Journal of Chromatography, 245 (1982) 45–56
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,947

LIQUID CHROMATOGRAPHY OF SUBSTITUTED PHENOXYPROPANOLAMINES ON DYNAMICALLY COATED SILICA USING AQUEOUS MOBILE PHASES

SVEN-OLOF JANSSON*, INGER ANDERSSON and MARIE-LOUISE JOHANSSON
Department of Analytical Chemistry, AB Hässle, S-431 83 Mölndal (Sweden)
(Received March 23rd, 1982)

SUMMARY

The retention behaviour of amines was investigated in systems with silica gel (LiChrosorb Si 60) as the support and an aqueous phosphate buffer containing organic modifiers as the mobile phase. N,N-Dimethyloctylamine and N,N,N-trimethyloctylamine were used as cationic modifiers, with 3,5-dimethylcyclohexylsulphate as the ion-pair forming counter ion. Adsorption isotherms were determined for the amines used as modifiers.

At low concentrations of modifier a monolayer is formed on the solid phase and the retention times of the solutes decrease with increasing modifier concentration. Higher concentrations give rise to the formation of a multilayer or bulk phase of modifier, leading to rapidly increased retention of the most hydrophobic solutes and a change in retention order. The hydrogen-bonding properties of the solute seem to be of major importance for the retention on unmodified silica, while the hydrophobicity of the solute determines the retention to the adsorbed phase of the modifier.

INTRODUCTION

Non-polar bonded phases are commonly applied as supports in reversed-phase chromatography but, despite the wide use of alkyl-bonded silica, there is doubt about the consistency of the packing material. Recently, attention has been paid to the use of aqueous mobile phases in combination with bare silica as an alternative to the chemically bonded material. In such systems, the retention of the solutes seems to be due to hydrogen bonding to the silica support, but ionic interaction and partition to a stationary phase dynamically coated on the silica may also contribute.

The hydrogen-bonding properties of silica have been thoroughly investigated by Iler[1–3]. Amines are supposed to be bound to polysilicic acid at low pH by a reverse type of hydrogen bonding.

Crommen[4,5] studied the retention behaviour of organic compounds on bare silica and used a retention model similar to that described for alkyl-bonded phases, which includes ion-pair partition[6]. In a study on the separation of biogenic amines on silica columns, Svendsen and Greibrokk[7] suggested a combination of two different retention mechanisms.

0021-9673/82/0000–0000/$02.75

Ghaemi and Wall[8–10] used silica dynamically coated with surfactants as stationary phases for reversed-phase chromatography. The versatility of this method was demonstrated by use of different types and concentrations of modifiers in the aqueous mobile phase. A similar approach was used by Hansen and co-workers[11,12].

In this study, the effect of organic ionic modifiers and counter ions was examined in the liquid chromatography of hydrophobic amines on silica using aqueous mobile phases. The modifiers were found to compete with the solutes for the available capacity of the silica support, but at low concentrations the hydrophobic counter ions had very little influence on the retention. When the mobile phase contained hydrophobic ion-pair forming agents at higher concentrations, a stationary hydrophobic phase was adsorbed on the silica. The degree of coating influences the retention mechanism and a change in the retention order of the solute amines occurs.

EXPERIMENTAL

Apparatus

The liquid chromatograph consisted of two Altex solvent-metering pumps (Models 100 and 110A), an LDC SpectroMonitor III UV detector, operated at 270 nm, a Rheodyne Model 70-10 sampling valve with a sample loop of 20 μl and an Altex Model 421 system controller. The stainless-steel columns (150 × 4.0 mm I.D.) were equipped with Swagelok zero-volume connectors and were packed with LiChrosorb Si 60, 7 μm (E. Merck, Darmstadt, G.F.R.).

Chemicals and reagents

N,N-Dimethyloctylamine (DMOA) was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and distilled before use. N,N,N-Trimethyloctylammonium bromide (TMOA), potassium 3,5-dimethylcyclohexylsulphate (DMCHS) and the solute amines as chlorides (Table I) were supplied by the Department of Organic Chemistry, AB Hässle (Mölndal, Sweden). All other chemicals were of analytical-reagent grade and used without further purification.

Liquid chromatographic system

Aqueous phosphate buffers of pH 2.2 were used as solvents for the mobile phases. Increasing amounts of organic modifiers were added to the mobile phase by means of the system controller. Capacity ratios were determined under isocratic conditions at a flow-rate of 1 ml/min by repeated injections of the compounds under study. The volume of mobile phase in the column, V_m, was determined by injection of mobile phase slightly diluted with water. The temperature was 23°C.

