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STUDIES IN ION-PAIR CHROMATOGRAPHY

II. RETENTION OF POSITIVE AND NEGATIVE IONS AND NEUTRAL SOLUTES IN TETRABUTYLAMMONIUM BROMIDE-CONTAINING METHANOL-WATER ELUENTS ON LICHROSORB RP-18*

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SUMMARY

The retention of positively and negatively charged ions, as well as of neutral solutes, was studied in 0–70% methanol in 25 mM aqueous phosphate buffer (pH 2.1–3.4) eluents as a function of the tetrabutylammonium bromide ion pair reagent concentration. The $\log k'$ vs. mobile phase pairing ion concentration (P_m) plots (which showed P_m - and [methanol]-dependent maxima) could be transformed into $\log k'$ vs. stationary phase pairing ion concentration (P_s) curves by using the simultaneously determined adsorption isotherms of tetrabutylammonium bromide. While the shape of the $\log k'$ vs. P_m curves of oppositely charged solutes changed considerably with the concentration of methanol in the eluent, the $\log k'$ vs. P_s curves were more similar, and their maxima are in the 80–120 $\mu\text{mole/g}$ range. The $\log k'$ vs. P_m curves of similarly charged solutes were more alike. The methanol concentration of the eluent influences the retention of oppositely charged solutes more sensitively than that of the similarly charged solutes. At constant P_m the $\log k'$ vs. [methanol] curves of oppositely charged solutes were S-shaped, while the $\log k'$ vs. [methanol] curves at constant P_s values ran similar to the $\log k'$ vs. [methanol] curves obtained in the absence of ion-pairing reagents.

INTRODUCTION

Although the retention of ionic solutes in reversed-phase ion pair liquid chromatography depends on numerous factors, the primary control variable is the mobile phase pairing ion concentration. Recent experimental evidence^{1–3} shows that retention is fundamentally related to the stationary phase pairing-ion concentration which, in turn, depends on both the mobile phase concentration of the pairing ion and the organic modifier⁴.

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The aim of this work was to obtain solute retention data and pairing-ion adsorption isotherm data⁴ over sufficiently wide (but chromatographically meaningful) parameter ranges and to test the data against the major models of reversed-phase ion-pair chromatography.

The system selected consisted of a LiChrosorb RP-18 column and of mixtures (0–70%) of methanol in 25 mM orthophosphoric acid–25 mM sodium orthophosphate aqueous buffer eluents in which a constant Br⁻ ion concentration of 200 mM was maintained. The pairing ion used was tetrabutylammonium bromide.

The log capacity factor (k') vs. mobile (P_m) and stationary phase (P_s) pairing-ion concentration curves (at constant methanol concentrations) and also the log k' vs. [methanol] curves of oppositely and like charged ionic solutes and neutral components (at constant P_m and P_s) are presented here, while their analysis by the various retention models will be published in Part III⁵.

EXPERIMENTAL

Materials

Ethanesulphonic acid (EtSO₃⁻), sodium butylsulphonate (BuSO₃⁻), sodium pentanesulphonate (PeSO₃⁻), sodium hexanesulphonate (HexSO₃⁻), sodium heptanesulphonate (HepSO₃⁻), sodium octanesulphonate (OcSO₃⁻), N,N-dibenzylamine [(ArCH₂)₂NH] and tetrabutylammonium bromide (TBABr) were from Fluka (Buchs, Switzerland); *d,l*-10-camphorsulphonic acid (DCSA⁻), *p*-toluenesulphonic acid (PTSA⁻), hexylamine (HexNH₂), N-benzyl-N,N,N-triethylammonium chloride (ArCH₂Et₃N⁺), aniline (A), N,N-dimethylaniline (Me₂A), N,N-diethylaniline (Et₂A), benzene (B), toluene (T), methyl iodide (MeI), diphenyl iodonium chloride (Ar₂I⁺), NaBr, H₃PO₄, NaH₂PO₄ and methanol (MeOH) (reagent grade) were from Reanal (Budapest, Hungary).

Experimental method

The equipment, measurement conditions and eluent preparation method were described in ref. 4.

Test substances were dissolved in the eluents (10⁻⁷–10⁻⁵ M) and 20- μ l samples were injected. At least three measurements were averaged and used for the k' calculations ($\pm 1.5\%$ relative standard deviation). Eluents saturated with NaBr were injected for the dead volume determinations (refractive index detector trace).

RESULTS

The test substances were selected in such a manner that their degree of ionization did not change in the pH range of the eluents used (2.1–3.4). The k' values are listed in Tables I–VII.

