

Factors affecting the retention of quaternary ammonium ions in reversed-phase high-performance liquid chromatography

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Abstract: The influence of solute structure (charge and hydrophobic substitution), organic modifier (type and concentration) and ion-pairing agent on the retention of nine quaternary- and bis-quaternary ammonium ions has been investigated in reversed-phase HPLC on ODS-silica. A functional group approach was taken to elucidate the influences of substitution on the charged nitrogen and the addition of a second positive charge to the solute molecule. These and other factors contributing to solute retention are discussed within the context of solvophobic theory. Hydrophobic effects and the solvation of the charged centre are shown to be the major factors contributing to retention in non-ion pair eluents. In addition, various electrostatic interactions in the mobile and stationary phases may contribute to solute retention in eluents containing an ion-pairing agent. It was found that ternary eluents containing hexane sulphonic acid and two organic modifiers offer certain selectivity advantages when compared with binary eluents.

Keywords: *Reversed-phase HPLC; quaternary ammonium compounds; ion-pair chromatography; hexane sulphonic acid; ternary eluents; solvophobic theory.*

Introduction

From studies of the reversed-phase high-performance liquid chromatography (RP-HPLC) of quaternary ammonium ions [1-12], it is clear that the mechanism of retention is complex and controlled by a number of constitutional and environmental factors.

The RP-HPLC of quaternary ammonium compounds has been investigated on silica [6] and alkyl-bonded silica [5-7, 12] stationary phases with mixed organic eluents. The influence of ion-pairing agents on the retention of quaternary ammonium ions was investigated initially in liquid-liquid systems with totally aqueous eluents [1-4]. These studies were later extended to liquid-solid systems with silica [10, 11] and alkyl-bonded silica [8, 9] stationary phases using aqueous methanol or aqueous acetonitrile as mobile phase.

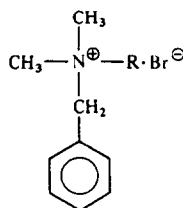
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The RP-HPLC of quaternary ammonium ions has often been associated with irreversible binding of the solutes to the stationary phase in the absence of electrolytes [5–7], or extreme peak tailing upon elution [4, 5, 7, 8, 12]. These effects have been attributed to interactions between the cationic solute and the residual silanols on the stationary phase [5–8]; several strategies have been proposed to overcome these effects [4, 5, 7, 8, 12, 13].

The present study is concerned with the effects of organic modifiers, solute structure and ion-pairing agent on the various interactions which contribute to the retention of quaternary ammonium ions on ODS-silica. The effects of adding 1-hexanesulphonate to the mobile phase were investigated to assess the contribution of ion-pairing to retention; and various mono- and bis-quaternary ammonium compounds (Scheme 1) were studied to evaluate the contribution of solute charge and hydrophobic substitution to retention.

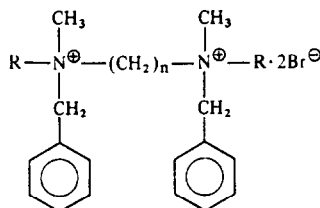
(A) *N*-alkyl-*N*-benzyl-*N,N*-dimethylammonium bromides



Series I :

R-	Abbreviation	Symbol
CH ₃ -	MMQ	○
CH ₃ (CH ₂) ₃ -	BMQ	□
CH ₃ (CH ₂) ₇ -	OMQ	△

(B) *bis*-(*N*-alkyl-*N*-benzyl-*N*-methyl)-1, *n*-methylene diammonium dibromides



Series II :

R-	<i>n</i>	Abbreviation	Symbol
CH ₃ -	2	DMBQ-2	●
CH ₃ (CH ₂) ₃ -	2	DBBQ-2	■
CH ₃ (CH ₂) ₇ -	2	DOBQ-2	▲

Series III :

CH ₃ -	2	DMBQ-2	●
CH ₃ -	3	DMBQ-3	◆
CH ₃ -	4	DMBQ-4	▼
CH ₃ -	6	DMBQ-6	+

Scheme 1 Structures of solutes

Experimental

High-performance liquid chromatography

The liquid chromatograph consisted of an Altex (Berkeley, CA, USA) Model 110A pump, a Rheodyne (Cotati, CA, USA) injector equipped with a 20- μ l sample loop and a Beckman (Berkeley, CA, USA) Model 153 UV detector (254 nm). The column and mobile phase temperatures were maintained at $40.0 \pm 0.2^\circ\text{C}$ with a circulating water bath (Haake, Saddle Brook, NJ, USA) and a column jacket (Alltech, Deerfield, IL, USA). The mobile phase was preheated by passing it through a short precolumn (50×4.6 mm, i.d.) and stainless steel tubing ($1 \text{ m} \times 0.009$ in., i.d.), immersed in a thermostated beaker ($40.0 \pm 0.2^\circ\text{C}$) and positioned between the pump and the injector. The analytical column (100×4.6 mm, i.d.) and the precolumn were slurry packed with 5- μm ODS-Hypersil (Shandon Southern, Sewickley, PA, USA) according to a method previously described [14]. The flow rate was maintained at 1.5 ml/min throughout.

