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## SEPARATION OF INORGANIC ANIONS BY LIQUID CHROMATOGRAPHY

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### SUMMARY

The influence of the presence of an organic counter ion, mobile phase pH and ionic strength on the retention of inorganic anions was studied in a reversed-phase chromatographic system with ODS-silica gel as the stationary phase. Retentions of all tested anions could be reached by acidifying the aqueous solutions of inorganic salts used as the mobile phase. The addition of tetrabutylammonium cations to the system led to the increase in retentions of all the studied anions within the whole investigated range of pH 7-3. However, it did not affect the elution order both inside the group of metal oxo anions ( $\text{CrO}_4^{2-}$ ,  $\text{MoO}_4^{2-}$ ,  $\text{WO}_4^{2-}$  and  $\text{VO}_3^-$ ) and non-metallic anions ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ ). The ionic strength of the mobile phase influenced retention, selectivity and elution order. Retentions of anions in systems without organic counter ion were explained by Coulombian interactions of anions with protonized residual silanols. Differences in the behaviour of oxo anions of Cr, Mo, W and V from the behaviour of non-metallic anions were explained by the formation of complexes between hydrated metal oxo anions and residual silanols.

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### INTRODUCTION

The technique of ion-pair chromatography, developed for the separation of organic compounds which dissociate in aqueous media, can also be used to separate inorganic ions<sup>1-5</sup>. Cetyltrimethylammonium bromide as an ion-pairing agent added to the mobile phase proved successful, particularly in combination with cyano-bonded silica<sup>1</sup>. In combination with common ODS-silica it provided systems suitable for the least strongly retained ions, e.g., iodates and bromates<sup>5</sup>. More strongly retained ions, such as, e.g., iodide, give in this system retention times that are too long<sup>5</sup> and the peak shapes of strongly retained solutes are poor<sup>1</sup>. The combination of ODS-silica and tetrabutylammonium (TBA) ions, obtained from an aqueous solution of tetrabutylammonium hydroxide (TBAOH) adjusted to pH 6.7 with  $\text{K}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$ , is more suitable<sup>3</sup>. The aqueous solution can be used as the mobile phase without the addition of organic modifiers. When chromatography is performed on ODS-silica gel, octylamine, neutralized to pH 6.5 with the aid of mineral acids (HCl,

$\text{H}_3\text{PO}_4$ ,  $\text{H}_2\text{SO}_4$ )<sup>4</sup>, is also a suitable ion-pairing agent. In these systems, the retention can be influenced by both the organic modifier and the mineral acids used for neutralization of the amine.

The previous work cited above deals with the separation of anions of common inorganic acids in almost neutral media. In the present work we have investigated the influence of pH on the behaviour of anions of oxo acids of Mo(VI), W(VI), Cr(VI) and V(V), which tend to form isopolyanions in acidic media. Significant deviations from the behaviour of the common inorganic anions of non-metals that were studied earlier<sup>1-4</sup> were observed in preliminary experiments with these ions. Thus we studied not only the dependences on the mobile phase pH, the type and concentrations of the compounds used to control this pH and on the mobile phase ionic strength, but also on the presence of onium counter ion in the chromatographic system. For this study we chose the chromatographic system proposed by Molnár *et al.*<sup>3</sup>.

## EXPERIMENTAL

Silasorb 300 ODS irregular silica gel modified chemically with octadecyl groups (Lachema, Brno, Czechoslovakia), particle size 10  $\mu\text{m}$ , served as the stationary phase. Stainless-steel columns, 100  $\times$  4 mm I.D., were packed by the viscosity variant of the high-pressure filtration technique with cyclohexanol-methanol (95:5) as the suspension medium. Water or a neutralized solution of TBAOH, concentration  $1 \cdot 10^{-3}$ – $2 \cdot 10^{-3}$  mol l<sup>-1</sup>, containing compounds to adjust the pH to 3–7 [phosphate buffer, tris(hydroxymethyl)aminomethane (Tris), phosphoric acid, sulphuric acid or hydrochloric acid] and to adjust the ionic strength ( $\text{Na}_2\text{SO}_4$ ), served as the mobile phase. Anions of acids were injected in the form of aqueous solutions of their sodium salts. A Variscan spectrophotometric detector, used to detect zones of acid anions, was operated at the wavelength of 210 nm. Column dead volumes were measured with the aid of the injection of <sup>2</sup>H<sub>2</sub>O (ref. 6). Other experimental conditions, including the procedure for column preparation, were as reported earlier<sup>7</sup>.

