Journal of Chromatography, 149 (1978) 377–390

CHROM. 10,759

CHROMATOGRAPHIC BEHAVIOUR OF BASIC AMINO COMPOUNDS ON SILICA AND ODS-SILICA USING AQUEOUS METHANOL MOBILE PHASES

K. SUGDEN, G. B. COX and C. R. LOSCOMBE

Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ (Great Britain)

SUMMARY

The effects of the variation of pH, methanol concentration, ionic strength and nature of the ions in aqueous methanol mobile phases on the capacity ratios of amprolium (INN), amylocaine, benzocaine, butacaine (INN) and cocaine using both silica and silica surface-modified by treatment with trichloro(octadecyl)silane (ODS-silica) have been investigated. The differences in retention of the solutes on the two columns are related to the partition coefficients between 2,2,4-trimethylpentane and the mobile phase over the pH range studied. Amprolium, a quaternary ammonium salt, had similar retention characteristics on both silica and ODS-silica when a mobile phase containing 0.05 *M* ammonium formate was used. The retention mechanisms of the species are complex and involve ion exchange with the proton of a surface silanol site, particularly with amprolium, partition of ion pairs and salting-out effects.

Applications of these observations to the analysis of erythromycin (INN) and various toxic diamines are given.

INTRODUCTION

The addition of a salt to an aqueous methanol mobile phase employed in reversed-phase high-performance liquid chromatography has been shown to have a significant effect on the capacity ratios of amines[1,2]. Both the nature and concentration of the added salt influence retention characteristics and, with careful control of pH, the separation of species can be optimized. The retention mechanism is complex but may involve both ion-pair partition and participation of unsilanized surface silanol groups[3]. Indeed, the weak cation-exchange properties of silica gel have been successfully exploited in the separation of weak protolytes[4–6] and aprotic ions[7].

The purpose of this work was to investigate the effect of pH, methanol concentration, ionic strength and nature of the added counter ion in aqueous methanol mobile phases on the retention behaviour of various amino compounds on both silica and silica chemically modified with trichloro(octadecyl)silane stationary phases.

EXPERIMENTAL

Solutes

Ethanolic solutions of cocaine hydrochloride, amylocaine hydrochloride, butacaine sulphate, benzocaine and amprolium were used.

Column packings and eluents

Silica gel (LiChrosorb Si 100, average particle size 5 μm, BDH, Poole, Great Britain) was refluxed with 2 *M* hydrochloric acid for 4 h, washed with water and dried at 200° for 16 h.

Five grams of the dry acid-washed material (AW-silica) were refluxed with 1.5 ml of trichloro(octadecyl)silane in 50 ml of 1,4-dioxan for 4 h, washed with 1,4-dioxan and acetone and dried on a water pump.

The AW-silica and ODS-silica phases were packed into two 150 × 5.0 mm I.D. stainless-steel tubes by a slurry technique previously described[8].

Mixtures of 95% methanol–water containing either ammonium formate, ammonium nitrate or sodium formate were used as the mobile phase for the chromatography of amprolium, and similar phases utilizing 70% methanol–water were used for the other solutes. The pH of these phases was adjusted by the addition of either ammonium hydroxide or nitric acid.

Chromatographic apparatus

A Haskel air-driven constant-pressure pump (Olin Energy Systems, Sunderland, Great Britain) was used to provide mobile phase flow and a variable-wavelength ultraviolet detector (Cecil Instruments, Milton, Cambridge, Great Britain), operated at 270 nm for amprolium and 240 nm for the other solutes, was employed to monitor column eluent.

Injection was by the stop-flow technique.

Determination of solute partition coefficients

Approximately 0.0100 g of the solute was accurately weighed and dissolved in 10 ml of the mobile phase. An equal volume of 2,2,4-trimethylpentane was added and the mixture was shaken for 5 min. The two layers were allowed to separate and the amount of solute remaining in the aqueous phase was determined by chromatographic analysis.

Determination of dissociation constants

Approximately 0.1 g of the solute was dissolved in 40 ml of 70% methanol–water and titrated with 0.1 *M* sodium hydroxide solution. The titration was followed by measurement of pH and the dissociation constant was determined from the equivalence point.

RESULTS AND DISCUSSION

Amylocaine, benzocaine, butacaine and cocaine were employed because, as weak protolytes, their amino functional group ionizes in acidic solution. Amprolium was used because, as a quaternary ammonium salt, it also exhibits ionic properties.

Carbon and hydrogen microanalyses of the ODS-silica phase revealed that 0.56 mmole per gram of the silane had bonded to the adsorbent. Although calculations from crystal study data[9] suggest that there are up to eight silanol groups on each square nanometer of silica surface, chemical tests[15] indicated that only five of these groups appear to be available for reaction. This means that for LiChrosorb Si 100, with a surface area of 400 m^2/g, the concentration of labile surface hydroxy groups is 3.32 mmole/g. If two of the available three chlorine atoms in the ODS molecule are reactive[16], 66% of the silanol groups remain unreacted and, provided that there is no steric hindrance, are available for interaction with eluting solutes.

