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INVESTIGATION OF THE EFFECT OF ADDED ORGANIC AMINE ON THE CHROMATOGRAPHY OF TRICYCLIC ANTIDEPRESSANT DRUGS USING REVERSED-PHASE CHROMATOGRAPHY ON OCTADECYLSILICA WITH SODIUM LAURYL SULPHATE AS PAIRING ION

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SUMMARY

The chromatography of a group of tricyclic antidepressant drugs has been investigated using reversed-phase chromatography on ODS Hypersil with sodium lauryl sulphate as pairing ion both in the presence and absence of various organic amines. The amines investigated ranged in hydrophobicity from methylammonium to cetrimide. The general effect of such added amines is to decrease retention of basic solutes and to alter the selectivity of the stationary phase. The results are interpreted on the basis of the ion-exchange desolvation process previously proposed and optimised systems for the separation of clinically relevant solute pairs in this drug class are demonstrated.

INTRODUCTION

Currently, the most widely used high-performance liquid chromatographic (HPLC) mode is that of reversed phase using chemically bonded stationary phases with octyl- and octadecylsilanes being far the most commonly used modifiers of the silica surface[1]. Such stationary phases, together with suitable choice of aqueous mobile phase incorporating methanol or acetonitrile as organic modifier, will produce retention and usually excellent separation in most chromatographic problems involving electrically uncharged solutes.

The retention of ionogenic species can be achieved in the case of acidic solutes by control of pH[2,3]. Ion suppression, at low pH, will often allow chromatography of weak acids in the undissociated state. For basic solutes ion suppression is less advantageous due to deterioration of the silica support material at high pH[4,5].

Both types of electrolyte have been successfully chromatographed by addition of hydrophobic pairing ions, opposite in charge to the solute, to the mobile phase[6–9].

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In such cases the pH is adjusted so that the solute species is completely in the ionised condition. This procedure has been studied by very many workers and various terms have been coined to describe the processes leading to retention and separation[6–12]. In spite of the confusion produced by terminology, this technique has augmented the ion suppression method for the chromatography of acids and has extended chromatographic possibilities to strong acids where the pH required for complete suppression would be so low as to adversely affect the silica support[6]. Its major application has been to the chromatography of organic bases at low pH. Using such systems the chromatographic conditions can be controlled extensively by modification of the mobile phase, in particular the type and concentrations of pairing ion and associated counter ion used[13]. Recently the variation of retention and separation of several solutes has been shown to be complex with respect to mobile phase pairing ion concentration; the capacity factors of all solutes studied showing a maximum with pairing ion concentration. These results have been shown to be general for groups of both acidic and basic solutes and have been interpreted on a quantitative basis by an ion exchange model which also involves desolvation on the silanised silica surface[8,9]. On this model, counter ion concentration, *i.e.*, an ion of similar charge to that of the solute, is also involved in the retention equilibrium. It has been shown that the effect of increasing ionic strength and thus counter ion concentration has the effect of reducing retention in such systems[13,14]. Previous work on such counter ion dependence has been limited to inorganic counter ions where interaction is largely confined to the mobile phase.

The literature contains several reports of organic amines being added to the mobile phase during the chromatography of basic drugs, in particular the tricyclic antidepressant group of bases[4,15–18]. The effect of such addition is generally to reduce tailing of eluted peaks. This has been explained on the basis of masking active silanol groups[19] or of suppressing dissociation of ion pairs in the organic phase[17,18]. Most of the data on such separations have been obtained in liquid–liquid systems in which higher alcohols have been used to modify the C-18 or C-8 surfaces. Little information is available on the effect of such amine addition on the chromatography of basic drugs on modern systems involving an octadecylsilica surface without the addition of higher alcohols. Neither has there been any systematic study as to the relative effectiveness of different organic bases in improving the chromatography of basic solutes.

Because of the clinical importance of the tricyclic antidepressant drugs together with the poor chromatography generally associated with these compounds[20], they are used as a model set of compounds in the present investigation. It is intended to examine the effect of several amines and quaternary ammonium salts on the chromatography of the major drugs in this group and to attempt to explain the mode of action in the light of the ion exchange desolvation model of hydrophobic pairing ion retention previously proposed[8,9].

