Journal of Pharmaceutical & Biomedical Analysis
Vol. 2, No. 2, pp. 165–172, 1984
Printed in Great Britain

0731–7085/84 $3.00 + 0.00
Pergamon Press Ltd.

Dynamically-modified silica — an alternative to reversed-phase high-performance liquid chromatography on chemically bonded phases*

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Abstract: An alternative to the well known reversed-phase separations on chemically-bonded phases has been developed. The approach is based on a dynamic modification of bare silica with long chain quaternary ammonium ions. The influence of the concentration and type of the quaternary ammonium ion, the pH value and the ionic strength of the eluent on the selectivity towards test solutes has been investigated. The large number of parameters that can be varied in the system offers numerous possibilities by which the desired selectivity can be attained. Once established, a high degree of reproducibility of the selectivity between solutes is obtained even when using different brands of silica; this is in contrast to the situation when using chemically-bonded phases, such as, for example, different brands of octadecylsilyl-bonded silica materials.

Keywords: *Dynamically modified silica; reversed-phase high-performance liquid chromatography; bare silica; selectivity; quaternary ammonium salts.*

Introduction

Separation methods based on reversed-phase high-performance liquid chromatography (HPLC) on column packing materials with chemically-bonded phases have been used with ever increasing success during the last decade. This increased use has been particularly pronounced in the field of analysis of samples of biological origin; this success will doubtless continue in the years to come. Despite all their indisputable advantages, these packing materials still suffer from one serious drawback, namely the lack of reproducibility as regards the selectivity obtained when using packing materials of theoretically the same nature from different sources. These problems have recently been discussed by Engelhardt and Ahr [1] and by Goldberg [2]. Furthermore, several reports

* Presented at the Symposium on Liquid Chromatography in the Biomedical Sciences, June 1984, Ronneby, Sweden.
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in the fields of pharmaceutical analysis [3–5], clinical chemistry [6, 7] and environmental analysis [8, 9] have appeared showing great variations in the selectivity of octadecylsilyl (ODS) bonded materials, when the same sample mixture is analysed on packing materials from different manufacturers and even on different batches of material from the same manufacturer. Using the dynamically-modified silica approach the reproducibility of the selectivity of packing materials from different manufacturers is considerably improved, as discussed below.

Experimental

Apparatus

Testing of the individual chromatographic systems was performed on a Waters (Milford, MA, USA) liquid chromatograph consisting of a model 6000 A pump, a model 710 A WISP autoinjector, a model 440 ultraviolet absorbance detector (operated at 254 nm), a model 730 data module and a model 720 system controller; alternatively a liquid chromatograph was assembled, consisting of a Kontron model 410 LC pump, a Pye–Unicam (Cambridge, UK) LC–UV detector (operated at 254 nm) and a Rheodyne model 7125 injection valve. Chromatograms were recorded on a Kipp and Zonen Model BD-8 recorder. Retention data were collected on a Waters model 730 data module or on a Hewlett–Packard Model 3353 A laboratory data system.

Procedures

Determination of the amounts of cetyltrimethylammonium (CTMA) bromide adsorbed on the column material by the breakthrough method or by the elution method was performed as previously described [10, 11].

Chromatography

All experiments were performed on 120 × 4.6 mm i.d. columns from Knauer (Berlin, FRG), packed by the dilute slurry technique, unless otherwise indicated, with 5-μm LiChrosorb Si-60 (E. Merck, Darmstadt, FRG). The eluent was methanol–water–phosphate buffer (50:45:5, v/v/v) with the addition of various types and concentrations of quaternary ammonium compounds.

All pH values stated are those measured in the buffers *before* dilution 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, followed by dilution to the final concentration of 0.2 M. During chromatography the column was protected by a silica pre-column situated between the pump and the injection device to saturate the eluent. The chromatographic system was equilibrated with eluent overnight. Following each adsorption experiment the column was brought to its initial status by eluting with methanol–0.05 M orthophosphoric acid (1:1, v/v) and finally with methanol.

Chemicals

Stearyltrimethylammonium (STMA) bromide was prepared as described previously [12]. Dodecyltrimethylammonium (DTMA) bromide and tetradecyltrimethylammonium (TTMA) bromide were obtained from Sigma (St Louis, MO, USA). Cetyltrimethylammonium (CTMA) bromide, tetrabutylammonium (TBA), tetrapentylammonium (TPA) bromide and all other reagents were of analytical grade from E. Merck. All chemicals were used as received from the manufacturers.