The effect of pH and the type and concentration of the counter ion (A^-) in the mobile phase on the nature of an amine (NR_3) can be expressed by the equilibria

$$NR_3 + H^+ \overset{K_a}{\rightleftharpoons} NR_3H^+ \quad (1)$$

and

$$NR_3H^+ + A^- \rightleftharpoons NR_3HA \quad (2)$$

which involve protonation and ion-pair formation (NR_3HA). The dissociation constants (K_a) of amylocaine, butacaine and cocaine were determined and the pK_a values were found to lie above the pH range of 5–9 for the 70% methanol–water mobile phases used in this study (Table I). Consequently, except at high pH, the species exist predominantly in the protonated form. Benzocaine has a $pK_a = 2.5$ (ref. 10) and is therefore a free base over this pH range.

TABLE I

DISSOCIATION CONSTANTS (pK_a) OF COCAINE, AMYLOCAINE AND BUTACAINE IN 70% METHANOL–WATER SOLUTIONS

Compound	*pK_a value*
Cocaine	9.5
	9.7
Amylocaine	9.2
	8.8
Butacaine	9.6
	9.6

Further equilibria involving ion exchange at the surface silanol groups may occur, *viz.*

$$SiOH \rightleftharpoons SiO^- + H^+ \quad (3)$$

$$SiO^- + C^+ \rightleftharpoons SiOC \quad (4)$$

and

$$SiO^- + NR_3H^+ \rightleftharpoons SiONR_3H \quad (5)$$

The surface hydroxyl site (SiOH) will ionise above pH 4 (ref. 11) and creates active sites for which the protonated amine and any cations (C^+) in solution will compete. These competing equilibria, together with the partition and adsorption mechanisms that may occur, can have a great effect upon retention.

Amylocaine, benzocaine, butacaine and cocaine

Initial experiments revealed that in the absence of a salt in the mobile phase at pH 7.0, amylocaine, butacaine and cocaine display a high affinity for both the AW- and ODS-silicas and have capacity ratios (k') greater than 15. As the partition coefficients of these solutes from the salt-free mobile phase into a hydrocarbon solvent (2,2,4-trimethylpentane) are low (Table II), it can be assumed that their affinity for the chemically bonded ODS molecule is also low. Consequently, the high k' values observed on the ODS phase must arise from a strong interaction between the protonated species and the unsilanized surface silanol groups. This interaction does not involve ion exchange as similar results were obtained below the isoelectric point of silica gel at pH 3.3. Benzocaine has the properties of a free base over the pH range studied and elutes near the solvent front.

TABLE II

PARTITION COEFFICIENTS OF COCAINE, AMYLOCAINE AND BUTACAINE BETWEEN 2,2,4-TRIMETHYLPENTANE AND THE 70% METHANOL–WATER MOBILE PHASE WITH AND WITHOUT ADDED SALT

Cocaine			*Amylocaine*			*Butacaine*		
NH_4CO_2H *(moles)*	*pH*	$K\left(\frac{org}{aq}\right)$	NH_4CO_2H *(moles)*	*pH*	$K\left(\frac{org}{aq}\right)$	NH_4CO_2H *(moles)*	*pH*	$K\left(\frac{org}{aq}\right)$
Absent	4.5	0.02	Absent	4.4	0.05	Absent	4.4	0.00
Absent	8.4	0.07	Absent	8.3	0.07	Absent	8.3	0.00
0.01	9.1	0.49	0.025	9.1	5.33	0.01	8.8	0.17
0.05		0.48	0.05		5.27	0.05		0.09
0.25		0.48	0.10		5.18	0.10		0.18
0.50		0.50	0.20		7.00	0.50		0.25
0.01	6.9	0.03	0.02	6.7	0.40	0.01	6.9	0.06
0.05		0.06	0.05		0.38	0.05		0.00
0.25		0.07	0.20		0.70	0.20		0.02
0.50		0.07				0.50		0.04

On the addition of a salt to the mobile phase, k' values for the three protonated amines decrease dramatically on both AW- and ODS-silicas (Tables III and IV). With a mobile phase at pH 6.9, the partition coefficients of cocaine and butacaine into the hydrocarbon solvent are similar to those with salt absent (Table II). The species, therefore, will have little affinity for the ODS molecule and their retention must still be governed by unsilanized hydroxy groups. For amylcocaine, partition into 2,2,4-trimethylpentane is much greater with a salt present and suggests that the ODS moiety influences retention either by partition of ion pairs or by a salting-out effect. At low hydrogen ion concentration, such that the pH of the mobile phase approaches the pK_a of the amine, the concentration of the free base increases and, for amylocaine and cocaine, is

TABLE III

EFFECT OF AMMONIUM NITRATE CONCENTRATION IN A 70% METHANOL–WATER MOBILE PHASE AT pH 7.0 ON THE k' VALUES OF COCAINE, AMYLOCAINE AND BUTACAINE ON AW–SILICA

$[NH_4NO_3]$ moles/l	k'		
	Cocaine	*Amylocaine*	*Butacaine*
0.01	2.20	1.12	1.03
0.05	0.71	0.35	0.24
0.10	0.58	0.30	0.17
0.20	0.30	0.17	0.04