EXPERIMENTAL

Chromatography was carried out using a variety of equipment including Altex (Model 110A) and Waters Associates (M6000A) constant flow pumps. Detection at 254 nm was by Cecil (CE2012) and Pye Unicam (LC3) detectors. The wavelength of

measurement was not optimised in this study. Injection was by a Rheodyne 7125 valve fitted with 20- or 100-μl loops. Columns of conventional design and incorporating Swagelok fittings were either 100 mm or 70 mm long (4.6 mm I.D.) slurry packed at 600 bar with 5-μm ODS Hypersil (Shandon Southern Products). Retention time data were measured directly from chromatograms as recorded on a Servoscribe potentiometric recorder.

The tricyclic antidepressant drugs amitriptyline, nortriptyline, imipramine, desipramine, doxepin, maprotiline, mianserine, nomifensine, protriptyline, trimipramine, clomipramine and dothiepin were kindly donated by their manufacturers. The drugs were used as the hydrochlorides at a concentration of 50 μg cm^{-3}. Sodium lauryl sulphate (SLS), dimethylamine (DMA) and triethylamine (TRIEA) were obtained from Fisons and used as supplied. Methylammonium chloride (META), trimethylamine (TRIMA) and propylamine (PPLA) were obtained from BDH and tetramethyl-(TMA), tetraethyl-(TEA) and tetrabutylammonium (TBA) bromides were obtained from Aldrich. Cetrimide (CETA) was obtained from ICI Pharmaceuticals. Water used in chromatography was distilled before use and acetonitrile (HPLC grade) was obtained from Rathburn Chemicals and Fisons. All other reagents were of AnalaR or similar grade.

RESULTS

The separation obtained among twelve tricyclic antidepressant drugs is summarised in Fig. 1, which shows the variation of capacity factor, k', with the mobile phase concentration of sodium lauryl sulphate used as highly adsorbed pairing ion. While other pairing ions of lower hydrophobicity would affect the magnitude of the k' values obtained, previous work indicated that no substantial improvement in resolution could be obtained[9]. Fig. 1 shows that the capacity factors go through the expected maxima and that for most solutes maximum k' and thus optimum separation is obtained at a SLS mobile phase concentration of 80 mM. While considerable separation is obtained among several of the test compounds at the maximum in k', the clinically relevant separations, namely between imipramine and desipramine and between amitriptyline and nortriptyline, are not adequate. The separations achieved are further demonstrated in Fig. 2 which shows representative chromatograms of selected groups of drugs from among the compounds shown in Fig. 1. Fig. 2 indicates that the addition of pairing ion alone to the mobile phase is adequate to separate certain but not all compounds in the class. It also indicates that good chromatographic peak symmetry is achieved with little evidence of the peak tailing problems claimed to be associated with such compounds[17,18].

In order to evaluate the effect of counter ion type and concentration, various concentrations of different organic base salts, ranging in hydrophobicity from methylammonium chloride to cetrimide, were added to the mobile phase of 80 mM SLS in acetonitrile–buffer (50:50). Fig. 3 shows that the effect of such counter ion addition was general for a given test compound, namely to decrease the capacity factor. This effect was found to be general for all other compounds in the series but the extents of the decrease differed. Fig. 3 also indicates that the effect is greater the greater the apparent hydrophobicity of the added counter ion. The inverse relationship between k' and counter ion concentration previously noted for inorganic counter ions and

