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PEAK BROADENING IN ION-PAIR LIQUID CHROMATOGRAPHY OF SUBSTITUTED PHENOXYPROPANOLAMINES ON DYNAMICALLY MODIFIED SILICA

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SUMMARY

N,N,N-Trimethyloctylamine and octylsulphate have been used as ion-pairing modifiers in an aqueous mobile phase of pH 2.1 for the separation of some phenoxypropanolamines on silica (LiChrosorb Si 60). The modifiers improve the chromatographic behaviour of the amine solutes and at increased concentration form a stationary phase. A change in peak dispersion for the most hydrophobic solutes was observed at intermediate modifier concentrations. The peak broadening was found not to be affected by chromatographic parameters such as flow-rate, column temperature, co-elution of mobile phase components or solute concentration. A non-linear Van't Hoff plot indicates a mixed retention mechanism.

INTRODUCTION

In recent years, silica modified by ionic interaction has frequently been used in reversed-phase liquid chromatography as an alternative to chemically bonded stationary phases. Several studies have shown that the silica surface may acquire hydrophobic properties by the adsorption of surface-active agents from aqueous mobile phases of pH 6–8[1–4]. Phenoxypropanolamines have been separated on such silica columns, modified by long-chain alkyltrimethylammonium compounds in mobile phases of pH 8[5].

The retention behaviour of ionic compounds on silica in acidic mobile phases was studied by Crommen[6] by means of a model based on ion-pair distribution to the stationary phase. We have used aqueous phosphate buffers of pH 2.1 and, in a different way, modified silica by use of ion-pairing reagents[7,8]. In our systems, hydrophobic aliphatic amines added to the mobile phase are adsorbed as ion pairs and compete at low concentrations with the amine solutes, thus decreasing the retention and improving the chromatographic performance. Higher concentrations, in the presence of hydrophobic alkylsulphates, give an adsorbed stationary phase and a changed retention pattern similar to that obtained with non-polar bonded phases[7].

Under conditions where the retention pattern changes, we have found that hydrophobic solutes give abnormally broad peaks[8]. In this study, the influence of different parameters on peak broadening was investigated.

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EXPERIMENTAL

Chemicals and reagents

Sodium octylsulphate was obtained from E. Merck (Darmstadt, F.R.G.). N-Methylimipramine bromide, N,N,N-trimethyloctylammonium bromide and the phenoxypropanolamines (Table I), mainly used as chlorides, were supplied by the Organic Chemistry Department of AB Hässle (Mölndal, Sweden).

Apparatus

The chromatographic system consisted of an Altex 110A pump (Beckman, Berkeley, CA, U.S.A.), a Rheodyne 7010 sample injection valve equipped with a 20-μl loop (Rheodyne, Cotati, CA, U.S.A.) and an LDC Spectromonitor III UV detector (270 nm) (LDC/Milton Roy, Riviera Beach, FL, U.S.A.). The refractive index (RI) detectors were ERMA, ERC-7511 (ERMA, Chiyodaku, Tokyo, Japan) or Waters R-401 (Millipore, Milford, MA, U.S.A.). The chromatographic columns (150 × 4 mm I.D.) were packed in the laboratory with LiChrosorb Si 60 (7 μm) (E. Merck).

Chromatographic conditions

The mobile phase contained 0.008 *M* sodium octylsulphate in a phosphate buffer of pH 2.1 (I = 0.1). N,N,N-Trimethyloctylammonium (TMOA) bromide and potassium bromide were added in a total concentration of 0.008 *M* of bromide in order to maintain a constant ionic strength. The flow-rate was generally 1 ml/min.

Determination of chromatographic parameters

The dead volume, t_0, was determined by injection of mobile phase diluted with water in equal volumes. The reduced plate height was calculated from measurements made at 13.4% of the peak height and the asymmetry factor was calculated at 10% of the peak height[9,10].

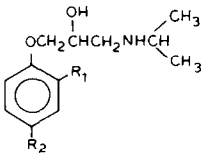
The amount of dynamically adsorbed modifiers was determined by elution of the modifier and determination by reversed-phase liquid chromatography with N-methylimipramine as ion-pairing reagent according to principles given by Hackzell and Schill[11].

