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ULTRA-VIOLET VISUALIZATION OF INORGANIC ANIONS BY REVERSED-PHASE ION-INTERACTION CHROMATOGRAPHY; FACTORS THAT CONTROL RETENTION AND SELECTIVITY

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SUMMARY

This paper describes the development and characterization of a separation and detection system for the analysis of mixtures of UV-transparent inorganic anions. Retention and separation occurs when a hydrophobic, positively charged paired-ion chromatography (PIC) reagent or an ion-interaction reagent (IIR) is added to the mobile phase of a reversed-phase system. Detection of UV-transparent ions results from a perturbation of the distribution equilibria of the UV-absorbing IIR upon injection of the sample ions. The effect of factors such as the concentration and nature of the buffer, co-ions and IIR as well as an organic modifier are described.

The major advantages of this method are that the system is nearly completely nonspecific, the separation system takes advantage of highly efficient reversed-phase columns, rapid separations of 4–6 anions in approximately 6–7 min and good sensitivity with detection limits of less than 1 nmole injected. In addition, no special equipment is required to perform ion analysis by this technique. Only conventional high-performance liquid chromatography pumps, detectors and reversed-phase columns are required.

INTRODUCTION

The determination of inorganic ions has undergone substantial changes in the past ten years. Significant advances have been made in the area of chromatographic separation and determination of ionic species. The major problem in this area has until recently not been in the separation but in the detection of the eluting species. In 1975 Small *et al.*[1] introduced a chromatographic technique based on the suppression of the eluent buffer species to improve conductometric detection of the sample ions. Fritz and co-workers[2,3] have pioneered work in the development of ultra-low capacity ion-exchange (IEX) resins so that only very low-ionic-strength mobile phases are needed to elute sample ions. High sensitivity conductivity detectors can then be

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used without suppressing the eluent's ionic content. Small and Miller[4] recently used low capacity resins in conjunction with UV-absorbing buffer ions to elute the samples. Vacancy (negative) peaks are produced upon injection of sample ions. These vacancy peaks are monitored as the samples elute. Others have used direct UV monitoring at short wavelengths (205–220 nm) with IEX[5], reversed-phase[6] and cyano bonded-phase[7,8] columns for the determination of species such as nitrate, nitrite and bromide.

In the past few years an ion-pair technique utilizing UV-absorbing paired-ion chromatography (PIC) reagents has been developed in order to detect UV-transparent organic ions such as alkane sulfonates. The first reports of this technique entailed normal-phase separations[9–12] but recently the technique has also been applied to reversed-phase systems[13–18]. Currently this general principle is being applied to the detection of inorganic anions[19,20]. The present report elaborates further on this work and deals specifically with those factors that control retention and selectivity of inorganic and a few organic anions as well as the system peak in reversed-phase ion-interaction systems.

This work was carried out because it is not only important to know the effect of all factors which can alter retention in this form of ion chromatography in order to achieve the desired separation, but also because the retention of the sample peak relative to the system peak can profoundly alter the analytical sensitivity of the methodology[14,16,19–22].

EXPERIMENTAL

The chromatographic system used in this work has been described in detail in an earlier report[19,20]. Ion-interaction reagents (IIRs) which were not commercially available are easily prepared as either the chloride or bromide salts[20]. IIRs in the chloride or bromide form were converted into the hydroxide form by passing an aqueous solution of the IIR over Bio-Rad AG 1-X8 anion-exchange resin in the hydroxide form. Since capacity factors are very strongly dependent on ionic strength (see below), extreme care is required in preparation of the eluent. To prepare an eluent that contains an IIR and 10 m*M* acetic acid–sodium acetate buffer one adds exactly a two-fold molar excess (relative to the IIR concentration) of acetic acid to the IIR (hydroxide form at 1–5 m*M*). The solution is then adjusted to a total ionic strength of 10 m*M* by addition of 1 *M* acetic acid–sodium acetate buffer of pH 4.75. Eluents containing phosphate buffers must be dealt with in an analogous manner.

RESULTS AND DISCUSSION

There are many factors that control retention of ionic samples and the system peak in ion-pair or ion-interaction chromatography (IIC). These factors include the nature of the sample anion, the concentration and nature of organic modifier, buffer, IIR and counter ion to the IIR.

Table I contains a list of a number of common inorganic and a few organic anions along with their capacity factors in three different eluent systems. The overall elution order is remarkably similar to that found in conventional anion-exchange systems[23] although the relative retentions in some cases are quite different, *e.g.*, iodide

TABLE I

CAPACITY FACTORS FOR COMMON ANIONS

Solvents (all contain 10 m*M* acetic acid–sodium acetate buffer, pH 4.75): A, 4 m*M* IIR, 0.25 m*M* hexanesulfonate; B, 4 m*M* IIR, 0.2 m*M* heptanesulfonate; C, 1 m*M* IIR, 0.5 m*M* octanesulfonate. –, No data. IIR = α-Naphthylmethyltributylammonium hydroxide.