## RESULTS

### *Systems without TBA*

In order to examine the possibilities of interaction of anions of different types with the surfaces of stationary reversed phases, the measurements were performed first on systems without onium ions. Phosphate buffers at pH 3–7 and at a concentration of 50 mM were employed as the mobile phase. By the addition of sodium sulphate, their ionic strengths were adjusted to the value of the phase prepared by neutralization of a 2 mM aqueous solution of TBAOH with phosphoric acid to which 50 mM phosphate buffer at pH 7 had been added.

The retention of all of the anions was found to increase monotonously (Table I) with decreasing pH. Non-metallic anions are not retained in neutral solutions (pH 6.9) or are slightly excluded. In accord with conclusions following from Knox's model of enthalpic exclusion of charged particles<sup>8</sup>, it was found that bivalent sulphate ions exhibit stronger exclusion than monovalent ions in neutral solutions. Measurable retentions of anions were observed at pH *ca.* 4.0. The slight tailing of nitrate and,

TABLE I

INFLUENCE OF THE MOBILE PHASE pH ON THE CAPACITY FACTORS OF ANIONS IN CHROMATOGRAPHIC SYSTEMS WITHOUT TBA

Anion	pH of phosphate buffer						pH of H <sub>2</sub> SO <sub>4</sub> solution	
	2.90	3.38	4.00	5.08	6.03	6.93	2.85	3.95
NO <sub>2</sub> <sup>-</sup>	0.45	0.32	0.08	0.01	-0.07	-0.08	0.54	0.16
NO <sub>3</sub> <sup>-</sup>	0.17	0.10	0.03	0.02	-0.02	-0.04	0.48	0.02
Br <sup>-</sup>	0.11	0.05	0.02	-0.03	-0.06	-0.06	0.32	0.05
I <sup>-</sup>	0.28	0.16	0.06	0.08	0	-0.02	0.67	0.09
SO <sub>4</sub> <sup>2-</sup>	-0.03	-0.04	-0.05	-0.03	-0.06	-0.12	-	-
CrO <sub>4</sub> <sup>2-</sup>	0.54	0.13	0.016	-0.01	-0.04	-0.14	0.8	0.1
WO <sub>4</sub> <sup>2-</sup>	0.48	0.17	-0.04	-0.04	-0.14	-0.10	8.1	1.3
MoO <sub>4</sub> <sup>2-</sup>	1.6	0.22	-0.01	-0.03	-0.15	-0.15	2.6	0.4

particularly, of nitrite peaks at pH 3 can be ascribed to the lower stability of the latter ion in a more acidic medium. The peak shape of other non-metallic ions was not influenced by the pH.

At pH 6.9, bivalent oxo anions of Cr, Mo and W were evidently excluded more than were monovalent non-metallic anions. Within the accuracy of these measurements, their exclusion can be compared to that of sulphate anions. The behaviour of vanadate, which was entirely different, is described later.

When the mobile phase was acidified to pH 4 or 3 with sulphuric acid, the retention of all of the anions was higher than was the case when phosphate buffers were used as the mobile phase. However, the peak shapes of individual anions were unaffected.

#### Systems with TBA

The retention of all injected ions increased at all tested acidities (*cf.*, Tables I

TABLE II

INFLUENCE OF THE MOBILE PHASE pH ON THE CAPACITY FACTORS OF ANIONS IN CHROMATOGRAPHIC SYSTEMS WITH TBA

H = Capacity factor greater than 30.

Anion	pH of phosphate buffer						pH of H <sub>2</sub> SO <sub>4</sub> solution		pH of Tris buffer 6.89
	3.07	3.40	4.09	5.04	6.10	6.91	2.96	3.97	
NO <sub>2</sub> <sup>-</sup>	0.9	1.8	1.9	1.8	1.2	0.7	1.4	2.7	0.78
NO <sub>3</sub> <sup>-</sup>	5.8	4.9	3.0	2.8	1.9	1.3	3.9	4.8	0.77
Br <sup>-</sup>	4.0	3.0	2.0	1.9	1.3	0.8	2.6	3.4	-
I <sup>-</sup>	14	12.5	9.2	8.8	6.3	4.6	10	13	-
SO <sub>4</sub> <sup>2-</sup>	2.6	1.6	1.3	1.2	0.7	0.2	-	-	-
CrO <sub>4</sub> <sup>2-</sup>	26	16	7.5	5.6	2.7	1.0	H	9	0.93
WO <sub>4</sub> <sup>2-</sup>	H	H	H	H	3	0.8	H	H	0.77
MoO <sub>4</sub> <sup>2-</sup>	H	H	H	17	2.5	0.9	14	H	0.78