Effects of the mobile and stationary phase concentration of the ion-pairing reagent

The log k' values of PTSA⁻ and DCSA⁻ are plotted in Figs. 1–4 against the mobile phase concentration (P_m) and stationary phase concentration (P_s) of TBABr. Initially, log k' increases with P_m , then passes a maximum¹ or tends to level off. The higher the methanol concentration, the higher the P_m where the leveling off occurs.

TABLE I
 k' VALUES IN PURE AQUEOUS PHOSPHATE BUFFER (pH 2.1) ELUENT

Solute	k'							
	P_m (mM)							
	0	0.5	1	2	3	5	10	20
PTSA	12.579	26.725	27.487	26.567	26.596	26.27	25.962	24.751
EtSO ₃	0.225	0.435	0.413	0.41	0.386	—	0.38	0.364
BuSO ₃	3.727	5.576	5.253	4.757	4.663	4.652	4.308	3.854
PeSO ₃	4.232	21.714	21.223	—	—	11.989	—	—
A	3.76	0.494	0.465	0.343	0.288	0.258	0.217	0.18
Me ₂ A	19.4	1.981	1.814	1.201	1.041	0.925	0.787	0.632
MeI	14.14	14.714	14.836	14.694	14.73	14.764	14.844	14.743
P_s (μ mole/g)	0	83	97	110	117	127	140	154

TABLE II
 k' VALUES IN 10% METHANOL IN PHOSPHATE BUFFER (pH 2.36) ELUENT

Solute	k'							
	P_m (mM)							
	0	1	2	3	5	10	20	35
PTSA	3.695	8.401	9.391	10.219	10.863	11.554	12.293	12.47
DCSA	10.093	13.057	13.215	—	12.457	11.493	11.059	10.254
BuSO ₃	1.369	2.452	4.491	2.53	2.777	2.815	—	2.668
PeSO ₃	5.93	0.616	10.032	10.1	9.457	9.475	—	8.985
A	1.104	0.401	0.30	0.276	0.223	0.196	0.167	0.16
Me ₂ A	3.251	0.878	0.656	0.62	0.50	0.413	0.356	0.343
P_s (μ mole/g)	0	55	70	80	93	111	131	144

TABLE III
 k' VALUES IN 25% METHANOL IN PHOSPHATE BUFFER (pH 2.5) ELUENT

Solute	k'						
	P_m (mM)						
	0	1	2	5	10	20	35
PTSA	1.446	2.25	2.818	3.475	4.157	4.838	4.968
DCSA	2.961	3.732	4.182	4.361	4.686	4.727	4.561
PeSO ₃	2.25	3.096	3.539	4.082	4.475	4.712	4.799
A	0.70	0.40	0.336	0.232	0.186	0.151	0.09
Me ₂ A	2.975	0.829	0.757	0.511	0.414	—	—
P_s (μ mole/g)	0	23	35	54	72	92	110

TABLE IV

 k' VALUES IN 37.5% METHANOL IN PHOSPHATE BUFFER (pH 2.75) ELUENT

Solute	k'							
	P_m (mM)							
	0	1	2	5	10	20	35	60
PTSA	0.629	0.771	0.878	1.158	1.433	1.85	2.151	2.498
DCSA	1.151	1.19	1.308	1.543	1.783	1.989	2.096	2.333
BuSO ₃	0.36	0.437	0.498	0.583	0.722	0.868	0.904	1.067
PeSO ₃	0.165	1.208	1.315	1.658	—	2.199	2.382	2.719
HexSO ₃	3.076	3.326	—	4.46	5.054	6.096	6.489	7.18
HepSO ₃	8.604	9.459	10.315	12.277	13.834	15.941	17.051	18.929
Et ₂ A	1.561	0.760	0.685	0.507	0.394	0.335	0.257	0.228
Ar ₂ I	2.928	1.305	1.09	0.838	0.679	0.61	0.496	0.416
ArCH ₂ Et ₃ N	1.241	0.591	0.524	0.363	0.264	0.21	0.162	0.135
B	13.029	12.701	12.699	12.604	12.729	12.835	12.39	12.094
P_s (μ mole/g)	0	7.7	13	25	38	55	72	90

