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SUPERCRITICAL FLUID CHROMATOGRAPHY WITH ION-PAIRING MODIFIERS

SEPARATION OF ENANTIOMERIC 1,2-AMINOALCOHOLS AS DIASTEREOMERIC ION PAIRS

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SUMMARY

Ionizable organic compounds have been separated by supercritical and subcritical chromatography using ion-pairing modifiers. The mobile phase was carbon dioxide mixed with an acetonitrile solution containing a counter ion and a competing ion. The retention and separating efficiency are controlled by pressure and temperature as well as by the concentration and nature of the ionic mobile phase components. These highly versatile systems are applicable to polar solutes of different structures. The technique has been applied to the separation of enantiomeric 1,2-aminoalcohols using N-benzoxycarbonylglycyl-L-proline as a chiral counter ion. The maximum resolution and the separating efficiency at higher flow-rates are superior to that obtained in high-performance liquid chromatography.

INTRODUCTION

Supercritical fluid chromatography (SFC) usually gives higher separating efficiency than high-performance liquid chromatography (HPLC) since it operates under more favourable kinetic conditions owing to an higher diffusivity and a lower viscosity of the mobile phase. This gives possibilities of significantly better resolution at higher flow-rates than that obtained with HPLC[1,2].

The improvement of the separating efficiency is of particular importance in the separation of enantiomeric compounds since it has been observed that chiral solutes often give wider peaks than achiral ones. SFC has been used for few chiral separations and exclusively with chiral solid phases[3–6], mainly due to the limited possibilities of applying the eluting properties of the mobile phase to polar solutes. However, this

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study shows that an important improvement of the selectivity and the versatility for ionizable solutes can be achieved by application of the ion-pairing principles that for a long time have been used in normal-phase HPLC[7].

The ion-pairing technique can be used for HPLC separation of enantiomers as demonstrated by Pettersson *et al.*[8–12]. Among the methods that have been developed, the resolutions of enantiomeric 1,2-aminoalcohols are of particular interest since compounds of this kind are of great pharmacological interest[12]. The chiral selector is an antipode of a dipeptide, N-benzoxycarbonylglycyl-L-proline (ZGP), which is added to the mobile phase. It gives diastereomeric ion pairs with the cationic solutes which are retained by an achiral solid phase. These separation principles have been applied to SFC in this study, using carbon dioxide mixed with a solution of the chiral selector in acetonitrile as the mobile phase. The ion-pair distribution principles have not previously been used in SFC and the objective of this work was to study the selectivity and the separating efficiency that can be achieved with SFC as compared to normal-phase HPLC.

EXPERIMENTAL

Apparatus

The SFC equipment used is shown in Fig. 1. The pumps for carbon dioxide

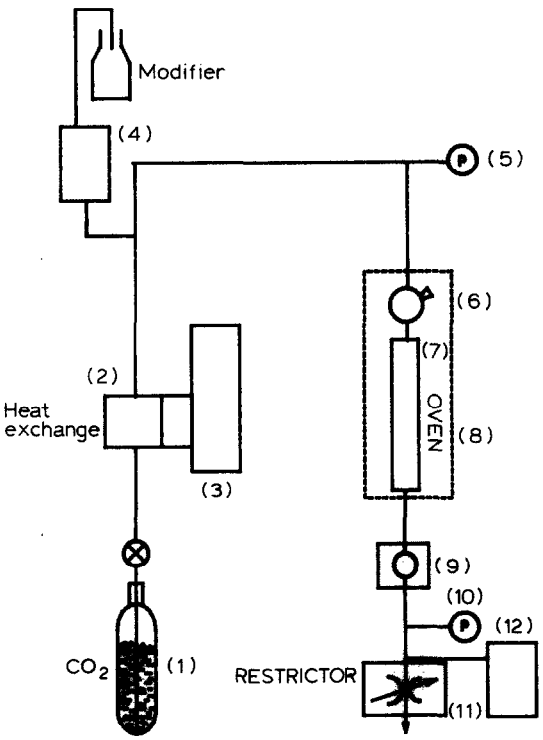


Fig. 1. Equipment for supercritical fluid chromatography: 1 = carbon dioxide cylinder; 2 = heat exchanger; 3 = carbon dioxide pump; 4 = modifier pump; 5, 10 = pressure monitors; 6 = injector; 7 = separation column; 8 = oven; 9 = UV detector; 11 = restrictor; 12 = wash pump.

