ELSEVIER

Journal of Chromatography A, 773 (1997) 115–121

JOURNAL OF
CHROMATOGRAPHY A

Anion-exchange chromatography with electrochemical detection of alditols and sugars at a Cu_2O–carbon composite electrode

Tommaso R.I. Cataldi[a,*], Diego Centonze[a], Innocenzo G. Casella[a], Elio Desimoni[b]

[a]*Dipartimento di Chimica, Università degli Studi della Basilicata, Via N. Sauro 86, 85100 Potenza, Italy*

[b]*Dipartimento di Fisiologia delle Piante Coltivate e Chimica Agraria, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy*

Received 28 December 1995; revised 12 February 1997; accepted 14 February 1997

Abstract

An anion-exchange column coupled with an amperometric sensor was used for the quantitative analysis of alditols and simple sugars. The sensing electrode is composed of cuprous oxide dispersed in a graphite powder–polyethylene composite matrix. The resulting Cu_2O–carbon composite electrode is stable in alkaline media and possesses good sensitivity, wide linear dynamic ranges and low detection limits for alditols, mono- and disaccharides. Alditols and carbohydrates are weakly ionizable compounds, so an anion-exchange column was employed for their chromatographic separation with an alkaline eluent. The separation problems due to the presence of low but uncontrolled amounts of carbonate in the alkaline mobile phase have been largely solved by the addition of Ca^{2+} or Ba^{2+} at a millimolar level and the consequent formation of carbonate insoluble salts. Using this strategy, the alkaline eluent provides improved separations without compromising the column's lifetime, electrode performance and chromatographic system. Under the optimal operating conditions, the detection limits of D-sorbitol, D-mannitol and D-glucose were 50, 40 and 80 pmol, respectively, with a linear concentration range up to 5 m*M*. Examples of applications, which include the separation and detection of D-sorbitol, D-mannitol and common sugars present in food samples, are illustrated.

Keywords: Electrochemical detection; Detection, LC; Electrodes; Alditols; Sugars

1. Introduction

Considerable efforts have been devoted to the development of chemically modified electrodes for achieving the detection and quantitation of non-absorbing UV–visible and scarcely electroactive compounds. Usually, modified electrodes prepared by attaching a modifier to the electrode surface by covalent bonds, adsorption or polymer film coatings, are not reproducibly obtained and their activity very often deteriorates during operation in flowing solutions. Transition metal oxides, such as nickel [1–8], cobalt [9–12], ruthenium [13–15] and copper [16–26] have attracted much interest as electrode materials or dispersed in carbon pastes because of their good electrocatalytic properties in alkaline solutions towards the oxidation of analytes of interest that lack a good electroactive group. Among the most challenging concerns of modified carbon paste electrodes are the simplicity of preparation, highly active surface and long stability, hence, a considerable number of approaches have been proposed for their construction [27–31].

Bulk modified carbon composite electrodes

---

*Corresponding author.

PII S0021-9673(97)00192-1

(CCEs) consist of a matrix of graphite powder and a water-immiscible solid binder doped with a proper catalyst. The resulting sensing electrodes are increasingly stable, sensitive and inexpensive. We previously demonstrated that a promising approach to the constant potential amperometric detection of alditols is the use of Cu_2O as an electrocatalyst, incorporated into a graphite powder–polyethylene composite matrix [26]. This sensing electrode, designed as Cu_2O–CCE, showed long-term stability coupled with significant activity towards the oxidation of hydroxyl-containing compounds in alkaline solutions. Notably, the preparation of the Cu_2O–CCE has some advantages, including a very simple procedure, the use of inexpensive synthetic graphite powder and an easy control of the catalyst loading in the carbon composite matrix. In this work, the application of the sensing electrode is pursued with the intention of using it for the detection of carbohydrates and alditols, following their separation on an anion-exchange column. To explore this possibility in real samples, some examples of application are illustrated. Amperometric detection by electrocatalytic oxidation has been shown to be advantageous in the flow injection determinations of D-mannitol [26]. The detection is based on the measurement of anodic current generated by the electrocatalytic oxidation of the substrate at the electrode surface, probably through the formation of Cu^{III} sites and the involvement of the adsorbed hydroxyl radical ($OH^{\cdot}$) as the oxygen transfer species [20]. Since electrode passivation is not present, a fixed potential can be applied in the detection mode.