TABLE V

 k' VALUES IN 50% METHANOL IN PHOSPHATE BUFFER (pH 3.0) ELUENT

Solute	k'							
	P_m (mM)							
	0	1	2	3	10	20	35	60
PTSA	0.280	0.318	0.343	0.392	0.496	0.645	0.761	0.9005
DCSA	0.456	0.468	0.521	0.561	0.627	—	0.833	0.963
HexSO ₃	1.224	—	1.364	1.558	1.775	2.043	2.272	2.524
HepSO ₃	2.836	2.989	3.164	3.464	3.88	4.652	5.007	5.495
Et ₂ A	0.477	0.389	0.35	0.313	0.257	0.214	0.178	0.161
Ar ₂ I	0.658	0.482	0.45	0.396	0.351	0.312	0.246	0.212
(ArCH ₂) ₂ NH	1.434	1.229	1.171	1.036	0.920	0.764	0.63	0.487
HexNH ₂	1.004	0.875	0.925	0.773	0.674	0.609	0.486	—
B	5.968	5.950	5.936	5.924	5.899	5.851	5.641	5.432
P_s (μ mole/g)	0	3.2	5.6	11.7	20	31	44	59

For different compounds (e.g. PTSA⁻ and DCSA⁻) the maxima occur at different [methanol] and P_m values.

When the $\log k'$ vs. P_m curves of PTSA⁻ and DCSA⁻ are replotted as $\log k'$ vs. P_s (using the simultaneously determined adsorption isotherm values of TBABr⁴⁺) the curves again show a maximum (or leveling off) in the 80–120 μ mole/g P_s range. Unlike the $\log k'$ vs. P_m curves, the $\log k'$ vs. P_s curves do not change significantly with the methanol concentration of the eluent. Increasing methanol concentration decreases the retention of oppositely charged solutes and slightly increases the initial slope of the $\log k'$ vs. P_s curves.

TABLE VI

 k' VALUES IN 60% METHANOL IN PHOSPHATE BUFFER (pH 3.12) ELUENT

Solute	k'							
	P_m (mM)							
	0	2	5	10	20	35	60	90
PTSA	0.178	0.173	0.209	0.239	0.297	0.362	0.436	0.519
DCSA	0.261	0.277	0.281	0.315	0.341	0.442	0.48	0.53
BuSO ₃	0.134	0.14	0.155	0.181	—	0.257	—	0.348
HexSO ₃	0.656	0.676	0.802	—	0.859	0.975	1.099	1.222
HepSO ₃	1.268	1.349	1.41	1.536	1.681	1.884	2.099	2.311
OctSO ₃	2.424	2.673	2.773	2.953	3.232	3.594	3.96	4.193
Ar ₂ I	0.315	0.284	—	0.241	0.181	0.159	0.147	—
(ArCH ₂) ₂ NH	0.638	0.55	0.507	0.471	0.409	0.348	0.3	0.252
B	3.147	3.176	3.137	3.134	3.076	3.0	2.86	2.719
T	6.221	6.227	6.140	6.123	5.964	5.757	4.454	—
MeI	1.739	1.723	1.716	1.725	1.707	1.67	1.645	1.593
P_s (μmole/g)	0	2.6	5.6	10.1	18	26	38	49

TABLE VII

 k' VALUES IN 70% METHANOL IN PHOSPHATE BUFFER (pH 3.4) ELUENT

Solute	k'						
	P_m (mM)						
	0	2	5	10	20	35	80
HepSO ₃	0.608	0.622	0.644	0.766	0.716	0.784	0.903
OctSO ₃	1.032	1.094	1.129	1.176	1.245	1.338	1.491
ArCH ₂ Et ₃ N	0.158	0.14	0.126	0.188	0.101	0.083	—
B	1.655	1.647	1.647	1.644	1.604	1.579	1.444
T	2.96	2.896	2.914	2.896	2.799	2.755	2.502
P_s (μmole/g)	0	1.25	3	5.6	10.4	16	30

In methanol-rich eluents the maxima of $\log k'$ could not be achieved even with fairly high concentrations of mobile phase TBABr.

Fig. 5 shows the $\log k'$ vs. P_s curves for various negatively charged ions, measured in 37.5% methanol eluents. For the sulphonic acids the trends of the curves are similar but the relative changes in $\log k'$ are different. Surface concentration changes of the pairing ion can even cause a reversal of the elution order (*cf.*, *e.g.*, PeSO_3^- , PTSA^- and DCSA^-).

As shown in Fig. 6, the $\log k'$ vs. P_s curves of the various *n*-alkyl sulphonates are almost parallel with only a slight decrease with increasing length of carbon chain.