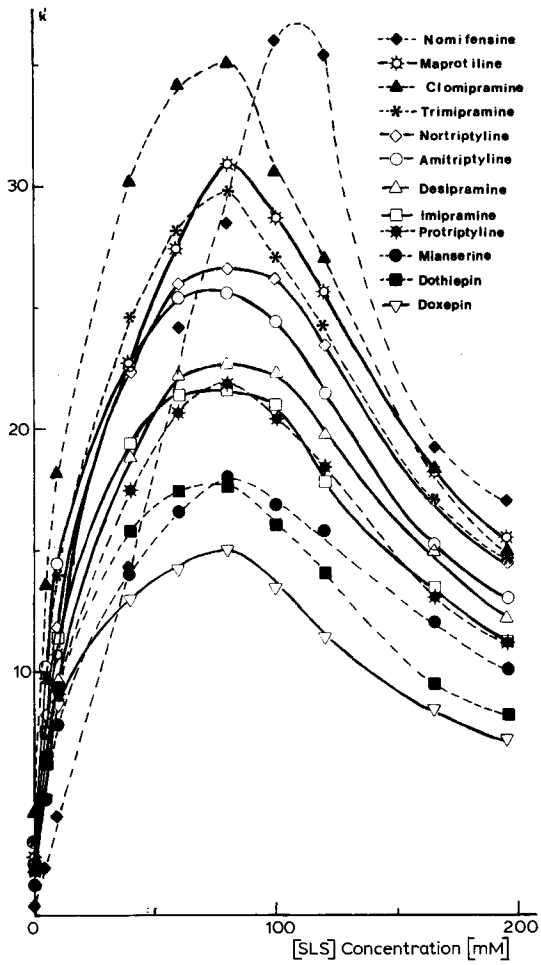

Fig. 1. Plots showing the variation of capacity factor, k', with mobile phase pairing ion (SLS) concentration for twelve tricyclic antidepressant drugs. Chromatographic conditions: column, 100 × 4.6 mm; mobile phase, acetonitrile–10 m*M* sodium dihydrogen phosphate (50:50) at pH 2; stationary phase, 5-μm ODS Hypersil; flow-rate 2.0 cm^3 min^{-1}.

predicted by the ion-exchange interpretation of ion pairing is not apparent from the present work with hydrophobic counter ions. It is also noted that while peak sharpening occurs, it is as a consequence of the decreased retention time. No real increase in plate number is obtained as a result of organic counter ion addition. Of greater significance is the effect of organic counter ion on the resolution between the pair amitriptyline and nortriptyline as shown in Fig. 4. The effect of adding amine to the mobile phase is to increase the resolution for this pair of compounds. The improvement is most marked in the case of cetrimide and least for methylammonium chloride. The effect of inorganic counter ion was observed to be minimal. These effects were identical for the imipramine–desipramine pair of compounds.

Although cetrimide shows by far the most noticeable effect on resolution both

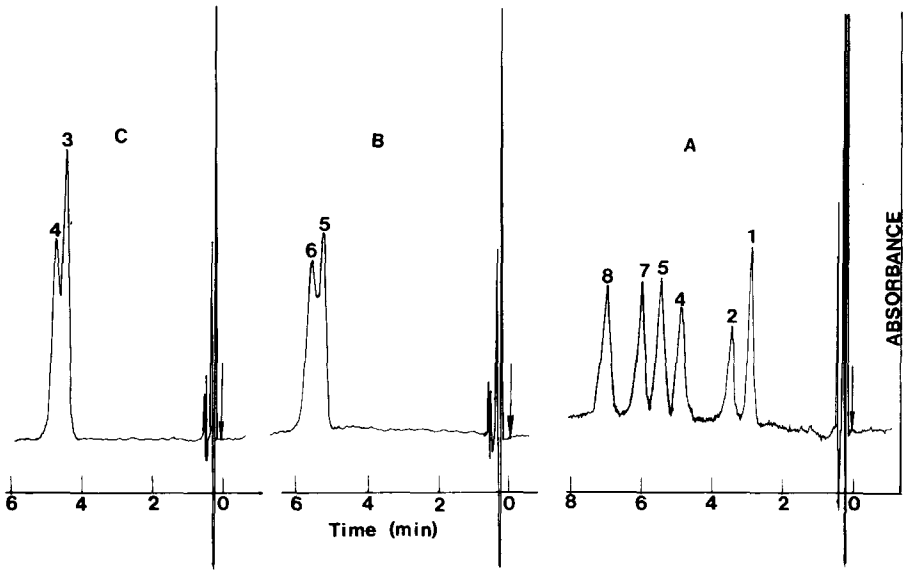

Fig. 2. Representative chromatograms showing complete and incomplete resolution obtained among certain tricyclic antidepressant drugs. Chromatographic conditions: column, 70 × 4.6 mm; mobile phase as in Fig. 1 with SLS concentration 80 m*M*. Compounds: 1 = doxepin; 2 = mianserine; 3 = imipramine; 4 = desipramine; 5 = amitriptyline; 6 = nortriptyline; 7 = trimipramine; 8 = clomipramine.

in the degree of resolution obtainable and in the minimum concentration required to achieve this, the retention times become very short and thus unacceptable for application to assay situations. Tetrabutylammonium (TBA) ion provides a better compromise between retention time and resolution for the above pair of compounds. Representative chromatograms are shown in Fig. 5, indicating the separations that can be achieved among selected members of the tricyclic antidepressant group of compounds by the addition of 5 m*M* TBA to 80 m*M* SLS. Comparison of Fig. 5 with Fig. 2 shows that although retention times are shorter, the peaks are considerably sharper and in fact measured plate numbers are comparable. It is also observed that resolution among the compounds has changed and that compounds previously separable without addition of added counter ion are now unresolved and *vice versa*. Complete data for the retention of all twelve compounds as a function of mobile phase pairing ion concentration in presence of a fixed TBA concentration of 5 m*M* are shown in Fig. 6. The maximum in k' with pairing ion concentration is still apparent but the relative magnitude of the capacity factors at the maximum is radically altered.

