ELSEVIER

Journal of Chromatography A, 692 (1995) 267–274

JOURNAL OF CHROMATOGRAPHY A

Determination of polycarboxylic acids by capillary electrophoresis with copper complexation

Jean P. Wiley

Unilever Research US, 45 River Road, Edgewater, NJ 07020, USA

Abstract

Reversed-polarity CE on unmodified silica, making use of copper sulfate as a combination complexation agent/electrolyte and myristyltrimethylammonium bromide as an electroosmotic flow modifier, has been shown to be a reliable and rapid method of analysis for polycarboxylic acid builders. Since separation is governed by a combination of charge and charge density, a number of similar polycarboxylic acids can be identified by unique retention times. Baseline resolution and symmetrical peak shapes are observed for a mixture of polycarboxylic acids including oxydisuccinic, ethylenediaminetetraacetic, nitrilotriacetic and diethylenetriaminepentaacetic acids. Using direct detection at 254 nm, the limit of detection for these acids approaches 100 ppb (ng/ml), making the technique viable for trace analysis.

CE results for nitrilotriacetic and citric acids in formulated products are comparable to results obtained by LC–UV detection. The CE method has been shown to be as sensitive as LC with amperometric detection, but less selective and easier to stabilize.

Other compounds which complex with copper(II) salts, such as ethylhydroxydiphosphonate can be determined at low levels in formulated products using this method.

1. Introduction

A rapid and sensitive method is required to analyze formulated detergent and personal care products for polycarboxylic acids (Fig. 1). Capillary electrophoresis (CE), utilizing copper complexation, has been identified as a technique that can sensitively separate and detect polycarboxylic acids in raw materials and finished products. Polycarboxylic acids act as builders in these formulations by complexing calcium ions in hard water. Builders enable surfactants to do the job of cleaning, rather than being complexed with calcium.

Early detergent formulations consisted of soap, which acted as both surfactant and builder. The introduction of sodium carbonate and sodium silicate to these formulations resulted in a "built" product at lower cost. Phosphate-built detergents enjoyed popularity up until the early 1970s, when environmental concerns resulted in a number of states banning their usage. Phosphates had been shown to cause the eutrophication of stagnant bodies of water.

Since 1970 much effort has been dedicated to finding a builder that is at once effective, biodegradable and affordable. Sodium citrate has been shown to be safe, effective and biodegradable, but is rather expensive. Nitrilotriacetate (NTA) seemed to be a viable replacement for the phosphates until 1971, when the US Surgeon General declared it to be unsafe on the basis of