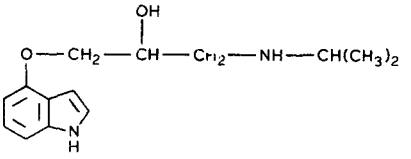
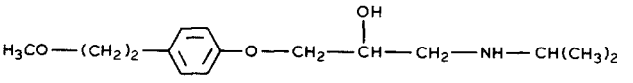
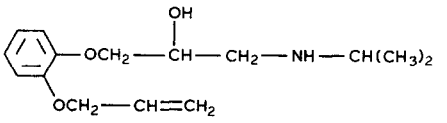
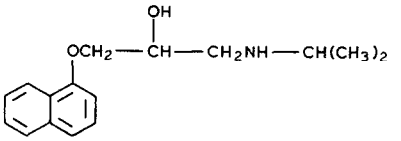
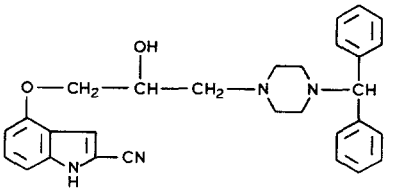
and the modifier were of Type LC240 (Kontron, Zürich, Switzerland). The pumphead for the carbon dioxide delivery was equipped with a heat exchanger which allowed the gas to be cooled below −10°C. The restrictor was continously washed with acetonitrile to prevent precipitation of the ion-pair reagents. The injector was a Rheodyne valve 70-100. An UV detector, UVIKON 720 LC from Kontron, equipped with a laboratory-made high-pressure resistant cell, was used. To maintain the desired system pressure a restrictor 26-1721 (Tescom, Elk River, MA, U.S.A.) was employed. The column inlet and outlet pressure, were measured using pressure monitors LCP 501 (Innovativ Labor, Wallisellen, Switzerland).

Chemicals

Carbon dioxide, 48 grade, was obtained from Carba Gas (Basle, Switzerland), acetonitrile from Rathburn (Walkerburn, U.K.), N-benzoxycarbonylglycyl-L-proline

TABLE I

STRUCTURES OF 1,2-AMINOALCOHOLS

Structure	*Name*
	Pindolol
	Metoprolol
	Oxprenolol
	Propranolol
	DPI 201-106

(ZGP) and triethylamine (TEA) from Fluka (Buchs, Switzerland). The racemic mixtures of the aminoalcohols, pindolol, metoprolol, propranolol, oxprenolol and DPI 201-106 (structures in Table I), were obtained from Sandoz (Basle, Switzerland).

Eluents

ZGP and TEA were dissolved in acetonitrile then mixed with liquid carbon dioxide in 1:4 (v/v) ratio. The concentrations of ZGP and TEA given refer to their concentrations in the modifier solution. This must be considered when comparing the results with those from HPLC measurements.

Columns

The cyano-bonded phase Type CS-GU was obtained from Brownlee Labs. (Santa Clara, CA, U.S.A.). For the thermodynamic measurements (k', α), the column dimensions were 30 mm × 4.6 mm, whereas for the kinetic measurements (H, N), 100 mm × 4.6 mm columns were employed. The void volume was measured with acetonitrile. The total porosity, ε_T, was calculated to be 0.72.

RESULTS AND DISCUSSION

The parameters that control the retention and the selectivity in HPLC separations using ZGP as the selector have been studied in detail by Pettersson and Jeffersson[12]. Some of the results are applicable to SFC, but a series of new problems arises owing to the different solubility and distribution conditions.

Modifier solution

The retention of the 1,2-aminoalcohols can be regulated by the concentration of the counter ion, ZGP, and by the nature and the concentration of a competing amine and a polar solvent in the mobile phase. The ion pairs are formed in the mobile phase and the coupling is disturbed by protic solvents since they compete for the hydrogen-bonding function of the counter ion. The modifier must therefore be aprotic as well as polar and acetonitrile was used in this study.

The solubilities of ZGP and the amine in this solvent are, however, fairly low. The carbon dioxide used as the mobile phase must furthermore have a density above 0.7 g/cm^3 in order to prevent precipitation of the reagent. Mobile phases of this kind gave good chromatographic behaviour of the ion pairs with cyano as well as with phenyl and C_2 phases, whereas DIOL phases gave very long retention times.

Concentrations of ZGP and amine

The addition of a competing amine to the mobile phase is necessary to reduce the retention and prevent peak tailing. Triethylamine was used as in the previous HPLC studies, always in a concentration lower than that of the acidic component, ZGP. The influence of ZGP and TEA on the separation of the enantiomers of the 1,2-aminoalcohol derivative propranolol is demonstrated in Fig. 2. The separation coefficient, α, increases with increasing ZGP concentration and decreases somewhat when the TEA concentration is increased. No chiral separation was obtained at a ZGP concentration below 10 mM.