2. Experimental

2.1. Reagents and materials

Sodium hydroxide, 50% (w/w) solution in water (d=1.515 g/ml), polyethylene medium density (d=0.940 g/ml), $Ca(OH)_2$ 95+%, D-sorbitol, xylitol, dulcitol, D-arabitol, *myo*-inositol, α-D-glucose, D-fructose, D-ribose, sucrose, and graphite powder (1–2 μm particle size) (Aldrich); carbon tetrachloride (Carlo Erba, Milan, Italy); D-mannitol, $Ba(OH)_2 \cdot 8H_2O \geq 98\%$ crystallized and cuprous oxide ≥97% (Fluka Chemika-BioChemika) were used as received without further purification. Stock solutions were prepared with deionized-doubly distilled water and were diluted in the supporting electrolyte just before use. Sodium hydroxide solutions, used as the mobile phase, of the desired concentration were prepared by diluting carbonate-free 50% (w/w) NaOH solution, because of the very low solubility of Na_2CO_3 in concentrated alkaline solutions. The exact concentration of the hydroxide ion in the mobile phase was determined, by titration against a standard solution of hydrochloric acid using phenolphthalein as an indicator in the neutralization reaction.

2.2. Apparatus

The chromatographic system consisted of a Varian pump (Model 2510) and an on-line vacuum degassing unit (Hewlett-Packard, Model 1050). The pump was equipped with a constant-volume injection valve Rheodyne (Berkeley, CA, USA), Model 7125, using a 50-μl sample loop. Amperometric detection was performed with an EG and G Princeton Applied Research (PAR) Model 400 electrochemical detector and a laboratory-made flow-through wall-jet electrochemical cell consisting of a Cu_2O–CCE as the working electrode, a Ag/AgCl reference electrode and a stainless steel auxiliary electrode. The detector time constant was set at 1 s. The output signal was recorded on an X-t JJ Instruments Model CR650S chart recorder. Chromatographic separations were effected with a CarboPac MA1 (Dionex) anion-exchange column (250×4 mm I.D.) coupled with the CarboPac MA1 (50×4 mm) guard column (8.5 μm bead diameter). The flow-rate was 0.4 ml/min and the pressure was about 94 bar. All experiments were carried out at room temperature (~22°C) unless noted otherwise. Alkaline mobile phase compositions are listed in the appropriate figures.

2.3. Electrode preparation

Copper(I) oxide, 50% (w/w), carbon composite electrodes were prepared by thoroughly mixing weighed amounts of catalyst, graphite powder and polyethylene in a ratio 2:1:1 (w/w/w). The mixtures were treated in boiling CCl_4 to dissolve the polyethylene and the resulting slurry was sonicated until the solvent had evaporated. Then, the mixture was heated to the melting point of polyethylene (about 150°C) and moulded. Finally, portions of the resulting paste were used to fill the end of a 4-mm I.D. PTFE cavity and were packed by applying slight pressure with a spatula. The surface was then smoothed on a piece of weighing paper and rinsed with double distilled water. The complete procedure takes about 1 h. In its design, the modified carbon paste was in direct contact with a glassy carbon disk and electrical contact was established via a copper wire. Prior to use as a sensing electrode, the Cu_2O–CCE was pretreated by continuous cyclic scans from 0.0 to +0.7 V vs. Ag/AgCl at 50 mV/s in a 0.5-*M* NaOH solution until a reproducible voltammetric profile was obtained. After normal operation each day, the electrochemical cell was disconnected and flushed with water, then the Cu_2O–CCEs were stored at room temperature without taking any particular precautions.

3. Results and discussion

In a previous paper [26], we reported on the preparation and performance of a CCE made of graphite powder and polyethylene modified with cuprous oxide, which acts as a powerful catalyst in the electrooxidation of alditols. Using D-mannitol as a test compound in flow injection amperometry, the Cu_2O–CCE, with an optimised catalyst loading of 50% (w/w), yielded the lowest background current, the highest sensitivity (ca. 11 μA/m*M*) and a linear range that extended over three orders of magnitude. Moreover, the proposed bulk-modified electrode provides good stability under flowing stream operation. After a working period of three weeks, the sensitivity was found to be reduced by less than 2%. Accordingly, such a sensing electrode was used throughout the present study and the effects of some experimental conditions were investigated to optimize the testing performance in anion-exchange chromatography with electrochemical detection (ED).

