A Potentiometric Detection Technique for Chelating Agents after Separation by Capillary Zone Electrophoresis

Wolfgang Buchberger[1,*]**, Dietmar Aichhorn**[1]**, Georg Niessner**[1]**, Paul R. Haddad**[2]**,** and **Declan Bogan**[2]

[1] Department of Analytical Chemistry, Johannes Kepler University, A-4040 Linz, Austria
[2] Department of Chemistry, University of Tasmania, Hobart, Tasmania 7001, Australia

Summary. The combination of capillary zone electrophoresis with potentiometric detection has been investigated for the determination of chelating agents such as diethylenetriaminepentaacetic acid. The detector consists of a metallic copper electrode with a diameter of $100\,\mu m$ arranged in an end-capillary configuration. The electrode potential depends on the concentration of copper ions at the electrode surface and therefore decreases when chelating agents pass the electrode. In combination with UV detection, both free ligands and metal complexes could be detected.

Keywords. Capillary electrophoresis; Potentiometric detection; Chelating agents; Aminopoly-carboxylic acids.

Potentiometrische Detektion von Komplexbildnern nach Trennung mittels Kapillarzonen-elektrophorese

Zusammenfassung. Für die Bestimmung von Chelatbildnern wie Diethylentriaminpentaessigsäure wurde die Kombination der Kapillarzonenelektrophorese mit einer selektiven potentiometrischen Detektionstechnik untersucht. Der Detektor beinhaltet eine metallische Kupferelektrode mit einem Durchmesser von $100\,\mu m$, welche in einer *end-capillary*-Anordnung positioniert ist. Das Elektrodenpotential hängt von der Konzentration freier Kupferionen an der Elektrodenoberfläche ab und sinkt daher, wenn komplexierende Analyte die Elektrode passieren. In Verbindung mit einer UV-Detektion ist die simultane Erfassung von freien Liganden und deren Metallkomplexen möglich.

Introduction

Capillary zone electrophoresis (CZE) has become a well-established technique for high-performance separations of inorganic as well as organic species. Unfortunately, detection techniques compatible with the small volumes encountered in CZE are limited; commercially available detectors often do not achieve detection limits (in terms of concentration) as low as in high-performance liquid chromatography. From this point of view it seems reasonable to investigate new

* Corresponding author

detection principles that might be complementary to commonly employed spectroscopic detection techniques.

Research in the field of potentiometric detection for ion chromatography has shown that a metallic copper sensing electrode can be used for the selective detection of species that are able to form complexes with copper ions such as aminopolycarboxylic acids [1]. The electric potential of the electrode depends on the concentration of free copper ions (both Cu(I) and Cu(II)) present in the diffusion layer established at the electrode surface. The precise potential built up at the electrode will depend on the nature of the eluent used (and especially on the type and concentration of any ligands present) as well as on the amount of dissolved oxygen. Under normal operating conditions, a stable background potential is established for an equilibrated system. When a chelating analyte is eluted from the column, the electrode potential will vary as a consequence of the difference in the complexing strengths of the eluent ligand and the analyte. In the present investigation, we have adapted this potentiometric detection principle to capillary zone electrophoresis and have used it to investigate applications concerning the analysis of diethylenetriaminepentaacetic acid (**1**).

Compound **1** and related aminopolycarboxylic acids are commonly used for masking metal ions that might interfere with various processes in pharmaceutical, food, and pulp and paper industry. The release of these chelating agents to the environment can affect the distribution of heavy metals within aquatic ecosystems by remobilization of metal ions from sediments. For this reason, there is a need for monitoring the levels of free aminopolycarboxylic acids as well as the corresponding metal complexes in surface, ground, and waste water.

Free aminopolycarboxylic acids can be determined by gas chromatography after derivatization of the carboxylic acid groups by esterification [2–5] or by high-performance liquid chromatography after pre-column derivatization of the analytes by adding an excess of a metal ion – usually Fe(III) – to the sample [3, 6–8]. Unfortunately, a differentiation between free and complexed analytes is difficult. Recently, we have demonstrated the benefits of CZE with UV detection for separation of **1** and some of its metal complexes [9]. The use of a selective potentiometric detector in combination with a UV detector adds another dimension of selectivity to the analytical system and should permit an improved detectability of these species.