0021-9673/95/$09.50
SSDI 0021-9673(94)00821-3

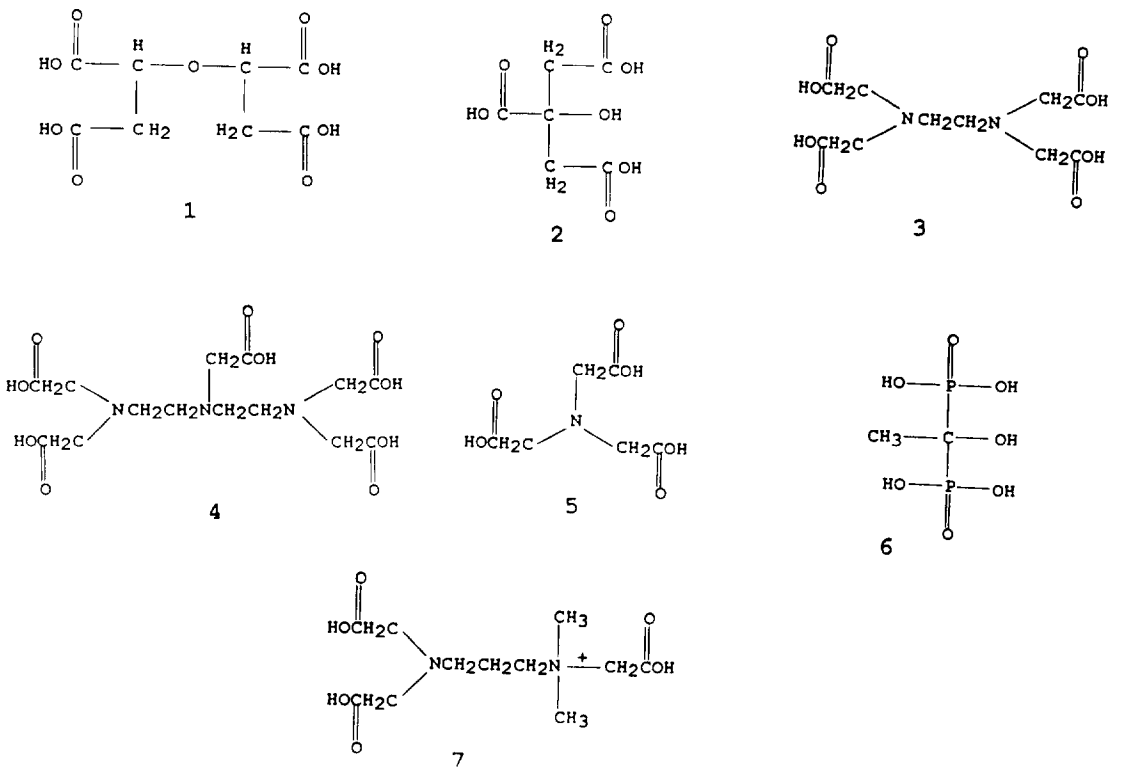

Fig. 1. Structures of compounds discussed in this paper. 1 = Oxydisuccinic acid; 2 = citric acid; 3 = ethylenediaminetetraacetic acid; 4 = diethylenetriaminepentaacetic acid; 5 = nitrilotriacetic acid; 6 = ethylhydroxydiphosphonate; 7 = aminopropyldimethylaminetriacetic acid.

suspected teratogenicity. NTA is used throughout Canada as a builder [1]. A number of other polycarboxylic builders have been studied over the years, including ethylenediaminetetracarboxylic acid (EDTA), oxydisuccinic acid (ODS) and diethylenetriaminepentaacetic acid (DTPA).

Many polycarboxylic acids do not have substantial UV absorptivities outside the low UV (200–220 nm). Even at these wavelengths, sensitivity is limited. This has been overcome for aminocarboxylic acids, using a liquid chromatographic method with pulsed amperometric detection (PAD) [2]. However, PAD detectors are highly selective and can be slow to equilibrate. Not all polycarboxylic acids will respond to PAD. Polycarboxylic acids, when complexed with copper, exhibit strong UV absorption and can be easily detected at low ppm levels at 254 nm.

The use of a negative power source, along with an electroosmotic flow (EOF) modifier, for anion analysis is referred to as "reversed-polarity CE" simply because the polarity is reverse to the traditional polarity. At low pH, an unmodified electroosmotic flow would carry anions to the negative electrode. When the electroosmotic flow is controlled, the bulk flow of electrolyte allows the anions to travel to the positive electrode. Varghese and Cole [3] demonstrated the use of cetyltrimethylammonium chloride as an EOF modifier in reversed-polarity CE. In our work we used a similar EOF modifier, myristyltrimethylammonium bromide.

The idea to use copper sulfate as the electrolyte in our CE method originated from the copper complexation (ligand-exchange) chiral LC technique utilized by Phenomenex in their Chirex CSP 3126 D-penicillamine bonded column [4]. The penicillamine column proved useful for

the separation of *R,R* and *S,S* isomers of oxydisuccinic acid. Analytical work on the penicillamine column conducted here at Unilever demonstrated that the ODS–copper complex absorbs so strongly in the UV that the limit of detection is in the low μg/ml range. A reversed-polarity CE method was then devised using copper sulfate as a combination complexation agent/electrolyte with detection by direct UV at 254 nm. The copper complexation CE method generated two peaks for oxydisuccinic acid. One was a combination of *R,R* and *S,S*, while the other was a separate peak for *meso*-ODS. The limit of detection was in the range of a fraction of a μg/ml. This low limit of detection makes the technique suitable for biodegradation studies which require the measurement of trace levels.

2. Experimental

All analyses were performed on the Millipore/Waters (Milford, MA, USA) Quanta 4000 CE instrument. The capillary was an uncoated silica from Polymicro Technologies (Phoenix, AZ, USA). It was 60 cm × 360 μm O.D. × 75 μm I.D. Data were collected on a Waters 746 data module.