From among the like-charged solutes, the $\log k'$ vs. P_m and $\log k'$ vs. P_s relationships for diphenyl iodonium (Ar_2I^+) chloride are shown in Figs. 7 and 8 for

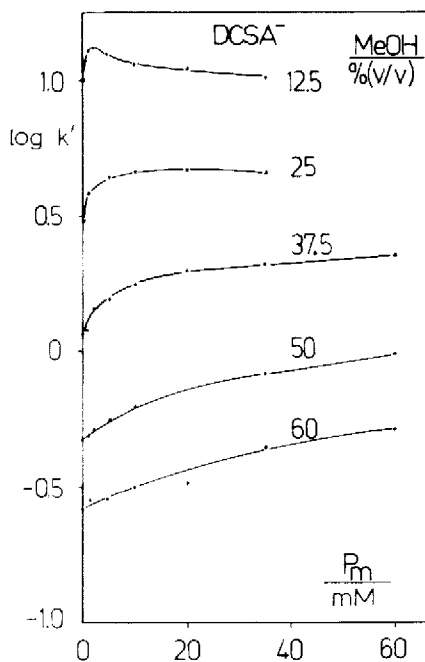
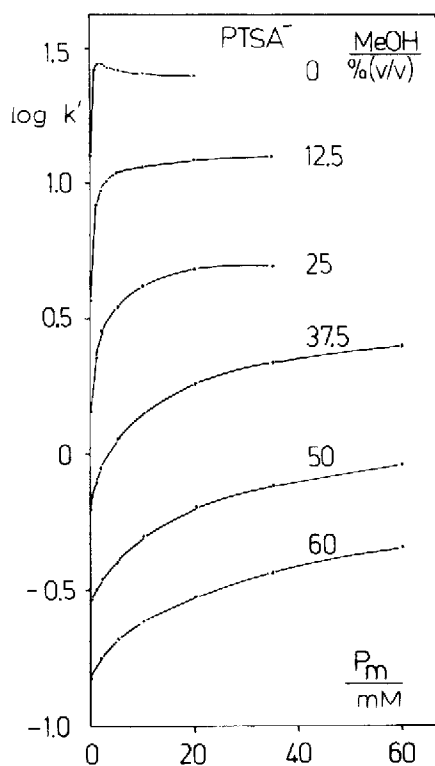


Fig. 1. Retention of *p*-toluenesulphonic acid as a function of the mobile phase concentration of tetrabutylammonium bromide in various methanol-phosphate buffer eluents.

Fig. 2. Retention of *d,l*-10-camphorsulphonic acid as a function of the mobile phase concentration of tetrabutylammonium bromide in various methanol phosphate buffer eluents.

various methanol concentrations. Methanol influences the shape of the curves to a lesser extent than for the oppositely charged solutes.

In Fig. 9 the $\log k'$ values for various positively charged solutes are plotted against the stationary phase TBABr concentration, at a constant (37.5%) methanol concentration. Again, although the curves are similar, the extent of the change varies with the type of solute.

In methanol-rich eluents (e.g. 60%) the neutral solutes which have no strongly polar functional groups (such as benzene, toluene and methyl iodide) show a slight, almost linear, decrease in $\log k'$ with increasing mobile phase pairing-ion concentration (cf. Fig. 10). On the other hand, when the methanol concentration of the eluent is zero, then the $\log k'$ value for methyl iodide is practically constant over the entire P_m range tested (cf. Table I). This apparent discrepancy will be dealt with in Part III⁵.

Effects of the concentration of the organic modifier

Several papers have dealt with the effects of the organic modifier upon the retention of oppositely charged solutes (e.g. refs. 6–9). The P_m of the pairing ion was

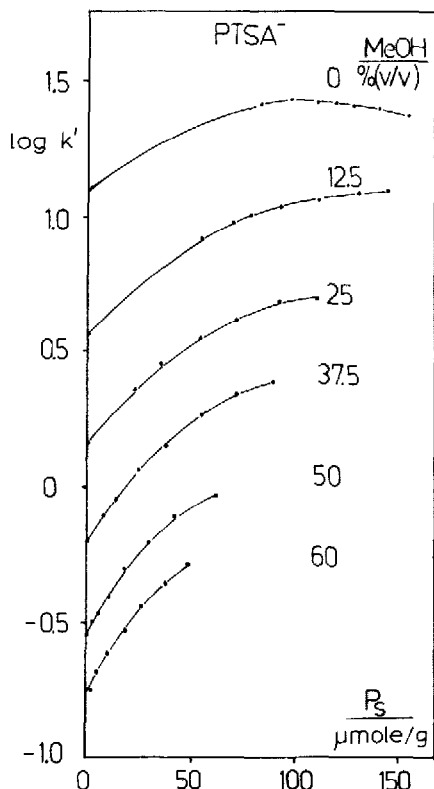


Fig. 3. Retention of *p*-toluenesulphonic acid as a function of the stationary phase concentration of tetrabutylammonium bromide in various methanol-phosphate buffer eluents.