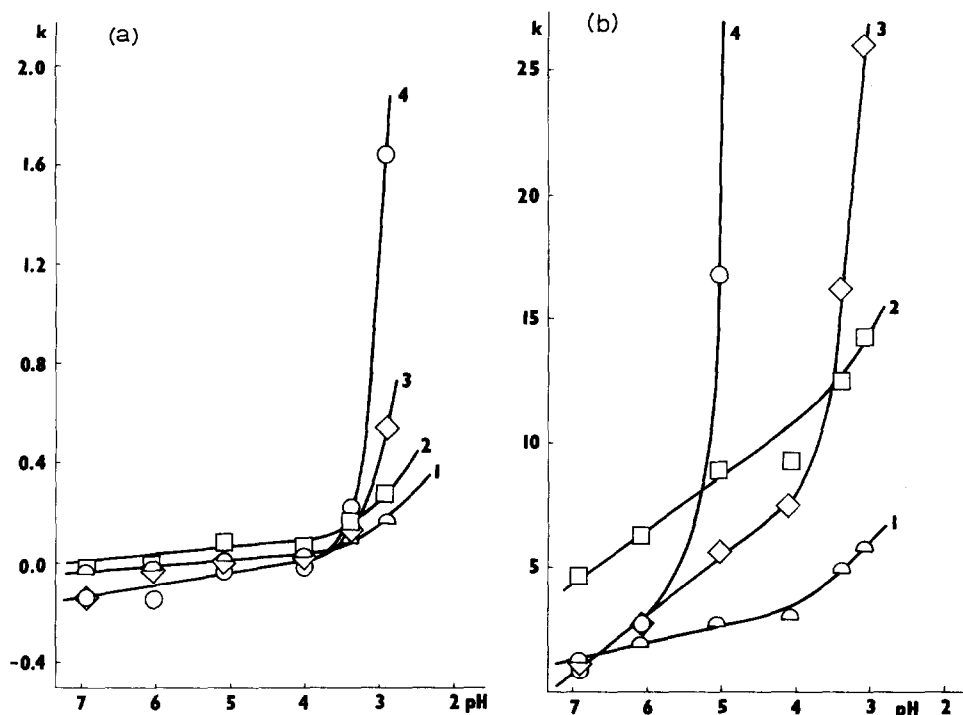


Fig. 1. Characteristic dependences of the capacity factors of anions of different types on pH without (a) and with (b) TBA in the mobile phase. Mobile phase: 50 mM phosphate buffer. Anions: 1, nitrate; 2, iodide; 3, chromate; 4, molybdate.

and II) after the addition of 2 mM TBAOH neutralized with  $\text{H}_3\text{PO}_4$  to a 50 mM solution of phosphate buffer, used as the mobile phase. Individual differences in retentions and the dependence of retention on pH became more marked. However, neither the chromatographic behaviour of the ions of both types (non-metallic anions and metal oxo anions) nor the elution sequence of ions of the same type changed qualitatively.

With non-metallic anions, a marked increase in retention, caused by the increase in mobile phase acidity, had already begun at  $\text{pH} \leq 7$ . However, it remained a linear function down to pH 4 for all anions of this type and was not associated with an anomalous increase in peak widths or with other distortions of peak shapes of stable anions even at  $\text{pH} < 4$ . The increase in retention was substantially more marked for oxo anions of Cr, Mo and W than for non-metallic anions, but commenced at higher pH values. At the same time, both the shift in pH and the slope of the retention increase depended on the chemical properties of the central metal ion (*cf.*, Fig. 1a, b). Commencing at pH 6, the retention increase of the metallic oxo anions was associated with an anomalous increase in peak widths (Fig. 2), which was reflected in a substantial decrease in column efficiency (Table III). A change of pH by four units resulted in 25-fold increase in the retention of chromate; for molybdate and tungstate the increase was still higher.

When the pH of a 2 mM solution of TBAOH was adjusted with sulphuric

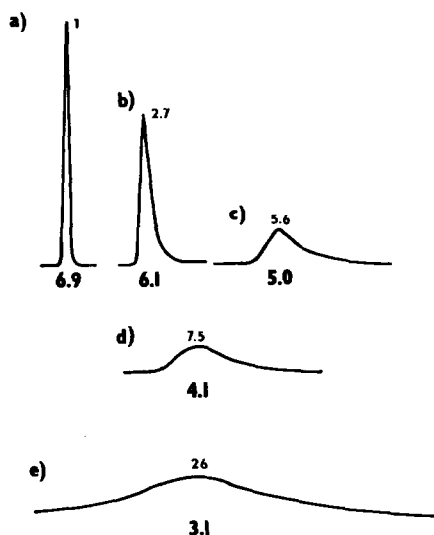


Fig. 2. Influence of pH on the shape of the chromate peak. Mobile phase as in Fig. 1b. Mobile phase pH: a, 6.91; b, 6.10; c, 5.04; d, 4.09; e, 3.07. Figures at peak maxima indicate values of capacity factors.