The cupric sulfate used in preparing the electrolyte solution was certified anhydrous from Fisher Scientific (Fairlawn, NJ, USA). The myristyltrimethylammonium bromide was 99%, obtained from Aldrich (Milwaukee, WI, USA). The sodium citrate dihydrate used as a standard was certified from Fisher Scientific. The EDTA was diluted from a certified 0.1 *M* solution from Fisher Scientific. The DTPA standard was 98% pure from Aldrich. Oxydisuccinic acid was generated internally (E. Gutierrez and D. Wu); aminopropyldimethylaminetriacetic acid was custom synthesized by Goldschmidt Chemical Co. (Hopewell, VA, USA).

Formulated products were dissolved in water and passed through a conditioned (methanol,

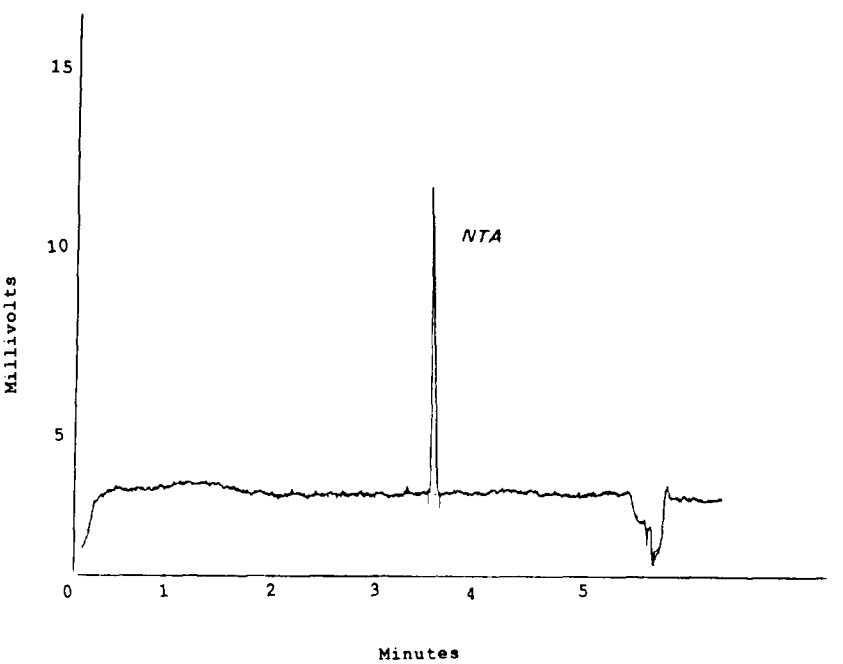

Fig. 2. Analysis of nitrilotriacetate (NTA) in formulated product by CE. Silica capillary: 60 cm × 75 μm. Electrolyte: 5 m*M* cupric sulfate, 1 m*M* myristyltrimethylammonium bromide. Negative power supply: 20 kV. Injection: hydrostatic, 20 s. Detection: 254 nm, direct. Sample: Canadian hard surface cleaner: 0.035 g/100 ml water; NTA content 7.9%. Sample filtered prior to injection.

water) Waters C_{18} Sep-Pak prior to analysis. Individual experimental conditions are described in the captions to the figures.

3. Discussion

Reversed-polarity CE on unmodified silica, making use of copper sulfate as a combination complexation agent/electrolyte and myristyltrimethylammonium bromide as an EOF modifier, has been shown to be a reliable and rapid method of analysis for polycarboxylic acid builders. Since separation is governed by a combination of charge and charge density, a number of similar polycarboxylic acids can be identified by unique retention times. For the series of polycarboxylic acids examined in this paper, the elution time is before the "water dip", indicating a net negative charge on the acid. In the case of EDTA, for example, the molecules must not be totally complexed with copper, or they would behave like neutral species and elute along with the water peak.

The copper complexes are readily detected by direct UV at 254 nm, with a limit of determination near 0.1 μg/ml. The limit of detection may well be an order of magnitude lower (0.01 μg/ml), particularly if the sampling time is extended in the hydrostatic injection mode. External standard quantitation was employed in all cases. The CE approach can obtain limits of detection comparable to the pulsed amperometric detector. CE analysis of a solution of 100 ng/ml ODS resulted in peaks with a signal-to-noise level > 10. The CE method has the added advantage over PAD that it can be used for polycarboxylic acids not containing an amine functionality, such as ODS and citrate. PAD systems can be difficult to stabilize; CE with direct UV detection is quite rugged. The response curve for hydrostatic injection appears linear over a wider range of concentration than for electromigration.