TABLE IV

EFFECT OF AMMONIUM FORMATE CONCENTRATION IN A 70% METHANOL–WATER MOBILE PHASE ON THE k' VALUES OF COCAINE, AMYLOCAINE AND BUTACAINE ON ODS–SILICA

$[NH_4CO_2H]$ (moles/l)	k'					
	Cocaine		*Amylocaine*		*Butacaine*	
	pH 6.7	*pH 8.6*	*pH 6.7*	*pH 8.6*	*pH 6.7*	*pH 8.6*
0.02	3.4	4.1	3.6	4.4	1.1	5.9
0.05	3.0	3.2	3.7	4.0	1.1	4.6
0.10	1.6	2.6	1.9	3.6	0.5	3.0

accompanied by an increase in both the partition coefficient and k' values, suggesting that the species salt out into the ODS phase. On increasing the ionic strength of the mobile phase, however, the partition coefficients do not decrease. Indeed, there is some increase in these values while retention times decrease (Table IV), implying that partition of ion pairs also occurs. Further evidence for a mixed retention mechanism is given by the non-linear decrease in k' values with decrease in the inverse of the molar ionic strength[12] (Fig. 1). For butacaine, partition into the hydrocarbon solvent does increase with pH but is still low and suggests that the ODS molecule has less effect on retention.

On AW-silica, k' values are much lower than those on the modified phase and a more linear relationship with the inverse of ionic strength is observed (Fig. 1). In addition, whereas the AW-silica column efficiency is constant with changes in ionic strength, the ODS-silica column efficiency decreases as the ionic strength increases, possibly owing to the competing retention mechanisms (Table V).

The effect of varying the apparent pH of the mobile phase containing various ions on the k' values of amylocaine, butacaine and cocaine on both AW- and ODS-silica was also investigated (Fig. 2). As the hydrogen ion concentration increases, the concentration of the amine in the protonated form also increases, according to equilibrium 1. This, in turn, makes formation of the ion pair more favourable and is accompanied by a reduction in k' values on both columns.

The k' values with nitrate ions in the mobile phase are lower than those with formate ions present on the ODS phase, owing to the greater ease with which the former species can couple with a cation[13] to form an ion pair.

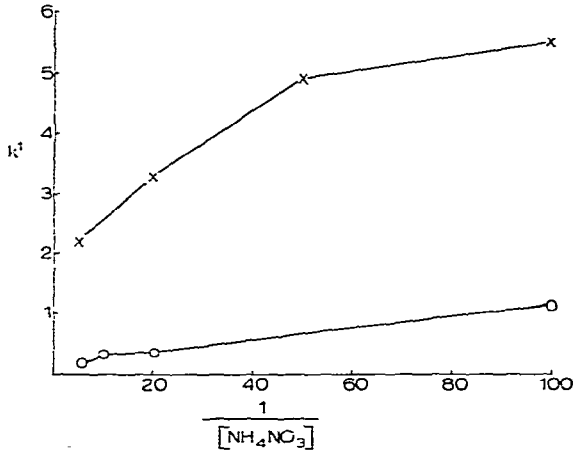

Fig. 1. Relationship between the capacity ratio (k') of cocaine and the inverse of the molar concentration of ammonium nitrate added to the 70% MeOH–H_2O mobile phase adjusted to pH 7.0. (○), AW-silica; ×, ODS-silica.