acid, it was found that the increase in the concentration of sulphate ions, corresponding to a decrease in pH from 4 to 3, resulted in a decrease in retention of all anions except chromate, *i.e.*, with respect to the retentions in the mobile phase at pH 4 containing sulphate ions and the mobile phase at pH 3 containing phosphate buffer. The behaviour of tungstate could not be evaluated because of the too high retentions. This experiment was the only one to show a decrease in anion retention upon increasing the concentration of hydrogen ions in the mobile phase. In accord with observations by Skelly<sup>4</sup>, this decrease indicates a strong competitive ability for sulphate ions.

The tests of the influence of organic modifiers in the mobile phase on the elution of inorganic ions showed that additions of 20% methanol or 10% acetonitrile result in almost identical decreases in retention. However, the separation selectivity

TABLE III

REDUCED HEIGHT EQUIVALENT TO A THEORETICAL PLATE,  $h$ , FOR DIFFERENT ANIONS AT DIFFERENT pH VALUES OF THE MOBILE PHASE CONTAINING TBA

Anion	pH of phosphate buffer					pH of H <sub>2</sub> SO <sub>4</sub> solution	
	3.07	4.09	5.04	6.10	6.91	2.96	3.97
NO <sub>3</sub> <sup>-</sup>	8.8	8.9	9.1	3.8	3.7	9.8	4.7
I <sup>-</sup>	6.8	51	4.0	4.5	4.1	4.1	4.2
CrO <sub>4</sub> <sup>2-</sup>	210	170	160	57	3.9	I*	290
WO <sub>4</sub> <sup>2-</sup>	I	I	I	320	6.6	I	I
MoO <sub>4</sub> <sup>2-</sup>	I	I	1300	100	5.2	I	I

\* The measurement could not be evaluated because of the too high retention.

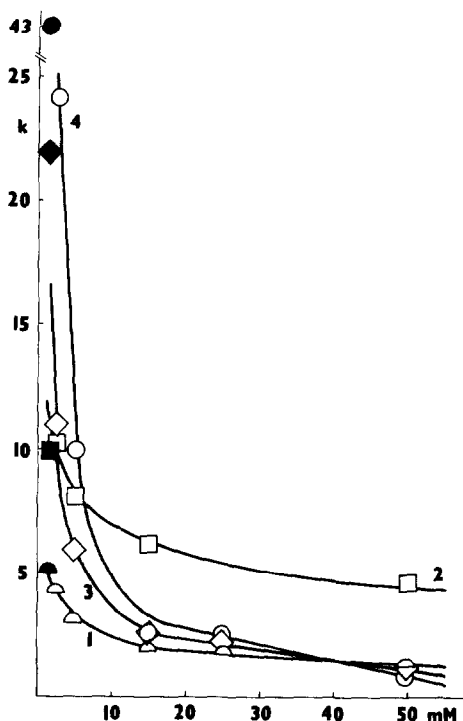


Fig. 3. Characteristic dependences of capacity factors of anions of different types on the concentration of phosphate buffer in a mobile phase containing 2 mM TBAOH neutralized to pH 6.9 with  $H_3PO_4$ . Solutes as in Fig. 1. Full symbols indicate capacity factors measured in a mobile phase with 1 mM TBAOH neutralized to pH 6.9 with HCl.

did not change and there was no improvement in the broadened peaks of metallic oxo anions.

The dependence of retention on the concentration of so-called indifferent electrolytes was tested with mobile phases containing different concentrations of phosphate buffer at pH 7 in addition to 2 mM TBAOH neutralized with phosphoric acid. The retention of all anions was found to increase monotonously with decreasing buffer concentration, most rapidly at buffer concentrations less than 15 mM (Fig. 3). This increase is accompanied by an improvement in the selectivity of the separation system, which was observed also with the tungstate-molybdate pair. Despite the fact that both oxo anions are eluted as narrow symmetrical peaks under these conditions, the difference in the values of the capacity factors in the mobile phase without addition of phosphate buffer is too small to provide a good mutual separation of these anions.

An approximately two-fold increase in the retention of metallic oxo anions was observed in a mobile phase prepared by neutralization of a 1 mM solution of TBAOH with hydrochloric acid. However, the difference between the capacity factors of tungstate and molybdate was unchanged from that in the mobile phase containing 2 mM TBAOH neutralized with phosphoric acid. The retention of non-metallic anions also remained almost unchanged (Fig. 3).

