

Short communication

Manipulation of separation selectivity for inorganic anions in capillary zone electrophoresis using control of electrolyte pH

Anthony H. Harakuwe, Paul R. Haddad*

Department of Chemistry, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania 7001, Australia

Received 7 August 1995; revised 23 November 1995; accepted 28 November 1995

Abstract

The influence of the pH of chromate–tetradecyltrimethylammonium bromide-based electrolytes on the separation selectivity of anions using capillary zone electrophoresis was studied. Changes in migration order over the pH range 8–13 were marked for weak acid anions at or near their pK_a due primarily to changes in effective charge. Precipitation problems encountered for electrolyte pH values less than 8 were prevented by the incorporation of small ($\leq 7.5\%$ v/v) amounts of 1-butanol as additive. Using this approach the pH could be lowered to 7. Manipulation of electrolyte pH was used to increase the resolution between fluoride and phosphate, making possible the fully resolved separation of trace fluoride ($1 \mu\text{g ml}^{-1}$) in the presence of excess phosphate ($>800 \mu\text{g ml}^{-1}$). The separation of fluoride from phosphate in toothpaste is also shown.

Keywords: pH effects; Electrolyte pH; Inorganic anions; Fluoride; Phosphate

1. Introduction

In capillary zone electrophoresis (CZE) the extent of dissociation and thus the charge on the solute depends predominantly on the pH of the background electrolyte (BGE) [1]. Since the migration rate of a solute ion is directly proportional to net charge, pH variation is an obvious parameter for manipulation of anion separation selectivity in CZE [2–6]. Pronounced selectivity changes occur at or near the pK_a or pK_b values of the solutes [1,3,5,6] where the rate of change of ionisation is greatest. Variations in pH of the BGE can be instituted in sequential steps or as a gradient [6–8] and for this study, the former approach was employed. The range over which the pH can be varied depends on the anion(s) of interest,

the desired resolution and analysis time, electrolyte composition and practical limitations such as precipitation with various electro-osmotic flow (EOF) modifiers or UV-absorbing probes [9].

Use of chromate as both the electrolyte and UV-absorbing probe for the separation and indirect detection of inorganic anions is commonplace in CZE due to its excellent UV absorbance characteristics [2,9] and its high mobility [10] which matches that of many inorganic anions. For rapid separation of anions, a surfactant such as tetradecyltrimethylammonium bromide (TTAB) is incorporated into the BGE so that the EOF is in the same direction as the migration of the solute anions. Separation of inorganic anions by CZE using chromate BGE containing TTAB at $\text{pH} \geq 8$ has been reported widely (see, e.g., Refs. [3,9,11–13]). On the other hand, separation of inorganic anions using chromate–

*Corresponding author.

TTAB at $\text{pH} < 8$ has not been possible due to the formation of precipitates between electrolyte components. While other probes and EOF modifier systems that do not suffer precipitation at $\text{pH} \leq 8$ have been used, these are inferior to chromate in terms of detection sensitivity (except for the trimellitic and pyromellitic acids [3,14,15]), separation efficiency and speed of inorganic anion separation.

In CZE, it is difficult to separate adjacent solutes with similar mobilities [6], such as fluoride and phosphate [16], especially when they are present in significantly disparate amounts. To improve resolution, dilution is usually the first option but is unsuitable when the minor component is present at trace levels. Manipulation of separation selectivity is a better approach and the weakly acidic nature of phosphate suggests that pH variation could be exploited to maximise the migration time difference between phosphate and fluoride. This paper describes the successful implementation of this approach and its application to the determination of fluoride in toothpaste.

2. Experimental

2.1. Instrumental

A Quanta 4000 CZE instrument (Waters, Milford, MA, USA) interfaced with a Waters Maxima 820 data station was employed for all separations. Sample introduction was in the hydrostatic mode by raising the sample vial to a height of 10 cm and holding for 30 s. Separations were performed using a polyimide-coated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) measuring 52 cm to detector $\times 75 \mu\text{m}$ I.D. and 60 cm total length, with 20 kV of applied voltage from a negative power source. Indirect detection was achieved at the anodic side by the measurement of absorbance at 254 nm with chromate as UV-absorbing probe.