Anion	*k'*		
	A	*B*	*C*
Fluoride	0.6	0	0
Iodate	1.8	1.4	0
Arsenate	–	1.5	0
Methanesulfonate	–	2.2	0
Chlorite	–	2.4	0
Chloride	3.5	2.6	0
Bromate	3.8	2.7	0
Ethanesulfonate	–	2.7	0
Nitrite	5.0	3.5	0
Cyanate	–	4.4	0.8
Bromide	7.8	5.1	0.5
Nitrate	11.5	7.8	1.3
Oxalate	–	8.0	0
Thiosulfate	–	9.9	0
Chlorate	14.7	9.9	1.6
Tartrate	15.3	6.5	0
Sulfate	16.4	6.6	0
Sulfite	17.0	7.3	0
Citrate	18.9	10.1	0
Butanesulfonate	24.1	10.7	1.6
Pentanesulfonate	–	35.3	4.6
Iodide	56	41	6.4
Hexanesulfonate	High	High	15.6
Thiocyanate	High	High	25.7
Perchlorate	High	High	–
Perbromate	High	High	55
Periodate	High	High	58
Heptanesulfonate	High	High	69
System peak	2.4	2.2	0.8

relative to chloride. Some ions such as thiocyanate and the perhalogenates are highly retained in these ion-interaction systems relative to most common anions. This enhanced retention is probably due to the ability of these specific ions to pair strongly with hydrophobic ions of opposite charge and for this reason species such as perchlorate are commonly used as extraction agents for organic cations. It is therefore likely that this extraction ability contributes to the overall retention for these specific ions in IIC and accounts for their enhanced retention.

Fig. 1 shows, for those ions where retention data are available under at least two conditions in Table I, that a plot of *k' versus k'* is approximately linear for all univalent inorganic anions (see closed circles, ●). Clearly divalent and organic anions (■) do not respond to a change in concentration of the IIR or the competing ion, *i.e.*, the sulfonates, in the same fashion. The observation of linearity in such plots is

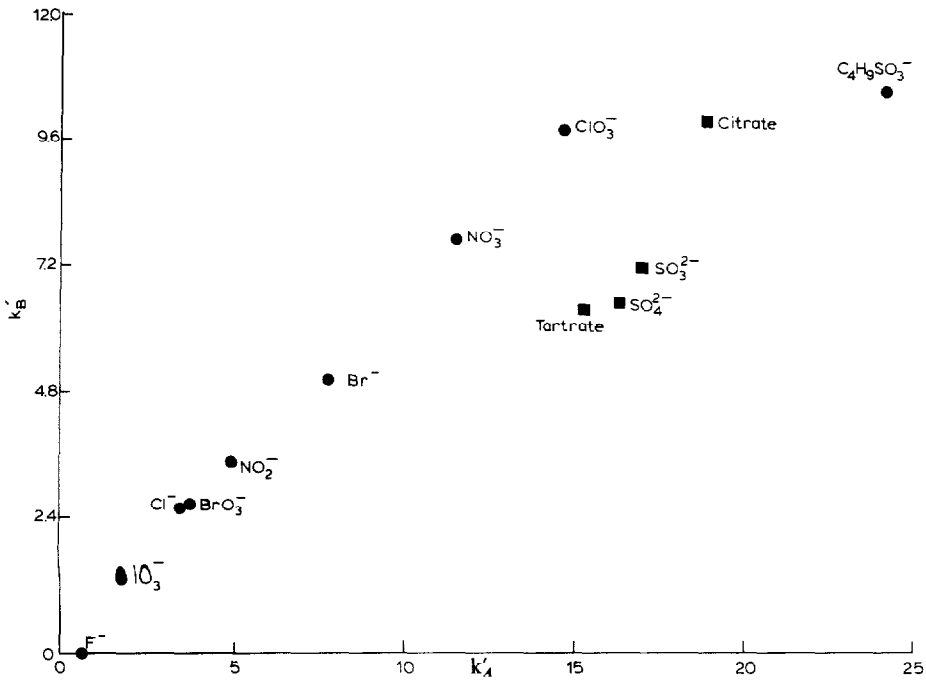

Fig. 1. Plot of k' *versus* k' for monovalent and divalent ions in two different eluents: A = 0.25 m*M* hexanesulfonate; B = 0.20 m*M* heptanesulfonate; both contain 4 m*M* α-naphthylmethyltributylammonium hydroxide and 10 m*M* acetic acid–sodium acetate buffer (pH 4.75).

quite convenient because it allows prediction of an anion's behaviour if its capacity factor is known under one set of conditions and the line has been established with at least two anions under two sets of conditions of analytical interest.