### Anomalies

Vanadate samples in the chromatographic systems described exhibited anomalously high retentions even when compared with molybdate, tungstate and chromate. For instance, with a mobile phase containing 2 mM TBAOH and 0.1 mol of Tris buffer, adjusted to pH 6.9 by the addition of sulphuric acid, vanadate could not be eluted from the column even at volumes corresponding to more than fifty times the column dead volume. The capacity factors,  $k$ , of all other anions were about 0.8 when this mobile phase was used as the eluent (Table II). Even the addition of 30% acetonitrile to this mobile phase did not result in elution of vanadate. When a mobile phase without TBA, pH 6.9, containing 25 mM phosphate buffer was used, the tested anions were not retained or were excluded. Only vanadate was eluted as a broad and heavily tailing peak with  $k \approx 5$ .

The above experiments demonstrate the anomalously strong sorption of vanadium(V) ions, even in systems without TBA. In order to evaluate whether interactions with the metal walls of the column and the joints or with the column packing are responsible for these anomalies, vanadate ions were injected into an empty 2 × 500 mm stainless-steel column and into a column with bed dimensions 4 × 100 mm, packed with glass beads, 60 μm in diameter. 1,2-Naphthoquinone and thiourea were used as reference solutes. The mobile phase was 50 mM phosphate buffer, pH 6.9, containing 2 mM TBA. The shape of the vanadate peak was similar to those of the peaks of the reference solutes, and the magnitude of its response did not depend on the number of injections when using the empty column. However, when eluted from the column packed with glass beads, the vanadate peak suffered more marked tailing than the peaks of the reference solutes (Fig. 4). When vanadate was repeatedly injected at constant time intervals of 3 min, the response of the photometric detector increased linearly to a limiting value. If the sequence of injections was temporarily interrupted, the response decreased, however, when the sequence of injections was restored the response again reached the same limit value (Fig. 5).

It is obvious from the above experiments that vanadate retention is caused by interaction with the surface of the glass beads. Changes in the peak height result

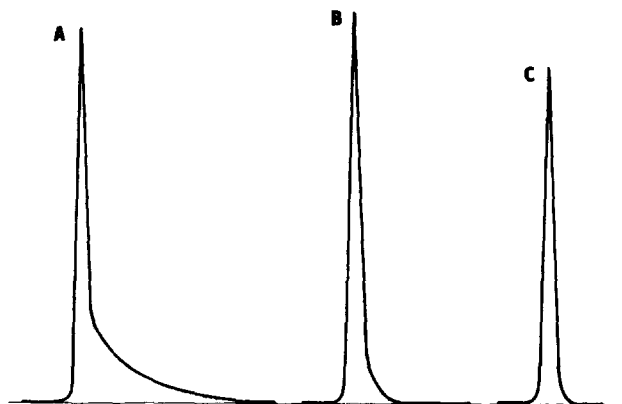


Fig. 4. Peak shapes of vanadate (A), 1,2-naphthoquinone (B) and thiourea (C) obtained by the elution from a column packed with glass beads. For other details see the text.

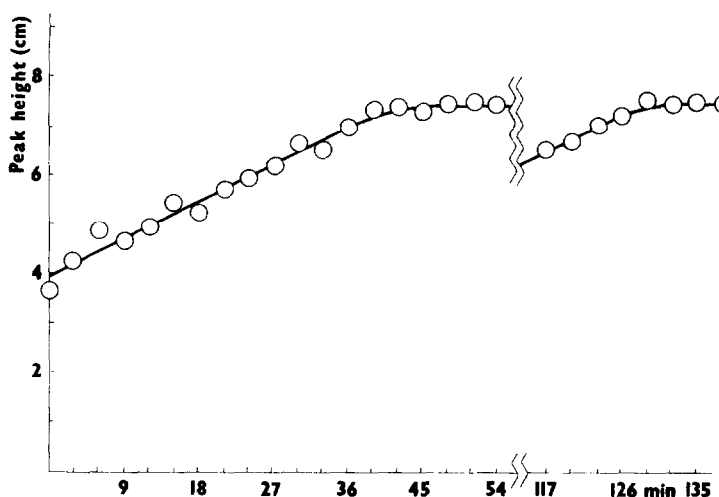


Fig. 5. Changes in the peak heights of vanadate, observed with repeated injections of  $4 \mu\text{g}$  of vanadate into a column packed with glass beads. For details see the text.

from slow saturation of the bead surface with vanadate ions and from slow elution of trapped ions.

Composite responses were obtained when sulphur ions were injected into mobile phases containing TBA, described as in refs. 3 and 9. There were several extremes corresponding to increases in absorbance and one extreme corresponding to a decrease in absorbance (Fig. 6) when elution was performed with a phosphate buffer containing TBA. As the pH was changed, not only the position of the response extremes but also the relative magnitude of some of them (the second and third extremes) varied. The position of the first absorbance maximum, detected at an elution volume corresponding to approximately 20% exclusion of eluted particles, did not change.