DISCUSSION

Previous explanations of the effect of added amine during the separation of basic drugs have suggested that the improvement in chromatographic behaviour is either as a result of decreased tailing due to the inactivation of unreacted silanol groups or alternatively, as a result of the suppression of secondary equilibria, namely the dissociation of ion pairs desolvated on the C-18 surface. Both of these expla-

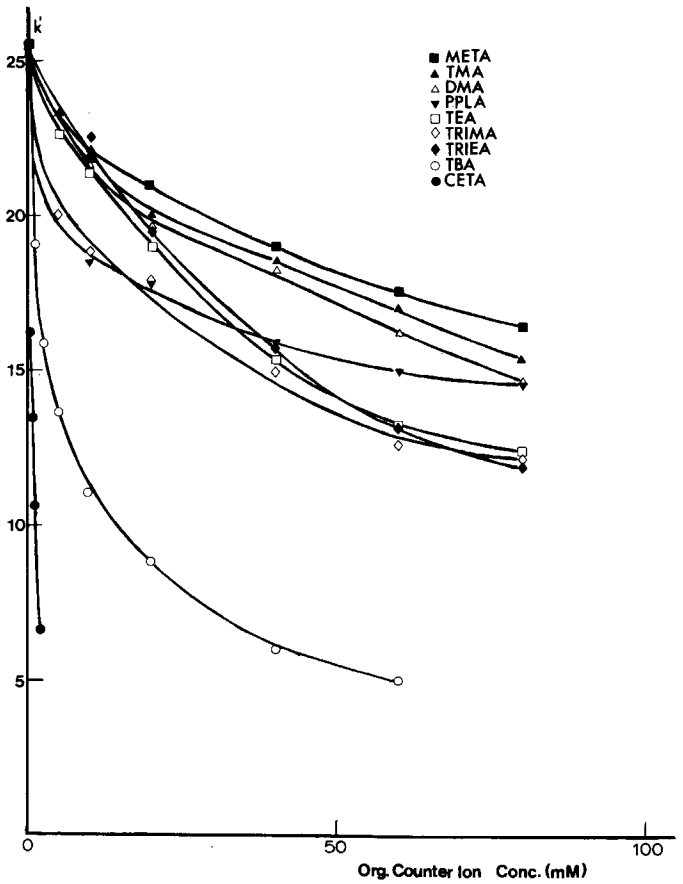

Fig. 3. Plots showing the variation in capacity factor, k', for amitriptyline as a function of various added counter ion concentrations. Chromatographic conditions as in Fig. 2, using a 100 × 4.6 mm column.

nations would require that the effect be general for all solutes and would result in a measurable increase in column efficiency for all basic solutes.

Any increase in efficiency in such systems involving added amine would be as a result of an increased rate of mass transfer. The mass transfer coefficient, measured as the slope of the HETP *vs.* mobile phase linear velocity curve at high linear velocities, was compared in presence and absence of 5 mM TBA for four solutes in a mobile phase of acetonitrile–water containing 80 mM SLS. Fig. 7 shows such plots for amitriptyline. No significant alteration in gradient was observed on addition of amine for any of the four solutes. This was taken as indicating constancy in the mass transfer rate and as further verifying the lack of real increase in column efficiency.

While the efficiency of the column is unaffected by addition of organic counter ion, resolution among the different solutes is markedly altered. The quantities contributing to resolution among any pair of compounds are included in the equation[21]

$$R = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1 + k'}\right)N^{\frac{1}{2}}$$