The retention change of the first enantiomer eluted is demonstrated in Fig. 2:

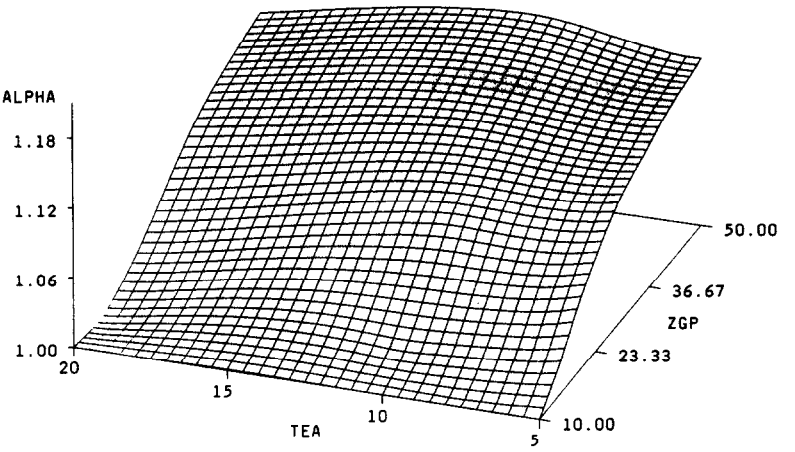


Fig. 2. Influence of ZGP and triethylamine (TEA) on the chiral selectivity, α, for antipodes of propranolol. Conditions: 70°C; 250 bar. Concentration of ZGP and TEA in m*M*.

k' increases with the concentration of the counter ion, ZGP, and decreases with the TEA concentration, in accord with the normal rules for normal-phase ion-pair HPLC.

Influence of pressure

The retention times decrease with increasing pressure as shown in Fig. 4 for four drug substances, all containing an 1,2-aminoalcohol moiety. The increase in the solvating ability of the mobile phase with increasing pressure represents a distinct advantage of SFC over HPLC, giving easy control of the retention. The versatility of the system is demonstrated in Fig. 5 which shows the resolution of two racemic

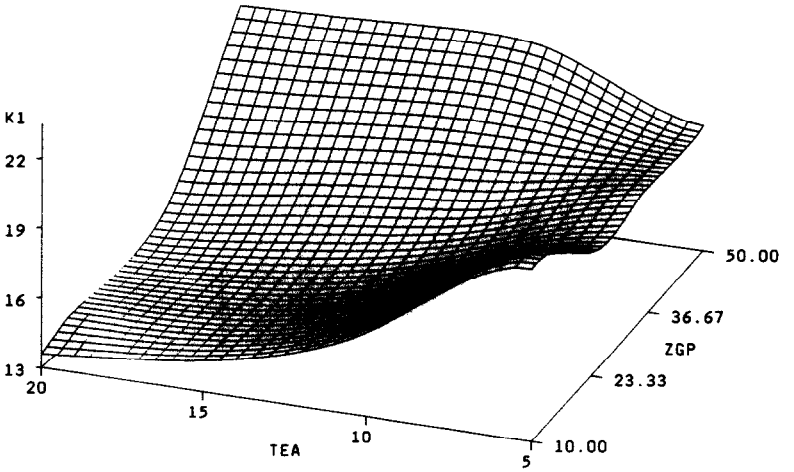


Fig. 3. Influence of ZGP and TEA on the retention of the first antipode of propranolol eluted. Conditions as in Fig. 2.