From a comparison of the magnitude on the response of the standard sulphate sample injected directly into the detection cell with the magnitudes of various absorbance maxima and from their pH dependence followed that the second maximum in Fig. 6 corresponds to the elution of sulphate ions. Considering the mobile phase composition, the absorbance minimum can be correlated with the decrease in concentration of phosphate ions after the sulphate peak. The origin of the first absorbance maximum is not clear.

## DISCUSSION

It is obvious from a detailed evaluation of the experimental results that the behaviour of the metal oxo anions studied differs considerably from that of the non-metallic ions selected as references. On the other hand, there is no significant difference between non-metallic oxo anions and halides. The chromatographic behaviour of oxo anions of Mo(VI), W(VI) and Cr(VI) and that of non-metallic anions will therefore be discussed separately.



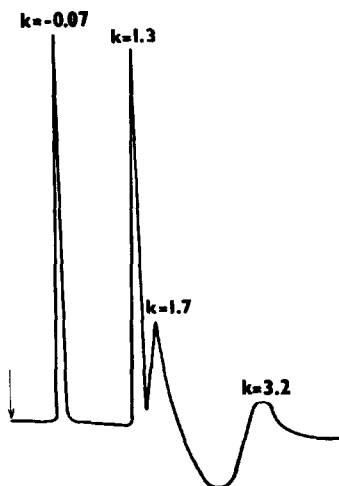


Fig. 6. Typical photometric response of sulphate ions in a mobile phase with 50 mM phosphate buffer, pH 4. TBA concentration: 2 mM.

#### *Anions of non-metals*

Our measurements showed that the increase in retention with decreasing pH observed by Skelly<sup>4</sup> for nitrite and nitrate in a chromatographic system with octylamine as counter ion probably occurs with all inorganic anions of non-metals and is independent of the presence of onium counter ion. Ion exchange on a pre-adsorbed layer of positively charged onium ions<sup>4</sup> or the formation of ion pairs with the onium counter ion<sup>3</sup> cannot therefore be considered as responsible for the retention of these anions. Knox's explanation of the retention of organic acids in terms of their hydrophobic interaction with chemically bonded alkyl groups<sup>8</sup>, whereby the increase in retention of acids with decreasing pH is assumed to be due to the decrease in the fraction of the ionized form of the acid, also cannot be applied. All the acids corresponding to the inorganic anions studied are completely ionized even at the lowest mobile phase pH used. It cannot be assumed that retention is caused by the interactions of relatively small, fully hydrated, anions with hydrocarbon groups bonded to the silica gel. A significant increase in the importance of this interaction with decreasing pH is also improbable.

On the basis of our experimental results and previously published data we cannot propose an unambiguous explanation of the retention of non-metallic anions on reversed stationary phases and with a mobile phase without onium counter ion, nor of the increase of this retention with decreasing pH. However, if account is taken of the results of Skelly<sup>4</sup> on the influence of the stationary phase on the separation of inorganic anions and of the knowledge obtained by studying polysilicic acids and silica gel<sup>10</sup>, it can be concluded that the retention of non-metallic anions is determined by their interactions with the silica gel matrix used for the preparation of the reversed phase. Coulombic interactions of the anions with protonated silanol groups can be considered as the retention mechanism proper. The increase in retention with decreasing pH can be ascribed to the change in the ratio of negatively charged, dissociated silanol groups to protonated, positively charged silanol groups.

Knox *et al.*<sup>9</sup> verified that the exclusion of small anions of organic acids from reversed stationary phases is caused by  $\equiv\text{Si-O}^-$  groups the concentration of which on the surface of sorbents is of the order of  $10^{-2} \mu\text{mol/m}^2$  when almost neutral aqueous mobile phases are used. These groups are formed by the dissociation of residual silanol groups unaffected by silanization, the concentration of which is estimated at  $3 \mu\text{mol/m}^2$ .

The acidity of silanol groups on the surface of amorphous silica gel was found to vary continuously over several orders of magnitude. According to measurements by different authors<sup>10</sup>, the minimum  $\text{p}K_a$  of surface silanols varies over the range 6.4–7.2. The isoelectric point of polysilicic acids<sup>10</sup> is, however, in the range of pH 1.5–2. It can therefore be assumed that the  $\text{p}K_a$  values of the surface silanols vary in the range of several units, possibly from 7.2 to 1.5. The proton-accepting abilities of residual silanols, through which positively charged  $\equiv\text{Si-O-H}_2^+$  ions are produced, will cover approximately the same range. The ratio of positively to negatively charged surface groups must vary significantly with changing pH, while their total concentration obviously remains an order of magnitude lower than the total concentration of underivatized surface silanols.

