Journal of Chromatography, 447 (1988) 315–322
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 546

ANION CHROMATOGRAPHY USING A COATED PRP-1 COLUMN AND ELUENTS OF pH > 7

E. PAPP* and A. FEHÉRVÁRI
Research Group for Analytical Chemistry, Hungarian Academy of Science, P.O. Box 158, H-8201 Veszprém (Hungary)

(First received November 19th, 1987; revised manuscript received February 8th, 1988)

SUMMARY

A non-polar stationary phase (PRP-1) modified with cetrimide was used for the high-performance liquid chromatographic separation of anions. The retention behaviour of common anions (F^-, Cl^-, NO_2^-, Br^-,NO_3^-, SO_4^{2-} and HPO_4^{2-}) and some weakly dissociated anions ($H_2AsO_3^-$, $HAsO_4^{2-}$, $H_2BO_3^-$, HS^-, HCO_3^- and CO_3^{2-}) was studied using aqueous eluents containing 24% (v/v) methanol, potassium hydrogenphthalate (suitable for indirect UV detection) and tris(hydroxymethyl)-aminomethane (pH > 7).

INTRODUCTION

One of the drawbacks of published methods for the chromatographic determination of weak acid anions is that the eluent is only suitable for fast-running components (*e.g.*, F^-, silicate, borate, arsenite) and is not good enough for bivalent anions (*e.g.*, SO_4^{2-}, arsenate) or, if the eluent is powerful enough for multivalent ions, it gives a poor resolution of anions with short retention times.

In our view, coated anion-exchange columns give more flexibility than anion-exchange columns with a fixed capacity and functional groups. With these columns eluents of pH < 7 have mostly been used[1–3]. Separations with eluents of higher pH were reported by Wheals[4] in 1987. Anions were separated with acetonitrile–citrate at pH 11 on a poly(styrene–divinylbenzene) stationary phase dynamically coated with hexadecyltrimethylammonium hydroxide. Detection was effected by measuring the UV absorbance (220 nm) and with electrochemical cells connnected in series.

The method presented here deals with the separation of anions on a PRP-1 column coated permanently with cetrimide and with indirect UV absorption detection. When a phthalate–Tris eluent of pH > 7 is used, then not only common anions but also anions of weak acids can be chromatographed and monitored with indirect detection.

In a previous paper[5] the reasons for the appearance of system peaks and their

0021-9673/88/$03.50

chromatographic behaviour were investigated. If the mobile phase contains tris-(hydroxymethyl)aminomethane (Tris) buffer in addition to potassium hydrogenphthalate, then only one system peak ($S_{\text{phthalate}}$) is obtained. In an eluent of pH 8.5 the retention time of $S_{\text{phthalate}}$ is small and, being near the dead volume, it does not affect the chromatographic detection of anions.

EXPERIMENTAL

The laboratory-made instrument used was described previously[5]. The analytical column (150 mm × 4.1 mm I.D.) contained PRP-1 (5 μm) poly(styrene–divinylbenzene) copolymer (prepacked) (Hamilton). The column was dynamically coated with cetyltrimethylammonium bromide (cetrimide).

The PRP-1 column was first washed with 25–30 cm^3 of eluent of pH 7, consisting of 24% (v/v) methanol containing 1 m*M* potassium hydrogenphthalate (KHP) and 1 m*M* Tris. Then 0.2 m*M* cetrimide was added to the eluent. The breakthrough curve of cetrimide was recorded with a refractive index detector connected in series with a UV detector and the amount of cetrimide adsorbed by the column was found to be 210 μmol. The column was then washed again with the cetrimide-free eluent until the retention of the sample ions (F^-, Cl^-, NO_2^-, Br^-, NO_3^- and SO_4^{2-}) did not change in a subsequent injection. (As long as the Br^- ions bound to the quaternary ammonium ions are exchanged with phthalate, the retention of the sample ions decreases.)

The column temperature was maintained as 25 ± 0.5°C by use of a water-bath (Type U10, MLW) and a water-jacket. The eluents were prepared as described earlier[6] with a constant 24% methanol concentration. The pH of the eluents was measured with an OP-208 precision digital pH meter (Radelkis) and a combined glass electrode calibrated with aqueous buffers of pH 4.0 and 7.0.

Standard sample solutions (0.01 *M*) were prepared from AnalaR-grade salts dissolved in deionized water.