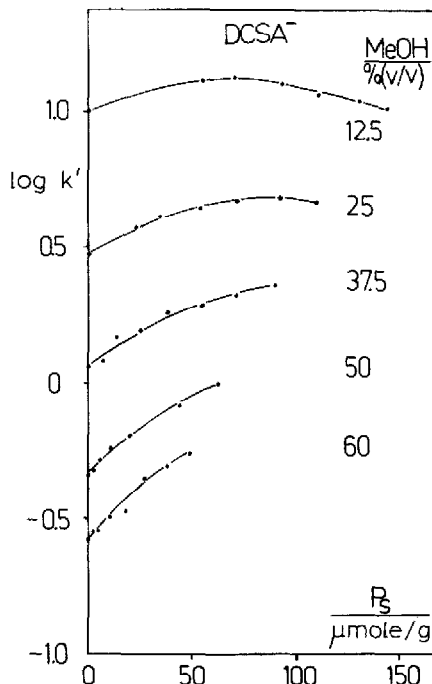


Fig. 4. Retention of *d,l*-10-camphorsulphonic acid as a function of the stationary phase concentration of tetrabutylammonium bromide in various methanol-phosphate buffer eluents.

maintained at a constant value, and the organic modifier was changed over a 20–30% range. With increasing modifier concentration, $\log k'$ decreased almost linearly^{7,8}.

In Fig. 11 the $\log k'$ of PTSA^- and DCSA^- are plotted against the methanol concentration of the eluent for TBABr concentrations of 0 and 5 mM. The shapes of the $\log k'$ vs. [methanol] curves differ in the presence and absence of pairing reagent.

With the help of the $\log k'$ vs. P_s curves the methanol dependence of $\log k'$ of various solutes at constant stationary phase pairing-ion concentrations could also be determined. In order to encompass a methanol range as wide as possible, a value for P_s of 54 $\mu\text{mole/g}$ was selected for this plot. The results for PTSA^- and DCSA^- are shown in Fig. 12, while those for the various *n*-alkylsulphonic acids are shown in Fig. 13.

It can be seen that in this representation the curves of all the compounds tested follow a similar trend, with their shapes being very similar to those obtained in the absence of ion pairing reagents. This suggests that the increased retention of oppositely charged solutes is due, primarily to the pairing ion which is adsorbed on the surface of the stationary phase.

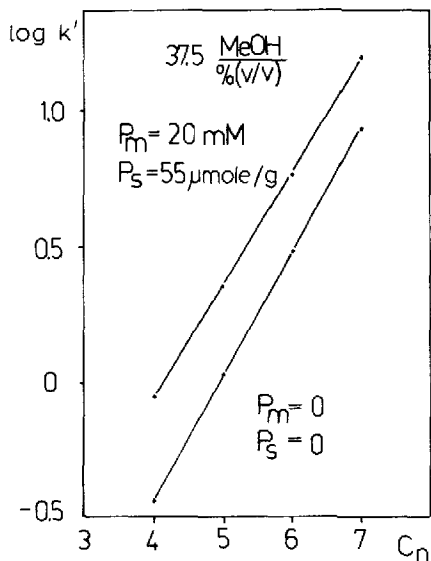
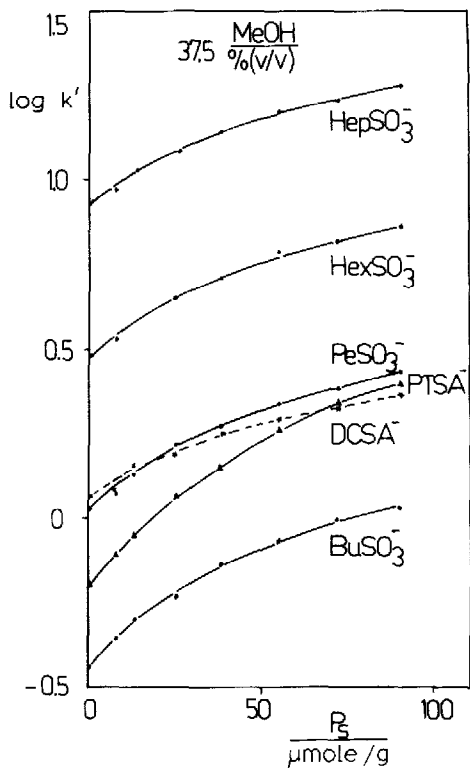


Fig. 5. The retention of various sulphonic acids (for abbreviation see Experimental section) as a function of the stationary phase concentration of tetrabutylammonium bromide in 37.5% methanol in phosphate buffer eluent.

Fig. 6. Retention of *n*-alkyl sulphonic acids as a function of their carbon number (C_n) in the presence (upper curve) and absence (lower curve) of tetrabutylammonium bromide in 37.5% methanol in phosphate buffer eluent.