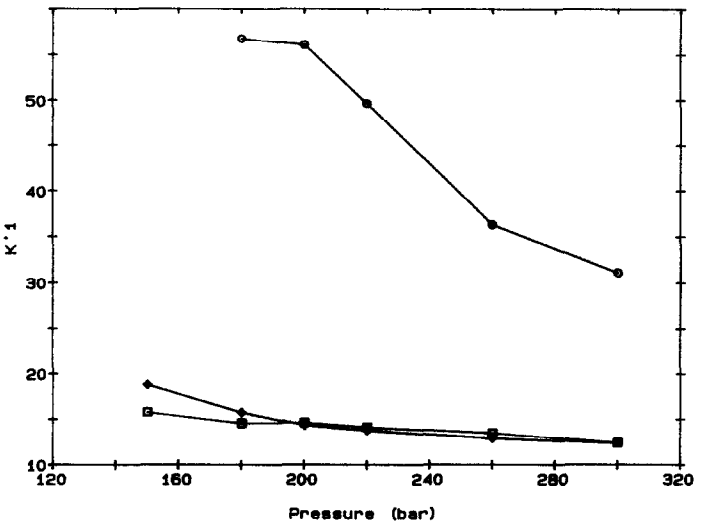


Fig. 4. Influence of pressure on the retention of the first antipode of 1,2-aminoalcohols eluted. Conditions: 30°C; 35 m*M* ZGP; 5 m*M* TEA. ○, Pindolol; □, propranolol; ◇, metoprolol.

aminoalcohols, propanolol and DPI 201-106, under subcritical conditions. The separating efficiency is also positively influenced by high flow-rates as will be discussed later.

The chiral selectivity is somewhat larger at lower than at higher pressure (Fig. 6). This might be due to changes in the stability of the ion pairs with the pressure-induced changes in the hydrophobicity of the mobile, or to changes in the distribution of ZGP in the solid phase.

Influence of temperature

The chiral selectivity decreases with increasing temperature (Fig. 7). The retention mechanism of the 1,2-aminoalcohols in this ion-pairing system has so far not been elucidated. Studies by HPLC[12] have shown that the chiral selectivity might be due to differences in the stabilities of the ion pairs in the mobile phase, as well as to differences in the retetion of the diastereomeric ion pairs by the achiral solid phase. If a similar complex retention mechanism is valid for SFC, both processes are affected by temperature changes. Further studies will be presented in a later paper.

Separating efficiencies in SFC, HPLC and subcritical chromatography

The relationships between the plate height, H, and the linear flow velocity, u, under supercritical, subcritical and HPLC conditions are shown in Fig. 8. The minimum, u_{min}, increases, as expected, drastically since the diffusivities are about 10 times higher in SFC than under HPLC conditions.

The H *vs.* u curves for supercritical and subcritical conditions cannot be well described by the van Deemter equation, probably due to the fact that isocratic conditions do not prevail in SFC. The eluting power of the mobile phase in SFC increases with increasing pressure, giving a shorter retention at the top than at the bottom of