The columns were stable for several months with unchanged efficiency, indicating that dissolution or chemical degradation of the silica could be disregarded[13]. The columns were washed and stored filled with methanol when not in use.

Determination of adsorbed DMOA and TMOA

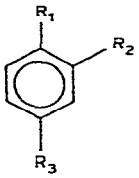
Adsorbed DMOA and TMOA were eluted from the liquid chromatographic column with 100 ml of 0.05 *M* phosphoric acid. The assay of DMOA was performed with a Perkin-Elmer 3920B gas chromatograph equipped with a flame-ionization detector, as described before[14]. TMOA was assayed spectrophotometrically by the picrate method[15].

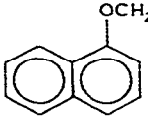
RESULTS AND DISCUSSION

The structures of the compounds studied are shown in Table I. They are secondary aliphatic amines used as β-adrenoreceptor antagonists or related intermediates from their synthesis.

TABLE I

STRUCTURES OF THE COMPOUNDS STUDIED



Name	R_1	R_2	R_3
Acebutolol	$OCH_2CH(OH)CH_2NHCH(CH_3)_2$	OCH_3	$NHCOCH_2CH_2CH_3$
Alprenolol	$OCH_2CH(OH)CH_2NHCH(CH_3)_2$	$CH_2CH=CH_2$	H
Metoprolol	$OCH_2CH(OH)CH_2NHCH(CH_3)_2$	H	$CH_2CH_2OCH_3$
Oxprenolol	$OCH_2CH(OH)CH_2NHCH(CH_3)_2$	$OCH_2CH=CH_2$	H
Pafenolol	$OCH_2CH(OH)CH_2NHCH(CH_3)_2$	H	$CH_2CHNHCONHCH(CH_3)_2$
H 177/06	$OCH_2CH(OH)CH_2NHCH_2CH_2CH_3$	H	$CH_2CHNHCONHCH(CH_3)_2$
H 138/01	OH	H	$CH_2CHNHCONHCH(CH_3)_2$
H 162/14	$OCH_2CH(OH)CH_2OH$	H	$CH_2CHNHCONHCH(CH_3)_2$
Propranolol			

Equilibration of columns

The adsorption capacity of silica is influenced by organic solvent components, such as 1-pentanol[4], methanol or acetonitrile[7], present in the aqueous mobile phase. A similar effect can be obtained by adsorption of ion-pairing organic modifiers to the solid phase[4]. As a rule the equilibration time is short but is to some extent depending on the concentration and the equilibrium constant for the adsorption of the modifier. In our study, constant retention times were normally obtained within 1 h. Considerably longer times (>8 h) were needed for systems containing high concentrations of hydrophobic ion-pair forming modifiers, such as DMOA in combination with DMCHS.

Effect of cationic modifiers

The effect of TMOA on the retentions of some of the compounds studied is illustrated in Fig. 1. The capacity ratios decrease initially with increasing concentration of modifier and reach an almost constant level in the region of 0.015–0.03 *M* of TMOA. On increasing the TMOA content from 0.03 to 0.04 *M*, an increase in the capacity ratios of all solutes is observed. This effect is obtained only with a hydro-

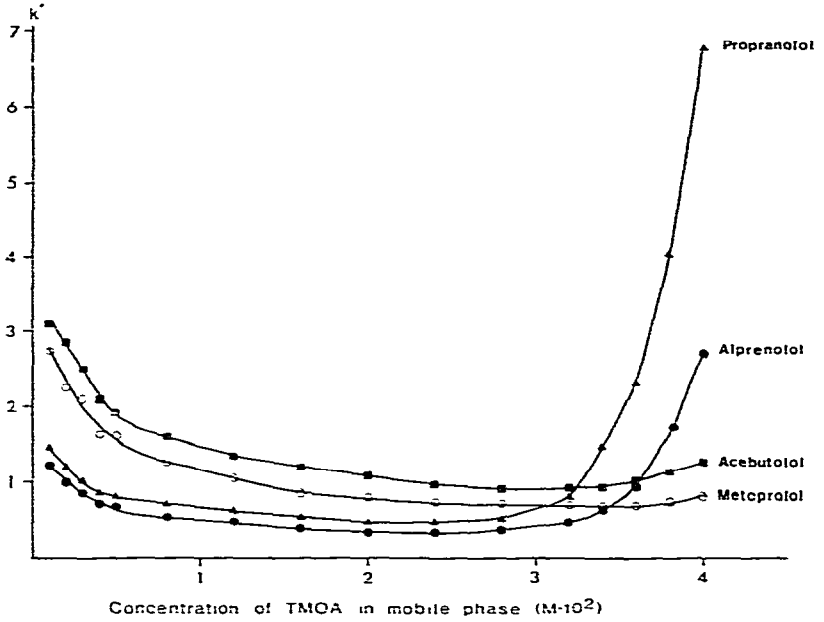


Fig. 1. Regulation of the retention by the concentration of TMOA in the mobile phase. Mobile phase: TMOA and potassium bromide in a total concentration of 0.04 M and $1 \cdot 10^{-2}$ M DMCHS in phosphate buffer (pH 2.2). Support: LiChrosorb Si 60.