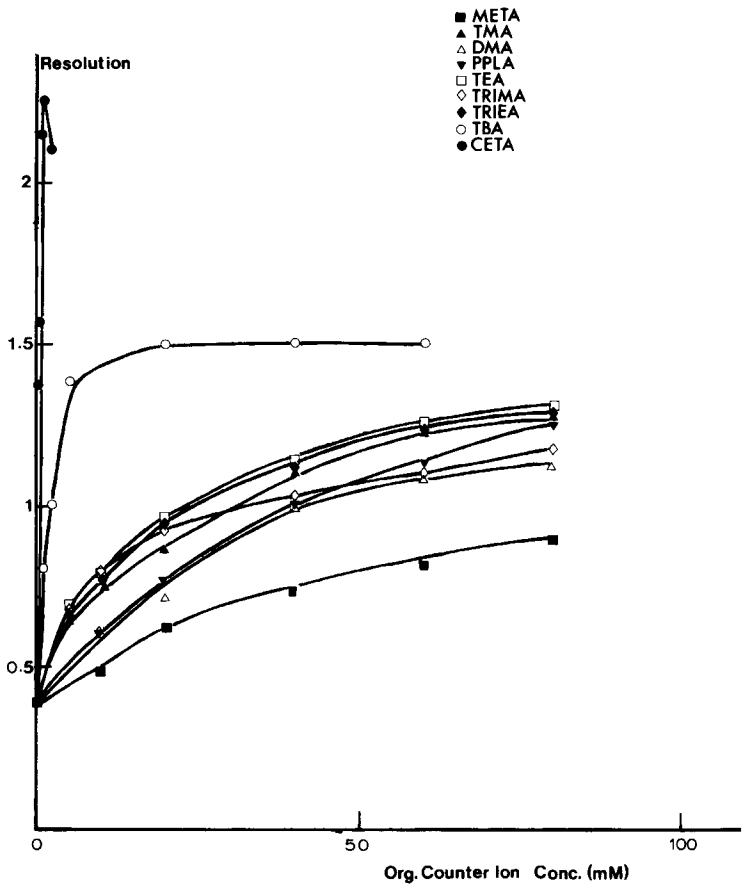

Fig. 4. Plots showing resolution between amitriptyline and nortriptyline as a function of various added counter ion concentrations. Chromatographic conditions as in Fig. 3.

where α represents the relative retention of the pair of compounds, k' the larger capacity factor and N the measured number of theoretical plates. In the present investigation, N is constant and the effect of added amine is to reduce k' so that any increase in R would appear to be as a consequence of a change in the selectivity term $(\alpha - 1)/\alpha$. The variation of $(\alpha - 1)/\alpha$ with concentration of added counter ion is shown in Fig. 8 and the overall shape of the plots appears to parallel that of the resolution as shown in Fig. 4. The ion exchange desolvation model formulated for hydrophobic pairing ions on octadecylsilica surfaces using aqueous mobile phases[8] can be used to explain the present findings.

This model represents retention of a basic solute in a completely ionised form by a hydrophobic anionic pairing ion adsorbed to an equilibrium extent on the C-18 surface. The basic equilibrium is one of ion exchange between the solute and the counter ion reinforced by desolvation of the solute on the C-18 surface. In the present investigation the hydrophobic nature of the counter ions will modify the behaviour from that observed with inorganic counter ions such as buffer salts, the interaction of which with the stationary phase pairing ion is purely electrostatic. When counter ions

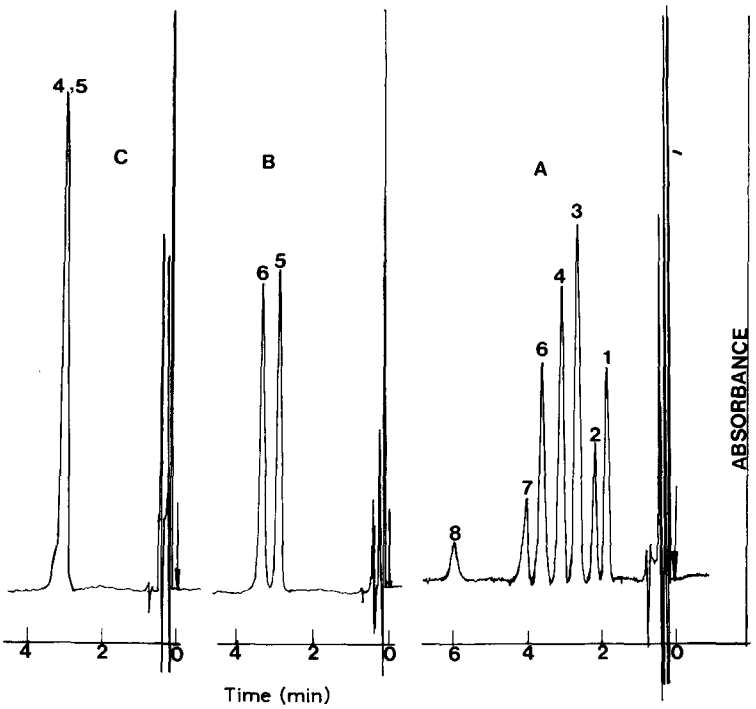

Fig. 5. Representative chromatograms showing complete and incomplete resolution obtained among certain tricyclic antidepressant drugs in presence of added organic counter ion. Chromatographic conditions as in Fig. 2 with the addition of 5 m*M* TBA to the mobile phase. Compounds: 1–6 as in Fig. 2; 7 = maprotiline; 8 = nomifensine.