The concentration and nature of an organic modifier is one variable that can be used to modify the system peak and sample retention. An experiment summarized in Table II was executed in order to determine the relative strength of methanol and acetonitrile. It is evident that acetonitrile has a stronger effect on the system peak

TABLE II

EFFECT OF ORGANIC MODIFIER ON RETENTION

Chromatographic conditions: 1 m*M* α-naphthylmethyltributylammonium hydroxide, 10 m*M* acetic acid–acetate buffer (pH 4.75), 5% organic modifier.

Anion	k'		
	None	*Methanol*	*Acetonitrile*
System	9.7	10.9	14.1
Chloride	17.1	13.3	14.1
Nitrite	26.2	18.8	19.6
Bromide	40.9	27.2	27.8
Nitrate	61.2	37.8	38.4

TABLE III

k' OF SAMPLES AND THE SYSTEM PEAK *vs.* SURFACE CONCENTRATION OF IIR

A = α-Naphthylmethyltributylammonium hydroxide; B = β-naphthylmethyltripropylammonium hydroxide; C = benzyltributylammonium hydroxide. All contained 10 m*M* acetic acid–sodium acetate buffer (pH 4.75).

IIR	*Eluent concn. (M)*	*μmol adsorbed*	*k'*				
			Cl^-	NO_2^-	Br^-	NO_3^-	*System*
A	0.002	72.4	30.5	47.4	76.8	117.5	7.22
	ratios =	1.69	1.78	1.81	1.88	1.92	1.35
A	0.001	42.8	17.1	26.2	40.9	61.2	9.73
	ratios =	1.23	1.66	1.74	2.02	2.15	1.31
B	0.001	34.8	10.3	15.1	20.3	28.5	12.8
C	0.001	29.0	8.98	12.8	16.6	22.8	9.71

than does methanol. The system peaks in both cases are more strongly retained in the presence of the modifier than in totally aqueous eluents. This unusual result, in the context of reversed-phase chromatography, is interpreted to indicate that the organic modifier shifts the adsorption isotherm so that less IIR is adsorbed on the surface causing the slope of the isotherm to be larger than in the absence of the modifier. This results in larger k' values for the system peak. Acetonitrile decreases the k' values of the samples slightly less than does methanol. Acetonitrile is generally considered to be a stronger solvent than methanol in reversed-phase chromatography and thus it removes more of the hydrophobic IIR from the surface than does methanol. Since the hydrophilic samples have a high charge density, the more polar, hydrogen-bonding solvent, *i.e.* methanol, acts as a stronger solvent for this type of species.

Retention of the sample anions and the system peak is strongly affected by the concentration of the quaternary ammonium IIR in the eluent. More correctly, the retention of sample anions is roughly proportional to the net charge on the surface of the column packing[24]. Table III contains data pertaining to the extent of loading of the column with IIR. The table also contains retention data for a series of sample ions as a function of concentration. When a column is first equilibrated with eluent, "breakthrough" of the IIR does not occur for many column void volumes. This is due to the adsorption of the IIR onto the column packing material surface according to the adsorption isotherm for the particular IIR.

Since the isotherms are normally not linear (usually they are convex) at the eluent concentrations used for most chromatographic work, the ratio of surface concentrations of IIR for two different eluents is not expected to be equal to the ratio of their mobile phase concentrations. Indeed the data in Table III show that for 2 and 1 m*M* α-naphthylmethyltributylammonium in 10 m*M* acetate buffer the ratio of the surface concentrations is only 1.69, not 2.0.

Furthermore, it might be expected that for a separation mechanism that depends primarily on surface interaction between sample ions and surface charge if the surface concentration of IIR were to increase (and concomitantly the surface charge), then the retention of sample ions should increase in proportion to the surface con-

centration of IIR. The data in Table III show, that this is true to a certain extent. Note that the ratio of an anion's k' at 2 mM to that at 1 mM is slightly greater than the ratios of surface concentrations of IIR at the same mobile phase concentrations. In addition, the ratios show a definite increasing trend towards ions with larger retention. Specifically, the ratio increases from 1.78 for chloride to 1.92 for nitrate. This might indicate that there is some other process contributing to the overall retention of the ions that is a function of the k' or extraction constant of the ions. This could very well be related to the extraction ability of the ion with an IIR into a hydrophobic media or its ion-pairing ability. The retention order of the ions listed in Table I does, in general, follow the increase in extraction constants for these ions[25].