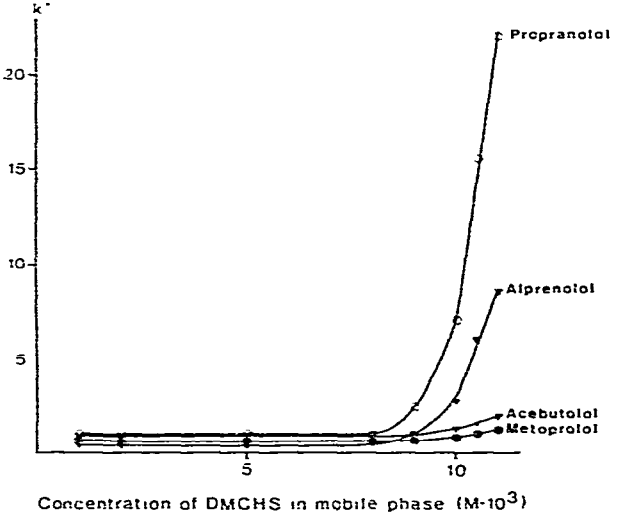


Fig. 2. Influence of DMCHS on the retention. Mobile phase: DMCHS and $4 \cdot 10^{-2}$ M TMOA in phosphate buffer (pH 2.2). Support: LiChrosorb Si 60.

phobic counter ion, such as DMCHS, present in the mobile phase. In this and other respects TMOA and DMOA were found to be equivalent.

The initial decrease in capacity ratios is assumed to be due to competition between the modifier and the solutes for the limited adsorption capacity of the solid phase. In the region of constant retention, the adsorption sites may be almost completely covered with TMOA and the solutes mainly retained by ion exchange with adsorbed TMOA[16]. The increasing capacity ratios at high concentrations of TMOA are probably due to the formation of an adsorbed stationary phase of the TMOA–DMCHS ion pair to which the solutes are distributed. A further indication of the formation of a layer of adsorbed TMOA–DMCHS ion-pair is given in Fig. 2, which shows that increasing concentrations of DMCHS at constant TMOA concentration ($4 \cdot 10^{-2}$ *M*) also give a rapid increase in capacity ratios when the DMCHS concentration exceeds $8 \cdot 10^{-3}$ *M*.