Procedures

Individual solutions (10^{-3} M) of the quaternary ammonium salts were prepared by diluting 250 μ l of stock solution (8×10^{-3} M) in HPLC grade water to 2.0 ml with mobile phase. The t_0 values were determined for each eluent composition, taking the first deviation from baseline after injection of the solutes. Solute injections (10 μ l) were made at least in duplicate, and retention times (t_r) were measured using a Model 8678 Digital Stopwatch (Cole-Parmer, Chicago, IL, USA). Both t_r and t_0 could be determined with a precision of ± 0.2 sec. The capacity factors (k') were calculated according to the conventional equation and t_r measured at the peak maximum in all cases.

An attempt was made to minimize the cation exchange contribution of the residual silanols to solute retention by adjusting the aqueous component of the mobile phase to pH 2.0 with H_2SO_4 [13]. The mobile phases were prepared on a volume fraction (ϕ) basis, by mixing known volumes of the organic modifier (V_{om}) and sulphuric acid solutions (pH 2.0, V_{aq}) (equation 1).

$$\phi = V_{\text{om}} / (V_{\text{om}} + V_{\text{aq}}) \quad (1)$$

Sodium 1-hexanesulphonate (HSA) or sodium nitrate (NaNO_3) was dissolved in mobile phase to give a final concentration of 15 mM.

Reagents

Benzyltrimethylammonium bromide was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and used as received. Methanol, acetonitrile, tetrahydrofuran and sodium 1-hexanesulphonate (Fisher, Springfield, NJ, USA) were all HPLC grade and used without further purification. All water was distilled in glass following mixed-bed deionization. All other chemicals were of reagent grade.

Synthesis

The quaternary ammonium compounds (I, Scheme 1) were prepared by direct alkylation of *N,N*-dimethylbenzylamine with the respective *n*-alkyl bromides and the bis-quaternary ammonium compounds (II and III, Scheme 1) were prepared by direct alkylation of the respective tertiary aliphatic diamines with benzyl bromide. Specific details concerning these syntheses have been presented elsewhere [15]. The identity and

purity of the products were confirmed by elemental analysis (within $\pm 0.4\%$ of theoretical) and chromatographic analysis.

Results and Discussion

The effects of methanol (MeOH), acetonitrile (ACN) and tetrahydrofuran (THF) concentrations ($\phi = 0.0\text{--}0.9$) and sodium 1-hexanesulphonate (HSA, 15 mM) on the retention of quaternized amines were investigated in binary and ternary mobile phases. To isolate the influence of ion-pairing, studies were also performed in the absence of HSA, in which case NaNO_3 (15 mM) was added to the mobile phase to minimize the influence of ionic strength. The other variables (i.e. stationary phase (ODS Hypersil), mobile phase pH (2.0) and column temperature (40°C)) were maintained constant.

The effects of (a) solute structure (charge and substitution) and (b) organic modifier (type and concentration) on the retention of quaternary ammonium ions may be conveniently described by the application of a functional group approach [16] and solvophobic theory [17]. According to solvophobic theory [18–20], the individual contributions to retention may be assessed, and the capacity factor (k') for a given solute is described by equation (2):

$$\ln k' = \frac{\Delta G_{\text{nh}}^\circ}{RT} + \frac{B\gamma}{RT} + \frac{N\gamma\Delta A}{RT} + \ln \frac{V_s}{V_m} + \text{constant} \quad (2)$$

where the theory [18–20] has been extended to include the various non-hydrophobic contributions to retention. ΔA is the decrease in hydrophobic surface area on binding of the solute to the stationary phase, γ is the surface tension of the mobile phase, N is Avogadro's number, R is the universal gas constant, T is the absolute temperature and B is a constant related solely to the properties of the mobile phase. $\Delta G_{\text{nh}}^\circ$ reflects the non-hydrophobic contribution to retention and, for the purpose of this study, can be defined as

$$\Delta G_{\text{nh}}^\circ = \Delta G_s^\circ + \Delta G_{\text{e.m.}}^\circ + \Delta G_{\text{e.s.}}^\circ + \Delta G_{\text{SiOH}}^\circ \quad (3)$$

where ΔG_s° is the contribution of solute solvation to retention, $\Delta G_{\text{e.m.}}^\circ$ and $\Delta G_{\text{e.s.}}^\circ$ reflect the contributions of ion-pairing in the mobile phase and cation exchange involving the solute and adsorbed pairing ion, respectively, and $\Delta G_{\text{SiOH}}^\circ$ accounts for solute interactions with residual silanols on the stationary phase [13, 21, 22]. It should be noted that $\Delta G_{\text{e.m.}}^\circ$ represents the overall contribution of ion-pairing in the mobile phase and is comprised of two terms related to the formation and distribution constants of the ion pair.

The second and third terms (in equation 2) describe the contribution of hydrophobic repulsion, which is related to the mobile phase surface tension. The influence of the stationary phase on retention is restricted to the effects of changes in phase volume ratio (V_s/V_m) (equation 2).