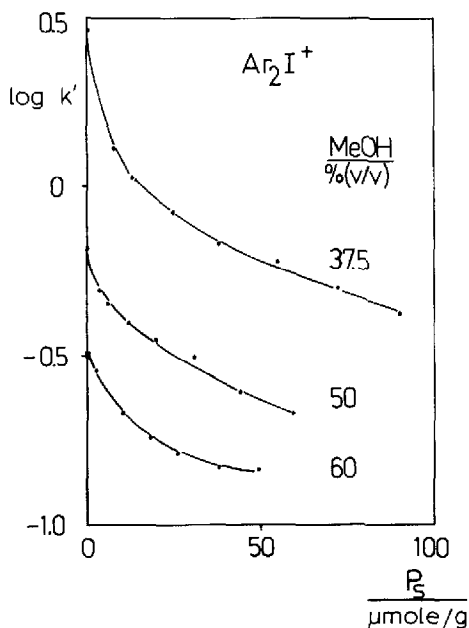
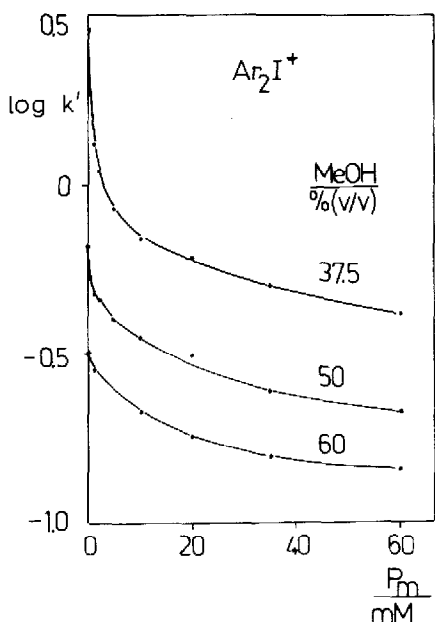


Fig. 7. Retention of diphenyl iodonium chloride as a function of the mobile phase concentration of tetrabutylammonium bromide in various methanol-phosphate buffer eluents.

Fig. 8. Retention of diphenyl iodonium chloride as a function of the stationary phase concentration of tetrabutylammonium bromide in various methanol-phosphate buffer eluents.

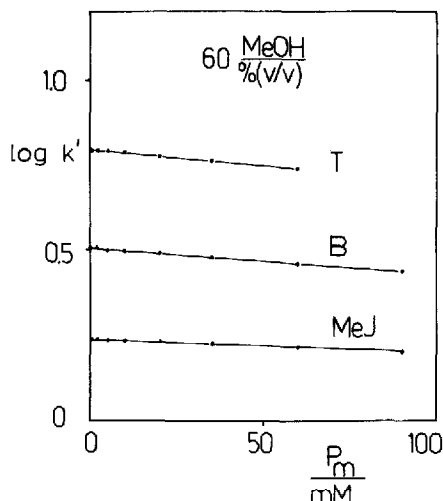
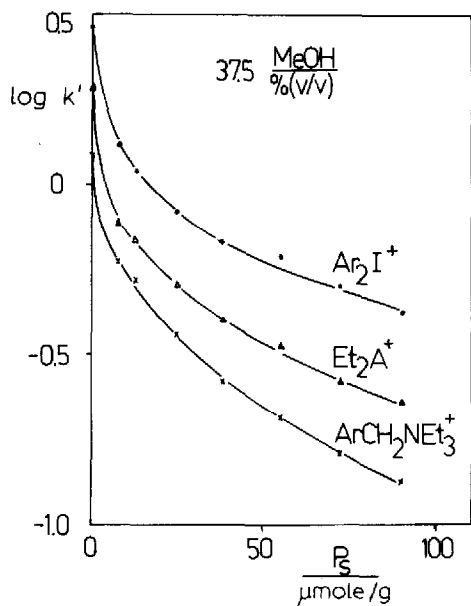


Fig. 9. Retention of various positively charged organic ions (for symbols see Experimental section) as a function of the stationary phase concentration of tetrabutylammonium bromide in 37.5% methanol in phosphate buffer eluent.

Fig. 10. Retention of neutral solutes with no strongly polar functional groups as a function of the mobile phase concentration of tetrabutylammonium bromide in 60% methanol in phosphate buffer eluent (for symbols see Experimental section).