Adsorption of DMOA on LiChrosorb Si 60

The amounts of DMOA and TMOA adsorbed on LiChrosorb Si 60 were

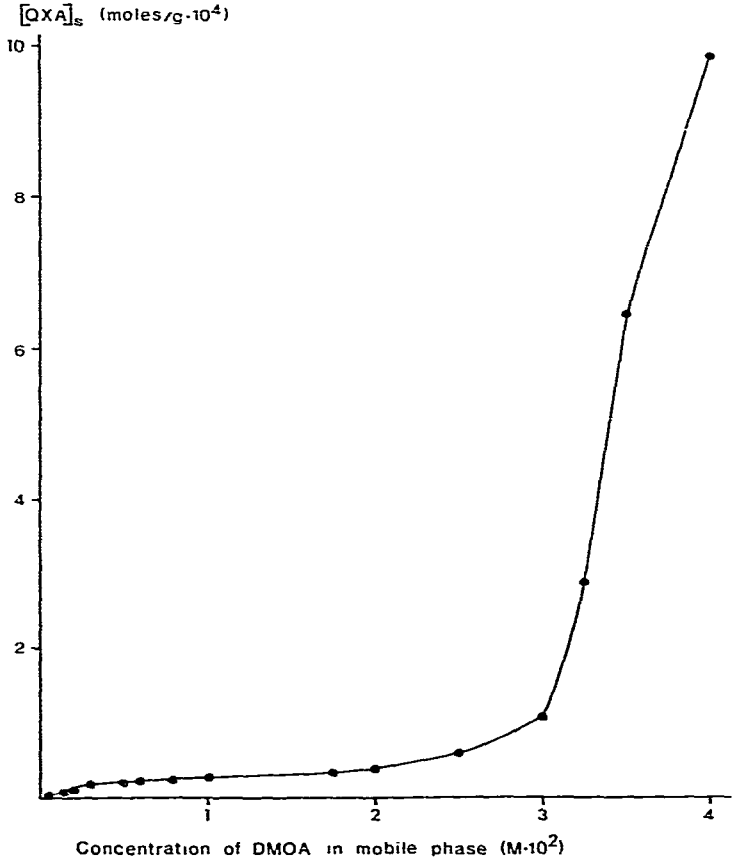


Fig. 3. Adsorption isotherm of DMOA. Mobile phase: DMOA and $1 \cdot 10^{-2}$ *M* DMCHS in phosphate buffer (pH 2.2). Support: LiChrosorb Si 60.

measured in order to elucidate the retention behaviour of the solutes, and the adsorption isotherm for DMOA is given in Fig. 3. Buffer solutions of pH 2.2, containing 0.057 *M* dihydrogen phosphate, 0.01 *M* DMCHS and various concentrations of DMOA, were used as mobile phases. The first part of the adsorption isotherm (DMOA concentration <0.02 *M*) was evaluated by means of the Langmuir isotherm for monolayer adsorption, defined according to

$$[QXA]_s = \frac{K_0 K_{QX} [Q^-]_m [X^-]_m}{1 + K_{QX} [Q^-]_m [X^-]_m} \quad (1)$$

where $[QXA]_s$ (mole/g) is the amount of a cationic modifier Q^+ adsorbed as an ion pair with a counter ion X^- on the adsorption sites A_s, as defined by the equilibrium

$$Q_m^- + X_m^- + A_s \rightleftarrows QXA_s \quad (2)$$

with the corresponding equilibrium constant K_{QX}. The total adsorption capacity of the solid phase, K_0 (mole/g), is given by

$$K_0 = [A_s] + [QXA_s] \quad (3)$$

Inversion of eqn. 1 gives

$$\frac{1}{[QXA]_s} = \frac{1}{K_0 K_{QX} [Q^-]_m [X^-]_m} + \frac{1}{K_0} \quad (4)$$

A plot of $1/[QXA]_s$ *vs.* $1/[Q^-]_m$, in accordance with eqn. 4, gave a linear relationship in the concentration range $[Q^-]_m = 0.0015$–0.01 *M* (Fig. 4). A monolayer capacity of $4.8 \cdot 10^{-5}$ mole/g was estimated from the intercept, the magnitude being similar to that reported by Crommen[5] in adsorption studies on Nucleosil 100. A value of $K_{QX} [X^-]_m$ was calculated from the slope of the line (Table II).

The second step in the isotherm (Fig. 3) at $[Q^-]_m = 0.03$–0.04 *M* indicates adsorption of a multilayer or bulk phase of the DMOA–DMCHS ion pair. The adsorption of DMOA–DMCHS cannot be studied at concentrations higher than that given in Fig. 3 owing to the limited solubility of DMOA in the mobile phase.

Monolayer adsorption of solutes

The solutes are assumed to be adsorbed as ion pairs on silica according to the same principle as discussed for monolayer adsorption of DMOA. The competing effect of a cationic modifier Q^- on the adsorption of an amine solute HB^+ is in accordance with eqn. 3, given by

$$K_0 = [A_s] + [QXA_s] + [HBXA_s] \quad (5)$$

An expression for the capacity ratio of the solute, k'_{HB}, can then be derived, as described elsewhere[4,6,14], giving