RESULTS AND DISCUSSION

The pH of the eluent was changed by increasing of the concentration of the Tris buffer component (2, 10 and 20 m*M* Tris, pH = 7.0, 8.7 and 9.1, respectively) Table I gives the retention volumes (V_R) at these three pH values. As can be seen, the retention volumes of anions which are present in the same form at these pH values do not change. However, the retention volume of the phosphate changes dramatically (from 2.25 to 4.1). The log k' *vs.* pH graph obtained in the pH range 3–9 shows a minimum (Fig. 1). At lower pH values phosphate ions are detected as $H_2PO_4^-$ ions [the increase in retention below pH 5 is due to the decrease in the phthalate ion (HP^-) concentration in the eluent]. At higher pH of the eluent the $H_2PO_4^-$ ions are converted into HPO_4^{2-} ions, which have a higher retention value.

The retention of the arsenate ions increases similarly when the pH of the eluent is increased from 7.0 to 8.7. At pH 7.0, 61.3% of the arsenic is present as $HAsO_4^-$ and 38.7% as $H_2AsO_4^-$, whereas at pH 8.7, 98.5% is present as $HAsO_4^{2-}$ and 1.3% as $H_2AsO_4^-$ ($pK_1 = 11.23$, $pK_2 = 6.77$)[7].

At pH 7 the arsenic elutes as arsenous acid near the dead volume. On increasing

TABLE I

RETENTION, V_R (ml), OF INORGANIC ANIONS AS A FUNCTION OF THE ELUENT pH

Stationary phase: PRP-1 coated with cetrimide (0.21 m*M* cetrimide per column). Mobile phase: 24% (v/v) methanol + 2 m*M* KHP + 1–20 m*M* Tris.

Anion	*Tris (mM)*		
	2 (pH 7.0)	*10 (pH 8.7)*	*20 (pH 9.1)*
Fluoride	1.75	1.75	1.75
Chloride	2.35	2.40	2.40
Nitrite	2.75	2.80	2.80
Bromide	3.45	3.50	3.50
Nitrate	3.90	4.15	4.05
Phosphate	2.25	4.10	4.10
Sulphate	5.35	5.50	5.50
Thiosulphate	8.85	9.45	9.30
Iodate	1.82	1.9	1.70
Bromate	2.75	2.85	2.70
Chlorate	5.74	5.80	5.80
Arsenite	–	1.40	1.50
Arsenate	2.45	4.00	4.25

the pH of the eluent the percentage of the $H_2AsO_3^-$ form increases and so its retention also increases.

Fig. 2 shows the chromatogram of some simple anions obtained at pH 9.3. The peaks occurring between the F^- and Cl^- peaks are positive system peaks due to the HCO_3^-/CO_3^{2-} content of the eluent. Similar peaks were obtained by Ishibashi *et al.*[8]

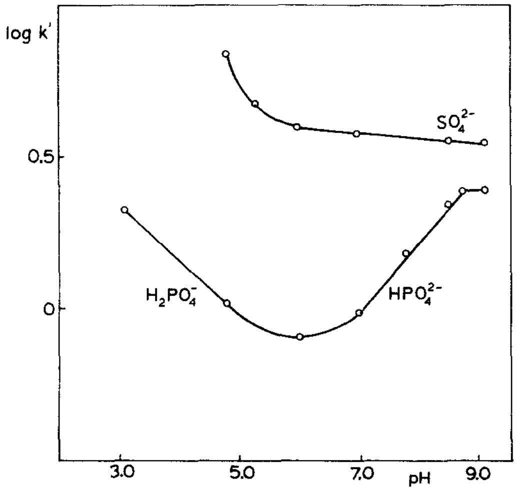
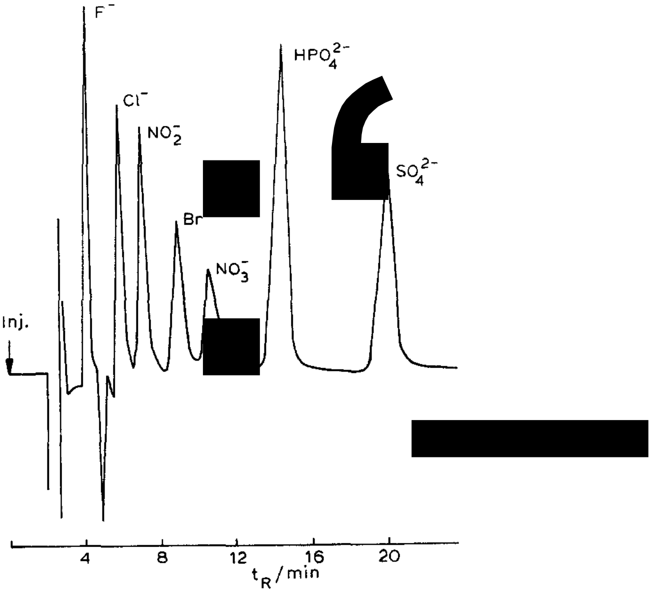