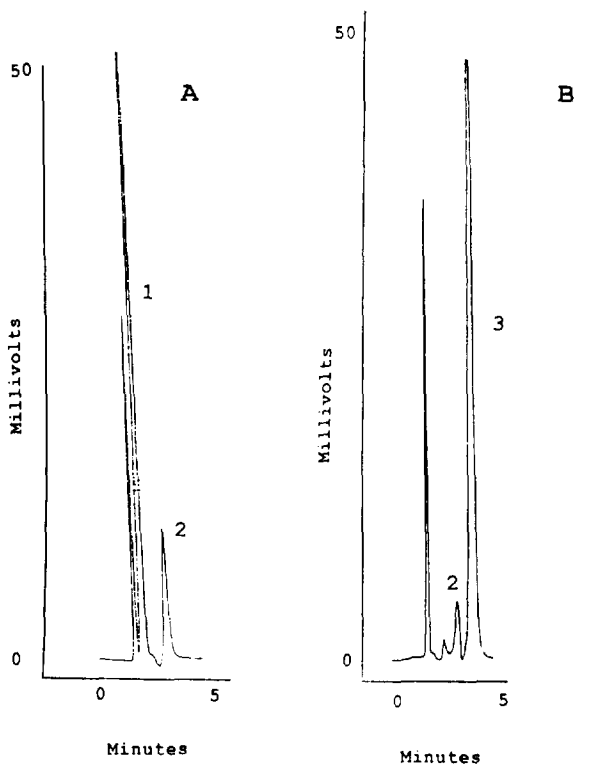

Fig. 3. Analysis of NTA and citrate in formulated products by liquid chromatography. Column: Hamilton PRP-1 (25 cm × 4.6 mm). Mobile phase: 0.1% (v/v) trifluoroacetic acid in water. Flow-rate: 1 ml/min. Injection volume: 50 μl. Detection: direct UV at 230 nm. (A) Canadian liquid hard surface cleaner. (B) Liquid laundry detergent. Peaks: 1 = NTA; 2 = trifluoroacetic acid; 3 = citrate.

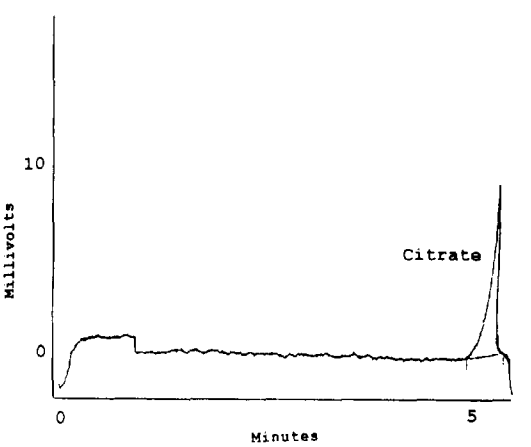

Fig. 4. Analysis of citrate in formulated product by CE. Conditions of analysis as in Fig. 2. Sample: liquid laundry detergent, 0.037 g/100 ml water; citrate content 8.8%. Sample filtered prior to injection.

3.1. Nitrilotriacetate and citrate

Nitrilotriacetate, used in Canada as a builder, can be easily quantitated in formulated products by the new complexation CE technique (Fig. 2). Results have been shown to be comparable to those obtained using a modified literature LC method [2] (Fig. 3) which calls for a polymeric reversed-phase column and trifluoroacetic acid as mobile phase modifier. The level of NTA in the hard surface cleaner in Figs. 2 and 3 is 7.9% (w/w). The literature reference for the LC method requires PAD to achieve a 100 ng/ml detection limit for aminopolycarboxylic acids. Our modified procedure takes advantage of UV detection as a more stable detection system for higher levels of analyte.