2.2. Reagents and procedure

Inorganic anion salts (AR grade as sodium) were sourced from Ajax (Sydney, Australia) and dried at 100°C overnight. From $1000 \mu\text{g ml}^{-1}$ stock stan-

dards, a standard mixture containing $10 \mu\text{g ml}^{-1}$ each of iodide, carbonate, iodate, nitrate and thiocyanate; $12 \mu\text{g ml}^{-1}$ phosphate; $8 \mu\text{g ml}^{-1}$ each of chloride and sulfate; $7 \mu\text{g ml}^{-1}$ bromate; $6 \mu\text{g ml}^{-1}$ nitrite; and $5 \mu\text{g ml}^{-1}$ fluoride was made up accurately. Water purified on a Milli-Q (Millipore, Bedford, MA, USA) system was used throughout. BGEs were prepared freshly on a daily basis, filtered to pass $0.45 \mu\text{m}$ and degassed by ultrasound before use. Apart from the differences in pH, all BGEs contained 5 mM chromate (LR, Ajax, NSW, Australia), 2.5 mM TTAB (Aldrich, Milwaukee, WI, USA), varying amounts of 1-butanol (LR, Ajax, Sydney) and adjusted to pH with dilute NaOH, HNO_3 or HCl (AR, BDH, Victoria, Australia). The relatively high concentration of TTAB (2.5 mM) used in this study was found to be necessary in order to achieve reproducible migration times. Measurement of pH was performed with a Model 8520 pH meter (Hanna Instruments, Singapore).

The toothpaste sample (Total gel, Colgate Palmolive, Sydney, Australia) was prepared by dissolving a 1.13-g sample in water using ultrasonification, followed by dilution to 100 ml. Aliquots of this sample were filtered to pass $0.45 \mu\text{m}$ (Millex-HA, Millipore) before analysis.

Prior to separation with each BGE, the capillary was conditioned as follows: 5 min each with water, ethanol and water; 10 min with 0.5 M KOH; 5 min with water; and 10 min with BGE. A blank sample of pure water was then separated (in triplicate) under the running conditions for 1 min. This routine was necessary to avoid poor phosphate response [17] and has the effect of stabilising the layer of TTAB adsorbed on the surface of the capillary. Anion electrophoretic mobilities were calculated according to the equation of Altria and Simpson [1] using an EOF determined by the water peak in the electropherograms.

3. Results and discussion

3.1. Effect of electrolyte pH on separation selectivity

Fig. 1 shows the effect on anion separation selectivity of BGE pH over the range 8.09–12.61 for

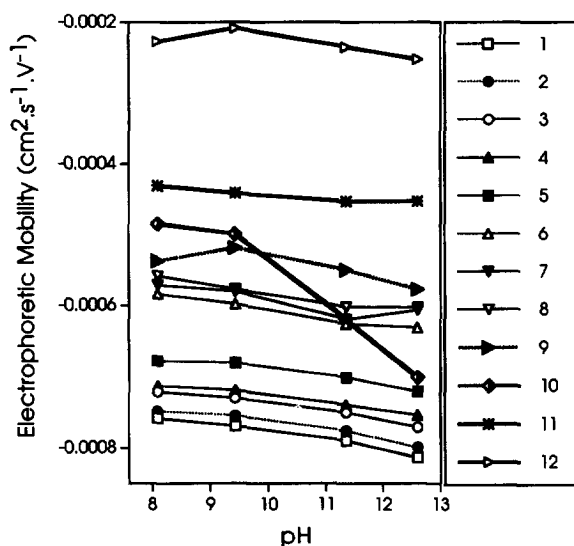


Fig. 1. Effect of electrolyte pH on electrophoretic mobility of inorganic anions. Conditions: all BGEs had 5 mM chromate and 2.5 mM TTAB; sampling was in the hydrostatic mode; indirect detection at 254 nm; separation voltage was -20 kV; fused-silica capillary (52 cm to detector $\times 75$ μm I.D.). See Section 2 for concentrations of anions. Negative mobilities denote migration to the anode. Key: 1=chloride, 2=system peak, 3=nitrite, 4=sulfate, 5=nitrate, 6=fluoride, 7=bromate, 8=hydrogenphosphate, 9=iodide, 10=hydrogencarbonate, 11=iodate, 12=thiocyanate.

a BGE containing chromate and TTAB only. Bicarbonate-carbonate showed marked changes in migration time, going from position 10 at pH of ca. 8 to position 6 at pH of ca. 12.5, due to the change in charge from -1 (as bicarbonate at pH of ca. 8) to -2 (as carbonate at pH > 10.33). Changes in migration time are most pronounced at or near the $\text{p}K_{\text{a}2}$ value of 10.3 for carbonic acid and the plot of mobility versus pH resembles a titration curve. In contrast, there was no change in the migration order of fluoride and phosphate at pH > 11 (i.e., in the vicinity of $\text{p}K_{\text{a}3} = 12.36$ for phosphoric acid) in this study, unlike the migration order changes noted for these two anions elsewhere [3]. This could be due to ion-pairing effects arising from the different amounts of TTAB used or errors in pH measurement caused by the presence of organic surfactant in the BGE [18].