Results and Discussion

Typically, the diameter of copper electrodes used for potentiometric detection in liquid chromatography is in the range of 1–2 mm. In CZE, electrodes with diameters less than 100 μm are necessary if end-capillary detection is to be used. Potentiometry should be particularly suited to application in combination with small electrode diameters because the response should be independent of the size provided that equilibrium conditions are established at the electrode surface and the *Nernst* equation is obeyed. However, this behaviour was not evident for copper electrodes in flowing systems as can be seen from Table 1; the data were obtained with electrodes coupled to a capillary used in the flow-injection mode. The results of the analysis of **1** show a significant decrease in sensitivity when the diameter of

Table 1. Dependence of the potentiometric response at copper electrodes on the diameter of the sensing electrode

Diameter of electrode (μm)	Relative response
50	0.15
100	0.26
500	0.35
1000	0.59
2000	1.00

the electrode was reduced. The typical non-*Nernstian* behaviour was also apparent from the calibration curves which were linear instead of logarithmic. From these experiments, 100 μm electrodes were considered to be a good compromise between sensitivity and need for miniaturization and were used throughout this work.

The signal-to-noise ratio was found to depend on the distance between the end of the separation capillary and the copper disk electrode. From the viewpoint of maintaining separation efficiency, a very short distance would be advantageous. On the other hand, the results obtained indicated that the lowest detection limits could be obtained for a distance of 80 μm as long as separation capillaries of 25 μm i.d. were used (Fig. 1). If low detection limits are to be attained, it is clearly important that the effects on the detection electrode arising from the high voltage applied to the separation capillary must be minimized. This suggests that the electrode should be moved away from the end of the capillary as far as possible without sacrificing separation efficiency. The optimum distance between the capillary and the electrode is smaller for capillaries with an internal diameter smaller than 25 μm, but such capillaries have not been used for detailed investigations as the small diameter results in poor detection limits for the UV detection mode. When separation capillaries of inner diameters larger than 25 μm were used, the results
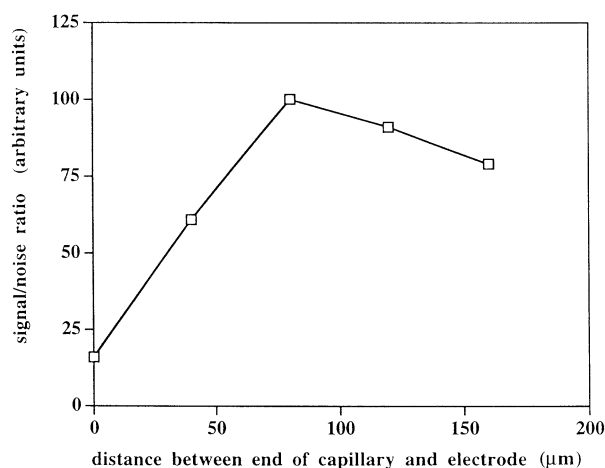
Fig. 1. Dependence of the signal/noise ratio on the distance between the end of the capillary and the electrode

obtained were unsatisfactory due to an unfavourably high influence of the separation potential on the electrode.

The *pH* of the carrier electrolyte was found to exert a significant effect on the detection sensitivity. For aminopolycarboxylic acids tested with the copper electrode in a flow-injection mode, optimum sensitivity was found to occur in a *pH* range between 5 and 6. On the other hand, the *pH* also affected the quality of the CZE separation, the peak shapes being strongly dependent on the *pH* of the carrier electrolyte as well as on the type of electrolyte. The best separation performance was obtained with a carrier electrolyte consisting of 5 m*M* sodium dihydrogenphosphate adjusted to *pH* 7 with 5 mM sodium tetraborate. In this case, the electrolyte *pH* was slightly more alkaline than required for best performance of the detector; however, this compromise was necessary for suitable separation to be achieved.

A comparison of UV and potentiometric detection was undertaken for a surface water sample spiked with *DTPA* and Fe*DTPA*. The only sample pretreatment consisted of passing the sample through a 0.45 μm filter and through a cartridge filled with 500 mg of C18 silica. As can be seen from Fig. 2, the UV detector gave a reasonable response for Fe*DTPA*, whereas the potentiometric detector allowed a highly selective detection of free *DTPA*. Obviously, some peak dispersion occurs in the potentiometric detector as the peak width was increased by a factor of 2, but this is still acceptable for the present application. Therefore, a combination of both detectors in series enhances the information and improves the identification of peaks in the electropherogram. The detection limit obtained using the potentio-
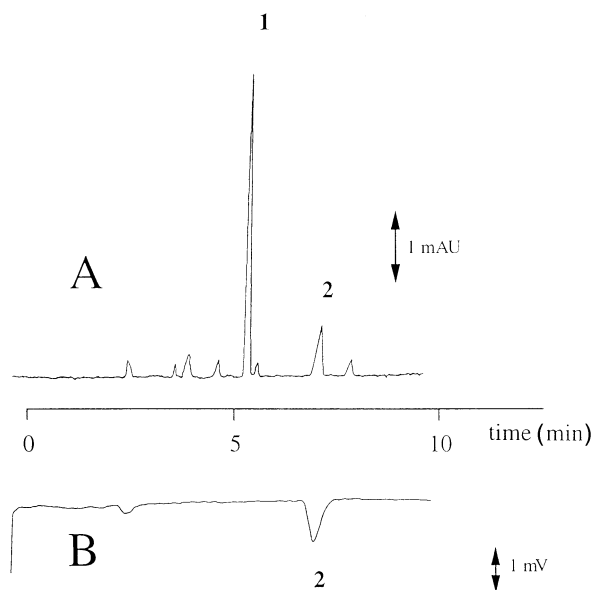