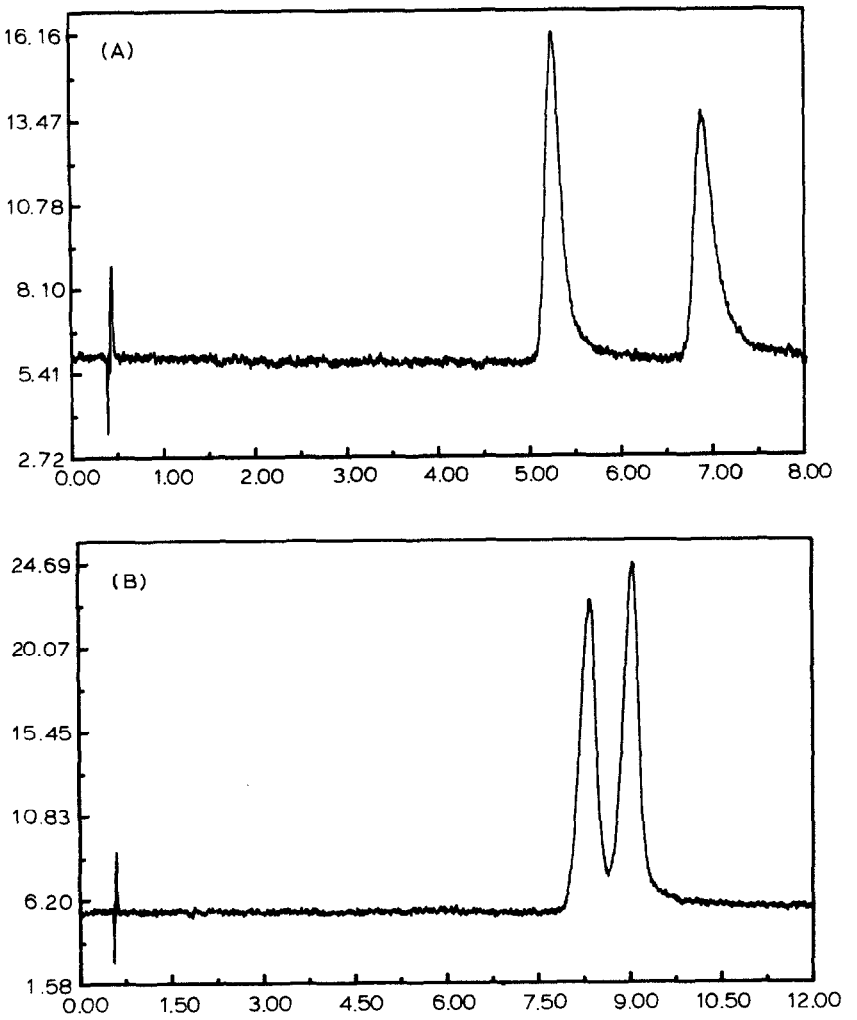


Fig. 5. Separation of antipodes of 1,2-aminoalcohols: (A) propranolol; (B) DPI 201–106. Conditions: subcritical, 21°C, 250 bar; 35 m*M* ZGP; 5 m*M* TEA; column 100 mm × 4.6 mm I.D.

the column. It seems that the peak broadening due to dispersion is superimposed by a peak compression effect similar to that in gradient elution HPLC. Further studies of this effect will be described in a later paper.

Resolution in HPLC, SFC and subcritical chromatography

The relationship between the flow-rate and resolution for the antipodes of propranolol calculated from

$$R_s = \frac{k'_2}{1 + k'_2} \cdot \sqrt{N} \cdot \frac{\alpha - 1}{4\alpha} \tag{1}$$

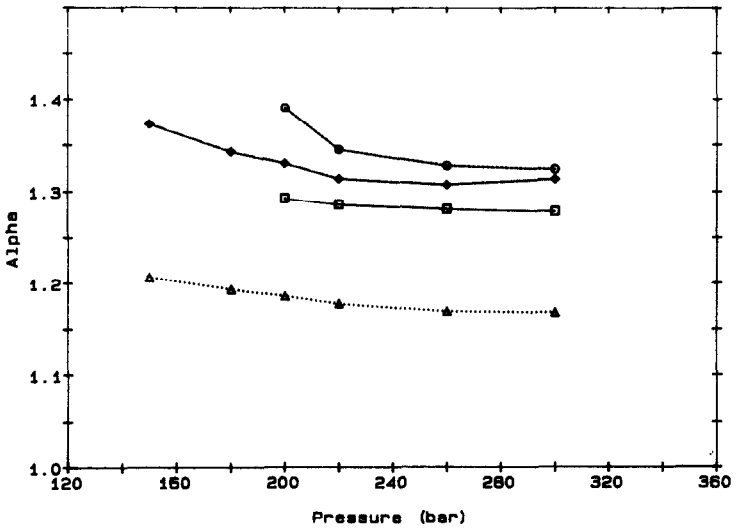


Fig. 6. Influence of pressure on the chiral selectivity for antipodes of 1,2-aminoalcohols. Conditions: 30°C; 35 m*M* ZGP; 5 m*M* TEA. ○, propranolol; ◇, metoprolol; □, pindolol; △, oxprenolol.

is demonstrated in Fig. 9. Subcritical chromatography is obviously superior not only to HPLC but also to supercritical chromatography. However, the difference between the subcritical and the supercritical modes seems to be mainly due to temperature differences since SFC at 32°C and 225 bar gives a curve similar to that obtained

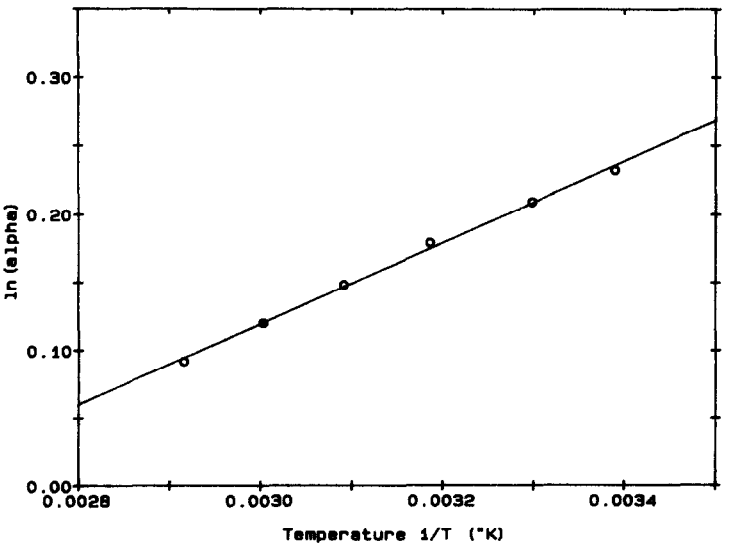


Fig. 7. Influence of temperature on the chiral selectivity for antipodes of propranolol. Conditions: 250 bar; 35 m*M* ZGP; 5 m*M* TEA.