With BGE containing 2.5 mM TTAB and chromate, the accessible pH range was 8–13. At pH > 13 ,

the baseline was very noisy whilst at pH < 8 , the BGE became opaque due to formation of sparingly soluble chromate-TTAB species. Precipitates did not form at pH < 8 for TTAB or chromate when present individually, suggesting that the observed precipitate involved both species. BGEs containing chromate with cetyltrimethylammonium bromide (CTAB) have been used to separate anions in standard mixtures at pH ≤ 7.5 [19], but the amount of CTAB used was less than 2% of the surfactant concentration used in this study. Our preliminary investigations have shown that at 2.5 mM, CTAB and dodecyltrimethylammonium bromide (both with 5 mM chromate) suffer also from precipitation problems at a pH of ca. 7.

Addition of small amounts of acetonitrile, 1-butanol or methanol to the BGE was found to redissolve the precipitate, even at pH values as low as 7. Of these solvents, 1-butanol was preferred after consideration of boiling points, UV absorption properties and effect on baseline, with relatively less of this solvent being required to generate a stable EOF [20], resulting in more precise migration times for anions than were obtained with the remaining solvents. A concentration of 7.5% (v/v) of 1-butanol was found to be optimal for both prevention of the precipitation at pH values as low as 7 and for stabilisation of migration times.

3.2. Effect of electrolyte pH on resolution of fluoride and phosphate

Access to BGE pH values in the range 7–8 creates the possibility of manipulation of separation selectivity for phosphate through acid-base dissociation of dihydrogenphosphate at $\text{p}K_{\text{a}2} = 7.20$ [21]. Fig. 2 shows the effect of BGE pH on resolution (R_s) between fluoride and phosphate and indicates that the protonation of hydrogenphosphate at pH 7 resulted in slower migration of this species and greatly improved resolution from fluoride. An alternative explanation for the observed migration behaviour in Fig. 2 is that 1-butanol influences the selectivity of the separation. However, additional experiments in which the concentration of 1-butanol was varied at constant pH showed that selectivity for phosphate was unaffected. Finally, it is noted that the maximum

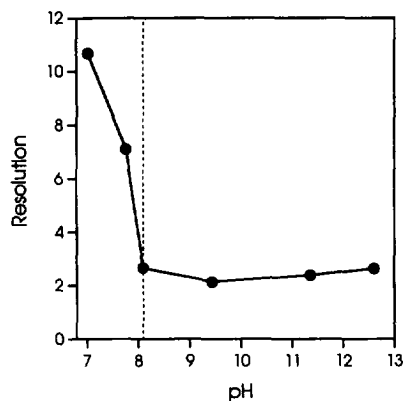


Fig. 2. Effect of pH on resolution between fluoride and phosphate. Conditions as in Fig. 1 for $\text{pH} \geq 8$. At pH values of 7.0 and 7.7, the BGEs also contained 7.5% and 2% (v/v) of 1-butanol, respectively. Resolution was calculated according to Ref. [22].

resolution (ca. 11) between fluoride and phosphate demonstrated in the present study is the best achieved to date for CZE using chromate-based BGEs.

Fig. 3 shows the separation of inorganic anions at pH 7.7 using the chromate–TTAB–1-butanol system. The utility of achieving such high resolution and selectivity is demonstrated by the separation of $1 \mu\text{g ml}^{-1}$ fluoride in the presence of $800 \mu\text{g ml}^{-1}$ phosphate (Fig. 4). The identity of the unknown peak in the electropherogram is most probably carbonate. The separation of fluoride from phosphate in toothpaste (Fig. 5) is shown as an example of an application of this approach. At pH 8.7 fluoride and phosphate were not resolved (Fig. 5a), but were separated at pH 7.5 (Fig. 5b). The addition of 1-butanol did not significantly alter the analysis time. It should be noted in Fig. 5 that the concentration of TTAB used in Fig. 5a was 0.5 mM whereas that for Fig. 5b was 2.5 mM, suggesting that the TTAB concentration might play a role in the observed changes in selectivity. However, increased TTAB concentrations normally lead to shorter migration times and in the example shown the migration time for phosphate increased substantially, indicating that the pH exerted the major influence. Additionally, it can be noted that although maximum resolution between fluoride and phosphate was achieved using a pH 7 BGE with 7.5% (v/v) 1-butanol, the improve-