Another indication that the extraction or ion-pairing ability may account for retention additional to that predicted by a strictly surface charge argument is revealed by changing the nature of the IIR. The surface concentrations and retentions of sample ions are compared for α-naphthylmethyltributylammonium and β-naphthylmethyltripropylammonium in Table III. The eluent concentrations of these two IIRs are equal. As expected, the breakthrough volume for the tributyl IIR is larger than that for the tripropyl IIR and, therefore, the surface concentration for the tributyl IIR is also greater. As discussed above, this implies that the sample k' values ought to be larger with the tributyl IIR and this is indeed the case. The ratio of the surface concentrations is 1.23. The increase in the k' ratios is again observed but in this case the magnitudes of the k' ratios relative to the surface concentration ratios are definitely larger. We interpret this as indicating that ion pairing or extraction is more important in terms of net retention for the larger, more hydrophobic tributyl IIR than for the tripropyl IIR. Again it should be noted that plots of k' *versus* k' (not

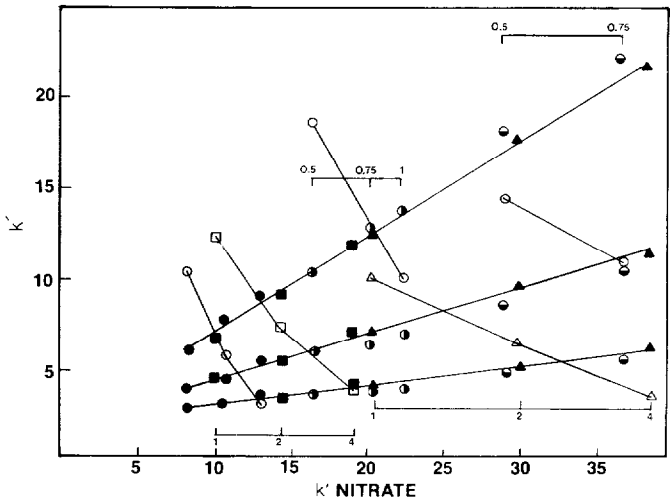

Fig. 2. Selectivity plot for four anions with IIRs in the hydroxide and chloride forms. The k' of chloride, nitrite, bromide and the system peak is plotted *versus* the k' of nitrate. ●, *p*-Nitrobenzyltributylammonium hydroxide; ■, β-naphthylmethyltripropylammonium hydroxide; ▲, α-naphthylmethyltributylammonium hydroxide; ◑, naphthylmethyltributylammonium chloride; ◒, naphthylmethyltripentylammonium chloride; open symbols indicate the system peak. Chromatographic conditions: concentrations of the IIRs as indicated below each column of points; 10 mM phosphate buffer (pH 7).

shown) for any two elution conditions shown in Table III are remarkably linear. Correlation coefficients for all four data sets exceeded 0.995.

The retention of sample anions is therefore observed to increase with increasing concentration and hydrophobicity of the IIR. These effects are summarized for a number of different IIRs in Fig. 2 for a phosphate buffer system. The closed symbols pertain to IIRs in the hydroxide form and the half-shaded symbols are for IIRs as the chloride salt. Data for three IIRs in an acetate buffer system were presented in previous reports[19,20].

Fig. 2 shows the k' of three anions *versus* the k' of another reference anion which in this case is nitrate. Nitrate is an arbitrary but reasonable choice for the reference ion because it is the most strongly retained ion of the four. Its capacity factor is very reproducible and never overlaps with the system peak as does that of chloride. This type of plot reveals another important characteristic of the data. As the concentration of a single IIR is increased or as the nature of the IIR is changed, the relative retention data for a single ion fall on a single straight line. The slope of this line is related to the selectivity, α, between the ion of interest and the reference ion. This type of plot shows very clearly that the concentration and nature of the IIR do not markedly affect the selectivity of the sample ions. In addition the linearity of the data indicates that the retention mechanism is not grossly altered and the retention process is similar for all anions shown. In Fig. 2 not only is the nature of the IIR changed by varying the alkyl chain length from propyl to butyl to pentyl but the aromatic group is also changed from *p*-nitrobenzyl to α-naphthylmethyl. Therefore it does not matter where the increase in hydrophobicity comes from, whether it be an alkyl or aryl group, the selectivity (slope) remains constant.

Fig. 2 also contains sets of data for the chloride salts of the two IIRs. These data follow nearly the same pattern as for the hydroxide form of the IIRs although some slight differences are apparent. No clear conclusion may be drawn from these data as to whether these differences are significant.