differing in hydrophobic character are used, the counter ion, as well as being bound to the pairing ion by coulombic forces, is also desolvated on the adjacent C-18 surface. This equilibrium situation is identical in nature with the transient equilibria, producing retention of any hydrophobic solute. For retention of a solute to be achieved when organic counter ions are employed, not only must ion exchange occur between solute and counter ion, but the counter ion must be resolvated into the mobile phase. Thus, the more hydrophobic the counter ion, the more difficult will the resolvation process be and the greater will be the reduction in retention for a given concentration of added counter ion. Fig. 3 is interpreted on this basis as representing the effect of gradual replacement of inorganic counter ion by organic amine. The equilibrium reaction producing retention in the situation involving organic counter ion may be represented as before by the ion exchange reaction

$$(P^-C^+)_{org} + A^+_{aq} \rightleftharpoons (P^-A^+)_{org} + C^+_{aq} \quad (1)$$

where $(P^-C^+)_{org}$ represents the adsorbed pairing ion together with its associated counter ion and A^+ represents the fully ionised solute. The equilibrium constant, K_{IE}, will depend upon which species predominates as the counter ion C^+. That is, K_{IE} will vary from a maximum when the organic counter ion concentration is negligible to a

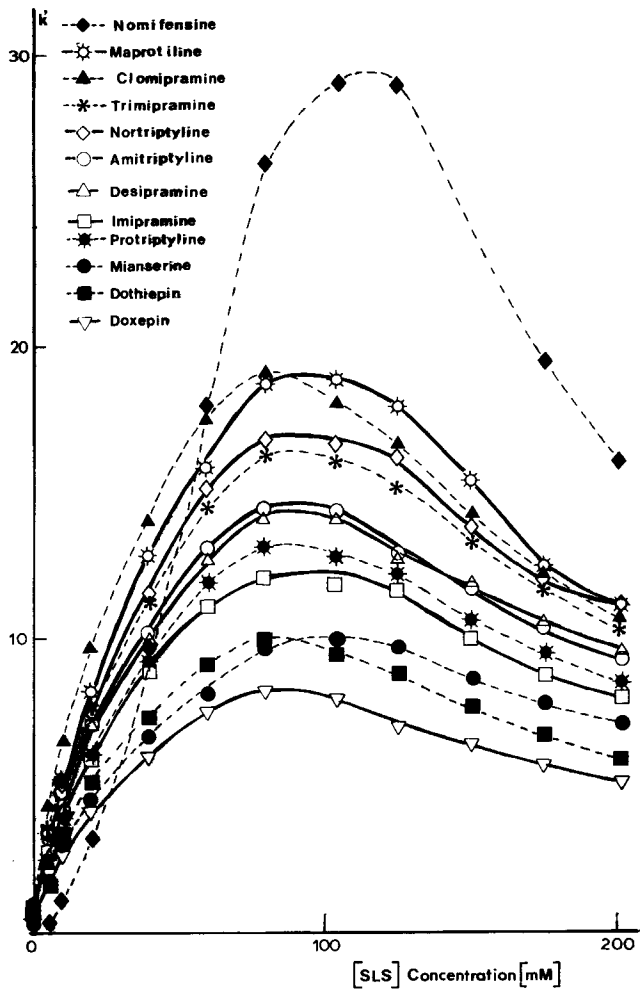

Fig. 6. Plots showing variation in k' with mobile phase pairing ion (SLS) concentration for antidepressant drugs. Chromatographic conditions as in Fig. 1 with the mobile phase modified to include 5 mM TBA.

minimum when all of the inorganic counter ion has been replaced by the added amine. Thus two limiting forms of the above equilibrium may be written:

$$(P^-Na^+)_{org} + A^+_{aq} \rightleftharpoons (P^-A^+)_{org} + Na^+_{aq} \ (K_{IE} \text{ large}) \quad (2)$$

$$(P^-TBA^+)_{org} + A^+_{aq} \rightleftharpoons (P^-A^+)_{org} + TBA^+ \ (K_{IE} \text{ small}) \quad (3)$$

At intermediate conditions, where not all of the sodium has been replaced due either to the limited hydrophobicity of the added amine, or to its low concentration, the value of K_{IE} will be intermediate between the two extremes.