$$k'_{HB} = \frac{q K_0 K_{HBX} [X^-]_m}{1 + K_{QX} [Q^-]_m [X^-]_m} \quad (6)$$

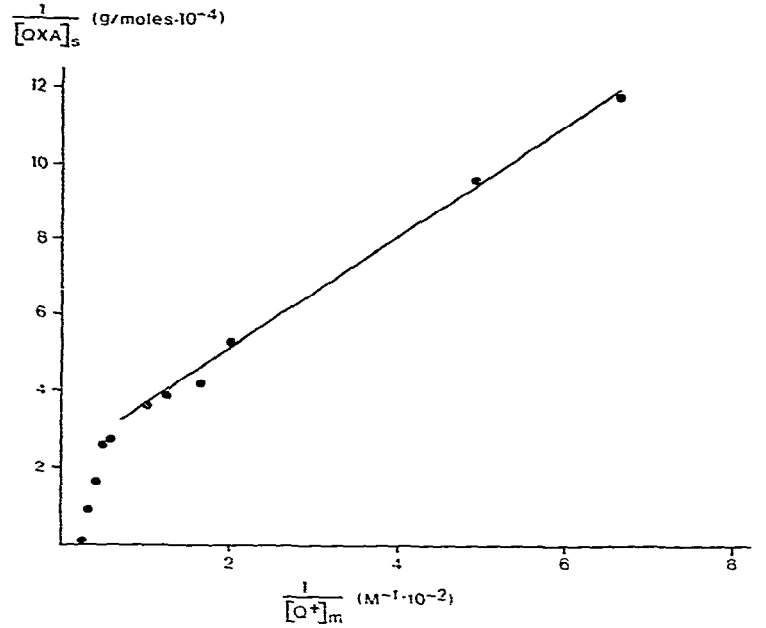


Fig. 4. Test of monolayer adsorption of DMOA according to eqn. 4. Conditions as in Fig. 3.

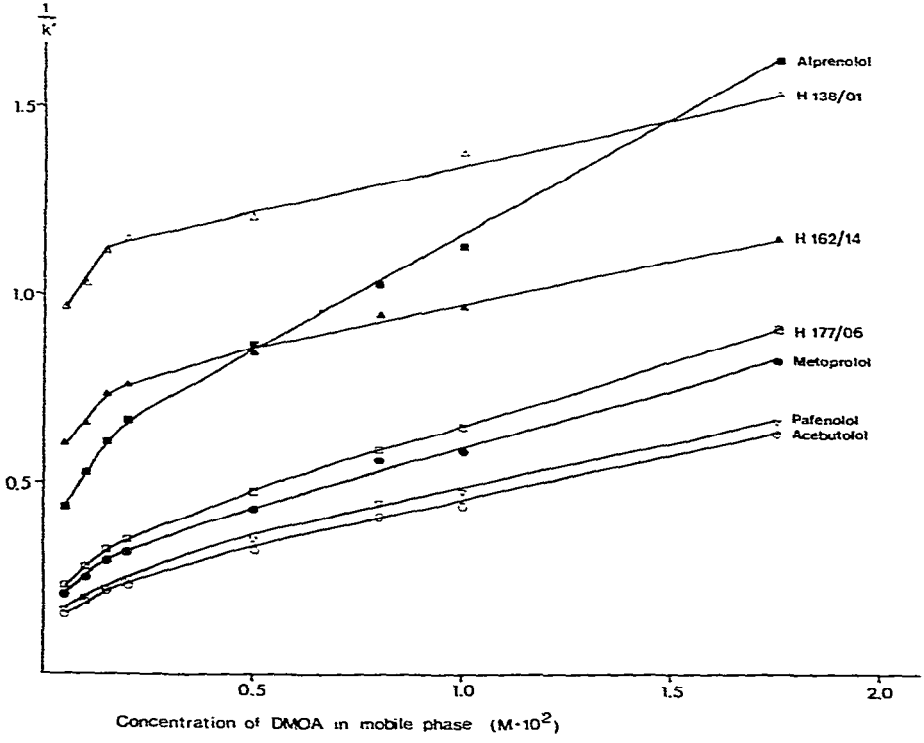


Fig. 5. Effect of DMOA on the retention. Conditions as in Fig. 3.

The term q expresses the ratio of weight (gram) of solid phase to volume (litre) of eluent in the column. Inversion of eqn. 6 gives

$$\frac{1}{k'_{HB}} = \frac{1}{qK_0K_{HBX}[X^-]_m} + \frac{K_{QX}[Q^-]_m}{qK_0K_{HBX}} \quad (7)$$

The capacity ratios found in retention studies with DMOA as modifier in the region of monolayer adsorption were used in a plot based on eqn. 7, as demonstrated in Fig. 5.

The deviation from linearity at low concentrations of DMOA indicates a stronger competing effect in this region. This has been further discussed by Crommen[5] as an influence of a second type of adsorption sites with high affinity for amine modifiers but with very low capacity. The influence of these sites seems to be almost negligible at concentrations of DMOA >0.002 M and fairly linear correlations were obtained, from which equilibrium constants were evaluated according to eqn. 7. The results are given in Table II.

TABLE II

EQUILIBRIUM CONSTANTS FROM RETENTION STUDIES WITH DMOA AS COMPETING CATIONIC MODIFIER

Mobile phase: 0.002–0.02 M DMOA (Q^-) and 0.01 M DMCHS in aqueous phosphate buffer, pH 2.2. ($[H_2PO_4^-] = 0.057$ M).