Citrate in formulated products can be quantitated by CE (Fig. 4), with results comparable to those found for liquid chromatography. The level of citrate in the liquid laundry detergent in Figs. 3 and 4 is 8.8% (w/w), expressed as disodium citrate dihydrate. By CE, the peak shape for citrate exhibits a good deal of fronting. Mikkers et al. [5] have explained that fronting can be predicted when the mobility of the analyte is greater than the main co-ion in the electrolyte. However, peak deformations gener-

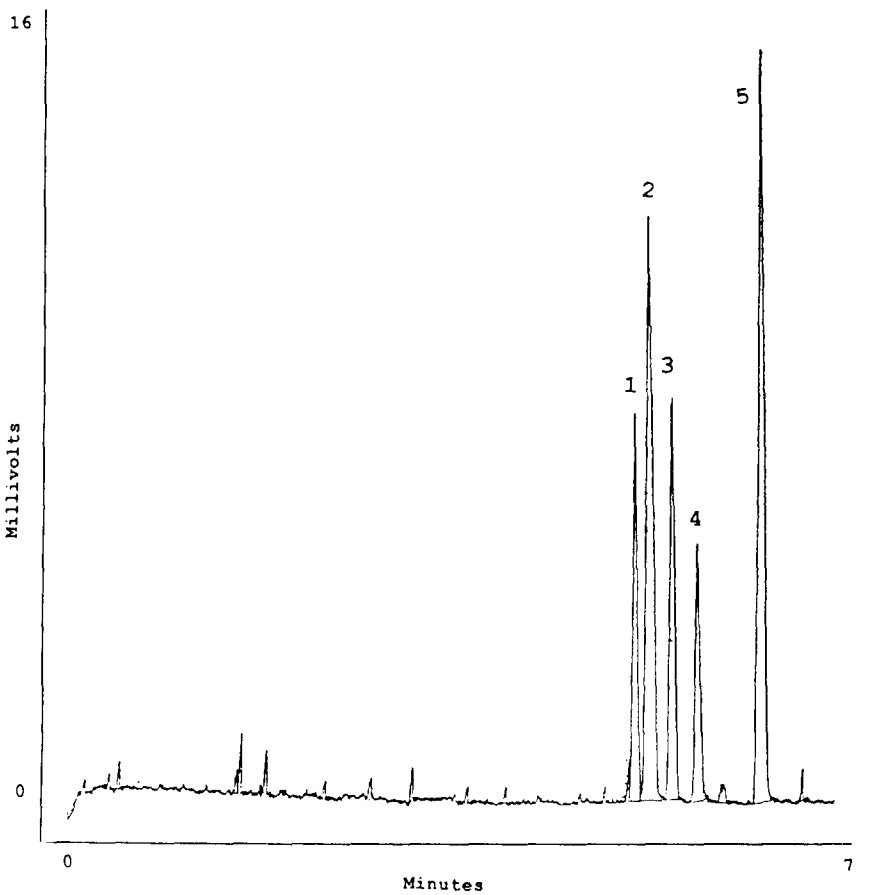

Fig. 5. Analysis of a mixture of polycarboxylic acids by CE. Silica column and electrolyte as in Fig. 2. Negative power supply: 13 kV. Injection: hydrostatic, 30 s. Peaks: 1 = ODS, *R,R* and *S,S*, 12.5 ppm; 2 = EDTA, 33.6 ppm; 3 = NTA, 20.6 ppm; 4 = ODS, *meso*, 8.9 ppm; 5 = DTPA, 21.2 ppm.

ally have more than one cause. At low pH, weak acids can demonstrate fronting. The pH of the electrolyte solution in Fig. 4 is 5.3. As long as a considerable amount of acid is in the protonated form, a high concentration of hydronium ions can result in the conductivity of the sample zone being higher than that of the electrolyte.

3.2. Mixture of polycarboxylic acids

Unique retention times and baseline resolution were demonstrated for a series of polycarboxylic acids analyzed using the complexation CE method. They include ODS, EDTA, NTA and DTPA (Fig. 5). Dilutions of this series were first introduced onto the capillary by hydrostatic injection. Then the entire series was run using the electrokinetic mode of injection. The range of linearity using hydrostatic injection was greater than electrokinetic injection (Fig. 6). Using hydrostatic injection, the area responses of the builders were linear between approximately 2 and 30 μg/ml; using electrokinetic injection, non-linear responses were obtained for this concentration range. However, the electrokinetic injection mode may be advantageous for trace