The k' of the system peak is of prime importance when attempting to optimize this type of analytical system. For reasons given in previous reports[19,20], the system peak should elute at a $k' \leqslant 2$ with the sample ions eluting in a k' window of 2–10. The retention of the system peak, as well as of the sample peaks, is strongly affected by the concentration of the IIR on the surface and in some cases (to be discussed in a subsequent report) by the nature of the IIR. From a theoretical point of view this is easily understood by considering that the adsorption isotherm for a quaternary ammonium salt is either of a Langmuir or Freundlich shape (convex)[26–28]. The isotherm either is or becomes non-linear at high concentrations. Since the slope of the isotherm is defined as the k' for the IIR the k' will vary as a function of mobile phase concentration of IIR at high concentrations. The k' of the system peak (IIR) will therefore decrease as the eluent concentration of IIR increases, the rate of decrease being defined by the shape of the adsorption isotherm.

Fig. 2 presents retention data for the system peak as well as for the sample ions discussed in the previous paragraphs. The system peak data are denoted by the open symbol in each column of points. It is evident from these data that the relative decrease in the k' of the system peak is smaller than the relative increase in the k' values of the samples, especially for the strongly retained ions such as bromide and nitrate. Another interesting feature is that the system peak k' at a specified IIR con-

centration is remarkably independent of the nature of the IIR. The k' values for the different IIRs obtained by varying the aryl group are essentially identical at the same concentrations. Changing the alkyl group, *i.e.*, from butyl to propyl, does have a slight effect as seen in Fig. 2. The surface concentration of the tripropyl IIR was seen to be less than that for the tributyl IIR (Table III) and therefore it should have a larger k' as discussed above. However, data presented in Table III show that that the benzyltributylammonium system also has much less IIR on the surface than α-naphthylmethyltributylammonium and yet the k' of the system peak for each IIR is essentially identical at the indicated concentration. This suggests that at very low eluent concentrations the slopes of the isotherms of different IIRs are different (the more hydrophobic IIR having the largest slope) and that above a certain eluent concentration the isotherm curves become orthogonal. This hypothesis is also consistent with the larger k' values for sample ions with the more hydrophobic IIRs.

In order to test this hypothesis the adsorption isotherms for three IIRs were determined. The data for these isotherms were obtained by measuring the "breakthrough" volumes for 8–10 eluent concentrations ranging from 0.05 to 4 mM for each IIR. These data are shown in Fig. 3. Within experimental error, these curves agree with the above hypothesis. A more detailed analysis of adsorption isotherms including curve-fit analysis will be the subject of a subsequent report.

The concentration and nature of a buffer ion can also have a significant effect on the retention of sample ions but have a much smaller effect, if any, on the retention of the system peak. The data plotted in Fig. 4 show very clearly that, as the concentration of buffer ions is increased, the retentions of all sample ions decrease. The basis for this effect is two-fold. First, the ionic strength of the eluent increases, which will tend to shield the sample ions from the IIR ions on the surface thus decreasing its retention. Secondly, the buffer ions, which would normally be less strongly re-

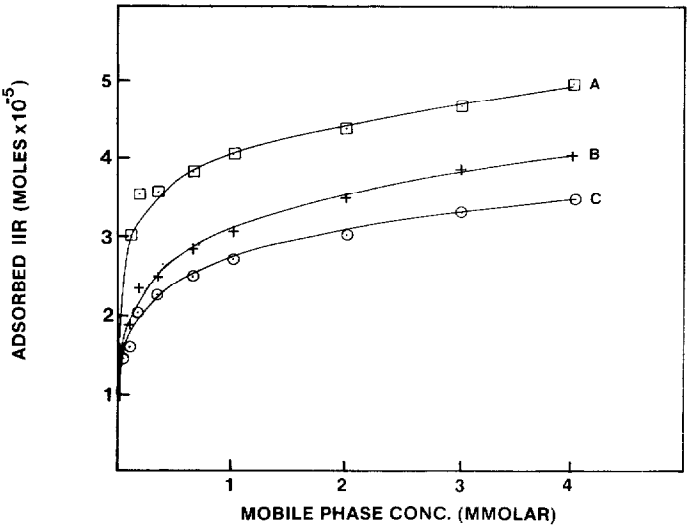

Fig. 3. Experimentally determined adsorption isotherms. Curves: A, α-naphthylmethyltributylammonium hydroxide; B, β-naphthylmethyltripropylammonium hydroxide; C, benzyltributylammonium hydroxide. Chromatographic conditions: 10 mM acetic acid–sodium acetate buffer, pH 4.75.