Fig. 1. Retention of phosphate ion as a function of the elutent pH. Mobile phase: 24% methanol in 2 m*M* aqueous phthalate, pH varied with Tris. Stationary phase: PRP-1 coated with cetrimide (210 μmol cetrimide per column).



[redacted]

[redacted]

[redacted]

[redacted]

Fig. 2. Chromatogram of inorganic anions. Mobile phase: 24% methanol–1 m*M* KHP–20 m*M* Tris (pH 9.3). Stationary phase: PRP-1 coated with cetrimide. Eluent flow-rate: 0.5 ml/min. Sample size: 10 μl of 10^{-4}–$5 \cdot 10^{-4}$ *M* solute. λ = 282 nm.

with a two-column ion chromatographic system with $NaHCO_3$–Na_2CO_3 as the eluent. They found that the heights, direction and retention of the peaks between the F^- and Cl^- signals depend on the CO_3^{2-}/HCO_3^- concentration ratio in the eluent.

For the identification of our system peaks, HCO_3^-/CO_3^{2-} sample solutions were injected. These ions can be detected as negative peaks as sample ions (Fig. 3a and b).

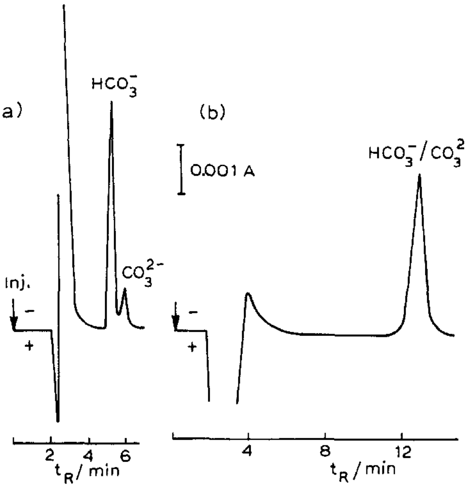


Fig. 3. Chromatogram of HCO_3^- and CO_3^{2-} ions. Mobile phase: 24% methanol–20 m*M* Tris containing (a) 0.8 m*M* KHP (pH = 9.2) and (b) 0.25 m*M* KHP (pH = 9.7). Stationary phase: PRP-1 coated with cetrimide. Eluent flow-rate: 0.5 ml/min. Sample size: 10 μl of 10^{-3} *M* $KHCO_3$. λ: (a) 282 nm; (b) 254 nm.

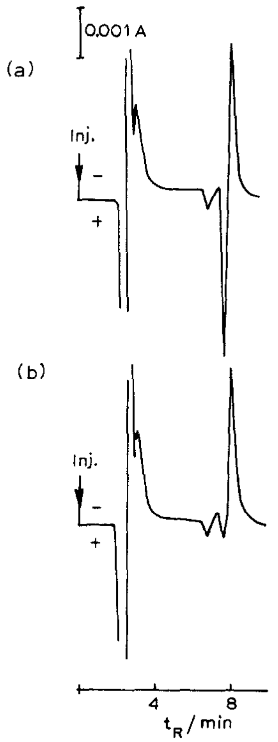


Fig. 4. Chromatogram of HS^- ion. Mobile phase: 24% methanol–0.4 m*M* KHP–20 m*M* Tris. (a) Eluent absorbed carbon dioxide. (b) Eluent prepared with carbonate-free water and measurements were made in a nitrogen atmosphere. Sample size: 10 μl of *ca.* $5 \cdot 10^{-4}$ *M* Na_2S. λ = 262 nm.

Using an eluent of lower pH (9.2) with a higher phthalate concentration (0.8 m*M*), two negative peaks are obtained. At higher pH (9.7) with a lower phthalate concentration (0.25 m*M*), only one peak is obtained. The retention is highly influenced by both the pH of the eluent and its phthalate concentration.