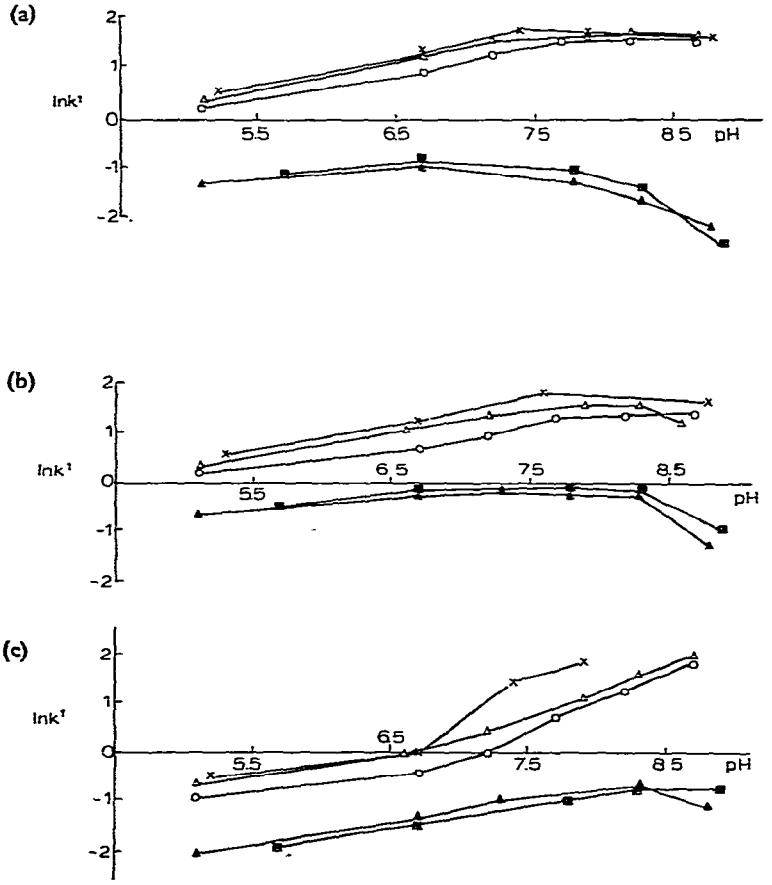

Fig. 2. Relationship between the logarithm of the capacity ratios (ln k') of amylocaine (a), cocaine (b) and butacaine (c) and the apparent pH of a 70% MeOH–H_2O mobile phase containing 0.05 *M* $NaCO_2H$ (×), 0.05 *M* NH_4CO_2H (△) and 0.05 *M* NH_4NO_3 (○) on ODS-silica and containing 0.05 *M* $NaCO_2H$ (■) and 0.05 *M* NH_4CO_2H (▲) on AW-silica.

The effect of pH and the change in the cation on the partition of the amines from the methanol–water phase into 2,2,4-trimethylpentane was also studied and the results were compared with the chromatographic data. Similarities between the data were most evident with amylocaine (Fig. 3); just as the k' values decreased with pH as ion pair formation became more favourable, so partition into the hydrocarbon solvent decreased and, except at high pH where the species tend towards the free base, was greater with formate ions present than with nitrate ions. Correlation between the partition coefficient and retention data for cocaine was much less marked and for butacaine was too low to measure.

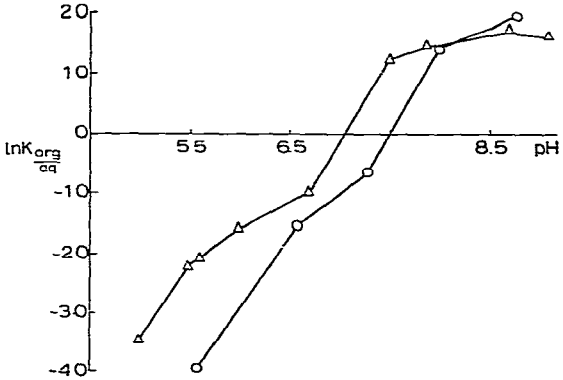

Fig. 3. Relationship between the logarithm of the partition coefficient [ln $K_{\frac{org}{aq}}$] of amylocaine, between 2,2,4-trimethylpentane and the mobile phase, and the pH of the mobile phase containing 0.05 M NH_4CO_2H (△) and 0.05 M NH_4NO_3 (○).

In order to investigate the possibilities of ion exchange occurring on the surface silanol sites (equilibria 3, 4 and 5), both sodium and ammonium salts were added to the mobile phase; the hydrated ammonium ion has a smaller radius than the sodium species and may therefore more readily exchange with the silanol to allow the amines to elute more readily. In fact, although retention data of the ODS phase suggests that some ion exchange of cocaine and, more particularly, butacaine may occur, this evidence is refuted by work on the untreated silica column, which shows that changing the cation has little effect.

The hydrogen ion concentration of the mobile phase was shown to have a great effect on the relative retention of the species (Fig. 4). By adjustment of the pH to increase the concentration of the amine in the form of the free base, cocaine and amylocaine could be resolved, and the k' value of butacaine significantly changed.

Increasing the methanol concentration of the mobile phase decreased the k' values of the amines on the ODS-silica. This is additional evidence for an ion-pair mechanism; as the methanol concentration increases the dielectric constant of the solvent mixture decreases and the electrostatic forces between a protonated amine and the counter anion are enhanced[14]. Ion-pair formation, therefore, is more favoured and the species elute more rapidly from the column. On AW-silica, increasing the methanol concentration has less effect on k' values, although the retention of cocaine also increases (Fig. 5). Ion exchange has already been shown not to occur, but the increase in k' values may be due to suppression of ionic activity.

