	0.64	5.9
Benzene	0.95	0.91	0.92	0.87	8.4
Phenol	1.06	1.02	1.01	0.96	9.4
Benzenesulphonic acid	1.19	1.08	1.04	0.92	22.7
Sorbic acid	1.57	1.36	1.32	1.18	24.8
Benzoic acid	1.63	1.50	1.44	1.29	20.9

three different acids when various concentrations of TBA are included in the eluent containing CTMA (2.5 mM). It appears that the TBA causes a decrease for all solutes, the decrease being most pronounced for the anionic compounds. Owing to an ion-exchange mechanism, TBA replaces a small part of the CTMA adsorbed on to the silica, resulting in a less hydrophobic surface and thus in a general decrease in retention. To some extent, however, TBA also forms ion pairs with the anions, but the hydrophobicities of these ion pairs are lower than those of the ion pairs between CTMA and the anions, thus leading to a further decrease in the retention of the acidic solutes.

Alkyl chain length

In reversed-phase HPLC on chemically bonded supports it has been demonstrated^{12,13} that the retention increases linearly with increasing amounts of bonded

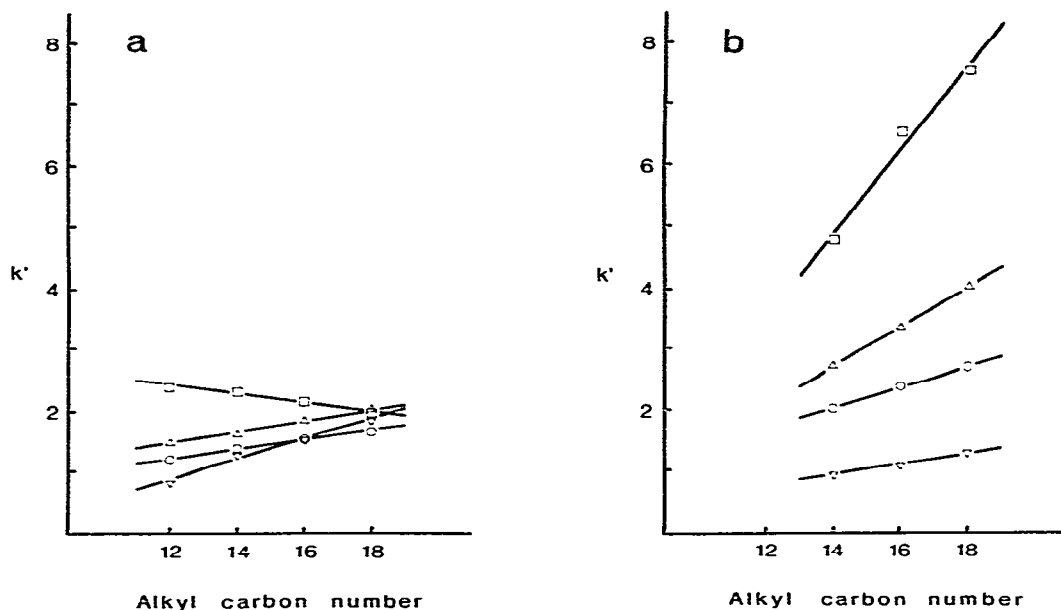


Fig. 3. Relationship between alkyl chain length (carbon number) and k' for four of the test substances at two different levels of coverage: (a) 0.4 mmol of surfactant per gram and (b) 0.6 mmol of surfactant per gram. k' values calculated from Figs. 1 and 2. O, Benzene; Δ , phenol; ∇ , phenethylamine; \square , benzoic acid.

phase, *i.e.*, there is a linear relationship between retention and alkyl chain length if the degree of coverage is kept constant. Figs. 3 a and b show the influence of the chain length on retention of four of the test compounds at two levels of adsorbed surfactant. It appears that the effect of increasing chain length at both levels is an increased retention of benzene and phenol, both of which are chromatographed mainly according to a reversed-phase partition mechanism. The retention of benzoic acid decreases with increasing chain length at low coverage (0.4 mmol/g), whereas the opposite is seen at a higher coverage (0.6 mmol/g). This may be explained by the fact that the surfactant concentrations needed to achieve the 0.4 mmol/g coverage are very low for the longer chain compounds (*cf.*, Fig. 1), and thus the possibility of ion-pair formation is drastically decreased with increasing chain length of the surfactant. For phenethylamine an increase in retention is found with increasing chain length of the surfactant, most pronounced at low coverage of the silica. Also in this instance the concentration effect is of vital importance as the ion-exchange mechanism, partly responsible for the retention of cationic compounds, is greatly influenced by the concentration of the concurrent ion.