The positive system peak due to the absorption of CO_2 by the eluent is the result of the competition between the sample ions and the HCO_3^-/CO_3^{2-} ions (this situation is very similar to that when bromide and acetate ions were components of the eluent[1]).

The HCO_3^-/CO_3^{2-} system peak may be especially confusing for ions which are eluted with approximately the same retention as the system peak. This occurs when HS^- ions are eluted (Fig. 4a). The HCO_3^-/CO_3^- system peaks can be avoided if provision is made to exclude the absorption of carbon dioxide (Fig. 4b).

Of the anions studied, the retention of some of them (arsenite, arsenate, HS^-, borate, phosphate) can be well controlled by adjusting the pH of the eluent, but the retention of all anions can be controlled by adjusting the phthalate concentration of the eluent. The log k' *versus* log c_{KHP} relationships obtained are shown in Figs. 5 and 6. Using the curves for the arsenite and arsenate ions in Fig. 6, a phthalate concentration and pH of the eluent can be selected that are suitable for the simultaneous detection of As^{III} and As^{V} ions (Fig. 7).

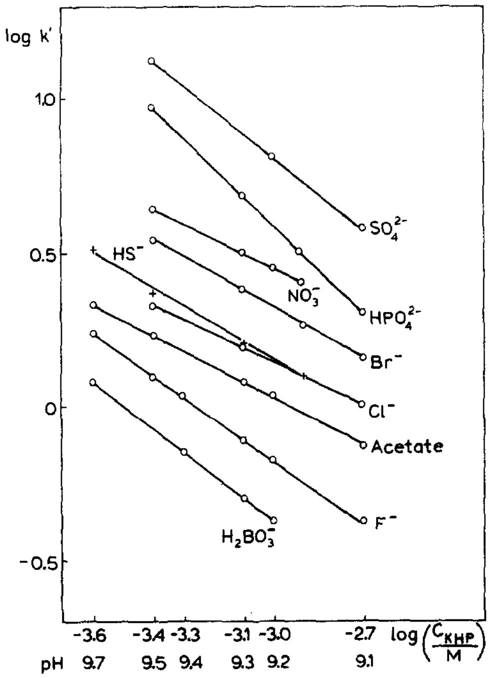


Fig. 5. Retention of inorganic anions as a function of potassium hydrogenphthalate concentration. Mobile phase: 24% methanol–0.25–2 m*M* KHP–20 m*M* Tris. Stationary phase: PRP-1 coated with cetrimide.

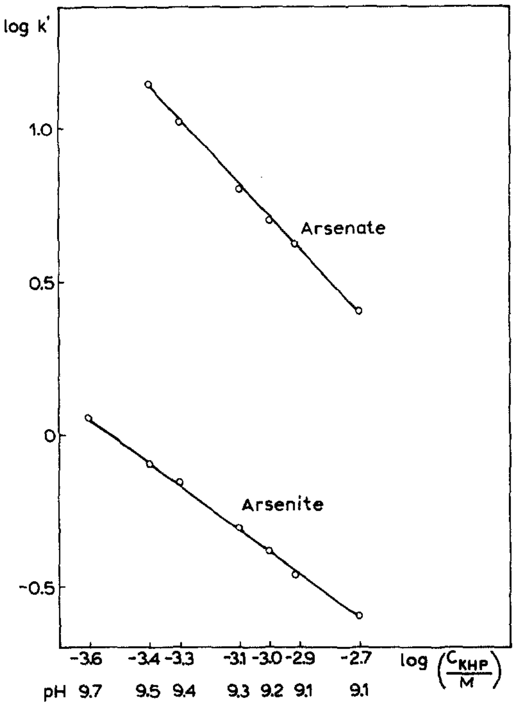


Fig. 6. Retention of arsenite and arsenate as a function of potassium hydrogenphthalate concentration. Conditions as in Fig. 5.