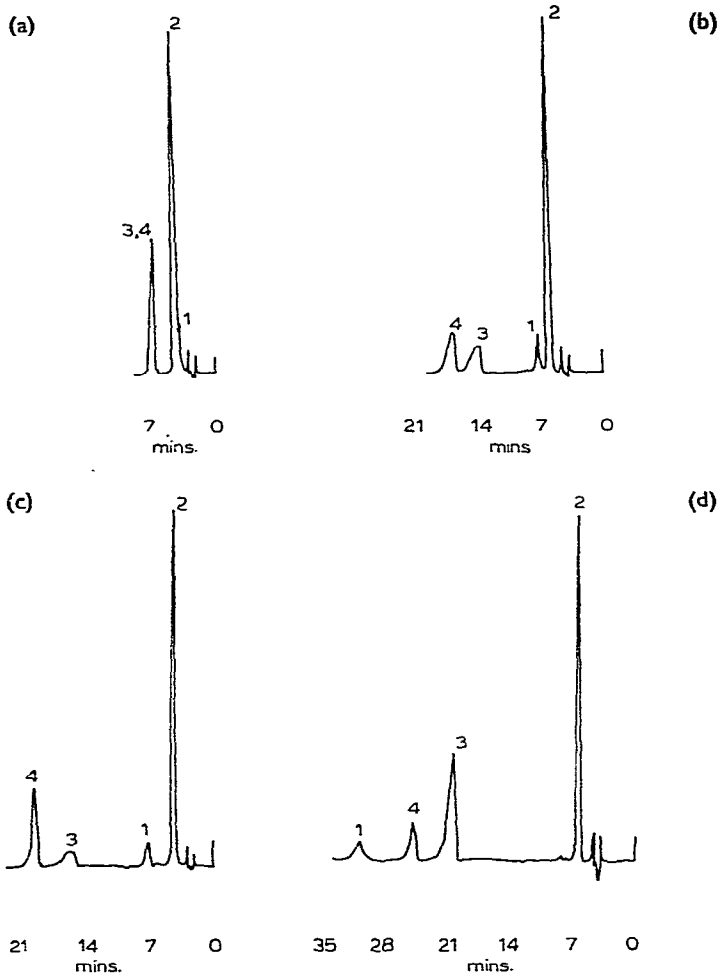

Fig. 4. Chromatograms of butacaine (1), benzocaine (2), cocaine (3) and amylocaine (4) on ODS-silica using 0.05 *M* NH_4CO_2H in 70% MeOH–H_2O at pH 5.1 (a), 6.6 (b), 7.2 (c) and 8.7 (d) as the mobile phase.

Amprolium

As with the other amines, the retention of the quaternary ammonium salt amprolium on AW- and ODS-silica gel columns increased with the pH and was dependent upon the presence and the nature of the salt in the mobile phase (Figs. 6 and 7). On AW-silica, a change in the counter anion from formate to nitrate had little effect on k' values, although the former species gave greater efficiency (Table VI). A more significant difference in retention was observed on changing the added cation; k' values with ammonium ions present were less than those with sodium ions and indicate an ion-exchange mechanism. The column efficiency was also greater with ammonium ions in the mobile phase (Table VI), an effect with was not found with the other amines.

On ODS-silica, the difference in retention times with these two cations is less

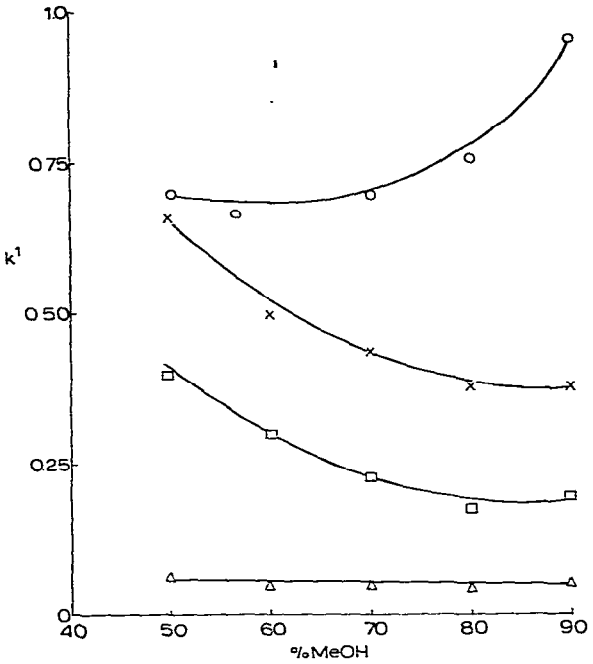

Fig. 5. Relationship between the methanol concentration of the mobile phase, containing 0.05 *M* ammonium formate at pH 6.5, and the capacity ratios (*k'*) of amylocaine (×), cocaine (○), butacaine (□) and benzocaine (△).

TABLE V

EFFECT OF THE CONCENTRATION OF ADDED SALT IN A 70% METHANOL–WATER MOBILE PHASE AT pH 7.0 ON THE CHROMATOGRAPHIC EFFICIENCY OF COCAINE ON BOTH AW- AND ODS-SILICA

[NH_4NO_3] (moles/l)	*HETP (μm)*	
	AW-silica	*ODS-silica*
0.01	25	241
0.05	30	83
0.10	26	99
0.20	24	59

TABLE VI

CHROMATOGRAPHIC EFFICIENCY FOR AMPROLIUM ON AW-SILICA WITH VARIOUS SALTS ADDED TO THE MOBILE PHASE

pH	*HETP (μm) with 0.05 M NH_4NO_3*	*pH*	*HETP (μm) with 0.05 M NH_4CO_2H*	*pH*	*HETP (μm) with 0.05 M $NaCO_2H$*
5.8	81	5.7	50	5.4	132
6.8	107	6.2	51	6.2	128
7.4	88	6.9	64	6.8	169
7.8	100	7.9	60	7.3	176
8.2	98	8.3	78	8.5	199
8.8	86	8.8	66		