Sample	K_{QX}	$X^-_m \times 10^{-1}$	qK_0K_{HBX}	X^-_m	K_{HBX}	$X^-_m \times 10^{-1}$*	qK_0K_s**	K_s*,**
Alprenolol	8.7		1.7		6.1			
H 177/06	17		3.8		14			
Metoprolol	18		4.4		16			
Pafenolol	20		5.8		21			
Acebutolol	18		5.8		21			
H 138/01	2.6						0.93	34
H 162/14	4.8						1.5	53
DMOA	14***							

* Estimates made with $q = 573$ and $K_0 = 4.8 \cdot 10^{-5}$.

** K_s = Equilibrium constant for partition of a neutral solute S between stationary and mobile phase[17].

*** Estimated from partition studies by elution of adsorbed DMOA (Fig. 4 and eqn. 4).

The estimated values of $K_{QX}[X^-]_m$ are in good agreement with the corresponding value obtained by adsorption measurement of DMOA. The neutral solutes H 138/01 and H 162/14 give considerably lower constants, which indicates a different retention mechanism than for the amines.

Effects of anions

Increasing concentrations of anionic counter ions usually give increasing capacity ratios. Hydrophilic counter ions are best suited to regulating the retention by monolayer adsorption, while hydrophobic counter ions contribute to the formation of a dynamically coated stationary phase, permitting the distribution of the solutes to this phase.

The effect of hydrophilic counter ions in monolayer adsorption is counteracted by the presence of even very hydrophilic cations, such as sodium, in the mobile phase, as discussed by Crommen[4]. The greatest effect was obtained when acids were used to regulate the pH of the mobile phase, while increasing concentrations of a sodium phosphate buffer (pH 2–3) gave decreasing capacity ratios. The competing effect was assumed to be due to the adsorption of sodium phosphate on the strongly adsorbing sites with low capacity.

In similar studies with 5% of methanol present in the mobile phase, we found that the capacity ratios were almost independent of the concentration of phosphate buffer. Further, when the influence of the second adsorption sites was eliminated by use of $5 \cdot 10^{-3}$ *M* DMOA as the competing modifier, increasing capacity ratios were obtained with increasing concentration of sodium dihydrogen phosphate. Equilibrium constants for DMOA and the solutes distributed as ion pairs with dihydrogen phosphate were evaluated from the linear plots according to eqn. 7. The results are presented in Table III.

TABLE III

EQUILIBRIUM CONSTANTS FROM RETENTION OF AMINES AS ION PAIRS WITH DIHYDROGEN PHOSPHATE

Mobile phase: 0.005 *M* DMOA (Q^+) in phosphate buffer (0.057–0.257 *M* sodium dihydrogen phosphate).

Sample	$qK_0K_{HBX} \times 10^{-1}$	$K_{QX} \times 10^{-3}$	$K_{HBX} \times 10^{-3}$*
Metoprolol	10.3	5.5	4.0
Propranolol	5.6	5.1	2.2
Alprenolol	4.9	5.0	1.9

* Estimates made with $q = 529$, $K_0 = 4.8 \cdot 10^{-5}$.

The equilibrium constants K_{QX} of DMOA and K_{HBX} of alprenolol and metoprolol, given in Table III, are of the same magnitude as those obtained in the study with both dihydrogen phosphate and DMCHS present as counter ions (Table II) if $[X^-]_m$ is assumed to be equal to the concentration of dihydrogen phosphate. This means that the hydrophobicity of the counter ion is not decisive for the adsorption, but the retention rather depends on the hydrogen bonding properties of the adsorbed ion pair.

The absence of additional effects of DMCHS was further seen by constant capacity ratios on changing the concentration of DMCHS within the concentration range 0.005–0.05 *M* in the presence of 0.002 *M* of TMOA in aqueous phosphate buffer. The results are probably due to the binding ability of the dihydrogen phosphate ion pairs being as strong as that of the DMCHS ion pairs.

Retention on multilayer coating

The formation of a multilayer coating by the presence of high concentrations of the hydrophobic anion and modifier causes a drastic change in the retention of the solutes, as illustrated in Fig. 6. The magnitude of the capacity ratios on coated silica seems to be dependent on the hydrophobicity of the solute and the retention follows