The equation derived previously relating capacity factor to adsorbed pairing

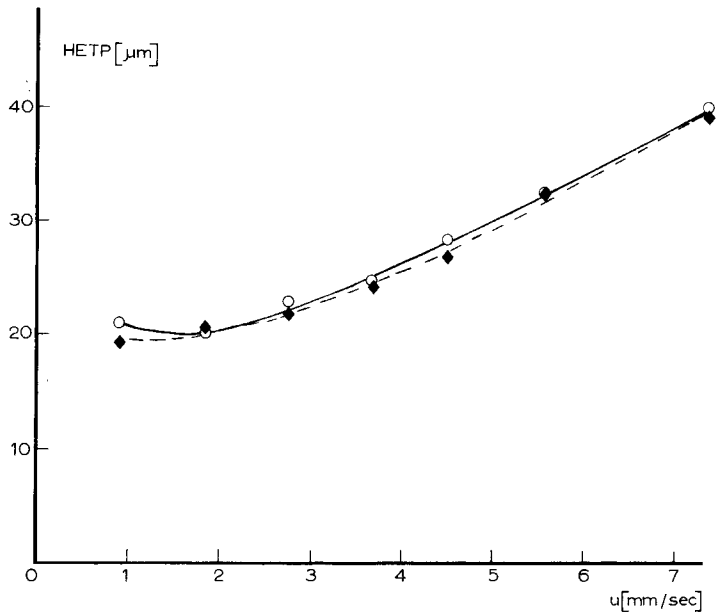

Fig. 7. Plot showing variation of HETP with linear mobile phase velocity for amitriptyline. Chromatographic conditions: O—O, as in Fig. 2, *i.e.*, with no added counter ion; ◆ ---◆, as in Fig. 5, *i.e.*, in presence of 5 m*M* TBA.

ion and aqueous counter ion concentration[8,9] will still apply for the condition where the counter ion concentration is held constant, *i.e.*:

$$k' = \frac{1}{V_m}\left(A_s K_1 - K_1 [P^- C^+]_{org} A_p + K_2 K_{IE} \frac{[P^- C^+]_{org}}{[C^+]_{aq}} - K_2 K_{IE} A_p \frac{[P^- C^+]^2_{org}}{[C^+]_{aq}} \right. \quad (4)$$

Eqn. 4 represents an improvement over the purely ion exchange processes represented above in that the ion exchange and desolvation tendency of a particular solute is represented by a combined constant $K_2 K_{IE}$. The term K_1 refers to the desolvation constant of the solute in the non-ion pairing situation and will be small in comparison with the $K_2 K_{IE}$ term when separation is obtained. A_s and A_p are the areas of the stationary phase and pairing ion on a molar basis respectively, and V_m the void volume of the column.

This form of equation is seen to apply both in the situation of pairing ion and buffer only for all the tricyclic antidepressant drugs investigated as shown by Fig. 1 and also for the case of a fixed concentration of TBA as organic counter ion in presence of SLS pairing ion as shown in Fig. 6. In this equation in the case of organic counter ion the $K_2 K_{IE}$ term will have the increased significance that it will reflect not only the desolvation of a given solute subsequent to ion exchange but it will provide an estimate of the desolvation tendency of a solute displacing an already desolvated organic counter ion. This is seen as providing an additional parameter of selectivity in such systems and may account for the alteration in elution order in Fig. 6 compared with Fig. 1. It is emphasised that while in this case the selectivity alteration has been