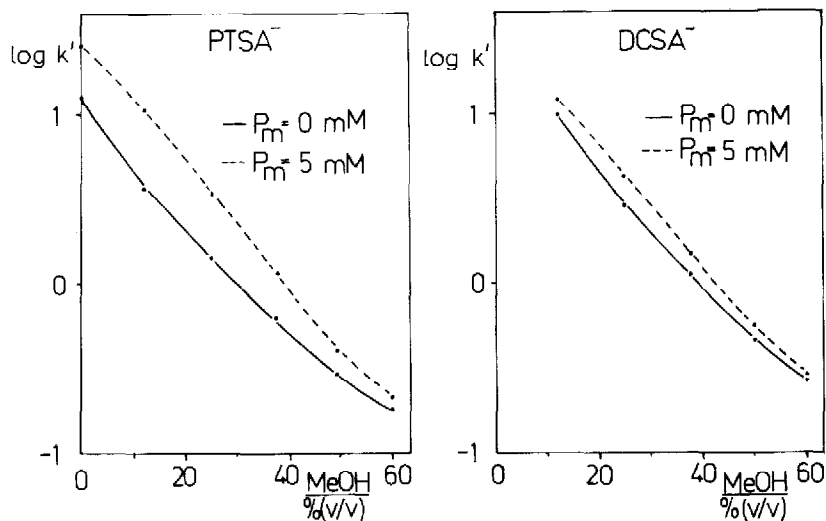


Fig. 11. Retention of *p*-toluenesulphonic acid and *d,l*-10-camphorsulphonic acid as a function of the methanol concentration of the eluent at constant tetrabutylammonium bromide mobile phase concentrations.

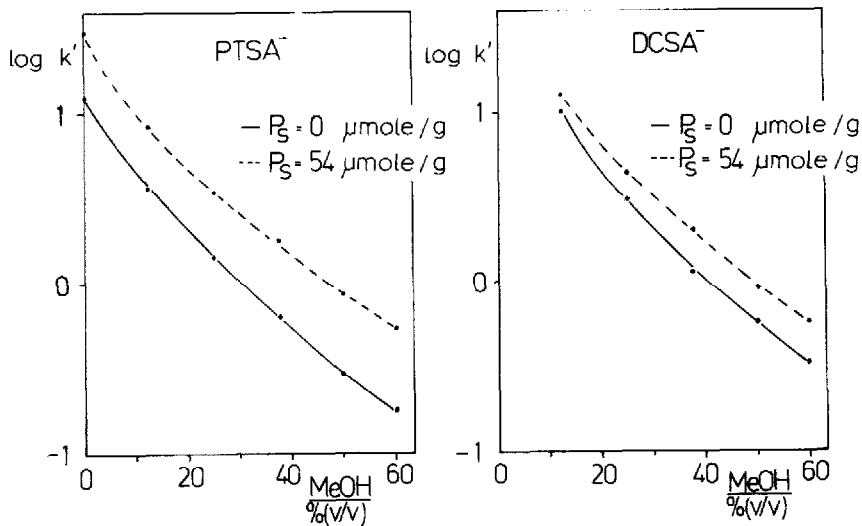


Fig. 12. Retention of *p*-toluenesulphonic acid and *d,l*-10-camphorsulphonic acid as a function of the methanol concentration of the eluent at constant tetrabutylammonium bromide stationary phase concentrations.