A functional group approach [16] was taken to evaluate the contribution of methylene substitution (CH_2) and the charged nitrogen (N^+) to retention. The selectivity factor (α) is related to the extra thermodynamic group contribution term τ by equation (4) [16]:

$$\tau = \log \alpha = \log k'_2/k'_1 \quad (4)$$

where subscripts 1 and 2 refer to the unsubstituted and substituted solutes, respectively.

By the application of solvophobic theory (equation 2), the various contributions to τ may be assessed (equation 5) [16].

$$\tau = \frac{\Delta(\Delta G_{\text{nh}}^{\circ}) + N\gamma\Delta(\Delta A)}{2.3 RT} \quad (5)$$

Equation (5) predicts that the selectivity in RP-HPLC under constant chromatographic conditions for quaternary ammonium ions arises from differences in reduction of exposed hydrophobic surface area (methylene substitution) and solvation of the charged nitrogens by the mobile phase.

Non-ion pair systems (containing NaNO₃)

Solute structure. For the simplest system in which no ion-pairing agent is added to the mobile phase, it is to be expected that $\Delta(\Delta G_{\text{nh}}^{\circ}) \approx \Delta(\Delta G_{\text{s}}^{\circ})$. Table 1 shows the k'

Table 1

The capacity factors of the mono- and bis-quaternary ammonium compounds and the τ values describing the effects of hydrophobic substitution, in mobile phases containing methanol and acetonitrile*

Solute†	Functional group difference	K'		τ^{\ddagger}		$\tau_{(\text{CH}_2)^{\S}}$	
		MeOH	ACN	MeOH	ACN	MeOH	ACN
I. MMQ		2.68	2.68				
a. BMQ	CH ₂ CH ₂ CH ₂	6.76	5.10	0.40	0.28	0.13	0.09
II. DFMBQ-2		0.59	0.94				
a. DBBQ-2	(CH ₂ CH ₂ CH ₂) ₂	1.84	3.91	0.49	0.62	0.08	0.10
III. DMBQ-2		0.59	0.94				
a. DMBO-3	CH ₂	0.79	0.13	0.13	0.08	0.13	0.08
b. DMBQ-4	CH ₂	0.95	1.28	0.08	0.05	0.08	0.05
c. DMBQ-6	(CH ₂) ₂	1.72	1.93	0.26	0.18	0.13	0.09

* Stationary phase: ODS Hypersil (100 × 4.6 mm i.d.); mobile phase: sulphuric acid (pH 2.0) and MeOH ($\phi = 0.4$) or ACN ($\phi = 0.364$) containing 15 mM NaNO₃; temperature: 40°C.

† See Scheme 1 for structures and classification.

‡ Calculated according to equation (4).

§ $\tau_{(\text{CH}_2)} = \tau/p$, where p is the number of CH₂ groups.

values for the solutes and the corresponding group contribution values calculated in mobile phases containing MeOH ($\phi = 0.40$) and ACN ($\phi = 0.364$) (where $\phi =$ volume fraction, equation 1). The values in Table 1 were calculated from the data in Figs 1 and 2, and the organic modifier concentrations were chosen such that $k'_{\text{MMQ}} = 2.68$ in both eluents. A similar analysis of the data for OMQ and DOBQ-2 was not possible since they did not elute from the column in these mobile phases.

The methylene increments ($\tau_{(\text{CH}_2)}$, Table 1) ranged between 0.08 and 0.13, and 0.05 and 0.10 in eluents containing MeOH ($\phi = 0.40$) and ACH ($\phi = 0.364$), respectively. The relationship between the τ values obtained from these two eluents is shown in Fig. 3, which is linear (with the exception of the data derived from DBBQ-2), having a slope of

Figure 1
Relationship between the logarithm of the capacity factor and methanol concentration (ϕ) for the nine quaternary and bis-quaternary ammonium ions. Key: MMQ (○), BMQ (□), OMQ (△), DMBQ-2 (●), DMBQ-3 (◆), DMBQ-4 (▼), DMBQ-6 (+), DBBQ-2 (■) and DOBQ-2 (▲). Stationary phase: ODS-Hypersil (100 × 4.6 mm i.d.). Mobile phase: sulphuric acid (pH 2.0) and methanol, containing 15 mM NaNO₃. Temperature: 40°C.

