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Improving sensitivity in simultaneous determination of copper carboxylates by nonaqueous capillary electrophoresis

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Abstract

A new method of nonaqueous capillary electrophoresis (NACE) with UV spectrophotometric detection was developed and optimized for the simultaneous determination of seven carboxylates (trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid (HEDTA), nitrilotriacetic acid (NTA), 1,3-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetic acid (PDTA) and triethylenetetraaminehexaacetic acid (TTHA)) as copper complexes. The method development was carried out by using a fused silica capillary. Background electrolyte (BGE) was optimized and the best separation achieved by using 30 mmol L^{-1} potassium bromide in *N*-methylformamide (NMF) at apparent pH (pH_{app}) 10.2. A voltage of +30 kV and direct UV detection at 280 nm were used in all measurements. Large-volume sample stacking using the electroosmotic flow pump (LVSEP) was tested in addition to basic capillary electrophoresis (CE) and observed to improve the separation of the analyte zones in the capillary. All the peaks in the electropherograms were properly separated, the calibration plots gave excellent correlation coefficients ($R^2 \geq 0.994$) and all seven copper carboxylate complexes were detected in less than 20 min using both the basic measurements and the large-volume sample stacking method. The new NACE method was tested with lake water and proved to be reliable.

Keywords: Chelating agents; Copper carboxylate complexes; Large-volume sample stacking; *N*-Methylformamide; Nonaqueous capillary electrophoresis

1. Introduction

There has been rapid development of aqueous (CE) and nonaqueous capillary electrophoresis (NACE) in recent years. Although aqueous methods have been more common, recently attention has been turned towards nonaqueous media. Several review papers [1–3] highlighted the unique properties of some solvent systems thus contributing to the fast development of NACE.

Nonaqueous capillary electrophoresis is based on the use of background electrolyte solutions (BGE) prepared from pure organic solvents or their mixtures [4]. Selecting and optimizing a suitable BGE for the analytes of interest is the most challenging part of method development in NACE. Composition of BGE determines the electrophoretic mobility of ionic analytes due to solvent and ion-ion interactions [5]. Unique properties of solvents as compared to water play the fundamental role in developing the separation procedure. A suitable liquid range, sufficient relative permittivity, relatively low viscosity, not too high volatility, chemical stability, availability, low cost and high purity are some of the important factors to consider on selecting the solvent for a particular separation [6]. Many properties of analytes may change in nonaqueous media; e.g., in methanolic medium pK_a values of basic analytes are higher than in water [1], and more generally pK_a values are larger in almost all the organic solvents used in NACE when compared to aqueous media [7].

NACE makes use of both amphiprotic and inert/aprotic solvents [2]. Alcohols (methanol (MeOH), etc.) belong to the class of water alike amphiprotic solvents. Formamide (FA), *N*-methylformamide (NMF) and dimethylformamide (DMF) are amphiprotic solvents exhibiting basic character (i.e., they enhance solute acidity [8]). Acetonitrile (ACN) represents the class of inert solvents [2]. As a consequence, NACE offers additional possibilities to the analyst. In aqueous media, the solvent

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remains the same while the additives are variable. In NACE both solvents and additives are variable thus increasing the practical possibilities for a good separation.

Methanol, either alone or in mixtures appear the most commonly used solvent in NACE [4,5,8–21,27] (e.g., halogenated phenolic and bisphenolic compounds [9], β-blockers [8,11], neutral surfactants [10], substituted benzoates and nitrophenolates [5] and small cations [12]). ACN [4,12,15–20,22–24], ethanol [12,20,21,25], propanol [12,20,21], butanol [21], FA and NMF [8,26] and DMF [12,26] have also been applied with satisfactory results.

On the other hand, as in aqueous capillary electrophoresis, the selection of appropriate electrolytes and additives is a key factor in optimizing the separations. Selection of additives is not generally straightforward. Acetic acid (HOAc) is common component of NACE media [1]. Also sodium tetraborate [9], ammonium acetate [1,15,17–20,24] and tris(hydroxymethyl)aminomethane (Tris) [27] appear frequently as salt additives in reported separations. Salts that have smaller molecular weight like sodium bromide are less commonly used in NACE.