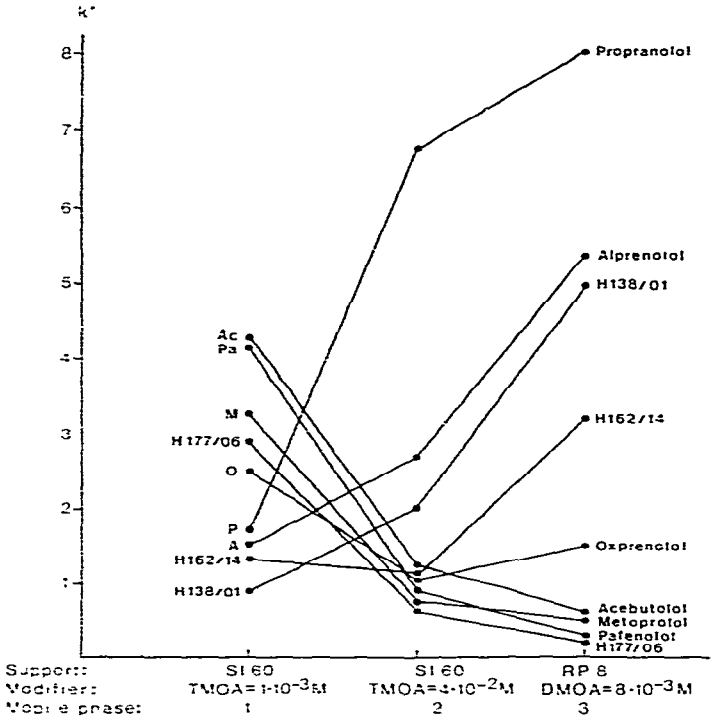


Fig. 6. Influence of the stationary phase on retention. Stationary phases: 1, LiChrosorb Si 60; 2, TMOA as ion pair with DMCHS on LiChrosorb Si 60; 3, LiChrosorb RP-8. Mobile phases: phosphate buffer (pH 2.2) with addition of (1) $1 \cdot 10^{-3}$ *M* TMOA, (2) $4 \cdot 10^{-2}$ *M* TMOA and $1 \cdot 10^{-2}$ *M* DMCHS, (3) $8 \cdot 10^{-3}$ *M* DMOA and 0.115 *M* 1-pentanol[14].

almost the same order as in reversed-phase chromatography on non-polar chemically bonded phases[14]. A comparison with retention on LiChrosorb RP-8 is made in Fig. 6.

The retention order on bare silica shows that the amines containing oxygen incorporated as O-methyl or urea groups in more than one side-chain on the benzene ring are eluted later than the other amines. A similar behaviour was observed by Svendsen and Greibrokk[7] and may be due to the ability to form hydrogen bonds with the silanol groups on the silica, as discussed by Iler[1-8].

The phenol H 138/01 and the diol H 162/14 show a lower affinity to bare silica than the structurally related amines, indicating the strong hydrogen-bonding ability of the amino group.

The different chromatographic behaviour on mono- and multilayer coated silica as regards retention order and separation factors gives additional possibilities. The composition of the mobile phase may then be adapted to the specific separation problem. In addition to a retention order mainly due to the hydrophobicity of the solutes, differences in hydrogen-bonding ability can be utilized. An illustration is given in Figs. 7 and 8, showing the separation of the same four components on bare silica and on silica coated with a multilayer of TMOA, respectively.