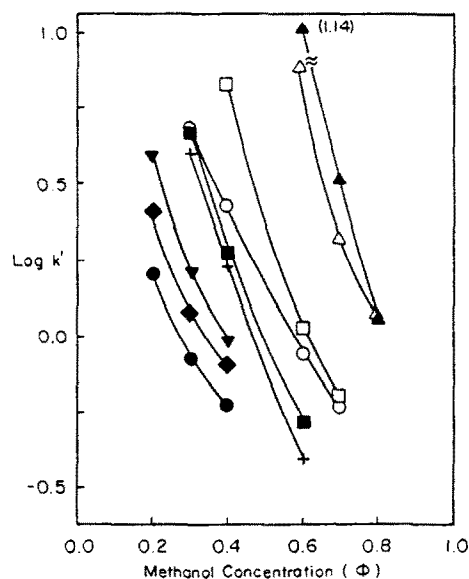
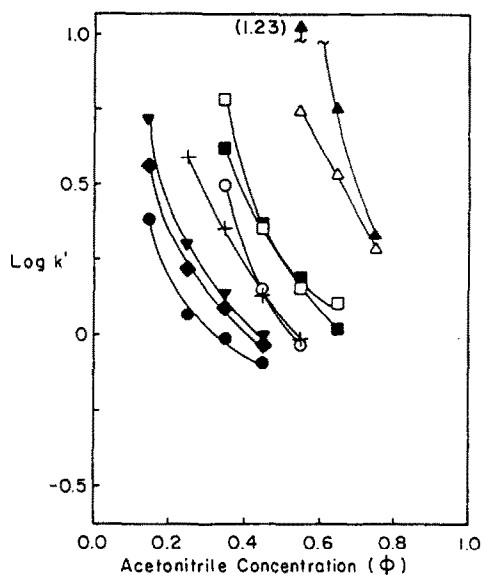
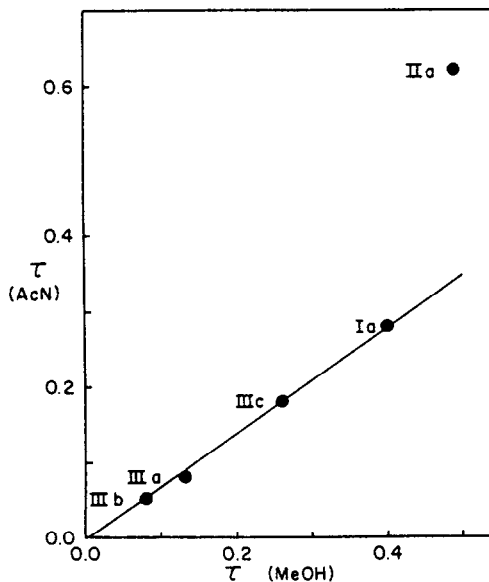


Figure 2
Relationship between the logarithm of the capacity factor and acetonitrile concentration (ϕ) for the nine quaternary and bis-quaternary ammonium ions. Key and other conditions as in Fig. 1.



0.73 and a negligible intercept. The deviation of this slope from unity reflects the differences in surface tension of the two mobile phases (equation 2) [23]. The intercept was not significantly different from zero, indicating that the contribution of hydrophobic substitution to $\Delta(\Delta G_{\text{nh}}^{\circ})$ is constant in the two eluents. The deviation of $\tau_{(\text{CH}_2\text{CH}_2\text{CH}_2)_2}$ from the general relationship (Fig. 3) presumably reflects a difference in the contribution of the two butyl groups to the solvation of the charged groups by the two eluents. No deviation from the relationship was observed for $\tau_{(\text{CH}_2\text{CH}_2\text{CH}_2)}$ derived from the mono-quaternary ammonium ions (MMQ and BMQ) (Fig. 3, Table 1), indicating that

Figure 3
The relationship between $\tau(\text{ACN})$ and $\tau(\text{MeOH})$. τ values were taken from Table 1 and are labelled accordingly.



increasing the size of one of the substituents on the charged nitrogen does not influence the value of $\tau_{(\text{N}^+)}$ ($\approx \Delta(\Delta G_{\text{nh}}^\circ)$).

The contribution of the positively charged nitrogen to retention ($\tau_{(\text{N}^+)}$) was calculated, and the influences of solvent and hydrophobic substitution on $\tau_{(\text{N}^+)}$ was determined as follows. When two solutes differ by one or more functional groups, the capacity factor of the more highly substituted solute (solute 2) can be predicted from that of the other (solute 1) from:

$$\log k_2 = \log k_1 + \sum_i \tau_i \quad (6)$$

where τ_i are the individual group contribution terms relating solutes 1 and 2 [24]. Riley *et al.* [16, 24] have shown that τ values may be obtained directly from chromatographic measurements. Alternatively, τ may be calculated from literature values since it is linearly related (equation 7) to the hydrophobic group contribution values π [25], which were calculated from octanol–water partition coefficients for substituted benzenes [25, 26]:

$$\tau = m\pi + c, \quad (7)$$

where m and c are the slope and intercept coefficients of the linear regression. The slope coefficient m is dependent on the chromatographic system and may be obtained from the literature [24].

Thus, the contribution of the positively charged nitrogen, $\tau_{(\text{N}^+)}$, to retention was calculated from the retention data for DMBQ-2 and MMQ (Table 1) using equation (8) [27]:

$$\tau_{(\text{N}^+)} = \log k'_{\text{DMBQ-2}} - \log k'_{\text{MMQ}} - \tau_{(\text{CH}_3)} - 3\tau_{(\text{CH}_2)} - \tau_{(\text{C}_6\text{H}_5)}. \quad (8)$$

The $\tau_{(\text{CH}_2)}$ values (Table 2) were taken from the means of those in Table 1 and those for CH_3 and C_6H_5 were obtained from literature values of π [25] and m (equation 8) [16, 24].