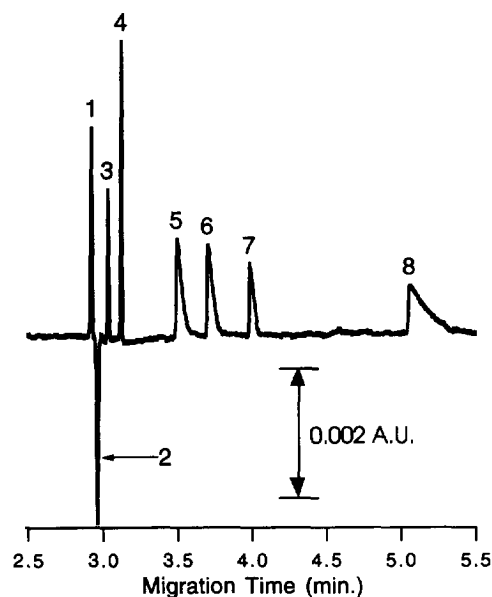


Fig. 3. Separation of inorganic anions at pH 7.7. Conditions: 5 mM chromate, 2.5 mM TTAB, 2% v/v 1-butanol. Other conditions as in Fig. 1. Anion ($\mu\text{g ml}^{-1}$): 1=chloride (8), 2=system peak, 3= nitrite (5), 4= sulfate (8), 5= fluoride (5), 6= phosphate (10), 7= hydrogencarbonate (5), 8= thiocyanate (10).

ment in resolution was such that slightly less 1-butanol (5%, v/v) and higher pH (7.5) were sufficient for separation.

At $\text{pH} \geq 8$, the presence of small amounts of 1-butanol in the BGE yielded stable EOF and solute migration times, as noted by Benz and Fritz [20]. This effect was also evident at pH 7.5, with absolute migration time precision (for five replicate injections) ranging from 1.2% relative standard deviation (R.S.D.) for chloride to 2.1% R.S.D. for carbonate.

4. Conclusions

The pH of the BGE can be used to create pronounced changes in separation selectivity for weak acid anions by altering their degree of ionisation and hence their effective charge and electrophoretic mobility. The resolution between fluoride and phosphate was improved greatly using a pH 7 chromate-based BGE containing 1-butanol. Small amounts of 1-butanol were needed to prevent pre-

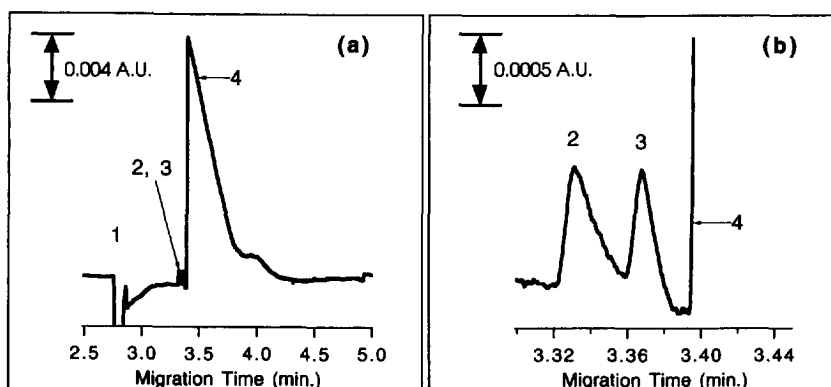


Fig. 4. Separation of $1 \mu\text{g ml}^{-1}$ fluoride in the presence of $800 \mu\text{g ml}^{-1}$ phosphate at normal (a) and expanded (b) scale. Conditions as in Fig. 3, except 5% (v/v) 1-butanol was used and BGE pH was 7. Key: 1=bromide (system peak), 2=fluoride, 3=unknown, 4=hydrogenphosphate.