Retention of non-metallic anions in the absence of an onium counter ion, as discussed above, can be considered as a fundamental anionic retention. The limited linearity of its increase with decreasing pH (Fig. 1a) may be caused by any of the following factors, or by their combinations:

(a) non-linearity of the decrease in the surface concentration of negatively charged groups

(b) non-linearity of the increase in the concentration of positively charged groups

(c) the shape or the distribution of pore sizes of the sorbent and associated non-linear changes in the fraction of the solid phase surface accessible to anions

(d) the influence of negative charges on the solid phase surface on the intensity of interactions of the anions with positively charged centres

The suppression of exclusion of anions caused by the so-called indifferent electrolytes can be explained by electrostatic shielding of negative surface charge by cations of salts. This shielding can increase the fraction of the internal pore volume accessible to anions of acids and hence also the fraction of the solid phase surface with which anions can interact. It can also affect interactions of chromatographed anions with positively charged centres on the surface. In the presence of onium ions, in addition to shielding effects similar to those observed with inorganic cations, the essential retention of anions increases due to mechanisms such as the formation of ion pairs<sup>3</sup> or ion exchange on a pre-adsorbed layer of onium cations<sup>4</sup>. With respect to Skelly's results on the effect of mineral acids on the retention of anions<sup>4</sup>, confirmed by the suppression of retention of anions in the presence of higher concentrations of sulphate ions (Table II), an ion-exchange mechanism is more probable. The higher efficiency of onium cations in comparison with inorganic cations is obviously associated with preferential sorption of onium cations on the reversed stationary phase even at their lower concentration in the mobile phase.

Differences in the retentions of anions with identical charges suggest that the interactions of anions with the stationary phase cannot be interpreted in terms of a mere electrostatic attraction. The existing experimental data are, however, insufficient for further discussion of these differences and for their interpretation.

### *Metal oxo anions*

In an almost neutral mobile phase, oxo anions of Cr(VI), Mo(VI) and W(VI) behave almost identically to non-metallic anions. Under these conditions, an identical retention mechanism can also be assumed. With decreasing pH, however, their behaviour changes markedly. A retention increase with decreasing pH in the absence of onium counter ion first appears as a more rapid suppression of the marked exclusion of bivalent anions. Starting from  $\text{pH} \leq 4$ , the retention of metal oxo anions increases much more rapidly than that of non-metallic anions (Fig. 1a) and is evidently associated with anomalous peak broadening. In systems with onium counter ions a more rapid increase in retention of the oxo anions of Cr, Mo and W is noted at higher pH values than in systems without onium ions (Fig. 1b). It commences at pH less than 6.9 and is connected with an anomalous peak broadening (Fig. 2).

A new, relatively slow, interaction with the stationary phase, qualitatively different from the interactions discussed for non-metallic anions, is responsible for the changes in the peak shapes of metal oxo anions. It follows from the rapid growth in retention, strongly differentiated for individual metal anions, and from the identical configuration and charge of  $\text{CrO}_4^{2-}$ ,  $\text{MoO}_4^{2-}$  and  $\text{WO}_4^{2-}$ , that this additional interaction must be relatively strong and dependent on the chemical properties of the central metal ion. As with non-metallic anions, it must be assumed that the retention of metal anions in the absence of onium ions is caused by their interaction with the silica gel matrix of the stationary phase. This must also be the reason for the anomalous peak broadening with decrease in pH.

Silanol groups of hydrated silica in both monomeric and polymeric forms are known to react with hydroxyl groups of other compounds with the elimination of water. Relatively stable compounds of complex structure are thus formed. Complexes with organic diols of aromatic character<sup>10</sup> can be used as an example. Analogous trends are known also with fully hydrated molybdenum trioxide, the composition of which can be expressed by the formula  $\text{Mo}(\text{OH})_6$ . Molybdate forms complexes with compounds having two hydroxyl groups in *ortho*-positions (polyphenols, sugars, etc.)<sup>10,11</sup> by the same mechanism as hydrated silica gel. Heteropolyacids produced from acidic solutions of molybdates in the presence of some other oxo anions (e.g.,  $\text{SiO}_4^{4-}$ )<sup>10</sup> have been described. It can therefore be assumed that the formation of complexes of completely hydrated metal oxo anions with silanol groups is responsible for the significantly higher increase in retention of metal oxo anions in comparison with non-metallic anions, associated with an excessive peak broadening. The non-linearity of the increase in retention with decrease in pH can be explained in an analogous manner to the increase in basic retention of anions.