Table 2

τ Values used in the calculation of $\tau_{(\text{N}^+)}$ in mobile phases containing MeOH ($\phi = 0.40$) and ACN ($\phi = 0.364$)

Functional group	τ^*	
	MeOH	ACN
CH_2	0.12 [†]	0.08 [†]
CH_3	0.29 [‡]	0.16 [‡]
C_6H_5	1.11 [‡]	0.60 [‡]

* Equation (4).

† Mean of values from Table 1.

‡ From π values [25, 26] after correction for mobile phase effects (equation 10) [24] and temperature effects [16].

The values for $\tau_{(\text{N}^-)}$ were -2.42 in MeOH ($\phi = 0.40$) and -1.46 in ACN ($\phi = 0.364$). Similar calculations using the data for the butyl-substituted mono- and bis-quaternary compounds (BMQ and DBBQ-2) gave values of -2.68 and -1.36 in eluents containing MeOH ($\phi = 0.4$) and ACN ($\phi = 0.364$), respectively. The differences in $\tau_{(\text{N}^-)}$ as calculated for the two model compounds ($\Delta(\Delta G_{\text{nh}}^\circ)$) (-2.42 vs -2.68 and -1.46 vs -1.36 in MeOH and ACN, respectively) most probably arise from the steric effects of the substituents on the accessibility of the solvent to the positively charged nitrogen (ΔG_s°); they may also arise from the accessibility of the solute to the silica support ($\Delta G_{\text{SiOH}}^\circ$). This explanation would be in accordance with the hypothesis presented above, namely that the size of the substituents on the positively charged nitrogen of the bis-quaternary ammonium ions influences the contribution ($\tau_{(\text{N}^-)}$) of the charged group to retention (Fig. 3).

Organic modifiers

Solute retention decreased with increasing concentrations of MeOH and ACN for all analytes in non-ion pairing systems (Figs 1 and 2). However, the dependence of retention on organic modifier concentration was less in eluents containing ACN compared with those containing MeOH. Thus, at low concentrations of organic modifier, the solutes were eluted more rapidly in the presence of ACN than in the presence of MeOH, whereas the reverse was true at higher concentrations. The differences in retention behaviour in the presence of these two organic modifiers presumably reflects the differences in the interactions of these organic modifiers with the solutes (equation 2).

If retention were governed solely by the surface tension of the mobile phase, then the solutes would be retained to a lesser extent in the presence of ACN than in the presence of MeOH at all concentrations over the range $\phi = 0.0$ – 0.7 (Figs 1 and 2) [28]. Figures 1 and 2 suggest that the overall decrease in retention with increasing organic modifier concentration arises from the resultant decrease in mobile phase surface tension. However, at higher concentrations of ACN and MeOH, the contribution of the organic modifier to the solvation of the solute (ΔG_s°) becomes more significant. This latter hypothesis is supported by the results previously presented in which the contribution of

the positively charged nitrogen ($\tau_{(N^+)}$) to k' was found to be about an order of magnitude different in eluents containing ACN ($\phi = 0.364$) compared with those containing MeOH ($\phi = 0.40$).

In mobile phases in which low concentrations of THF ($\phi \leq 0.05$) were used as organic modifiers, most of the solutes eluted rapidly ($k' < 2$, Fig. 4). Therefore, it was not possible to apply the same vigorous treatment to the data obtained in mobile phases incorporating this organic modifier as was applied to the data for the MeOH and ACN eluents. However, Fig. 4 suggests that THF may offer certain selectivity advantages, compared with MeOH and ACN (Figs 1 and 2), as borne out in subsequent studies with ion-pair systems, in which retention is prolonged.

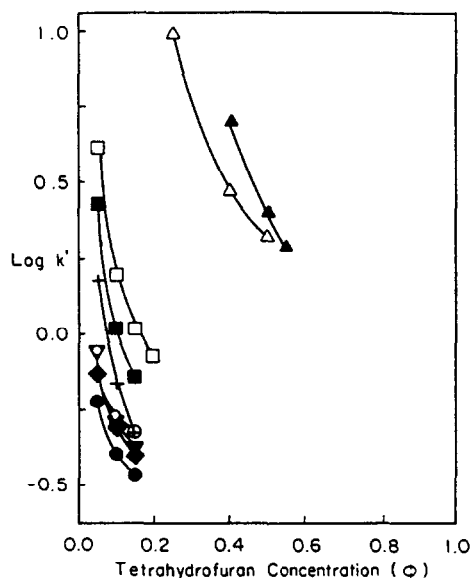


Figure 4
Relationship between the logarithm of the capacity factor and tetrahydrofuran concentration (ϕ) for the nine quaternary and bis-quaternary ammonium ions. Key and other conditions as in Fig. 1.

Ion-pair systems containing sodium 1-hexanesulphonate (HSA)

Binary systems. In general, the replacement of NaNO_3 by HSA (15 mM) in the mobile phase increased the retention of the solutes (Figs 5 and 6). However, the influence of the pairing ion (HSA) on retention was dependent upon the organic modifier (type and concentration) and solute structure (charge and substitution) (Figs 5–7). As for binary eluents containing NaNO_3 as the electrolyte, the retention of all the solutes decreased with increasing concentration of organic modifier. Previous studies [29] suggest that the ion-pair contribution from nitrate should be negligible in these systems.