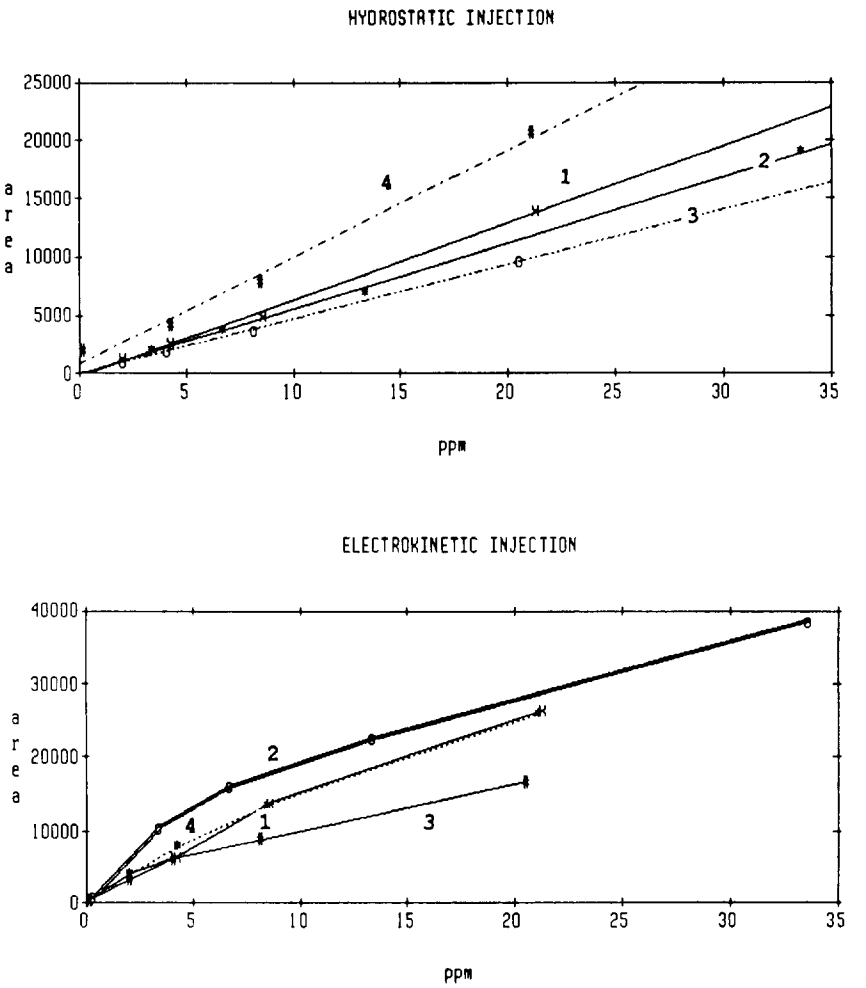

Fig. 6. Comparison of area responses based upon injection mode. Curves: 1 = ODS; 2 = EDTA; 3 = NTA; 4 = DTPA.

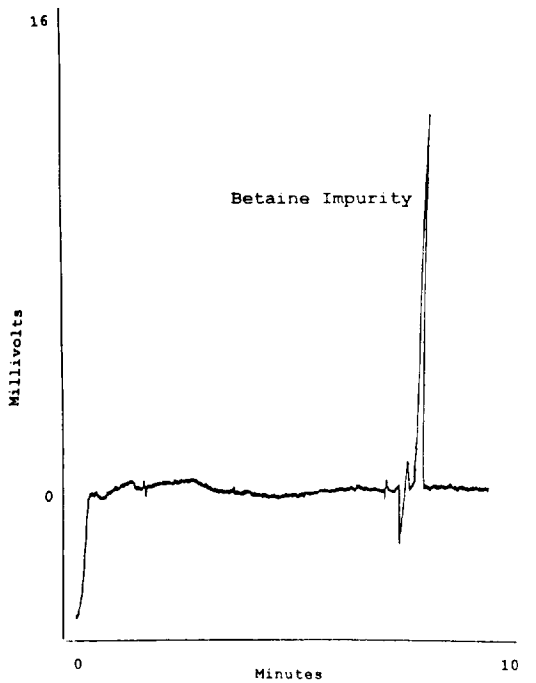

Fig. 7. Electropherogram of aminopropyldimethylamine-triacetic acid, a potential by-product in the commercial synthesis of cocoamidopropylbetaine surfactants. Conditions as in Fig. 2.

analysis as it allows for concentration of the anions in the sample at the head of the capillary [6].