The influence on retention of the degree of coverage for each of the four surfactants is shown in Fig. 4 for benzene, which is the only compound in the mixture that is assumed to be chromatographed by a pure reversed-phase partition mechanism, *i.e.*, without any ion-exchange or ion-pair effects. The linear relationship that appears is in accordance with what has been demonstrated between retention and carbon content for chemically bonded phases¹³.

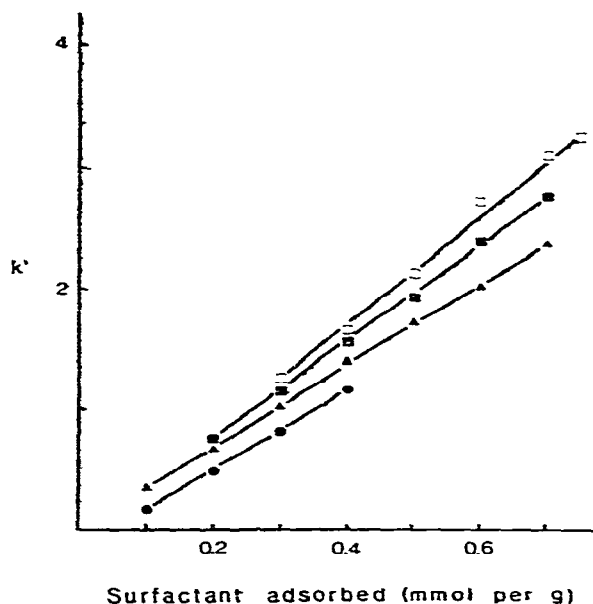


Fig. 4. Relationship between amount of surfactant adsorbed and retention of benzene. k' values calculated from Figs. 1 and 2. ●, DTMA; ▲, TTMA; ■, CTMA; □, STMA.

Selectivity

A change in selectivity in the separation of a mixture of compounds, when using the dynamically modified silica approach, may be accomplished in several ways. Changing the chain length of the quaternary ammonium compound has a pronounced effect (*cf.*, Fig. 3), the effect being different at different concentration levels. If a change in selectivity between ionic solutes of opposite charge is wanted, a change in the concentration of surfactant is to be preferred (*cf.*, Fig. 2). If a change in retention of compounds in a mixture without affecting the selectivity is wanted, a change in the accessible surface area of the column is a possibility. This can be achieved by using a different column length or by changing to another brand of silica².

Apart from the nature and concentration of the surfactant, the whole composition of the mobile phase is of vital importance for the selectivity, *e.g.*, the nature and concentration of the organic modifier and buffer components, the pH and the ionic strength. Work is in progress to clarify the influence of these parameters.

CONCLUSION

It has been demonstrated that the affinity to bare silica of quaternary ammonium compounds depends greatly on the structure and the size of the ions. Symmetrical quaternary ammonium ions are adsorbed to only a minor extent and have little influence on the retention of acidic and neutral solutes. For long-chain alkyl-trimethylammonium compounds it was found that the maximum amounts adsorbable are of comparable magnitude but, owing to increased affinity to the silica, this amount is reached at lower concentrations the longer is the alkyl chain. The reten-

tions of neutral and acidic solutes for increasing surfactant concentration reach a maximum that coincides with the critical micellar concentration for the individual surfactants. This is in accordance with what has been found previously for chemically bonded supports.

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REFERENCES

- 1 S. H. Hansen, *J. Chromatogr.*, 209 (1981) 203.
- 2 S. H. Hansen, P. Helboe, M. Thomsen and U. Lund, *J. Chromatogr.*, 210 (1981) 453.
- 3 B. Nielsen and K. Ebert, *Arch. Pharm. Chem., Sci. Ed.*, 2 (1974) 130.
- 4 R. N. Icke, B. B. Wisegraver and G. A. Alles, *Org. Synth.*, Coll. Vol. III (1955) 723.
- 5 A. C. Cope and R. D. Bach, *Org. Synth.*, Coll. Vol. V (1973) 315.
- 6 K. K. Unger, *Porous Silica — Its Properties and Use as Support in Column Liquid Chromatography*, Elsevier, Amsterdam, 1979, p. 104.
- 7 W. R. Melander and C. Horváth, *J. Chromatogr.*, 201 (1980) 211.
- 8 C. T. Hung and R. B. Taylor, *J. Chromatogr.*, 202 (1980) 333.
- 9 C. T. Hung and R. B. Taylor, *J. Chromatogr.*, 209 (1981) 175.
- 10 J. A. Graham and L. B. Rogers, *J. Chromatogr. Sci.*, 18 (1980) 614.
- 11 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.
- 12 H. Hemetsberger, W. Maasfeld and H. Richen, *Chromatographia*, 9 (1976) 303.
- 13 P. Spacek, M. Kubin, S. Vozka and B. Porsch, *J. Liquid Chromatogr.*, 3 (1980) 1465.