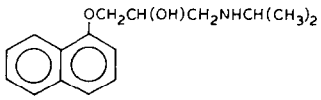
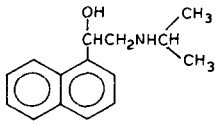
RESULTS AND DISCUSSION

In a previous paper[8], we showed that by combining a hydrophobic ammonium ion, TMOA, with a hydrophobic anion, octylsulphate, a change in retention pattern was obtained. With no hydrophobic cationic modifier present in the mobile phase the reduced plate height is high (h = 50–70) and so is the asymmetry factor (asf = 4–6) for most solutes tested. Addition of TMOA to the mobile phase improved the peak shape and, with a further increase in the modifier content acceptable plate numbers and asymmetry factors are obtained. However, in the concentration range $[TMOA]_m$ = 0.0010–0.0014 a drastic increase in peak dispersion, not correlated with the capacity factors, was observed for the more hydrophobic phenoxypropanolamines such as alprenolol, pronetalol and propranolol, while the performance of more hydrophilic solutes was not influenced. An illustration of the relationship be-

TABLE I

STRUCTURES OF THE COMPOUNDS STUDIED



Compound	R_1	R_2	*Solute*
Atenolol	H	CH_2CONH_2	1
Practolol	H	$NHCOCH_3$	2
Desmethylmetoprolol	H	CH_2CH_2OH	3
H 128/80	H	CHO	4
ortho-Metoprolol	$CH_2CH_2OCH_3$	H	5
Metoprolol	H	$CH_2CH_2OCH_3$	6
H 9/64	H	H	7
Oxprenolol	$OCH_2CH=CH_2$	H	8
Alprenolol	$CH_2CH=CH_2$	H	9
H 56/56	$CH_2CH_2CH_3$	H	10
Acebutolol	$COCH_3$	$NHCOCH_2CH_2CH_3$	–
Propranolol			
Pronetalol			

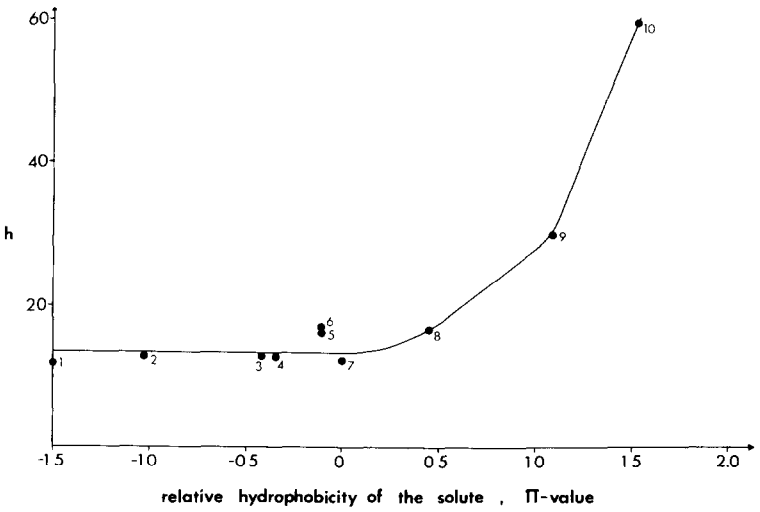


Fig. 1. Influence of hydrophobic properties of phenoxypropanolamines on reduced plate heights. Solid phase: LiChrosorb Si 60. Mobile phase: phosphate buffer (pH 2.1) with 0.008 *M* octylsulphate and 0.0014 *M* TMOA. Structures of tested solutes 1–10 as in Table I.