Figure 5 shows the effect of HSA on the retention of (a) MMQ and (b) DMBQ-2 as a function of organic modifier concentration (ϕ). The pairing ion has the greatest effect in eluents containing MeOH and the least effect in those containing ACN. In mobile phases containing ACN or THF, the effect of ion-pairing agent decreased with increasing concentration of organic modifier. This behaviour is consistent with an 'ion exchange' retention mechanism [30, 31], where k' decreases as the amount of adsorbed HSA available for interaction with the solute decreases at higher concentrations or organic modifier [32] (i.e. $\Delta G_{e.s.}^\circ$ decreases). The retention of MMQ and DMBQ-2 was independent of HSA at $\phi = 0.45$ and $\phi = 0.22$, respectively, in eluents containing ACN

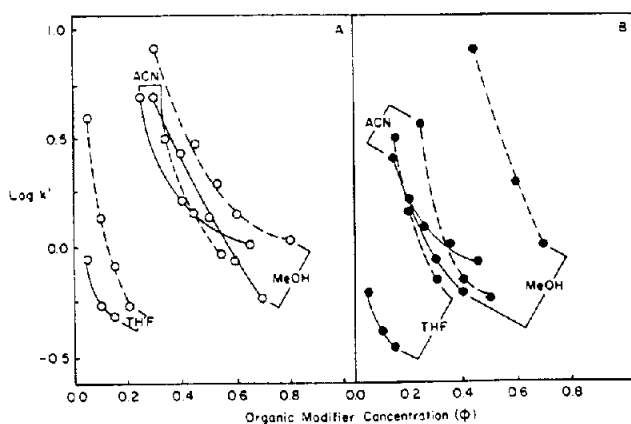


Figure 5
The effect of HSA (15 mM) on the retention of (a) MMQ and (b) DMBQ-2 as a function of organic modifier concentration (ϕ). Key: solid lines (—), non-ion pair eluents (15 mM, NaNO_3) and dashed lines (---), ion pair eluents (15 mM, HSA). Conditions as in Fig. 1.

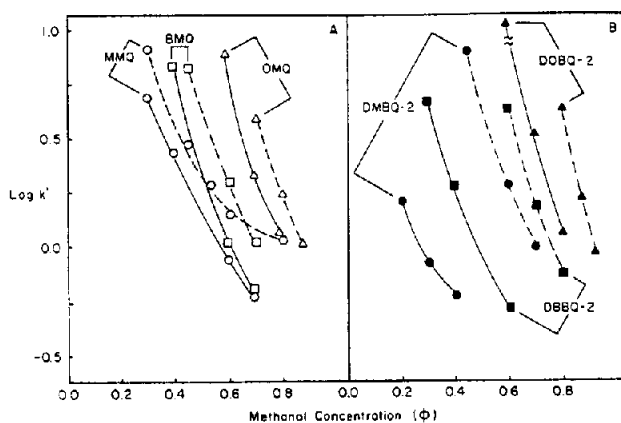


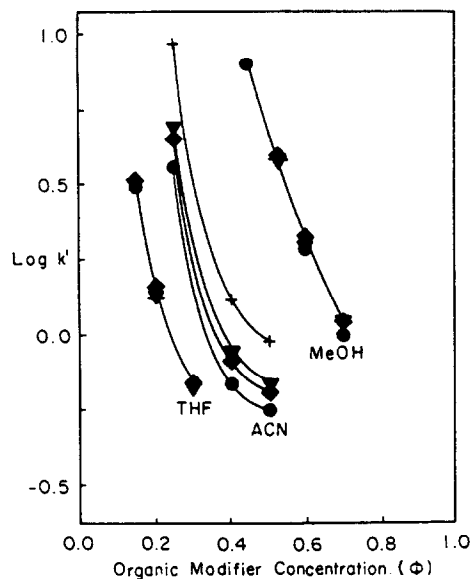
Figure 6
The effect of HSA (15 mM) and hydrophobic substitution on the retention of (a) series I and (b) series II solutes (scheme 1) as a function of methanol concentration (ϕ). Key: as in Fig. 5. Conditions as in Fig. 1.

(Fig. 5). At higher concentrations of ACN, HSA actually promoted elution, perhaps due to the effect of ion-ion interactions in the mobile phase decreasing the overall charge of the solute, which in turn results in a more negative contribution of ΔG° , (equation 3) to retention. This implies that ion pairs between HSA and quaternary ammonium ions are better solvated than the corresponding non-aggregated ionic species in eluents containing ACN [33], and are therefore less retained in the presence of HSA.

By contrast, the influence of HSA on solute retention increased with increasing concentration of MeOH (Fig. 5), which may reflect an increase in the resultant contribution of ion-pair formation and distribution ($\Delta G^\circ_{e.m.}$) and more than compensates for the decrease in retention due to the desorption of pairing ion from the stationary phase ($\Delta G^\circ_{e.s.}$) [32] at higher MeOH concentrations.

Figure 7

The effect of organic modifier type on the retention of the series III bis-quaternary ammonium ions as a function of organic modifier concentration (ϕ) in eluents containing 15 mM HSA. Key and other conditions as in Fig. 1. Some lines have been omitted for clarity.