The development of methods for the analysis of carboxylate ligands is important because the environmental impact of these compounds. Widely used in industry and agriculture, they enter the environment not only as diffuse pollutants but via industrial and domestic waste waters. In previous papers [28,29] dealing with the capillary electrophoretic separation of carboxylates only aqueous media have been considered and optimized. Aqueous CE procedures are cheap, simple and practically feasible, although lower detection limits (LOD) are needed to accommodate real environmental concerns. The background signal has a marked influence in LOD calculation when 3 × noise based calculations are used, and in our experience the background noise of aqueous CE is often higher than in nonaqueous media because the high salt concentrations applied and the interactions between salts and water. Our preliminary tests proved that NACE allows higher ionic strengths of the BGEs and higher voltages for the capillary electrophoresis (CE) methods resulting lower currents, which enable the use of wider i.d. capillary. Thus, NACE appears as a potential alternative in the development of sensitive separation and determination procedures of copper carboxylate complexes, although to the best of our knowledge only aqueous CE has been previously explored with this aim. The practical aqueous methods used for separation of chelating ligands are discussed in more detail in [28].

This paper presents the development of a NACE procedure to separate several carboxylates. Additionally, large-volume sample stacking using the electroosmotic flow pump (LVSEP) [27,30] has been explored to overcome the sensitivity limitations imposed by the widespread UV detection in CE. Finally, natural water sample was used to check the suitability of this NACE method for simultaneous separation of copper complexes of trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid (HEDTA), nitrilotriacetic acid (NTA), 1,3-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetic acid (PDTA) and triethylenetetraaminehexaacetic acid (TTHA). Through this study all chelating ligands used are considered as copper complexes, hence this is not mentioned every time they appear in the text.

2. Experimental

2.1. Instrumentation

Electropherograms were obtained on a Hewlett-Packard CE^{3D} G1600 AX apparatus (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector (DAD) and an air cooling unit for the capillary. Instrument control and data acquisition were performed with HP^{3D} Chemstation software (Hewlett-Packard, Rev 04.02.). Absorbances at 191, 210, 254, 280 and 290 nm were monitored for analyte detection. The uncoated fused silica capillary (Hewlett-Packard, Waldbronn, Germany) was of 75 μm i.d., 375 μm o.d. and had a total length 70 cm (61.5 cm to the detector). The temperature of the capillary cassette was adjusted to 25 °C by air cooling. Depending on the experiment runs were performed with reverse polarity (−30, −20 kV) or normal polarity (+20 and +30 kV) generated by the negative and positive power supply, resulting in electric currents from −229 to +60 μA. Standards and samples were injected hydrodynamically by overpressure (50 mbar = 5000 Pa). Depending on the experiment, the injection time was from 2 to 3 s. The instrument was placed in a special room with automated temperature control (+20 °C).

Apparent pH (pH$_{\text{app}}$) measurements were done with Orion 720A pH-meter by using Orion Ross combination pH electrode (8103BN), the filling solution of the electrode was 3 M KCl (Orion Cat. No 810007). The pH equipments were obtained from Orion (Cambridge, England).

2.2. Reagents and solutions

Ultra pure water with a resistivity greater than 18.2 MΩ/cm was obtained by passing distilled water through an ELGA Elgastat Maxima (Elga Ltd., UK). All the chemicals used were of analytical reagent grade. Diethylenetriaminepentaacetic acid, methanol, sodium tetraborate and strontium bromide hexahydrate were obtained from Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid disodium salt dihydrate was obtained from FF-Chemicals (Yli-II, Finland). Nitrilotriacetic acid trisodium salt monohydrate, 1,3-diaminopropane-*N*,*N*,*N*′,*N*′-tetraacetic acid and *N*-methylformamide were obtained from Fluka Chemika (Buchs, Switzerland). Tetradecyltrimethylammonium bromide, acetonitrile, sodium bromide and acetic acid were obtained from J.T. Baker (Deventer, Holland). Copper sulphate pentahydrate and potassium bromide were obtained from Riedel-de Haën (Seelze, Germany). Trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid hydrate, iminodiacetic acid (IDA) and triethylenetetraaminehexaacetic acid were obtained from Sigma–Aldrich (Steinheim, Germany) and *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid from Fluka BioChemika (Buchs, Switzerland). Tris(hydroxymethyl)aminomethane and formamide were obtained from Aldrich Chemical Company (Milwaukee, USA).