Results and Discussion

In the dynamically-modified silica approach the surface of the bare silica is covered with a layer of quaternary ammonium ions as shown schematically in Fig. 1. The amount attached (primarily by electrostatic forces) to the silica surface is dependent on the composition of the eluent [13]. It forms a dynamic coating in which the quaternary ammonium ions on the silica surface are in equilibrium with the quaternary ammonium ions that are continuously present in the eluent.

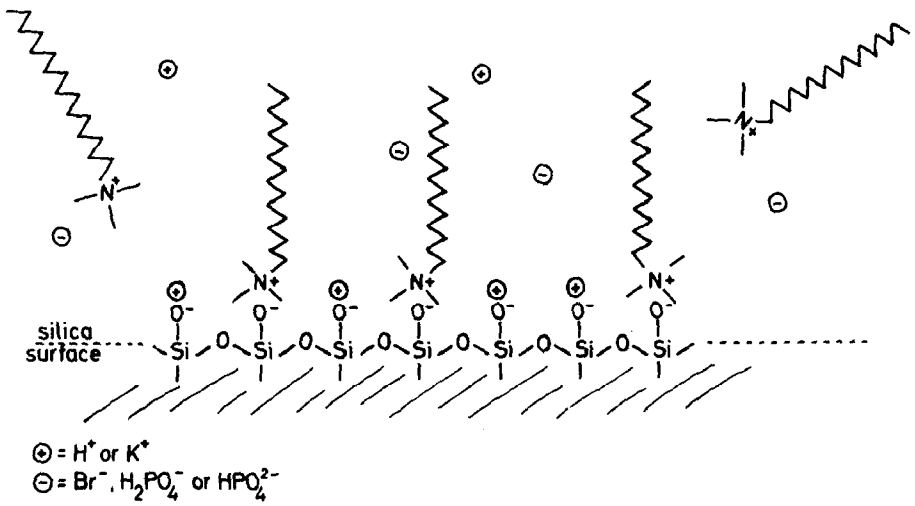

Figure 1
Bare silica dynamically modified with long chain quaternary ammonium ions. A typical system would be: solid phase, 5-μm LiChrosorb SI-60, 120 × 4.6 mm i.d.; mobile phase, methanol–water–0.2 M potassium phosphate (pH 7.5) (50:45:5, v/v/v) with 2.5 mM of cetyltrimethylammonium bromide added.

Amount and nature of the quaternary ammonium ion

The amount of quaternary ammonium ions adsorbed on the silica surface increases to a certain level with increasing concentration in the eluent [10, 12] (Fig. 2), the more hydrophobic ammonium ions having a higher affinity for the silica. The bulky symmetrical ammonium ions (TBA and TPA) exhibit only a slight affinity for silica under the selected conditions (50% v/v of methanol).

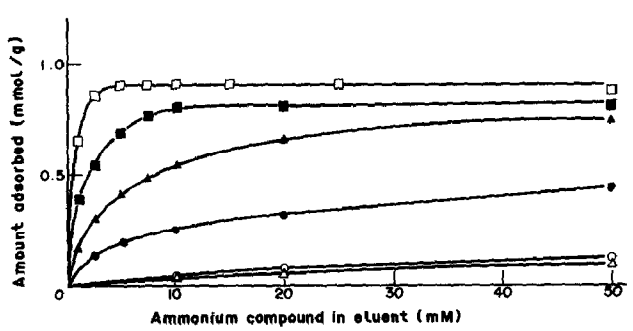

Figure 2
Amount of quaternary ammonium salt adsorbed per gram of silica vs concentration in eluent. Key: ○, TBA; △, TPA; ●, DTMA; ▲, TTMA; ■, CTMA; □, STMA. Eluent: Methanol–water–0.2 M potassium phosphate (pH 7.5) (50:45:5, v/v/v) (plus the appropriate concentration of quaternary ammonium compound as indicated).

The resulting retention values of some test solutes are shown in Fig. 3. When the critical micelle concentration (CMC) is reached as indicated in Fig. 3d, no more quaternary ammonium ions are adsorbed on to the silica surface (Fig. 2).

Concentrations of the quaternary ammonium ions above the CMC result in the formation of a secondary mobile phase — the hydrophobic phase inside the micelles — in the eluent itself; the total eluent thus becomes more hydrophobic and elutes more strongly.