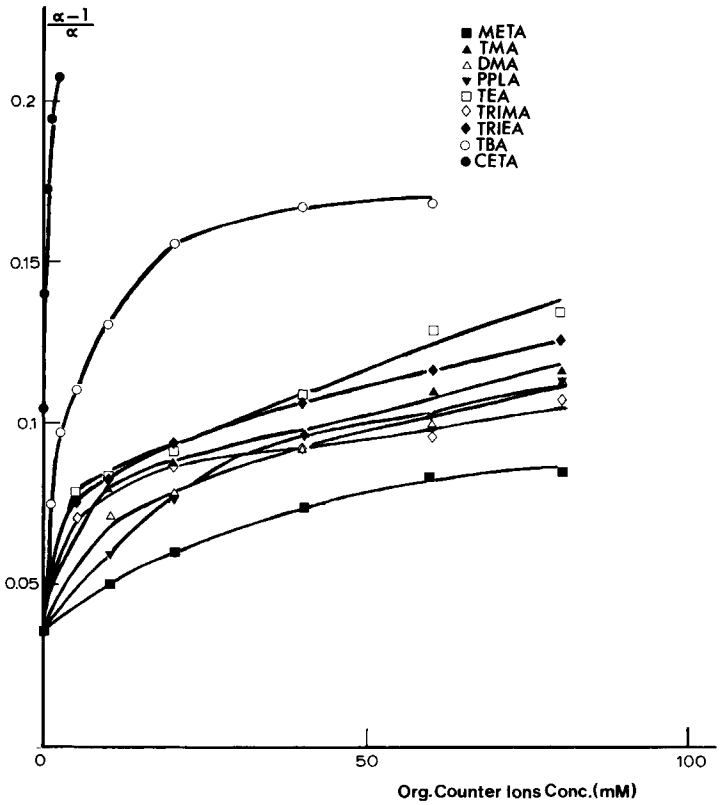

Fig. 8. Plots of the variation of selectivity $(\alpha - 1)/\alpha$ between amitriptyline and nortriptyline as a function of different organic counter ion concentrations. Chromatographic conditions as in Fig. 3.

to improve the resolution for the pairs desipramine and imipramine and amitriptyline and nortriptyline, its effect is also to reduce the resolution between other pairs of compounds as shown in Fig. 5C for desipramine and amitriptyline.

The applicability of the above equation on a quantitative basis for the above pairs of compounds can be assessed by determining the K_2K_{IE} constants for each compound in presence and absence of organic counter ion. The K_2K_{IE} constants were evaluated by fitting the curves for the appropriate compound as shown in Figs. 1 and 6 to the above equation by the method of least squares using the Hooke software system previously employed[8,9]. $[P^-C^+]_{org}$ values were obtained by interpolation of values from previous isotherms[9] measured at 30% and 60% acetonitrile concentrations and it is assumed on this model that the area available for desolvation of solutes will be unaffected by the presence of the more bulky organic counter ion. If the equation is applicable to such systems involving added organic counter ion, the numerical values of K_2K_{IE} should be seen to decrease in the presence of organic counter ion. In addition the separation factors, α, which can be measured by direct observation of the relative retention times between pairs of compounds should be identical with the ratio of the calculated K_2K_{IE} values for that pair of compounds obtained by the above curve fitting procedure. The results of such a comparison are shown in

TABLE I

OBSERVED SEPARATION FACTORS AND CALCULATED RATIOS OF ION EXCHANGE DESOLVATION CONSTANTS, K_2K_{IE}, DERIVED FROM EQN. 4 IN PRESENCE AND ABSENCE OF ORGANIC COUNTER ION FOR SELECTED PAIRS OF COMPOUNDS

Compound	*SLS (80 mM)*			*SLS (80 mM) + TBA (5 mM)*		
	K_2K_{IE}	K_2K_{IE} *ratio*	α	K_2K_{IE}	K_2K_{IE} *ratio*	α
Imipramine	0.179	1.04	1.05	0.0955	1.188	1.129
Desipramine	0.185			0.114		
Amitriptyline	0.208	1.06	1.04	0.109	1.250	1.123
Nortriptyline	0.219			0.136		
Desipramine	0.185	1.12	1.13	0.114	1.05	1.03
Amitriptyline	0.208			0.109		

Table I. It is seen that the expected decrease in K_2K_{IE} is observed and that good agreement is obtained between α values and K_2K_{IE} ratios calculated both for the situation where resolution has been improved and for that in which resolution has been decreased.

CONCLUSIONS

The present investigation using the closely related group of tricyclic antidepressant drugs as a model system indicates that the action of an organic amine when added to a mobile phase containing hydrophobic anionic pairing ion is that of a counter ion involved in an ion exchange desolvation process. Such species, because of their desolvation on the C-18 surface, act to reduce capacity factors for all basic solutes. They may also provide an additional degree of selectivity over that observed in such systems in absence of added amine. The effect may be utilised to increase resolution among certain compounds but may also act to reduce resolution among others. It has been found that the systems discussed can provide the required selectivity and sensitivity for the measurement of these drugs in serum at therapeutic levels. The quantitative results of such an assessment will be the subject of a future communication.

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