3.1. Optimization of the operating potential at the Cu_2O-CCE

To determine the optimum applied potential at the Cu_2O–CCE, a standard solution containing D-sorbitol, D-mannitol and D-glucose was injected. After complete elution, the applied potential was increased and the chromatogram was repeated at the new potential. Fig. 1 shows the resulting hydrodynamic voltammograms: D-sorbitol (A), D-mannitol (B) and D-glucose (C). Others alditols and carbohydrates exhibited similar current–potential curves and, although slightly pronounced, all the compounds show a peak-shaped profile. Such behaviour is probably related to the formation of O_2 at potentials higher than 0.5 V, which is propitiated by the generation of $HO^{\cdot}$ radicals at the Cu_2O surface lattice, and the concomitant increase in the background current [20]. An applied potential of +0.45 V was chosen for detection of alditols and carbohydrates in chromatographic separations. It represented the best compromise between maximum current response and minimum residual current noise.

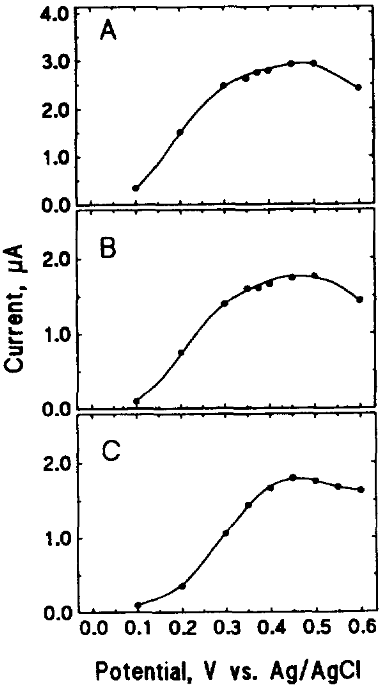

Fig. 1. Hydrodynamic voltammograms for (A) 1.6 m*M* D-sorbitol, (B) 0.8 m*M* D-mannitol, and (C) 0.8 m*M* glucose at the Cu_2O–CCE, loading 50% (w/w). Conditions: mobile phase, 0.48 *M* NaOH; flow-rate, 0.4 ml/min.

3.2. Effect of OH^- on retention time

Earlier work on the Cu_2O–CCE demonstrated the effect of pH on the electrocatalytic currents observed for D-mannitol, which increased substantially in magnitude under the more alkaline conditions [26]. Probably, this is because hydroxide ion is involved in the electrooxidation process of copper-based electrodes [20,22,32]. Indeed, the electrode activity for D-mannitol oxidation, and for other polyhydric compounds, was very pH-dependent, occurring with greater ease in solutions with higher pH values.

A particularly valuable technique for the separation at ambient temperature of weakly ionizable compounds, such as alditols and sugars, is ion chromatography using an anion-exchange column. Recently, a high capacity 8.5-μm macroporous resin, fully functionalized with anion-exchange sites, was introduced, which is particularly suitable for the separation of alditols and simple carbohydrates with alkaline eluents [33]. Fortunately, the requirement of strongly alkaline conditions for the chromatographic separations, using an anion-exchange column that works at high pH, has beneficial effects on the electrocatalytic activity of the Cu_2O–CCE. It needs to be considered, however, that another concomitant effect is also involved. The changes in the concentration of hydroxide ion provoke variations in the retention times and in the selectivity factors. Fig. 2 displays the OH^- effect on the retention time of *myo*-inositol (a), xylitol (b), D-sorbitol (c), dulcitol (d), D-mannitol (e), D-glucose (f) and D-fructose (g). As can be seen, the carbohydrates exhibit greater retention compared to alditols. Generally, each of these chromatographic peaks was shifted to shorter times at higher OH^- concentration, in essentially the same qualitative manner. The retention times observed for these compounds, however, varied over a considerable range. For example, the retention of *myo*-inositol was only slightly changed, as the concentration of NaOH in the mobile phase was increased from 410 to 630 m*M*, while the retention time for D-fructose was reduced by ca. 8 min using 610 m*M* NaOH instead of 410 m*M* NaOH. Mobile phases with hydroxide ion concentrations lower than 410 m*M* were not used because the column efficiency was considerably compromised, with large peak widths and, above all, pronounced tailing.