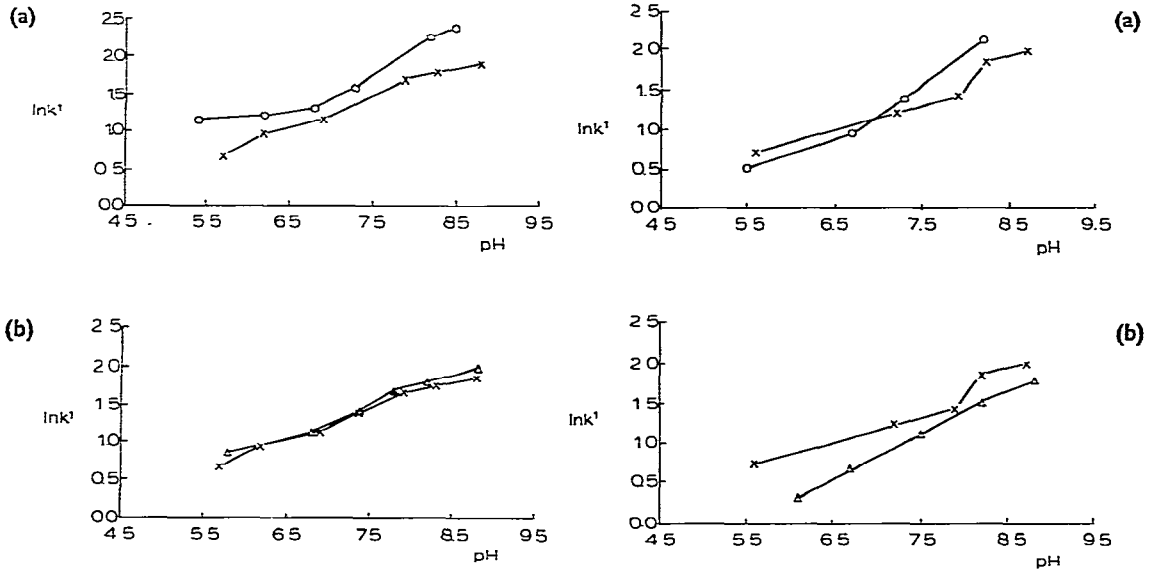

Fig. 6. Relationship between the logarithm of the capacity ratio (ln k') of amprolium on AW-silica and the apparent pH of the 95% MeOH–H_2O mobile phase containing (a) 0.05 M $NaCO_2H$ (○) and 0.05 M NH_4CO_2H (×) and (b) 0.05 M NH_4NO_3 (△) and 0.05 M NH_4CO_2H (×).

Fig. 7. As for Fig. 6 on ODS-silica.

marked, although some ion exchange on the residual hydroxy groups may occur above pH 6.7. On the other hand, the counter anion has more influence on the ODS phase than on AW-silica and shorter retention times are observed with nitrate ions present than with formate ions. As partition coefficient data revealed that the affinity of the hydrocarbon solvent for the species is low and independent of the nature and concentration of the added salt (Table VII), ion-pair partition can be ruled out. Indeed, the low affinity of the species for the ODS moiety is emphasized by the observation that with 0.05 M ammonium formate in the mobile phase, k' values on the AW- and modified silicas are very similar, suggesting that the retention mechanisms on the two columns are alike. The unmodified phase, however, has the advantage of having a much higher column efficiency (Table VIII). The ODS moiety interferes with the

TABLE VII

PARTITION COEFFICIENTS OF AMPROLIUM BETWEEN 2,2,4-TRIMETHYLPENTANE AND 95% METHANOL–WATER MOBILE PHASE WITH AND WITHOUT ADDED SALT

0.05 M NH_4NO_3		*0.05 M NH_4CO_2H*		*Salt absent*	
pH	$K_{(\frac{org}{aq})}$	*pH*	$K_{(\frac{org}{aq})}$	*pH*	$K_{(\frac{org}{aq})}$
5.6	0.18	5.9	0.04	4.8	0.08
6.5	0.07	6.8	0.13	6.8	0.10
7.9	0.13	7.7	0.06	7.6	0.13
8.3	0.07	8.3	0.12	8.8	0.03

TABLE VIII

CHROMATOGRAPHIC EFFICIENCY OF AW- AND ODS-SILICA COLUMNS WITH AMPROLIUM USING 0.05 *M* NH_4CO_2H IN 95% METHANOL–WATER AS THE MOBILE PHASE

AW-silica		*ODS-silica*	
pH	*HETP (μm)*	*pH*	*HETP (μm)*
5.7	50	5.6	253
6.2	51	6.7	425
6.9	64	7.2	300
7.9	60	7.9	360
8.3	78	8.2	397
8.8	66	8.7	393

chromatography when 0.05 *M* ammonium nitrate is present and k' values are lower than those found on AW-silica.

The dependence of retention on ionic strength is shown in Fig. 8. Non-linear relationships between k' values and the inverse of the molar ionic strength were found with AW-silica, even at pH 4.5 such that there is little ionization of the silica gel, and indicate a mixed retention mechanism. A similar relationship is observed with a mobile phase at pH 7.0 on the ODS phase, but at pH 4.4 the graph is linear and shows that the retention mechanism has changed. As with the other amines, k' values greater than 15 are observed on both columns when a salt-free mobile phase at pH 3.3 is employed.