The extraordinarily high retentions of vanadate anion, noticeable even at pH 7 in the absence of onium ions, can be explained in the same way. In accord with ref. 10, it is sufficient to assume that the interactions of fully hydrated vanadate anion with surface silanols, leading to the formation of complexes, are even stronger than the corresponding interactions of molybdate.

The formation of isopolyacids may be one of the reasons for the changes in retention of the metal oxo anions studied upon variations in pH. Experiments with chromate ions however show that these changes cannot be connected with anomalous peak broadening<sup>3</sup>. The formation of heteropolyacids of the anions studied with phosphate buffer at lower pH cannot result in anomalous peak broadening. This is ob-

vious from the anomalous broadening of peaks of oxo anions of W and Mo in mobile phases containing only sulphate anions (Table III). Neither can abnormal broadening of peaks of chromate, molybdate and tungstate be caused by the reaction of these ions with the wall of the stainless-steel column. There are several reasons for this. Molybdates and tungstates are so stable that their reduction can only result from the action of highly efficient reducing agents, such as  $\text{SnCl}_2$  or metallic zinc, and only in a strongly acidic medium, *e.g.*, in diluted HCl. Even the considerably more reactive chromate requires a medium of about 1 M HCl (pH  $\approx$  0) if it is effectively to reduce  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ . Abnormal peak broadening, however, appears at pH  $\approx$  6 and is least evident for the most reactive chromate (Table III). Column dimensions were selected such that the regime of infinite column diameter might apply during the elution in which the injected solute does not come into contact with the column wall.

After injection of either chromate, molybdate or tungstate only one peak was always obtained, disregarding the mobile phase pH. When positively charged TBA ions were added to these chromatographic systems, the retentions of peaks increased without exceptions. Such behaviour is possible only if no reaction of chromate, molybdate and tungstate with stainless-steel takes place. The stability of metal oxo anions was also supported by the fact, that the peak area did not decrease with increasing mobile phase pH.

## CONCLUSIONS

It was observed that a significant increase in retentions of inorganic anions occurred in a reversed-phase chromatographic system with silica gel chemically modified with alkyl groups, even in the absence of onium counter ions, by merely increasing the concentration of hydrogen ions. Anomalous peak broadening or distortion of peak shape (tailing) does not occur with anions of stable acids, derived from non-metals ( $\text{NO}_3^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{SO}_4^{2-}$ ) even at lower pH values. Coulombic interactions of anions with positively charged protonated silanol groups are considered to be responsible for this retention, called fundamental anionic retention. The increase in fundamental anionic retention with decreasing pH was ascribed to phenomena associated with changes in the surface concentration of negatively charged dissociated silanols and protonated silanol groups. The increase in retention of oxo anions of Cr(VI), W(VI) and Mo(VI) with decreasing pH, associated with anomalous broadening of their peaks, is substantially more expressive. It is explained by an additional retention mechanism based on the formation of complexes of the fully hydrated metal oxo anions with residual silanols. The extremely high retention of vanadate anion can be explained in the same way.

The retention of non-metallic anions in the presence of onium counter ions is explained by two concurrent mechanisms: fundamental anionic retention and the contribution resulting from the presence of onium ions. The latter can be called counter ionic retention. In the presence of onium counter ions, retention of metal oxo anions is considered to be a sum of three contributions: fundamental anionic retention, counter ionic retention and retention caused by complex formation. The existence of fundamental anionic retention and the retention caused by complex formation should be confirmed by measurements with pure silica gel as a stationary phase.

Study of the influence of pH on the chromatographic behaviour of anions of different types confirmed that systems with almost neutral mobile phases containing onium counter ions are suitable for routine analyses. An organic component in the mobile phase (methanol, acetonitrile) as well as tetrabutylammonium counter ion can significantly affect only the magnitude of retention. The separation selectivity can be controlled efficiently by the addition of the so-called indifferent electrolytes.

The system studied permits a good separation of tungstate or molybdate from chromate and from common non-metallic anions (not shown). Systems with minimum concentrations of buffer and so-called indifferent electrolytes seem to have potential for the separation of the tungstate-molybdate pair. For the separation of metal oxo anions in mobile phases at  $\text{pH} < 6.5$  and of metal oxo anions showing peak tailing even at  $\text{pH} \approx 7$  (vanadate), it will be necessary to transform the separated ions into chemical forms whose reactivity towards silica gel will be eliminated or, at least, significantly suppressed. The formation of simple, stable complexes of metals unable to react with silanol groups may be profitable here. From the analogy with the separation of cations, this step should improve also the selectivity of mutual separation of metal oxo anions.

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