The retention of quaternary ammonium ions in the presence of HSA was influenced by the charge number, since the pairing ion enhanced the retention of the bis-quaternary ammonium ions to a greater extent than for the mono-quaternary ammonium ions (Fig. 5). The data in Fig. 5 are consistent with higher order aggregates between the bis-quaternary ammonium ions (series II) and HSA, relative to the expected 1:1 interaction between the mono-quaternary ammonium ions (series I) and HSA. In previous reports [16, 33] concerned with ion-pair RP-HPLC, 2:1 interactions between di-anionic solutes and benzyltrimethylammonium pairing ions have been observed.

In addition to the effects of charge, the retention of quaternary and bis-quaternary ammonium ions (series I and II) in eluents containing HSA was influenced by the size of the hydrocarbonaceous functional group, R (Fig. 6). Retention increased with increasing size of R ; however, the influence of HSA on retention decreased as R increased. The latter effect most probably reflects the decreasing surface tension of the mobile phase (since $N\gamma\Delta(\Delta A)/RT$ decreases) with increasing organic modifier concentration and/or the increasing steric influence of R on the extent of ion-pair formation.

It appears that the hydrophobic selectivity observed in non-ion pair systems is maintained in eluents containing HSA when R is varied (Fig. 6). However, the hydrophobic selectivity arising from differences in the length of the carbon bridge (n) separating the charges is reduced or lost completely in the presence of HSA (Fig. 7). Very low hydrophobic selectivity ($0.01 < \tau_{(CH_2)} < 0.03$) was observed for the series III bis-quaternary ammonium compounds in eluents containing MeOH or THF. However, the selectivity increased with increasing concentration of organic modifier in these two systems (Fig. 7), which presumably reflects the decreasing contribution of ion-pairing with increasing concentration of organic modifier (see Fig. 5), allowing the hydrophobic contribution of the 'free' ions (Figs 1 and 4) to be expressed (Fig. 7). This explanation is supported by the data for the series III ions in ACN eluents, for which the hydrophobic selectivity observed for these ions in non-ion pair systems (Fig. 2) was only partially reduced in eluents containing HSA (Fig. 7), where ion-ion interactions were shown to contribute much less to retention (Fig. 5).

Ternary systems. Since solute selectivity was influenced by the nature of the organic modifier and its concentration in the mobile phase, it was of interest to study the combined effects of two organic modifiers in ternary eluents (Figs 8 and 9). These studies were restricted to mobile phases containing HSA, which enhanced retention and permitted the specific effects of the solvents to be observed over reasonable concentration ranges. Also, the addition of HSA to the mobile phase offers further selectivity advantages, particularly with respect to separations based on charge number.

As with the binary eluents, the aqueous component of the eluent was dilute sulphuric acid (pH 2.0) and the HSA concentration in the mobile phase was maintained at 15 mM. Two concentrations of THF were studied ($\phi = 0.1$ and 0.2), and the effects of ACN ($\phi = 0.0$ –0.6) and MeOH ($\phi = 0.0$ –0.7) on retention were examined (Figs 8 and 9). As seen

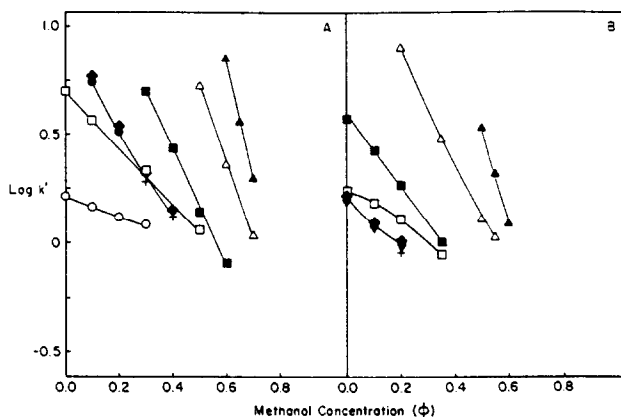


Figure 8

The relationship between the logarithm of the capacity factor and methanol concentration (ϕ) for the nine quaternary and bis-quaternary ammonium ions in ternary eluents containing 15 mM HSA and either (a) 10% THF or (b) 20% THF. Key and other conditions as in Fig. 1. Some lines have been omitted for clarity.

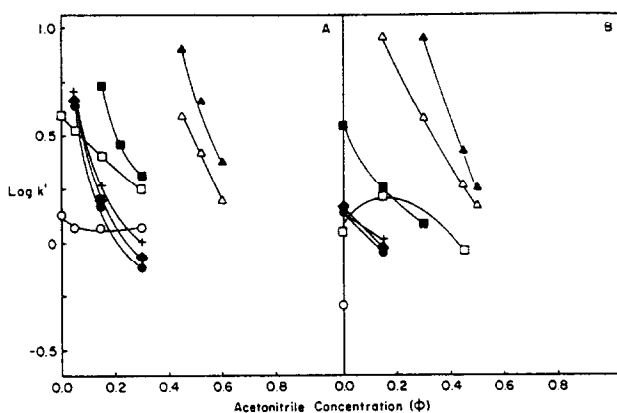


Figure 9

The relationship between the logarithm of the capacity factor and acetonitrile concentration (ϕ) for the nine quaternary and bis-quaternary ammonium ions in ternary eluents containing 15 mM HSA with either (a) 10% THF or (b) 20% THF. Key and other conditions as in Fig. 1. Some lines have been omitted for clarity.

previously by Schoenmakers *et al.* for neutral molecules [32], the results obtained in ternary eluents containing pairing ion (HSA) were consistent with and could be predicted from those obtained in the binary eluents.