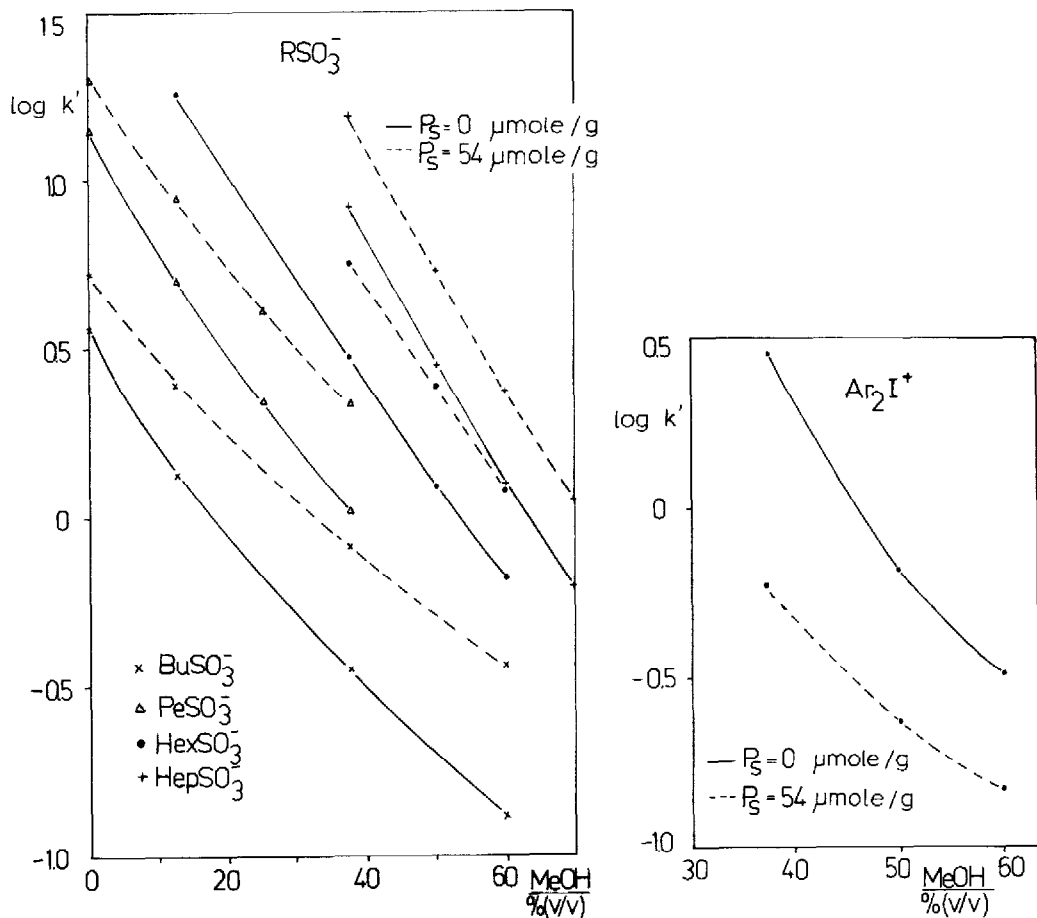


Fig. 13. Retention of *n*-alkylsulphonic acids as a function of the methanol concentration of the eluent at constant tetrabutylammonium bromide stationary phase concentration.

Fig. 14. The retention of diphenyl iodonium chloride as a function of the methanol concentration of the eluent at constant tetrabutylammonium bromide stationary phase concentration.

CONCLUSIONS

The capacity ratios of both positively and negatively charged ions, and of neutral solutes, were determined in methanol-aqueous phosphate buffer solutions on an RP-18 reversed-phase packing, in the absence and presence of TBABr. The phosphate buffer concentration was maintained at a constant value (25 mM H_3PO_4 -25 mM NaH_2PO_4) along with that of the bromide counter ion ($[\text{Br}^-] = 200 \text{ mM}$). Solutes were selected in such a manner that their ionization state did not change over the pH range used.

For oppositely charged solutes the $\log k'$ vs. P_m curves reached or approached a maximum, the position of which changed both with the structure of the solute and the concentration of methanol in the eluent.

The $\log k'$ values for like-charged solutes decrease rapidly with increasing P_m . The $\log k'$ values for neutral solutes which have no strongly polar functional groups decrease slightly (and linearly) with increasing P_m in methanol-rich eluents, and remain constant at zero methanol concentration.

Very similar curves are obtained when the $\log k'$ vs. P_s relationships for oppositely charged solutes are plotted. They have (or approach) a maximum in the 80-120 $\mu\text{mole/g}$ TBABr range. Unlike the $\log k'$ vs. P_m curves, the concentration of methanol in the eluent does not influence significantly the shape of the $\log k'$ vs. P_s curves. At any methanol concentration the relative change in $\log k'$ depends as well on the structure of the solute.

At constant mobile phase pairing-ion concentrations the shapes of the $\log k'$ vs. [methanol] curves for oppositely charged solutes differ significantly from those obtained in the absence of pairing agents. However, if the stationary phase pairing-ion concentration is maintained at a constant value, the $\log k'$ vs. [methanol] curves become more regular, and run almost parallel to those obtained in the absence of pairing ions.

An increase in the mobile phase concentration of the organic modifier results in a decreased relative permittivity of the eluent and, consequently, in an increased strength in the electrostatic interactions between the pairing ion and the solute ion. If the stationary phase concentration of the pairing ion is kept constant and the retention of the oppositely charged ionic solutes is controlled primarily by electrostatic interactions, then the retention of oppositely charged solutes should decrease more slowly with increasing methanol concentration in the presence of a pairing ion than when one is absent. Figs. 12 and 13 show that this is indeed the case for the oppositely charged solutes.

Similar reasoning predicts that the decrease in retention with increasing methanol concentration in the case of like-charged solutes is greater in the presence than in the absence of pairing ions. However, as is shown by Fig. 14, the experimental evidence contradicts this conclusion. Thus, the methanol dependence of the retention of ionic compounds cannot be explained solely by changes in the electrostatic interaction between the adsorbed pairing ion and the solute ion: other strongly polar and hydrophobic interactions have also to be taken into consideration⁵.

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