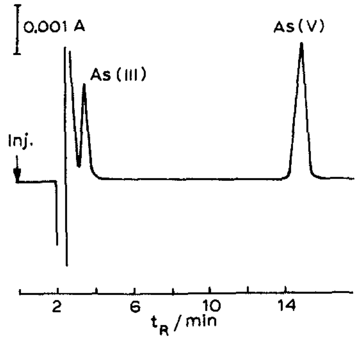


Fig. 7. Chromatogram of arsenite and arsenate ions. Mobile phase: 24% methanol–0.8 mM KHP–20 mM Tris. Stationary phase: PRP-1 coated with cetrimide. Eluent flow-rate: 0.5 ml/min. Sample size: 10 μl of $5 \cdot 10^{-4}$ M Na_2HAsO_4 and $NaAsO_2$. λ = 280 nm.

The study of the slopes of the log k' *versus* log c_{KHP} graphs makes possible a better understanding of the nature of the ion-exchange processes taking place in the system. Making use of the relationship derived by Haddad and Cowie[9]:

$$\log k' = \text{constant} - \frac{y}{x}[B^{x-}]_m$$

where k' is the capacity factor of the sample ion, $[B]_m$ is the concentration of the eluent ion (phthalate), $x = 2$, *i.e.*, the charge on the phthalate ion at pH > 7, and y = charge on the sample ion.

The experimentally obtained slopes of the log k' *versus* log c_{KHP} graphs are given in Table II. As can be seen, the agreement between the experimental and theoretical values (0.5 for monovalent and 1.0 for bivalent ions) is satisfactory for most of the ions studied (*e.g.*, Br^-, HPO_4^{2-}, Cl^-, NO_3^-, $HAsO_4^{2-}$, CH_3COO^-).The difference for the SO_4^{2-}, F^- and HS^- ions, however, is significant. No comparison can be made for the $H_2BO_3^-$ and $H_2AsO_3^-$ ions because the ratio of the neutral and monovalent forms changed as the pH of the eluent was altered.

For the SO_4^{2-} and Br^- ions the slopes were compared with the values published

TABLE II

OBSERVED SLOPES OF LOG k' *VS.* LOG c_{KHP} PLOTS

Anion	*Slope*	*Anion*	*Slope*
Chloride	0.47	HS^-	0.61
Bromide	0.56	$H_2BO_3^-$	0.75
Fluoride	0.69	$H_2AsO_3^-$	0.74
Acetate	0.48	$HAsO_4^{2-}$	1.05
Nitrate	0.48	SO_4^{2-}	0.81
		HPO_4^{2-}	0.96

by Small and Miller[10]. Using an anion-exchange column containing fixed functional groups and a phthalate–borate buffer system (pH 9), the slopes obtained were 0.47 and 0.98 for the Br^- and SO_4^{2-} ions, respectively.

The relatively good agreement between the experimental and theoretical results corroborates the view that the retention of anions on the cetrimide-coated column is governed by the ion-exchange equilibria between the bivalent phthalate and the sample ion when a phthalate–Tris buffer system is used as the eluent.

CONCLUSIONS

Common simple anions and the anions of weak acids can be separated efficiently on a PRP-1 column permanently coated with cetrimide if phthalate ions are used as eluent ions and the pH of eluent is controlled with Tris buffer. Owing to the absorption of carbon dioxide, eluent system peaks(s) occur on the chromatogram. These can be eliminated, however, by the use of carbonate-free eluent and by the use of a nitrogen purge and a nitrogen atmosphere during the measurements.

The column performance was checked after intensive use for 3 months. About a 5% decrease in the retention of Br^- and NO_3^- ions was observed using an eluent of pH 7.

REFERENCES

1 R. M. Cassidy and S. Elchuk, *Anal. Chem.*, 54 (1982) 1558.
2 R. M. Cassidy and S. Elchuk, *J. Chromatogr. Sci.*, 21 (1983) 454.
3 R. M. Cassidy and S. Elchuk, *J. Chromatogr.*, 262 (1983) 311.
4 B. B. Wheals, *J. Chromatogr.*, 42 (1987) 115.
5 E. Papp, *J. Chromatogr.*, 402 (1987) 211.
6 Á. Bartha and Gy. Vigh, *J. Chromatogr.*, 260 (1983) 330.
7 J. Inczédy, *Analytical Applications of Complex Equilibria*, Akadémiai Kiadó, Budapest, 1976.
8 W. Ishibashi and R. Kikuchi and K. Yamamoto, *Bunseki Kagaku*, 31 (1982) 207.
9 P. R. Haddad and C. E. Cowie, *J. Chromatogr.*, 303 (1984) 321.
10 H. Small and T. E. Miller, *Anal. Chem.*, 54 (1982) 462.