Since solute selectivities and the effects of HSA were similar in binary eluents prepared with MeOH or THF, ternary systems containing these two organic modifiers exhibited little advantage for the ion-pair separation of quaternary ammonium ions (Fig. 8). However, the selectivities arising from differences in charge number were potentiated by using mixtures of these two organic modifiers at lower individual concentrations. In binary eluents containing HSA, there was some indication that the retention of the mono-quaternary ammonium ions decreased less rapidly than the retention of the bis-quaternary ammonium ions with increasing concentrations of MeOH (Fig. 5). These differences were more pronounced (Fig. 8) when the mobile phase also contained low concentrations of THF ($\phi = 0.1$ or 0.2). As seen previously for binary systems, poor selectivity with respect to series III bis-quaternary ammonium ions was observed in ternary eluents containing both THF and MeOH with HSA (Fig. 8).

In ternary eluents containing ACN ($\phi_{\text{THF}} = 0.1$ and 0.2 , Fig. 9), the dependence of charge selectivity ($\tau_{(N^-)}$) on ACN concentration was more dramatic (Fig. 9). In these systems (Fig. 9), the retention of the smaller series I ions (MMQ and BMQ) was much less dependent on the concentration of ACN compared with the series II and III ions, and in one case (BMQ, Fig. 9b) retention even increased with increasing ACN concentration. As predicted from the studies in binary eluents, the ion-pair separation of the series III bis-quaternary ammonium ions was improved in the presence of ACN in the mobile phase and selectivity increased with increasing concentration of this organic modifier (Fig. 9).

Conclusions

The contribution of the various factors which influence the retention of mono- and bis-quaternary ammonium ions in RP-HPLC can be adequately described within the framework of solvophobic theory [18–20] and the application of a functional group approach [16]. In non-ion pair eluents, hydrophobic substitution on the positively charged nitrogen influences solute retention primarily as a result of hydrophobic effects ($N\gamma (\Delta A)/2.3$ RT, equation 5), whereas the influence of an additional positive charge (i.e. di-cationic solutes) on retention was predominantly controlled by $\Delta G^{\circ}_{\text{nh}}$ (equation 5), which reflects the extent of solvation of this positive charge in the respective eluents. Similarly, the influence of organic modifier type and concentration on retention results primarily from solvophobic factors, although at higher concentrations of organic modifier the influence of the solvation of the positive charge (ΔG°_s) by the eluent becomes an important contributor to solute retention.

At low concentrations of organic modifiers in eluents containing HSA, retention appears to be largely controlled by ion–ion interactions in the stationary phase ($\Delta G^{\circ}_{\text{e.s.}}$) and as the concentration of organic modifier increases, ion–ion interactions in the mobile phase ($\Delta G^{\circ}_{\text{e.m.}}$) become increasingly important. Since $\Delta G^{\circ}_{\text{e.m.}}$ reflects both the extent of ion-aggregation and its distribution, this term can result in either positive or negative contributions to retention as was observed for solutes in MeOH and ACN mobile phases, respectively, containing HSA [34].

The retention of quaternary and bis-quaternary ammonium compounds was also investigated in ternary eluents containing HSA. These eluents, however, offered little

advantage in solute selectivity when compared with binary eluents containing HSA, other than in separations based on solute charge.

Although binary eluents in the absence of HSA have shown a distinct advantage in the ability to resolve cationic solutes of similar structure (i.e. series III solutes), the peak tailing exhibited by series III solutes in these eluents greatly reduces their practical utility. Generally, organic modifiers improved solute peak shape in the order THF > ACN > MeOH. Although further improvements in peak shape were achieved in eluents containing HSA (at low concentration of organic modifier), these advantages must be weighed against the reduced selectivity of these eluents for closely related solutes (series III ions). As expected, ternary eluents containing HSA gave peak shapes intermediate to those observed in the corresponding binary systems.

It is concluded, therefore, that eluents comprising THF/aqueous H₂SO₄ (pH 2.0) together with an ion-pairing agent offer the best possibilities for the RP-HPLC separation of quaternary ammonium compounds when solute selectivity is large. In situations where additional selectivity is required, eluents containing HSA and either ACN or ACN and THF should be considered.

Although these studies were performed on quaternary and bis-quaternary ammonium ions, it remains to be seen whether or not these findings will be generally applicable to other singly- and doubly-charged cationic solutes, such as protonated amines.

Acknowledgement: This work was supported in part by NIH Training Grant CA-09242, awarded by the National Cancer Institute.

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[First received for review 14 January 1983; revised manuscript received 10 March 1983]