3.3. Aminopropyldimethylaminetriacetic acid

Aminopropyldimethylaminetriacetic acid is a possible non-surfactant byproduct of the commercial synthesis of cocoamidopropylbetaine surfactants. This tricarboxylic acid could potentially result from the addition of 3 mol of chloroacetic acid to residual dimethylaminopropylamine. We asked Goldschmidt Chemical Co. to synthesize it as a standard for analysis. We were unsuccessful in developing an ion chromatography method which would permit its detection, yet it is easily determined by the new CE copper complexation technique (Fig. 7). The figure is illustrative of the analysis of a 20 μg/ml aqueous solution of the standard. The compound elutes just after the "water dip", suggesting a slightly positive net charge under the conditions of analysis. This elution behavior is consistent with the mechanism postulated for polycarboxylic acids using the copper complexation CE approach.

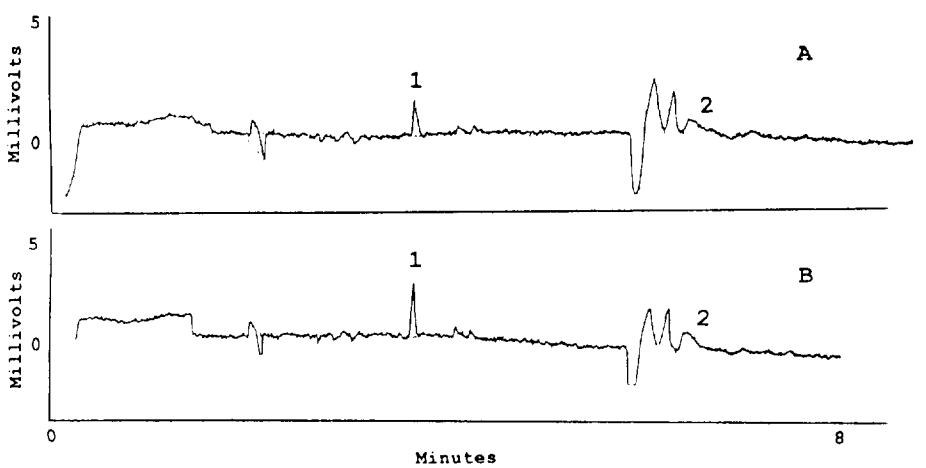

Fig. 8. Analysis of EDTA and EHDP in liquid hand soap by CE. (A) Liquid hand soap, 4 % (w/v) in water. (B) Liquid hand soap (4%, w/v) spiked with additional EDTA and EHDP. Peaks: 1 = EDTA; 2 = EHDP. Analysis conditions as in Fig. 2 except for mode of injection: electrokinetic, 20 s, 5 kV.

3.4. Additional applications

Preliminary CE work with EDTA and ethylhydroxydiphosphonate (EHDP), which can be present in liquid soaps as preservatives, indicates that this technique may also be viable for phosphonates (Fig. 8) and other species which complex copper(II) salts.

4. Conclusions

CE analysis of polycarboxylic acids on untreated silica, with copper complexation and electroosmotic flow modification, can be a useful technique to monitor these ingredients in formulated products as well as in studies which require trace (μg/ml) levels of detection. Both amine-containing carboxylic acids, such as EDTA and NTA, and non-amine-containing carboxylic acids, such as citric acid and ODS, can be analyzed by this procedure. Hydrostatic injection of these acids results in a greater linear response range than electromigration. Other species which complex copper(II), such as EHDP, may also be detected by this procedure.

Modifications of the electrolyte composition may be necessary to enhance the peak shape for the phosphonates.

Acknowledgements

The author acknowledges Unilever Research US for supporting the research described in this paper. The author also thanks Ms. Betty Bautista, Dr. Henry Kalinoski and Dr. Richard Montgomery of Unilever Research US for their technical assistance.

References

[1] A. Cahn, *Inform*, 5 (1994) 70–74.
[2] J. Dai and G. Heiz, *Anal. Chem.*, 60 (1988) 301–306.
[3] J. Varghese and R. Cole, *J. Chromatogr. A*, 652 (1993) 369–376.
[4] *Product Literature: Chiral Separations*, Phenomenex, Torrance, CA, 1993.
[5] F.E.P. Mikkers, F.M. Everaerts and T.P.E.M. Verheggen, *J. Chromatogr.*, 169 (1979) 11–20.
[6] X. Huang, M. Gordon and R. Zare, *Anal. Chem.*, 60 (1988) 375–377.