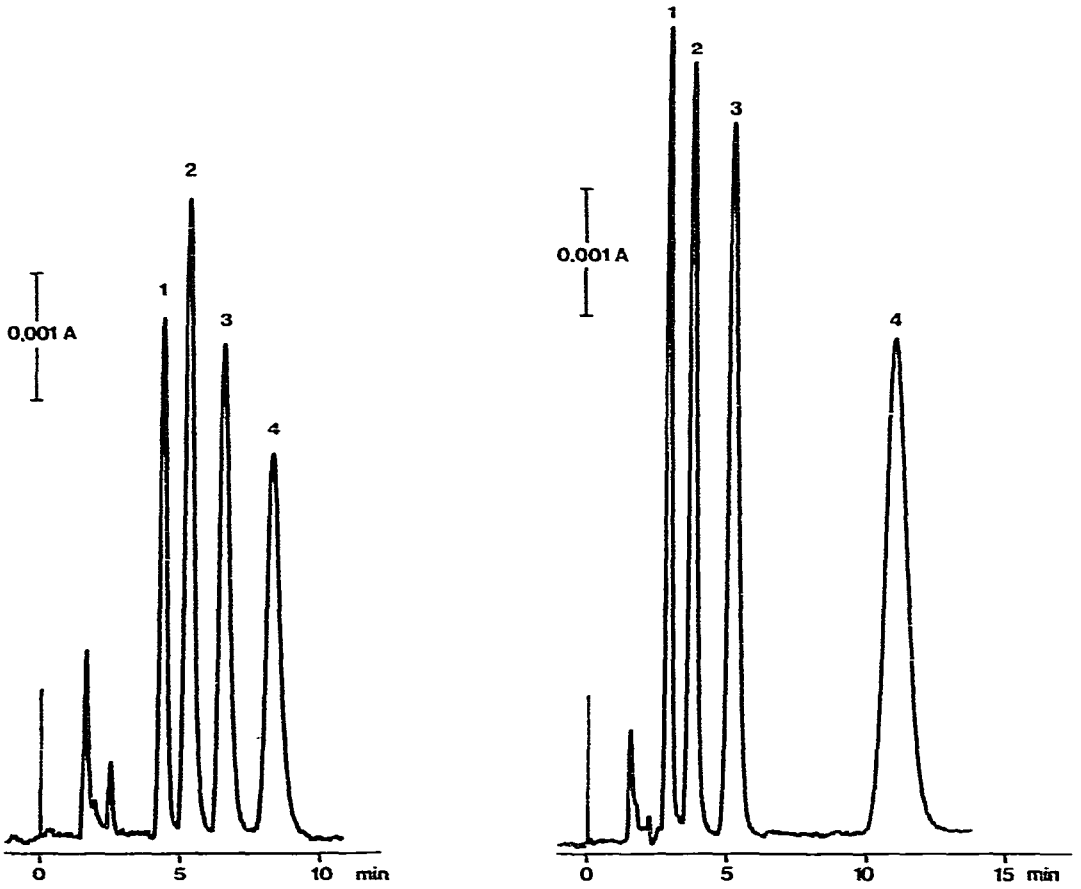


Fig. 7. Separation of β-adrenoreceptor antagonists. Mobile phase: $1.5 \cdot 10^{-3}$ *M* TMOA in aqueous phosphate buffer (pH 2.2). Solid phase: LiChrosorb Si 60. Flow-rate: 1.0 ml/min. Detection wavelength: 270 nm. Samples: 1, alprenolol; 2, propranolol; 3, metoprolol; 4, acebutolol. Amount injected: 0.5 μmole.

Fig. 8. Separation of β-adrenoreceptor antagonists. Mobile phase: $4 \cdot 10^{-2}$ *M* TMOA and $1 \cdot 10^{-2}$ *M* DMCHS in aqueous phosphate buffer (pH 2.2). Solid phase: LiChrosorb Si 60. Flow-rate: 1.0 ml/min. Samples: 1, metoprolol; 2, acebutolol; 3, alprenolol; 4, propranolol. Amount injected: 0.5 μmole.

ACKNOWLEDGEMENTS

We are very grateful to Dr. B. A. Persson for his interest in this work and to Professor G. Schill for valuable discussions.

REFERENCES

1 R. K. Iler, *J. Phys. Chem.*, 56 (1952) 673.
2 R. K. Iler, in G. Bendz and I. Lindqvist (Editors), *Biochemistry of Silicon and Related Problems*, Plenum Press, New York, 1978, p. 53.
3 R. K. Iler, *The Chemistry of Silica*, Wiley, New York, 1979.
4 J. Crommen, *J. Chromatogr.*, 186 (1979) 705.
5 J. Crommen, *Thesis*, Université de Liège, Liège, 1980.

6 A. Tilly-Melin, Y. Askemark, K. G. Wahlund and G. Schill, *Anal. Chem.*, 51 (1979) 93.
7 H. Svendsen and T. Greibrokk, *J. Chromatogr.*, 212 (1981) 153.
8 Y. Ghaemi and R. A. Wall, *J. Chromatogr.*, 174 (1979) 51.
9 R. A. Wall, *J. Chromatogr.*, 194 (1980) 353.
10 Y. Ghaemi and R. A. Wall, *J. Chromatogr.*, 212 (1981) 271.
11 S. H. Hansen, *J. Chromatogr.*, 209 (1981) 203.
12 S. H. Hansen, P. Helboe, M. Thomsen and U. Lund, *J. Chromatogr.*, 210 (1981) 453.
13 K. K. Unger, *Porous Silica —Its Properties and Use as a Support in Column Liquid Chromatography*, Elsevier, Amsterdam, Oxford, New York, 1979.
14 S. O. Jansson, I. Andersson and B. A. Persson, *J. Chromatogr.*, 203 (1981) 93.
15 K. Gustavii and G. Schill, *Acta Pharm. Suecica*, 3 (1966) 241.
16 S. O. Jansson, *J. Liquid Chromatogr.*, 5 (1982) 677.
17 S. O. Jansson and S. Johansson, *J. Chromatogr.*, 242 (1982) 41.