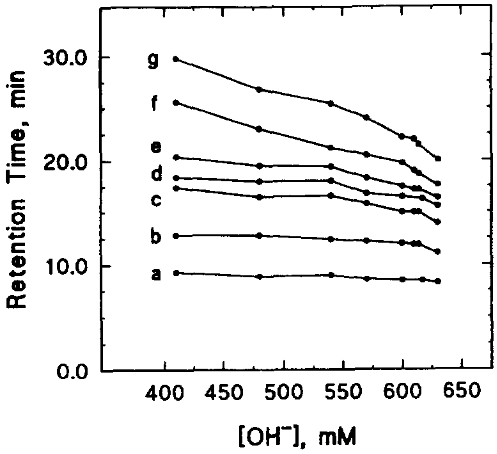

Fig. 2. Effect of hydroxide concentration on the retention times of (a) *myo*-inositol, (b) xylitol, (c) D-sorbitol, (d) dulcitol, (e) D-mannitol, (f) D-glucose and (g) D-fructose at room temperature. Column, Carbopac MA1 (250×4 mm) and guard (50×4 mm) (Dionex); flow-rate, 0.4 ml/min; $E_{app}=+0.45$ V.

Therefore, for the potential analytical applications of the Cu_2O–CCE, which forms the focus of this work, an alkaline eluent with a concentration ranging from 450 to 600 m*M* NaOH was chosen as being a good compromise between resolution and the magnitude of oxidation currents. Note that the alkaline eluents were normally prepared by using commercially available reagent-grade carbonate-free 50% (w/w) sodium hydroxide. The presence of carbonate in the mobile phase poses a practical disadvantage in terms of irreproducible effects on retention times and column efficiency when its concentration is unknown or frequently changed.

3.3. Carbonate-free mobile phases

A current strategy for minimizing the uptake of carbon dioxide, and thus of carbonate in the mobile phase, is to sparge the eluent and to pressurize the reservoir with an inert gas such as helium or nitrogen [33,34]. Whereas such a strategy is apparently consistent to apply, it is both inherently expensive and rather cumbersome. For example, the water used for preparing the mobile phases must be accurately degassed. Moreover, during storage of the basic eluent, the introduction of CO_2 is practically inevitable. Alternatively, the use of calcium or barium as precipitating ions is suggested, which is a simpler and more effective procedure to minimize contamination by carbonate. Fig. 3 compares the chromatograms of a sample mixture obtained with the same mobile phase (0.50 *M* NaOH) before (a) and after (b) the addition of $Ca(OH)_2$ at a concentration of 0.5 m*M*. The most noticeable feature in Fig. 3b is that the column provides a better separation upon the addition of Ca^{2+} to the alkaline mobile phase, with the resolution of D-sorbitol and D-mannitol being more affected. The increase in column efficiency demonstrates that this strategy was successful for obtaining a carbonate-free sodium hydroxide solution. More specific details on the benefits observed in terms of peak symmetry and column efficiency will be discussed elsewhere [35]. Hence, it is reasonable to assume that the solubility of calcium carbonate is considerably reduced and that its involvement in the chromatographic process is greatly minimized. The same effect was observed when $Ba(OH)_2$ was used instead of $Ca(OH)_2$. One major advantage of spiking the eluent with Ca^{2+} or Ba^{2+} is that no further precautions need to be taken for preventing the involvement of CO_3^{2-} in the chromatographic process.

3.4. Analytical performances of the Cu_2O–CCE

Considering the above experimental results, a mobile phase consisting of 0.45 *M* NaOH+0.5 m*M* $Ba(OH)_2$ was employed to evaluate the performance of the electrode. Fig. 4 shows a typical chromatogram obtained on injection of a solution containing 12.5 μ*M* *myo*-inositol, 90 μ*M* xylitol, 20 μ*M* D-sorbitol, 20 μ*M* D-mannitol, 25 μ*M* D-glucose, 50 μ*M* D-fructose, 20 μ*M* D-ribose and 100 μ*M* sucrose. Good peak separation was obtained and data on the peak-height calibration plots are summarized in