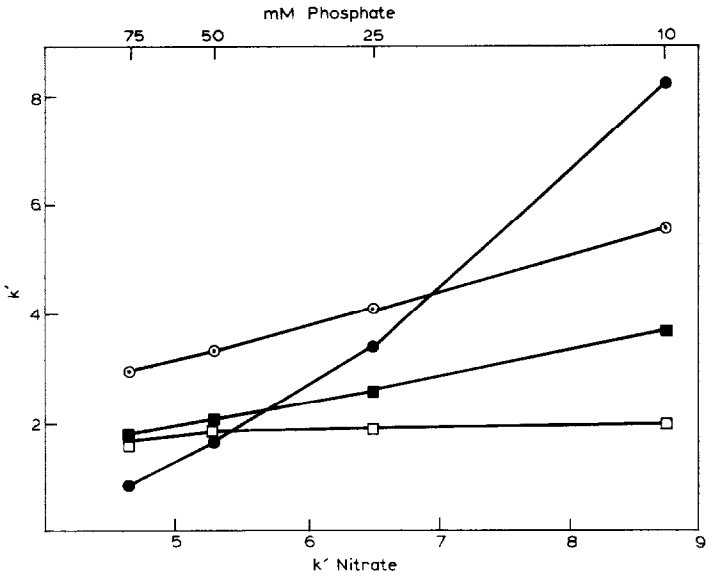

Fig. 4. Effect of buffer concentration on retention. ○, nitrate; ■, bromide; ●, sulfate; □, system peak. Chromatographic conditions: 5 m*M* benzyltributylammonium chloride; 10, 25, 50 and 75 m*M* phosphate buffer (pH 7).

tained than the sample, will compete more effectively with the sample ions for retention on the surface at high buffer concentrations. This is primarily due to the large excess of buffer ions over the concentration of sample ions.

The system peak is not, however, affected by the changes in buffer concentration. This must be due to the inability of the buffer ions to cause a significant change in the surface concentration of IIR. The buffer ions used in this work are either inorganic, *i.e.*, phosphate, or small organic ions such as acetate which are relatively hydrophilic and have relatively small extraction constants compared to long chain organic sulfonates or sulfates.

The data in Fig. 4 are plotted in a similar manner to those in Fig. 2. This type of plot is basically a selectivity plot and shows the relative changes in retention between a sample ion and a reference ion as a function of a chromatographic variable which in this case (Fig. 4) is the buffer concentration. The data indicate that for all the monovalent species shown in Fig. 4, the k' values at different buffer concentrations all fall on a single line indicating that the selectivities between sample ions do not change as a function of buffer concentration. Note, however, that the slope of the divalent sulfate data is much larger than that for the monovalent ions. This is consistent with the general observation in ion-exchange chromatography that divalent species are much more affected by changes in ionic strength than are monovalent species. In addition, the sulfate data in 10 m*M* buffer do not fall on the same line as the data at the three higher concentrations of buffer. Since the deviation occurs at low rather than at high buffer concentrations, the origin of the problem may lie with a relatively large change in the ionic strength within the sample zone when the divalent sulfate is injected. For a phosphate buffer concentration of 10 m*M* and an injected sample concentration of 1 m*M* the maximum increase in ionic strength when

a monovalent ion is injected is 5%, but when a divalent sample such as sulfate is injected the maximum increase is nearly three times greater, *i.e.*, 15%. This is a large relative change within the sample zone and would affect sulfate more than monovalent ions thereby changing the selectivity between them as indicated in Fig. 4. A non-linear isotherm is also consistent with this observation and explains the poor peak shape for sulfate in Fig. 3A in ref. 19.

The concentration of buffer ions is not the only variable related to the buffer that can affect retention of sample ions. The nature of the buffer can also be changed so that the buffer ion will compete more or less strongly with the sample ions for retention and thereby act as a strongly or weakly eluting "solvent" [by analogy to strong and weak solvents in reversed-phase high-performance liquid chromatography (RP-HPLC)]. Skelly[6] has reported a separation of a series of unsubstituted and substituted carboxylic acids using octylamine in acidic aqueous media on a reversed-phase column. One of the reported separations shows that glycolate is about 30% less strongly retained than acetate under his experimental conditions. This suggests that a glycolic acid–glycolate buffer would act as a "weaker" solvent for sample ions in our chromatographic system than an acetic acid–acetate buffer where all other variables are constant.

This hypothesis was tested by preparing eluents that were identical except for the buffer ion and determining the k' values for each sample ion. The results are presented in Table IV for comparison. The two important parameters that must remain constant in this experiment for a valid comparison are the pH and the concentration of the conjugate base form of the buffer. Since the pK_a for glycolic acid (3.83) is smaller than that for acetic acid (4.75), the amount of the acid form of the species must change relative to the amount of the conjugate base form to keep the pH constant. The total moles of the buffer species will, of course, be different in the two experiments. The pertinent concentrations are given in Table IV.