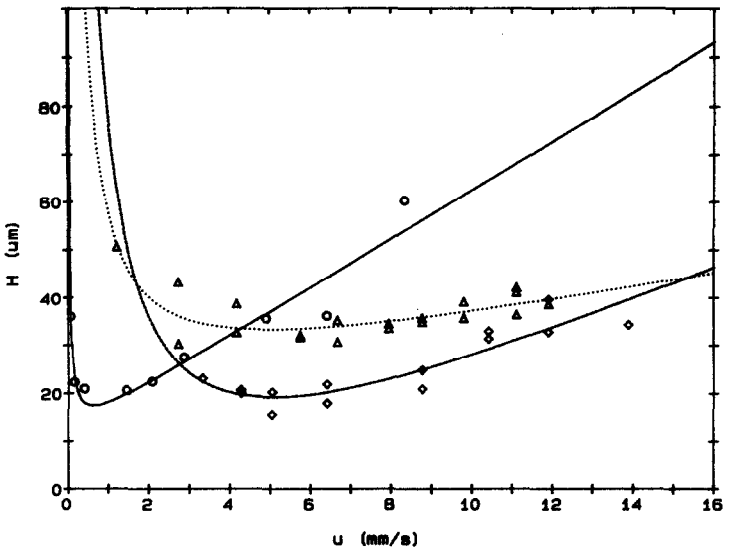


Fig. 8. Relationships between the plate height, H, and mobile phase velocity, u, for propranolol. ○, HPLC (dichloromethane; 2.5 mM ZGP, 1 mM TEA); △, subcritical (21°C, 250 bar; 35 mM ZGP, 5 mM TEA); ◇, supercritical (60°C, 225 bar, 35 mM ZGP, 5 mM TEA).

under subcritical conditions. The results indicate that it is the use of carbon dioxide and acetonitrile as the mobile phase that gives the great advantages of supercritical and subcritical chromatography over HPLC in the resolution of enantiomeric cations by the ion-pair technique.

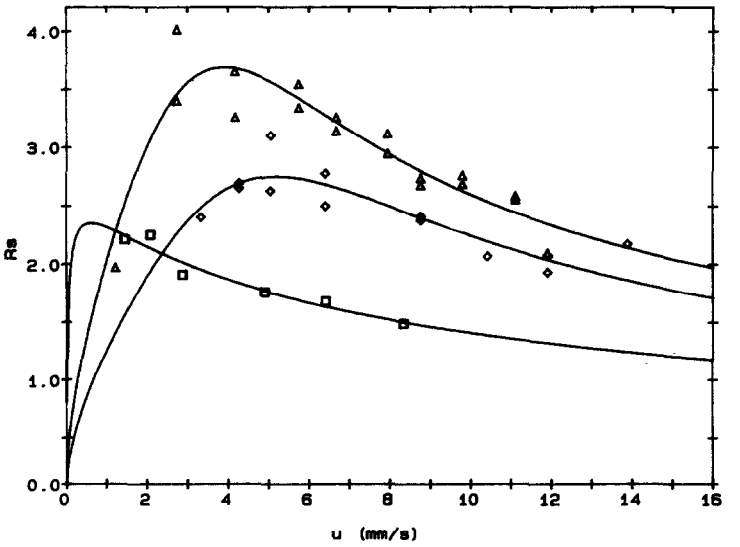


Fig. 9. Influence of the mobile phase velocity on the resolution, R_s, of antipodes of propranolol. □, HPLC (dichloromethane; 2.5 mM ZGP, 1 mM TEA); △, subcritical (21°C, 250 bar; 35 mM ZGP, 5 mM TEA); ◇, supercritical (60°C, 225 bar; 35 mM ZGP, 5 mM TEA).

CONCLUSIONS

Ion-pairing agents are suitable for the control of retention and separating efficiency of ionized organic solutes under supercritical and subcritical conditions in analogy with normal-phase HPLC. Variation of the nature and concentration of the counter ion and a competing ion, present in the mobile phase, makes the systems highly versatile and applicable to solutes of widely different structures.

On application of the technique to the separation of the antipodes of 1,2-aminoalcohols as diastereomeric ion pairs, the maximum resolution is obtained under subcritical and minimum-temperature, supercritical conditions.

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