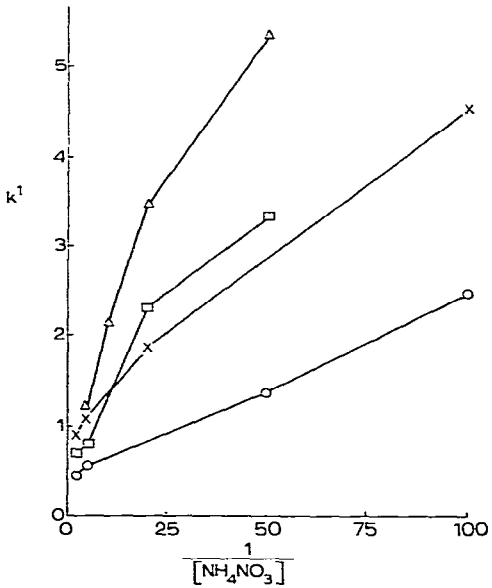

Fig. 8. Relationship between the capacity ratio (k') of amprolium and the inverse of the molar concentration of ammonium nitrate in a 95% MeOH–H_2O mobile phase. △, AW-silica, mobile phase at pH 7.0; ×, AW-silica, mobile phase at pH 4.5; □, ODS-silica, mobile phase at pH 7.0; ○, ODS-silica, mobile phase at pH 4.4.

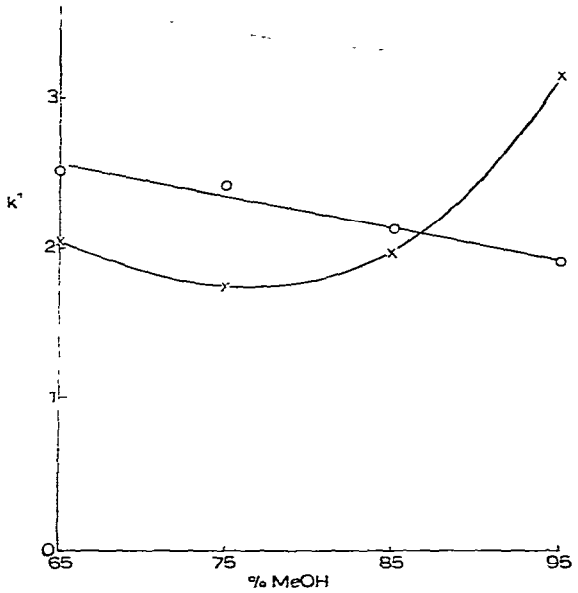

Fig. 9. Relationship between the capacity ratio (k') of amprolium, on (×) AW-silica and (○) ODS-silica, and the methanol concentration of the mobile phase containing 0.05 M NH_4NO_3 at pH 6.7.

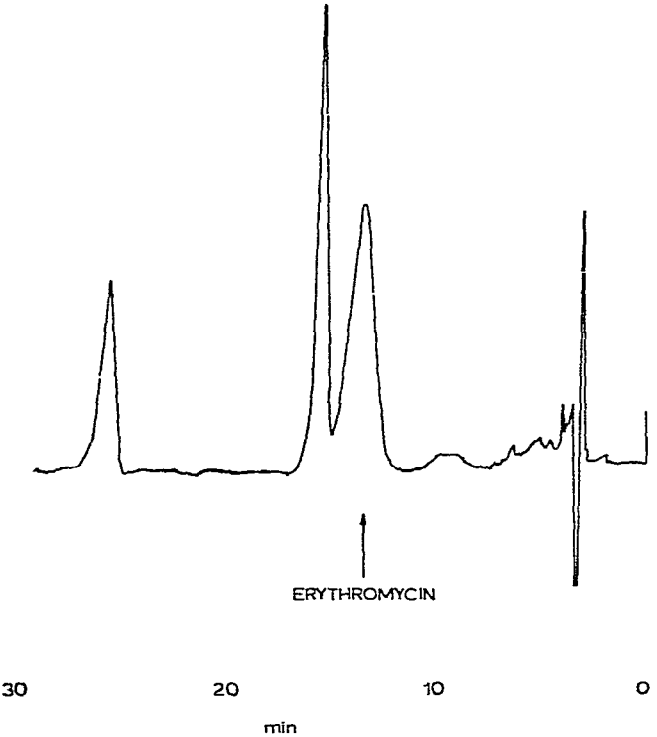

Fig. 10. Chromatogram of erythromycin in enteric tablets. Column, 150 × 50 mm I.D. Spherisorb S5 ODS; mobile phase, 1% NH_4OH (sp.gr. 0.880) in 70% MeOH–H_2O adjusted to pH 4.8 with orthophosphoric acid and delivered at 0.5 ml/min; λ = 202 nm. The peaks, other than that due to erythromycin, arise from impurities. These peaks do not interfere with the analysis and, therefore, their identities, although desirable from an academic point of view, are of no practical significance. Ambient temperature and a pressure of 700 p.s.i. were employed.