The k' data in Table IV show that our prediction is correct. The k' of each sample is larger in the glycolate buffer system. Since only two points for each ion were obtained under controlled conditions, a selectivity plot would not be revealing. However, the ratio of the k' values in each system is constant for all the sample ions

TABLE IV

EFFECT OF THE BUFFER NATURE ON RETENTION

Chromatographic conditions. (0.001 *M* α-naphthylmethyltributylammonium hydroxide in each case): A, 0.005 *M* acetic acid, 0.005 *M* acetate, pH = 4.75; B, 0.000602 *M* glycolic acid, 0.005 *M* glycolate, pH = 4.75; C, 0.005 *M* dihydrogenphosphate, monobasic, 0.005 *M* hydrogenphosphate, dibasic, pH = 7.00.

Anion	*k'*				
	A	*B*	*B/A*	*C*	*B/C*
System	9.7	10.1	1.04	10.0	1.01
Chloride	17.1	24.3	1.42	3.9	6.2
Nitrite	26.2	37.6	1.43	6.7	5.6
Bromide	40.9	58.3	1.43	12.5	4.7
Nitrate	61.2	87.8	1.43	20.4	4.3

indicating that the selectivities between each ion from one buffer system to another also remain constant.

The system peak is not strongly affected by the nature of the buffer. The ratio of k' values is very close to but slightly larger than 1.0 suggesting that acetate does indeed force slightly more of the IIR onto the surface causing a smaller k' for the system peak than does glycolate. This suggests that a longer alkyl chain length on the conjugate base might have a stronger effect on the system peak. This possibility is discussed below.

Data for a 10 mM, pH 7 phosphate buffer system are also included in Table IV. These data are not directly comparable since the pH is higher and the second component of the buffer system is not the fully protonated form of the buffer species but the divalent form. Given the information discussed above, the likely conclusion is that a phosphate buffer at pH 7 should be a much "stronger" eluent than either acetate or glycolate buffers and the sample k' values should be much smaller. This is indeed the case as indicated by the data in the table. Note, however, that the system peak k' is very close to that for glycolate indicating that inorganic buffers, in terms of their interaction with the IIR, are very similar to hydrophilic organic buffers.

The concentration and nature of the organic modifier, buffer and IIR have, as discussed above, been shown to affect significantly the retention of sample anions. The retention of the system peak can be reduced significantly only by increasing the concentration of the IIR. However, such an increase leads to a proportional decrease in analytical sensitivity which will be discussed in a subsequent report.

Iskandarani and Pietrzyk[29] have investigated the retention of quaternary ammonium salts on PRP-1 and the effect of the co-ion on the retention of the salts. They have clearly shown that in an electrolyte-free eluent the retention of an IIR is greater with a co-ion such as bromide or nitrate than with a co-ion such as hydroxide or fluoride on this very hydrophobic surface. This, at first glance, is inconsistent with the observations discussed above which indicated that the nature of the buffer did not affect the retention of the system peak to any great degree. However, in the experiments above, a silica-based C_{18} column was used, which does have some polar and ion-exchange sites, and in the previous work PRP-1, which is an essentially totally hydrophobic material, was used. This observation did suggest, however, that the surface might be saturated with IIR by introducing an anion into the eluent that would cause increased adsorption of the IIR at low eluent IIR concentrations.

From the data presented above, inorganic anions and short-chain alkyl organic anions such as acetate are not expected to have a large effect on adsorption of the IIR, whereas long-chain alkyl sulfonates or sulfates are likely to precipitate the IIR in aqueous media. Alkane sulfonates of intermediate length (4–8 carbons) were chosen to test this hypothesis. Retention data are presented in Table V for samples and the system peak as a function of IIR concentration, IIR type, chain length of the alkane sulfonate and concentration of the alkane sulfonate. Butanesulfonate reduces the retention of the sample peaks quite drastically while affecting the system peak very little. Hexanesulfonate on the other hand reduces the system peak k' by nearly half, as well as reducing the k' values of the samples under the same conditions as the butanesulfonate. Octanesulfonate, at an even lower concentration than in the previous two experiments, reduces the k' of the system peak to well below 2 but the samples are for the most part nearly unretained.

TABLE V

EFFECT OF ALKANE SULFONATES ON RETENTION

A = α-Naphthylmethyltributylammonium hydroxide; B = α-naphthylmethyltripentylammonium hydroxide.