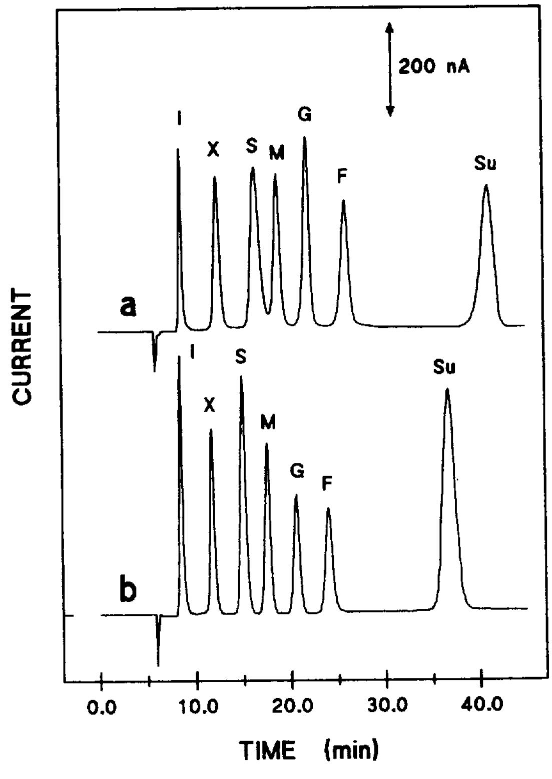

Fig. 3. Liquid chromatographic separation of a sample mixture of (I) *myo*-inositol, (X) xylitol, (S) D-sorbitol, (M) D-mannitol, (G) D-glucose, (F) D-fructose and (Su) sucrose carried out with a mobile phase of 0.50 *M* NaOH before (a) and after (b) the addition of 0.5 m*M* $Ca(OH)_2$. Other conditions are given in Fig. 2.

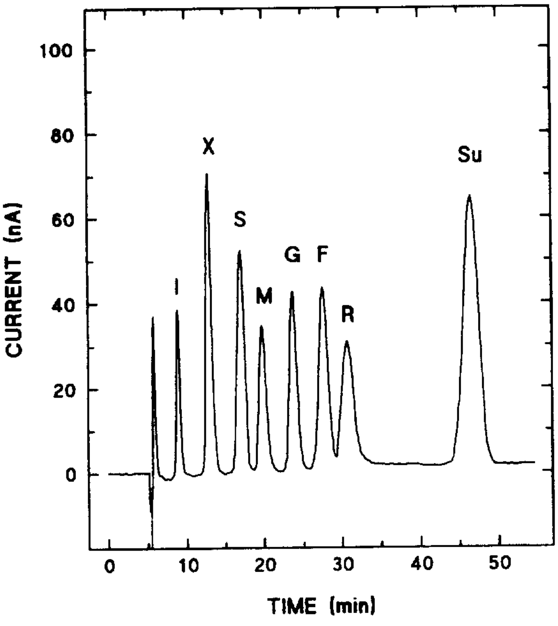

Fig. 4. Liquid chromatographic separation with electrochemical detection at a Cu_2O–CCE of a standard mixture containing (I) 12.5 μ*M* *myo*-inositol, (X) 90 μ*M* xylitol, (S) 20 μ*M* D-sorbitol, (M) 20 μ*M* D-mannitol, (G) 25 μ*M* D-glucose, (F) 50 μ*M* D-fructose, (R) 20 μ*M* D-ribose and (Su) 100 μ*M* sucrose. Isocratic elution at room temperature with 0.45 *M* NaOH+0.5 m*M* $Ba(OH)_2$. Other chromatographic conditions are given in Fig. 2.

Table 1
LC–ED quantitative parameters of alditols and sugars at a Cu_2O–CCE[a]

Compound	k'[c]	i (µA)=$a+bC$ (mM)[b]			Detection limit[d] (nmol)
		$a \pm t_{95}s_a$ (nA)	$b \pm t_{95}s_b$ (µA/mM)	r	
myo-Inositol	0.66	15±20	2.04±0.01	0.9998	0.04
Xylitol	1.40	10±20	0.65±0.02	0.9996	0.15
D-Sorbitol	2.20	15±20	1.75±0.03	0.9997	0.05
D-Mannitol	2.71	−10±30	2.25±0.02	0.9995	0.04
D-Glucose	3.46	−20±20	2.56±0.04	0.9994	0.08
D-Fructose	4.17	−10±20	1.12±0.04	0.9997	0.12
D-Ribose	4.76	15±20	2.07±0.04	0.9994	0.05
Sucrose	7.74	−10±10	0.75±0.05	0.9996	0.16

[a] Cuprous oxide (50%, w/w) in graphite–polyethylene composite matrix (1:1, w/w).