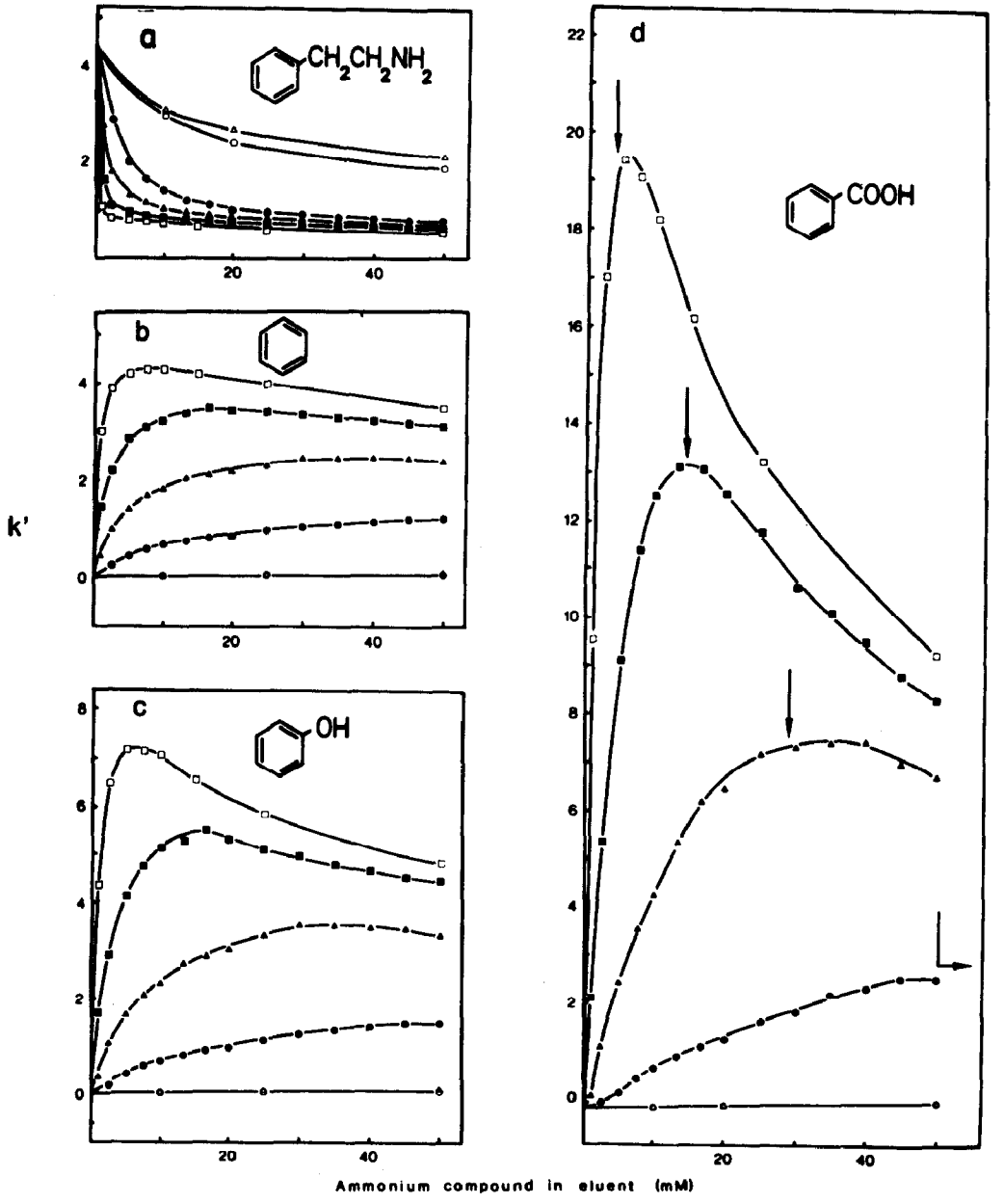

Figure 3
Phase capacity ratio (k') values for four test solutes vs the concentration of quaternary ammonium salt in the eluent: a, phenethylamine; b, benzene; c, phenol; d, benzoic acid. Symbols as in key to Fig. 2. Eluent composition as in Fig. 2. The arrows in d indicate the estimated critical micellar concentrations.

pH of the eluent

The amount of quaternary ammonium ions absorbed onto the silica surface also increases with increasing eluent pH [14], as shown for CTMA in Fig. 4. This is mainly due to the increased ionization of the silanol groups.

The retention of neutral and cationic solutes increases with increasing pH due to the reversed-phase effect. A cation-exchange mechanism will be of importance only for very hydrophobic cationic solutes, as the long chain quaternary ammonium ions in the eluent exhibit a very high affinity for the silica.

With quaternary ammonium ions the anionic solutes form ion pairs, which are retained by a reversed-phase partition mechanism. However, the retention of anions is influenced by various factors that result in a dependence on the eluent pH; this needs further investigation.