The effect of the methanol concentration of the mobile phase on the retention of amprolium was also investigated (Fig. 9). As with the other amines, the k' values decrease with increasing methanol concentration on the ODS phase and, as with cocaine, increase on AW-silica.

Applications to other analyses

By observing the effects of the various parameters on the retention of these amines, it has been possible to carry out hitherto difficult analyses. The determination of erythromycin, normally carried out by a time-consuming and computer-assisted microbiological technique, using high-performance liquid chromatography has now been accomplished. The species will not elute when using a salt-free methanol–water mobile phase under normal reversed-phase conditions, but by the addition of ammonium hydroxide to the mobile phase with subsequent adjustment of the pH to 4.8, a retention time of approximately 15 min is obtained (Fig. 10). This procedure has been successfully employed to determine the antibiotic in methanol extracts of enteric tablets.

Another problem that has arisen in this laboratory is the separation and determination of toxic amines in hair-colouring preparations. Salt-free aqueous mobile phases were again found to be unsatisfactory, but upon the addition of ammonium formate to the phase, *m*- and *p*-phenylenediamines and 2-methyl-*p*-phenylenediamine, 4-methyl-*m*-phenylenediamine and 4-methyl-*o*-phenylenediamine could be determined in a single chromatographic run (Fig. 11).

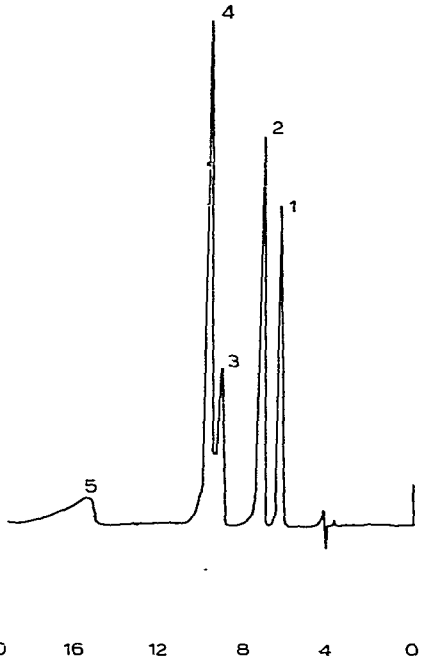

Fig. 11. Chromatogram of a methanolic solution of *m*-phenylenediamine (1), *p*-phenylenediamine (2), 2-methyl-*p*-phenylenediamine (3), 4-methyl-*m*-phenylenediamine (4) and 4-methyl-*o*-phenylenediamine (5). Column, 150 × 50 mm I.D. Spherisorb S5 ODS; mobile phase, 0.02 *M* NH_4CO_2H in 30% MeOH–H_2O adjusted to pH 6.4, delivered at 0.5 ml/min; $\lambda = 254$ nm. Ambient temperature and a pressure of 700 p.s.i. were employed.

CONCLUSION

The chromatographic behaviour of basic amino compounds on AW- and ODS-silica stationary phases has been studied and shown to be dependent upon the pH, methanol concentration and the nature and type of ions in the aqueous methanol mobile phase. These parameters are shown to affect both absolute and relative capacity ratios. Salting-out effects, partition of ion pairs and the residual surface hydroxy sites all play a role in the retention of the species on ODS-silica. Several retention mechanisms were also evident on the unmodified phase and some ion exchange, particularly of the quaternary ammonium salt, was observed.

REFERENCES

1 K. Krummen and R. W. Frei, *J. Chromatogr.*, 132 (1977) 27.
2 W. J. Rzeszotarski, W. C. Eckelman and R. C. Reba, *J. Chromatogr.*, 124 (1976) 88.
3 R. E. Hill, *J. Chromatogr.*, 135 (1977) 419.
4 I. Jane, *J. Chromatogr.*, 111 (1975) 227.
5 W. W. Dean, G. J. Lubrano, H. G. Heinsohn and M. Stastny, *J. Chromatogr.*, 124 (1976) 287.
6 H. G. Barth and A. Z. Conner, *J. Chromatogr.*, 131 (1977) 375.
7 G. B. Cox and K. Sugden, *Analyst (London)*, 101 (1976) 738.
8 G. B. Cox, C. R. Loscombe, M. J. Slucutt, K. Sugden and J. A. Upfield, *J. Chromatogr.*, 117 (1976) 269.
9 R. K. Gilpin and M. F. Burke, *Anal. Chem.*, 45 (1973) 1383.
10 N. W. Blacow (Editor), *Martindale, The Extra Pharmacopoeia*, Pharmaceutical Press, London, 26th ed., 1972.
11 R. K. Iler, *The Colloid Chemistry of Silica and Silicates*, Cornell University Press, New York, 1955.
12 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1974) 171.
13 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, London, Sydney, Toronto, 1974.
14 R. A. Robinson and R. H. Stokes, *Electrolyte Solutions*, Butterworths, London, 2nd ed., 1959.
15 K. Karch, I. Sebestian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.
16 H. Hemetsberger, W. Maasfeld and H. Ricker, *Chromatographia*, 9 (1976) 203.