Fig. 2. Electropherogram of a surface water sample spiked with 1 ppm Fe*DTPA* and 5 ppm *DTPA*; carrier electrolyte: 5 m*M* sodium dihydrogen phosphate adjusted to *pH* = 7 with 5 m*M* sodium tetraborate; A: UV detection at 220 nm, B: potentiometric detection at metallic copper; peaks: 1: Fe*DTPA*, 2: *DTPA*

metric detector was approximately 0.4 ppm *DTPA*. Peak heights increased linearly up to 20 ppm.

The stability of the electrode response was found to be dependent on a polishing procedure being applied before use. In addition, corrosion processes at the electrode were observed during prolonged use depending on the type of carrier electrolyte, although these phenomena have not yet been fully investigated. It is recommended that the electrode be polished at the beginning of each working day.

Experimental

Apparatus

The CZE instrument employed was a HP 3D Electrophoresis System (Hewlett Packard, Palo Alto, CA, USA) equipped with a diode array detector and interfaced to a 3D CE Chemstation (Hewlett Packard). Separations were carried out at 25°C in fused silica capillaries of 50 cm length with an outer diameter of 360 µm and an inner diameter of 25 µm using a voltage of 25 kV. Injection was performed electrokinetically at 10 kV for 10 seconds.

Copper microelectrodes were prepared from copper wires with a diameter of 100 µm inserted into short pieces of a fused-silica capillary (outer diameter 360 µm, inner diameter 150 µm) and sealed with epoxy glue. At one end, the protruding copper wire was cut, producing a copper disk electrode of 100 µm diameter. Before use, the electrode was polished with a slurry of alumina. The design of the cell (Fig. 3) used for potentiometric detection in CZE was similar to that described by *Fermier et al.* [10] for amperometric detection. A block of plexiglass (5 cm length, 3 cm width, 2 cm thickness) was used as base plate, and a straight groove of 0.15 mm width and 0.15 mm depth was machined into its surface. The end of the separation capillary and the detection electrode were placed into the groove (the distance between the two was varied in the range of 20–100 µm), and the assembly was fixed in place by putting another block of grooved plexiglass on top of it. The two blocks were kept together by four screws. The whole assembly was placed into a crystallizing dish filled with carrier electrolyte and equipped with the reference electrode as well as the high voltage electrode. The potentiometric detection cell was connected to a E510 mV-meter (Metrohm, Herisau,
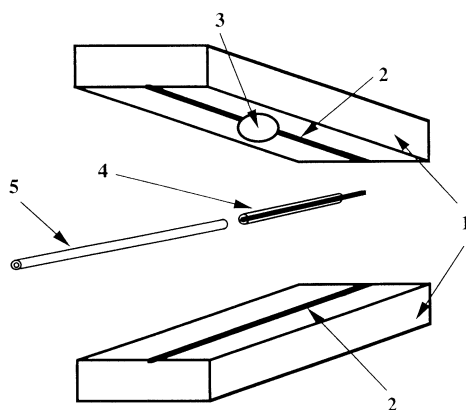

Fig. 3. Design of the potentiometric detection cell; 1: block of plexiglass, 2: groove (150 µm), 3: outlet into carrier electrolyte vessel containing reference and high voltage electrode, 4: 100 µm copper wire glued into fused-silica capillary (o.d. 360 µm), 5: fused-silica separation capillary (o.d. 360 µm, i.d. 25 µm)

Switzerland) in combination with a strip-chart recorder. The flow-injection experiments were carried out with a Ismatek peristaltic pump and a Metrohm EA1096 wall-jet detector which was equipped with copper disk electrodes of diameters between $50\,\mu m$ and $2\,mm$ prepared from copper disks embedded in rods made from epoxy resin.

Chemicals

All carrier electrolytes were prepared from analytical reagent grade chemicals using water purified with a Millipore (Bedford, MA, USA) Milli-Q water treatment system.

Acknowledgement

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