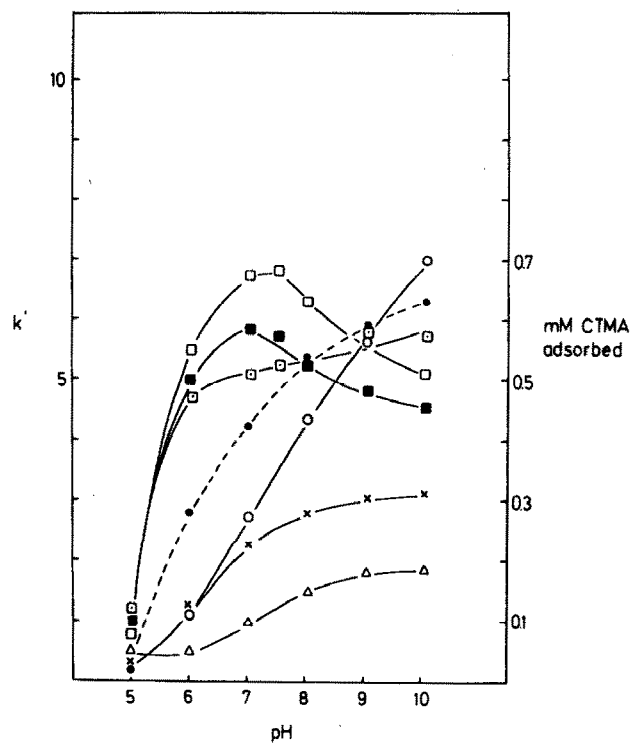

Figure 4
Relationship of the pH of the buffer used in the eluent with: (a) the amount of CTMA adsorbed; and (b) retention (k') of various test solutes. Eluent: methanol–water–0.2 M phosphate buffer (50:45:5, v/v/v) with 2.5 mM CTMA added. Symbols: ●, CTMA; △, phenethylamine; ×, benzene; ○, phenol; □, benzoic acid; ■, sorbic acid; □, fumaric acid.

Ionic strength

Increasing the buffer concentration or adding other ions to the eluent may also be used to control the selectivity of the system [14]. Table 1 shows the variations in k' value of some test solutes as a function of various added salts. The retention of anionic solutes can be reduced selectively by adding inorganic salts. The addition of sulphonates results in an even stronger decrease in the retention of anionic solutes, while the retention of non-ionic and cationic solutes increases. A decrease in the hold-up volume indicates the formation of a thicker layer of stationary phase, presumably caused by adsorption of ion

Table 1
Effects of addition of various anions to the eluent on the retention (k') of various test solutes on dynamically-modified silica*

Solute	No inorganic salts added	Inorganic salts				*n*-Alkanesulphonates	
		Phosphate (pH 7.5)		Bromide		Octane	Decane
		0.01 M	0.02 M	0.02 M	0.04 M	0.02 M	0.02 M
Benzene	1.94	2.18	2.19	1.88	2.01	2.45	3.21
β-Methylnaphthalene	13.7	15.0	15.2	13.9	13.7	23.0	21.9
Phenanthrene	28.9	31.4	31.4	30.0	29.2	50.8	47.2
Imipramine (IP)	22.1	22.0	20.5	21.2	19.8	27.1	41.7
Desmethyl-IP	13.4	12.4	11.1	11.1	9.4	21.8	51.2
N-Methyl-IP	3.00	2.49	2.53	2.46	2.35	7.74	22.0
Benzoic acid	4.26	3.54	3.12	3.44	3.36	0.65	0.49
Phthalic acid	13.3	5.81	3.72	5.92	3.71	0.47	0.23
1,3,5-Benzenetri-carboxylic acid	110	22.9	10.5	24.9	9.73	2.48	0.00
t_0† (water)	1.04	1.02	1.02	1.04	1.03	0.92	0.82

* Eluent: methanol–water–0.2 M potassium phosphate (500:487.5:12.5, v/v/v) containing 2.5 mM CTMA bromide. Column temperature, 39.6°C.
† t_0 = void volume (min).

pairs (formed by quaternary ammonium ions and sulphonates) onto the hydrophobic silica surface generated.

Reproducibility of the selectivity

Other parameters such as column temperature, nature and concentration of organic modifier, and nature of the buffer ions, may also influence selectivity in the system (cf. [14, 15]). However, one of the most obvious advantages of the proposed approach is the high reproducibility of selectivity from batch to batch and even between brands of bare silica from various manufacturers [10].

An example of this reproducible selectivity is given by the determination of impurities in propranolol [16]. The relative retention values of propranolol and some of its impurities on different brands of chemically bonded ODS-materials, and also on different brands of bare silica dynamically modified with CTMA, are given in Table 2. A chromatogram of the separation in the latter system is shown in Fig. 5.