Expt. No.	*IIR*	*Concn. (mM)*	*Alkane-sulfonate*	*Concn. (mM)*	*k'*				
					System	Cl^-	NO_2^-	Br^-	NO_3^-
1	A	1	–	–	9.73	17.1	26.2	40.9	61.2
2	A	1	Butane	0.5	9.31	6.92	10.9	17.3	24.8
3	A	1	Hexane	0.5	5.18	1.90	2.67	3.75	7.15
4	A	1	Hexane	0.75	4.02	1.51	2.13	2.98	5.80
5	A	1	Octane	0.25	1.36	0.16	0.58	0.74	2.05
6	A	2	–	–	7.22	30.5	47.4	76.8	117
7	A	2	Hexane	0.75	3.05	1.74	2.40	4.44	6.61
8	A	4	–	–	3.86	30.4	48.3	80.5	127
9	A	4	Hexane	0.5	1.86	2.40	3.52	5.76	8.54
10	A	4	Hexane	0.75	1.90	2.40	3.40	5.25	8.00
11	B	1	Hexane	0.75	4.68	2.24	3.32	6.76	10.8
12	B	1	Octane	0.75	1.47	0.35	0.74	0.89	2.47
13	B	4	Hexane	0.5	2.63	4.60	7.03	12.6	21.4
14	B	4	Hexane	0.75	2.28	3.32	5.22	7.38	16.1

This effect is most probably due to an increase in the stationary phase concentration (surface concentration) of IIR in equilibrium with a given eluent. Indeed, higher "breakthrough" volumes are obtained upon equilibration of a column when the eluent contains an alkanesulfonate. Actually, two "breakthrough" curves are observed when a column is equilibrated with an eluent containing a deficient quantity of alkanesulfonate, relative to the IIR. The first corresponds to the IIR only. The concentration of the IIR in the mobile phase eluting from the column at this point is the entering concentration less the steady state amount that is being adsorbed by the alkanesulfonate at the alkanesulfonate front. The alkanesulfonate is present in the eluent at lower concentrations than is the IIR so it takes a larger volume of eluent to equilibrate the column with alkanesulfonate than to equilibrate with the IIR. The second "breakthrough" curve corresponds to the alkanesulfonate completing its equilibration with the column. The second increase in detector absorbance is not due to the alkanesulfonate itself, since it is UV-transparent, but to the amount of IIR that was being adsorbed by the alkanesulfonate at the leading edge of the alkanesulfonate front.

This overall increase in the amount of IIR adsorbed would effectively alter the shape of the adsorption isotherm so that for a given eluent concentration of IIR the slope of the adsorption isotherm for the IIR would be less in the presence of the alkanesulfonate. This results in the lower k' for the system peak for a given eluent concentration of IIR. The presence of the alkanesulfonate, however, reduces the k' values of the samples as well even though there is more IIR on the surface than in its absence. This is because the alkanesulfonate competes with the inorganic ions very effectively for charged sites on the surface since it is much more hydrophobic.

This use of alkanesulfonates to reduce the retention of the system peak was first suggested and used in a previous report[19]. In this earlier report an eluent system

was optimized for the analysis of chloride, nitrite, bromide, nitrate and sulfate. The present work reports the use of alkanesulfonates such as heptane- and octanesulfonates to optimize separations of additional anions of widely different retentions.

It is important to note how easily the nature of the alkanesulfonate can be used to adjust the retention of the sample ions and of the system peak. Basically, the more hydrophobic is the alkane sulfonate, the lower is the retention for both the samples and the system peak. Table I contains retention data for a variety of both inorganic and organic anions. Slightly retained ions are separated using eluent A, *i.e.*, that containing hexanesulfonate, whereas ions with intermediate retention are best separated by eluent B which contains heptanesulfonate. The k' values of highly retained ions such as iodide or pentanesulfonate can be adjusted into the k' window of 2–10 by reducing the concentration of IIR and using octanesulfonate as in eluent C.

All ions, therefore, cannot be separated under a single set of conditions. A balance between the concentration of IIR and the concentration and nature of the alkanesulfonate needs to be determined for each particular separation of interest.

CONCLUSIONS

The data presented in this work define in a qualitative fashion how the retention of a sample anion, *i.e.* inorganic, can be adjusted by changes in the mobile phase composition. This is, of course, an important issue in any form of chromatography. It is particularly important in UV-visualization chromatography because we have observed[19,20] in agreement with Hackzell *et al.*[22] that the sensitivity of the method varies dramatically with the retention of the sample peak relative to the system peak.

In our previous communication[19] we introduced the use of a "retention map", *e.g.*, Fig. 2 of this paper, which indicates a linear relationship between the k' of any sample anion and a reference anion. Although quite good linear correlation coefficients are obtained, detailed statistical analysis shows that the maps are in fact slightly curved. One should not infer any detailed mechanistic interpretation of the retention process from this apparent linearity. However, these maps are important because they allow one to readily estimate the effect of a change in the analytical conditions for any species once the behaviour of that species has been determined under any two sets of conditions such as different concentrations of IIR, buffer or organic modifier.

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