[b] Regression lines were calculated by least-squares analysis; the confidence limits for the slope and intercept are given in columns three and four, respectively, where t is taken at the 95% confidence level.

[c] Capacity factor.

[d] The detection limits determined for a $S/N=3$ from the lowest injected concentration.

Table 1. Each data point ($n=6$–7 concentrations in the linear range) was generated from at least two injections. A linear response was generally obtained over a range approximately three orders of magnitude above the detection limits. Within the linear range, the correlation coefficients were better than 0.999 for all compounds. The standard deviations (SD) of the slope and the intercept were estimated at the 95% confidence level. As can be seen, the Cu_2O–CCE (50%, w/w) allows convenient quantitation of alditols and simple sugars at the micromolar level. For instance, the detection limits of D-sorbitol and D-mannitol, evaluated as $A(3\ S/N)$, where $N=$ noise, $S=$signal (peak height) and $A=$the lowest injected amount, were about 50 and 40 pmol, respectively (see Table 1).

3.5. Applications

Normally, the assay of complex samples requires a preliminary purification step. In view of the chromatographic results described above using the Cu_2O–CCE as an amperometric sensor, it seemed interesting to use it in the chromatographic separation of real samples. For example, the chromatograms obtained in the constant-potential amperometric detection of celery (A) and plum (B) juices are shown in Fig. 5. Celery was crushed and the mechanical suspension was filtered by passage through a 0.45-µm Millipore membrane. Upon dilution of the relatively concentrated sample with pure water, the solution was injected into the chromatographic system. The peaks were identified as (M) D-mannitol, (G) D-glucose and (F) D-fructose. Similarly, a weighed amount of plums, purchased from a

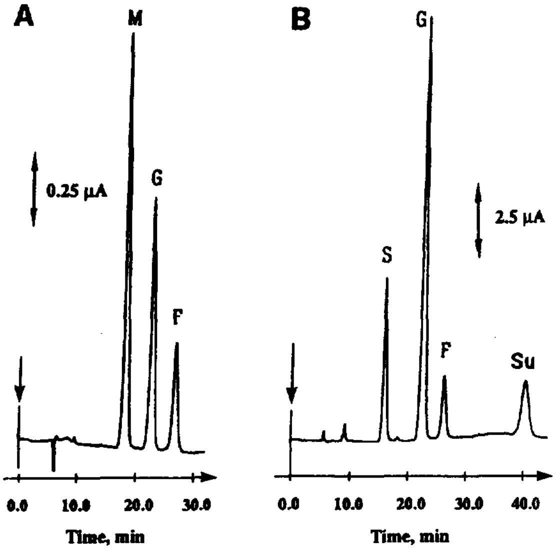

Fig. 5. Chromatographic separation with electrochemical detection at a Cu_2O–CCE of (A) celery juice and (B) plum juice. The mobile phase was 0.48 M NaOH+0.5 mM $Ca(OH)_2$, in the case of celery juice, and 0.50 M NaOH+0.5 mM $Ba(OH)_2$ in the case of plum juice.

local store, were homogenized, dissolved in water, sonicated for a few minutes and centrifuged; the extract was then filtered and injected. In the plum juice sample (S) D-sorbitol, (G) D-glucose, (F) D-fructose and (Su) sucrose were identified. On repetitive injection of both samples, the within-run precision for the separation of alditols and simple sugars was 0.4% R.S.D. ($n=3$), and no deterioration was apparent in the performance of the sensing electrode during a period of three weeks. Peaks were identified by matching retention times with compound standards, and quantification was performed using a calibration curve.

4. Conclusions

In conclusion, we have demonstrated that a carbon composite matrix, prepared with graphite powder and polyethylene, and modified by cuprous oxide, provides an effective electrochemical sensor for the quantitation of alditols and sugars at low and fixed applied potentials. It appears to be very useful for the determination of alditols and sugars in real samples following high-performance ion-exchange chromatographic separations. The addition of Ca^{2+} or Ba^{2+} has been shown to be an effective and straightforward procedure, which allows the carbonate ion content to be kept very low in the alkaline mobile phase.