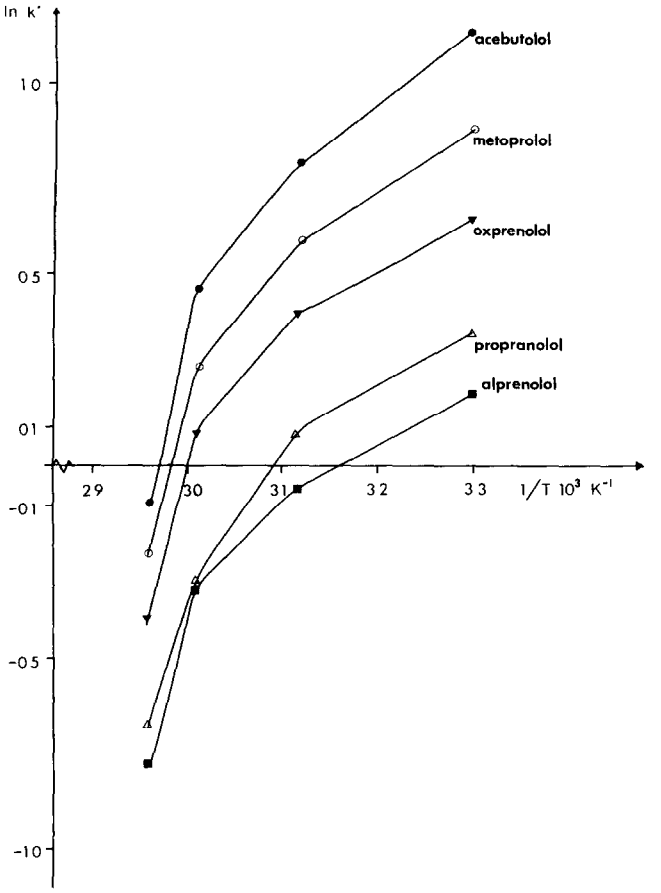


Fig. 2. Van't Hoff plots of capacity factors of phenoxypropanolamines. Chromatographic conditions as in Fig. 1.

tween the reduced plate heights and the relative hydrophobicity of some solutes, expressed by π-values[12], with compound H 9/64 (Table I) as a reference, is presented in Fig. 1. A further increase in the TMOA concentration (>0.0014 *M*) restores the peak performance of the hydrophobic solutes.

Influence of chromatographic parameters

The influence of the solute concentration on the peak broadening effect was studied for acebutolol and propranolol at concentrations in the mobile phase in the range 0.001–0.02 mg/ml. Neither the capacity factor nor the reduced plate height of the solutes was changed. When the flow-rate was increased from 0.6 to 1.0 ml/min, a small increase in plate height was seen for early eluting desmethylmetoprolol and metoprolol. Slow mass transfer between the mobile and stationary phases or overloading of the column seems not to be of importance for the peak broadening effect.

When the column temperature was varied in the range 30–65°C, a non-linear relationship between log k' (capacity factor) and the reciprocal of absolute temper-

TABLE II

INFLUENCE OF COLUMN TEMPERATURE ON REDUCED PLATE HEIGHTS AND CAPACITY FACTORS OF PHENOXYPROPANOLAMINES

Solid phase: LiChrosorb Si 60. Mobile phase: phosphate buffer (pH 2.1) with 0.008 *M* octylsulphate and 0.0014 *M* TMOA.

Solute	*h*				*k'*			
	30°C	*40°C*	*50°C*	*65°C*	*30°C*	*40°C*	*50°C*	*65°C*
Acebutolol	20	15	13	11	3.1	2.2	1.6	0.92
Metoprolol	15	14	12	12	2.4	1.8	1.3	0.80
Oxprenolol	16	14	13	13	1.9	1.5	1.1	0.67
Alprenolol	23	18	18	16	1.2	0.95	0.73	0.45
Propranolol	37	31	21	18	1.4	1.1	0.82	0.51

ature was obtained in a Van't Hoff plot (Fig. 2). The deviation from linearity indicates a mixed retention mechanism with different enthalpies[13]. The peak shape is improved for the most hydrophobic solutes, concominant with the decrease in capacity factors, as illustrated in Table II.