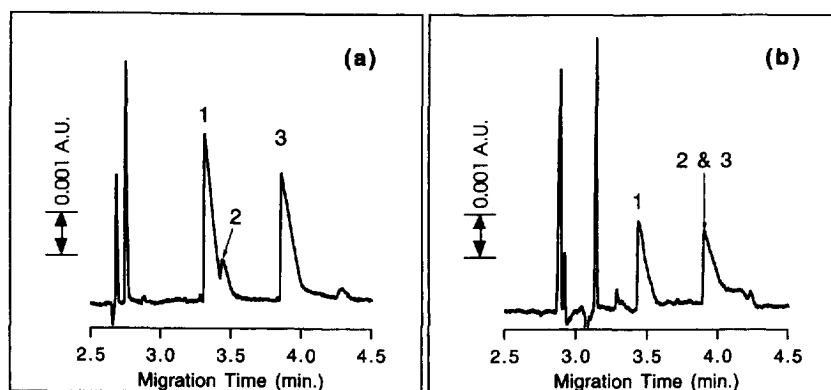


Fig. 5. Separation of fluoride and phosphate in toothpaste at pH 8.7 (a) and at pH 7.5 (b). For (a), the conditions were 0.5 mM TTAB, 5 mM chromate, pH 8.7 and 30 s injection time; for (b) the conditions were 2.5 mM TTAB, 5 mM chromate, 5% 1-butanol and pH 7.5. Other conditions for both (a) and (b) were as in Fig. 1. Key: 1=fluoride, 2=phosphate, 3=carbonate.

cipitation but did not affect the detection of anions. Separation of disparate levels of fluoride and phosphate was shown to be possible. The fully resolved separation of these anions in a standard mixture and toothpaste was demonstrated.

Acknowledgments

Financial support from Waters Corporation is gratefully acknowledged. Critical comments from Dr. Per Andersson during the preparation of this paper are also acknowledged.

References

- [1] K.D. Altria and C.F. Simpson, *Chromatographia*, 24 (1987) 527.
- [2] I.Z. Atamna, C.J. Metral, G.M. Muschik and H.J. Issaq, *J. Liq. Chromatogr.*, 13 (1990) 3201.
- [3] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
- [4] B.F. Kenney, *J. Chromatogr.*, 546 (1991) 423.
- [5] C.L. Ng, H.K. Lee and S.F.Y. Li, *J. Chromatogr.*, 598 (1992) 133.
- [6] H.-T. Chang and E.S. Yeung, *J. Chromatogr.*, 608 (1992) 65.
- [7] J. Pospíchal, M. Deml, P. Gebauer and P. Bocek, *J. Chromatogr.*, 470 (1989) 43.
- [8] V. Sustáček, F. Foret and P. Bocek, *J. Chromatogr.*, 480 (1989) 271.
- [9] P. Jandik and W.R. Jones, *J. Chromatogr.*, 546 (1991) 431.

- [10] P. Jandik, W.R. Jones, A. Weston and P.R. Brown, *LC·GC*, 9 (1991) 634.
- [11] J. Romano, P. Jandik, W.R. Jones and P.E. Jackson, *J. Chromatogr.*, 546 (1991) 411.
- [12] B.J. Wildman, P.E. Jackson, W.R. Jones and P.G. Alden, *J. Chromatogr.*, 546 (1991) 459.
- [13] W. Buchberger and P.R. Haddad, *J. Chromatogr.*, 608 (1992) 59.
- [14] M.P. Harrold, M.J. Wojtusik, J. Riviello and P. Henson, *J. Chromatogr.*, 640 (1993) 463.
- [15] S.M. Cousins, P.R. Haddad and W. Buchberger, *J. Chromatogr. A*, 671 (1994) 397.
- [16] M. Jimidar, M.S. Khots, T.P. Hamoir and D.L. Massart, *Quim. Anal.*, 12 (1993) 63.
- [17] A.H. Harakuwe, P.R. Haddad and W. Buchberger, *J. Chromatogr. A*, 685 (1994) 161.
- [18] M. Jimidar and D.L. Massart, *Anal. Chim. Acta*, 294 (1994) 165.
- [19] K. Li and S.F.Y. Li, *J. Liq. Chromatogr.*, 17 (1994) 3889.
- [20] N.J. Benz and J.S. Fritz, *J. Chromatogr.*, 671 (1994) 437.
- [21] G.H. Aylward and T.J.V. Findlay, *SI Chemical Data*, 2nd ed., Wiley, Milton, 1974, p. 123.
- [22] H.K. Jones and N.E. Ballou, *Anal. Chem.*, 62 (1990) 2484.