Acknowledgments

The financial support of the Italian National Research Council (C.N.R., Rome) and Ministero dell'Università e della Ricerca Scientifica (M.U.R.S.T., Rome) are gratefully acknowledged.

References

[1] K.G. Schick, V.G. Magearu, C.O. Huber, Clin. Chem. 24 (1978) 448.

[2] W. Buchberger, K. Winsauer, Ch. Breitwieser, Fresenius' Z. Anal. Chem. 315 (1983) 518.

[3] R.E. Reim, R.M. Van Effen, Anal. Chem. 58 (1986) 3203.

[4] I.G. Casella, E. Desimoni, T.R.I. Cataldi, Anal. Chim. Acta 248 (1991) 117.

[5] I.G. Casella, T.R.I. Cataldi, A.M. Salvi, E. Desimoni, Anal. Chem. 65 (1993) 3143.

[6] T.R.I. Cataldi, D. Centonze, G. Ricciardi, Electroanalysis 7 (1995) 435.

[7] T.R.I. Cataldi, D. Centonze, Anal. Chim. Acta 307 (1995) 43.

[8] P.F. Luo, T. Kuwana, Anal Chem. 66 (1994) 2775.

[9] L.M. Santos, R.P. Baldwin, Anal. Chem. 59 (1987) 1766.

[10] S. Mannino, M.S. Cosio, S. Ratti, Electroanalysis 5 (1993) 145.

[11] T.R.I. Cataldi, I.G. Casella, E. Desimoni, T. Rotunno, Anal. Chim. Acta 270 (1992) 161.

[12] T.R.I. Cataldi, A. Guerrieri, I.G. Casella, E. Desimoni, Electroanalysis 7 (1995) 305.

[13] J. Wang, Z. Taha, Anal. Chem. 62 (1990) 1413.

[14] D. Leech, J. Wang, M.R. Smyth, Analyst 115 (1990) 1447.

[15] Q. Chen, J. Wang, G. Rayson, B. Tian, Y. Lin, Anal. Chem. 65 (1993) 251.

[16] S.V. Prabhu, R.P. Baldwin, Anal. Chem. 61 (1989) 852.

[17] S.V. Prabhu, R.P. Baldwin, Anal. Chem. 61 (1989) 2258.

[18] S.V. Prabhu, R.P. Baldwin, J. Chromatogr. 503 (1990) 227.

[19] P. Luo, S.V. Prabhu, R.P. Baldwin, Anal. Chem. 62 (1990) 752.

[20] Y. Xie, C.O. Huber, Anal. Chem. 63 (1991) 1714.

[21] P. Luo, F. Zhang, R.P. Baldwin, Anal. Chem. 63 (1991) 1702.

[22] J.M. Zadeii, J. Marioli, T. Kuwana, Anal. Chem. 63 (1991) 649.

[23] K. Kano, M. Torimura, Y. Esaka, M. Goto, T. Ueda, J. Electroanal. Chem. 372 (1995) 137.

[24] M.Z. Luo, R.P. Baldwin, J. Electroanal. Chem. 387 (1995) 87.

[25] X. Huang, J.J. Pot, W.Th. Kok, Anal. Chim. Acta 300 (1995) 5.

[26] T.R.I. Cataldi, D. Centonze, Anal. Chim. Acta 326 (1996) 107.

[27] K. Ravichandran, R.P. Baldwin, J. Electroanal. Chem. 126 (1981) 293.

[28] D.E. Tallman, S.L. Petersen, Electroanalysis 2 (1990) 499.

[29] P. Luo, F. Zhang, R.P. Baldwin, Anal. Chim. Acta 244 (1991) 169.

[30] J. Wang, N. Naser, L. Angnes, H. Wu, L. Chen, Anal. Chem. 64 (1992) 1285.

[31] K. Kalcher, J.-M. Kauffmann, J. Wang, I. Svancara, K. Vytras, C. Neuhold, Z. Yang, Electroanalysis 7 (1995) 5.

[32] B. Wels, D.C. Johnson, J. Electrochem. Soc. 137 (1990) 2785.

[33] Installation Instructions and Troubleshooting Guide for the CarboPac MA1 Analytical Column; Document No. 034752, Dionex, Sunnyvale, CA, 1992.

[34] I.G. Casella, A. Destradis, E. Desimoni, Analyst 121 (1996) 249.

[35] T.R.I. Cataldi, D. Centonze, G. Margiotta, submitted for publication.