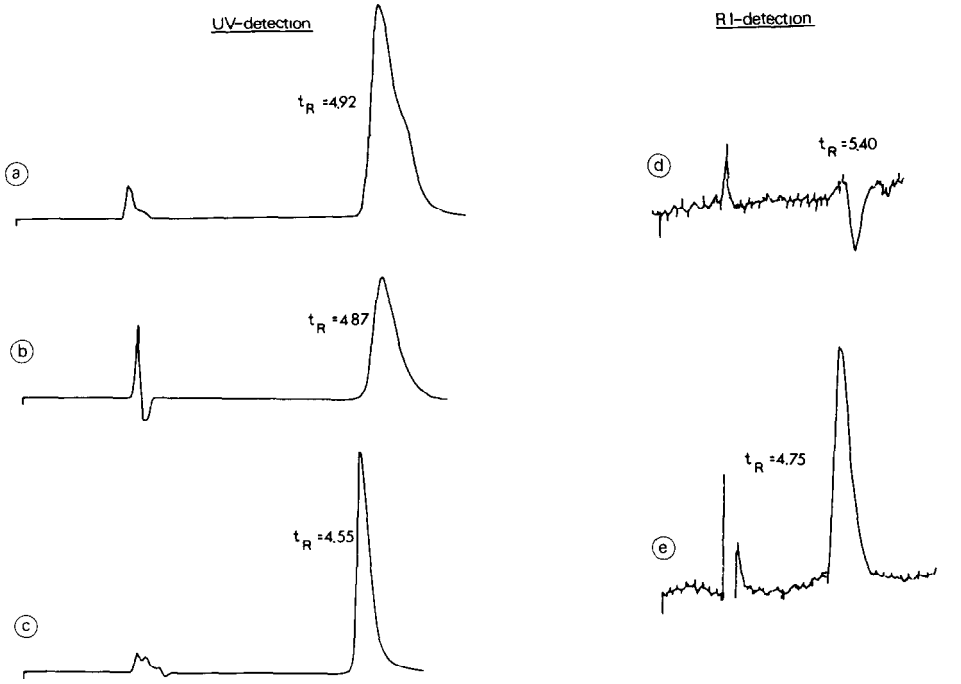


Fig. 3. Influence of TMOA concentration in injected solutes of acebutolol on peak performance. Solid phase: LiChrosorb Si 60. Mobile phase: phosphate buffer (pH 2.1) with 0.001 *M* TMOA and 0.008 *M* octylsulphate. (a), (d) Injection medium = mobile phase without TMOA; (b) Injection medium = mobile phase; (c), (e) injection medium = mobile phase with 0.008 *M* TMOA.

TABLE III

ADSORPTION OF TMOA AND OCTYLSULPHATE ON LICHROSORB Si 60

Mobile phase: TMOA bromide and 0.008 *M* sodium octylsulphate in phosphate buffer (pH 2.1).

$(TMOA)_m \cdot 10^4$	*Amount of modifier adsorbed (μmol/g of solid phase)*	
	TMOA	*Octylsulphate*
32	25	16
34	39	31
36	62	63
38.4	131	141
40	187	175

Influence of mobile phase components

In order to examine the influence of mobile phase components on the peak performance, various concentrations of TMOA were injected while keeping the concentration of TMOA in the mobile phase constant. By RI detection a negative peak was registered using a lower concentration of TMOA, compared with the mobile phase, and a positive peak appeared when the concentration of TMOA was higher.

The capacity factors of the negative and positive peaks decreased with increasing content of TMOA in the mobile phase. In the region where peak broadening appeared, acebutolol had almost the same retention as TMOA and gave (Fig. 3) a narrow peak with a reduced plate height even lower than that for the most hydrophilic solutes. However, other more hydrophobic solutes with retention times differing from the TMOA system peak appeared with unchanged peaks. Nilsson and Westerlund[14] have reported peak compression and dispersion in ion-pair chromatography of amine solutes on a bonded phase while simultaneously injecting non-UV-absorbing modifiers.

Ion-pair formation

The adsorption of amine modifiers on the silica surface, neutral at pH 2, is due to the formation of ion pairs. The data presented in Table III confirm that the two hydrophobic modifiers are adsorbed in equivalent amounts. The monolayer capacity of the silica surface ($5 \cdot 10^{-5}$ mol/g)[7] is exceeded at $[TMOA]_m > 0.0035$ *M*. Formation of a multi-layer and filling of the pores of the support must then be initiated at much lower concentrations of TMOA in the mobile phase and may coincide with the region where peak broadening of hydrophobic solutes appears. An ion exchange between the amine modifier and the hydrophobic solute may give rise to the disturbance observed as peak broadening when the binding capacity of a small secondary layer of the ion-pair modifier is exceeded.

We also investigated whether ion pair or micelle formation in the mobile phase by the hydrophobic ion-pairing agents could account for the effects observed at high concentrations. Distribution on a batch scale gave no evidence for this assumption, which, however, does not exclude such effects within the pores of the column packing.

CONCLUSIONS

We have shown that peak broadening was not dependent on the flow-rate, column temperature, co-elution of mobile phase components or solute concentration. The modifiers and solutes are adsorbed as ion pairs. Formation of a second layer of modifiers with a limited capacity for adsorbed hydrophobic solutes is assumed to contribute to the excessive peak dispersion. A non-linear Van't Hoff plot supports the theory of a mixed retention mechanism.

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