Drug analysis in biological samples

The dynamically-modified silica approach has also been used in studies on drug metabolism [17–19]. The determination of the toluene metabolite in urine [17], for example, emphazises the importance of the reproducibility of selectivity, since many peaks due to endogenous phenols are present in the chromatogram. Over a period of three years using several new columns for this assay, no problems in reproducing the selectivity have appeared.

Conclusion

Reversed-phase HPLC based on dynamic modification of bare silica with quaternary ammonium ions has been developed. The main advantage of the approach is high

Table 2
Separation factors between propranolol and three of its possible impurities, measured on eight different silica columns and eight different ODS-silica columns

Column packing material	Pore size (nm)	Separation factor* II	III	IV
Silica				
LiChrosorb Si 60	6	0.26	1.86	2.22
Nucleosil 50–5	5	0.26	1.76	2.13
Zorbax SIL	7	0.24	1.76	2.08
Partisil 5	7–8	0.25	1.66	1.98
Spherisorb S 5W	8	0.28	1.74	2.01
LiChrosorb Si 100	12	0.31	1.39	1.60
Nucleosil 100–5	10	0.26	1.43	1.66
Hypersil	10	0.26	1.40	1.66
ODS-silica				
LiChrosorb RP-18		0.40	2.43	3.81
Nucleosil-5 C_{18}		0.61†	2.40	4.69
Hypersil ODS		0.38	2.62	3.34
Zorbax ODS		0.38	2.97	4.38
Partisil 10 ODS		1.19†	2.05	4.48
Partisil 10 ODS 2		0.61	3.21	6.53
Partisil 10 ODS 3		0.56	2.42	3.71
Spherisorb S5 ODS		0.60	1.84	2.68

* Separation factor is defined as relative retention time with respect to propranolol.
† The broad propranolol peak overlaps the peak in the chromatogram.

Substances	R = 1 - naphthyl
I (Propranolol)	R—CH_2—CHOH—CH_2—NH—$CH(CH_3)_2$
II	R—CH_2—CHOH—CH_2OH
III	R—CH_2—CHOH—CH_2—N(—$CH(CH_3)_2$)—CH_2—CHOH—CH_2—O—R
IV	R—CH_2—CHOH—CH_2—O—R
V	R—CH_2—CH(O)—CH_2
VI	R—CH_2—CHOH—CH_2Cl

reproducibility of the selectivity. This is especially important, when the same analysis has to be performed in various laboratories. The solubility of the silica is a disadvantage that can be readily overcome by installing a saturation column between the pump and the injection device.

Acknowledgement: This work was supported by the Danish Medical Research Council, grant No. 12-0649.

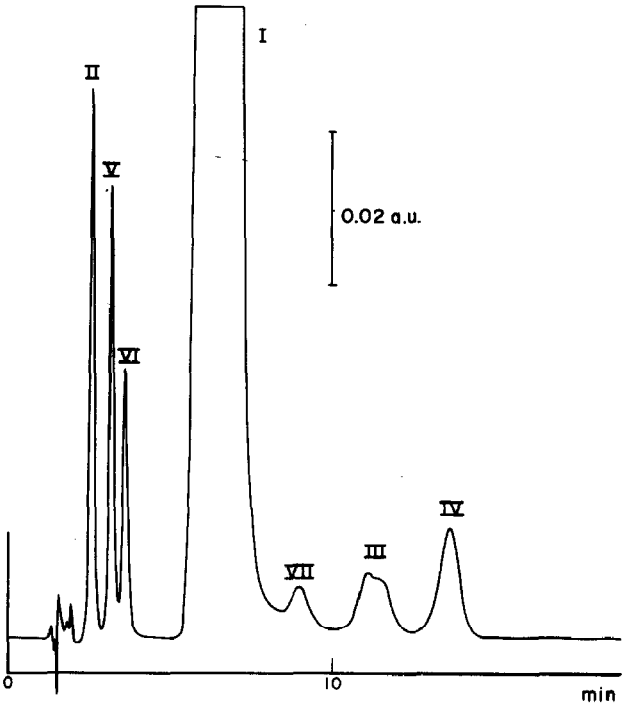

Figure 5
Chromatogram of propranolol spiked with 0.3% m/m of each possible impurity. Column, Zorbax SIL (120 × 4.6 mm i.d.); eluent, methanol–water–0.2 M potassium phosphate buffer (pH 8.0) (70:25:5, v/v/v), containing 2.5 mM CTMA; detection wavelength, 292 nm; flow rate, 1 ml/min. Peak identification: as in Table 2, except for VII, which is an unknown impurity.

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[*Received for review 17 July 1984*]