Determination of Metal Ions in Algal Solution Samples by Capillary Electrophoresis

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Abstract

Trace metals determination in aqueous samples can be readily accomplished by capillary electrophoresis (CE) via indirect absorbance detection. A method for the separation of metal ions is presented and applied to the determination of seven metals in algal solution samples. 2-Hydroxyisobutyric acid background electrolyte (BGE) containing UV CAT-1 (an ultraviolet-absorbing amine) is used to perform capillary ion analysis. Acetic acid is used to adjust the pH value of BGE to 4.4. All ions can be separated in less than 15 min. All peaks are well separated and baseline resolved (i.e., no peaks overlapped). This work presents the applicability of CE to the quantitative analysis of algal solution samples and shows the adsorption process of seven metals in solution (Mn, Cd, Cr, Ni, Zn, Pb, Cu) to *Chlorella vulgaris*. The innovation of the application of CE in the determination of metals bound by *Chlorella vulgaris* is shown to be an improvement of the pH over what has been published previously. The detection limit is in the range of 13 (Mn) to 102 (Pb) ppb with electrokinetic injection mode (15 kV, 7 s). Reproducibility was 1.4% for the migration time, better than 5% for peak area for four of the metal ions (Cr, Mn, Cd, and Cu), and lower than 5% for the other three (Ni, Zn, and Pb). Calibration curves are linear for most ions in the 10^{-7}–10^{-5}M range (correlation coefficient r^2 = 0.9933–0.9986) using electrokinetic injection mode.

Introduction

Metal-ion separations are of great interest in different areas of research such as the pharmaceutical industry, power-generating industry, and in environmental impact assessments (1). Ion chromatographic and spectrochemical techniques have been used for the routine determination of metal ions in a variety of samples. However, both methods have limitations for certain samples. Consequently, capillary electrophoresis (CE) has recently attracted attention as a promising alternative analytical technique for small-ion separations, having several advantages especially in the analysis of inorganic ions. Its high resolution results in very short analysis times, and operating costs are significantly lower in comparison with classical techniques.

Because of its obvious advantages, CE is widely used for the determination of ions in biological (2) and environmental samples (3–6), as well as in food (7), industrial (8,9), and pharmaceutical (10) products. Romano and Krol (5) demonstrated the ability of CE to analyze primary and secondary anionic contaminants as well as other ions of environmental concern in drinking water, groundwater, and wastewater. Rapid, highly efficient separations related to ion chromatography with different selectivities were obtained.

Lee and Lin (11) investigated the role of complexing agents and pH on the CE separation of alkali and alkaline earth metal ions. CE methods for the analysis of a mixture of nineteen metal ions via indirect ultraviolet (UV) absorbance detection, with pyridine or imidazole as the background electrolyte (BGE) and glycolic acid as the metal-complexing agent, have been recently reported (11). Calibration curves were linear for most ions in the 10^{-5}–10^{-3}M range using hydrodynamic injection mode (HD).

Yang et al. (4) studied the applicability of CE to the quantitative analysis of real complex mixtures. A comparison with a well-established method, flame atomic spectrometry (FAS), in terms of sensitivity, limit of detection, linearity, accuracy, and precision was carried out. The accuracy and precision of CE were acceptable with the imidazole–H_2SO_4 BGE and hydrostatic injection, but those of FAS were better. Although CE is comparatively more susceptible to matrix interferences and a sample pretreatment may be necessary, it is possible to detect different elements simultaneously.

Pascucci (12) studied the effects of a multielement solution of four metals on the binding process of *Chlorella vulgaris*. Samples were analyzed by a multi-element flame atomic absorption spectrophotometer.

This paper presents a CE method for the analysis of a mixture of seven metal ions in algal solution samples. The binding of seven metals (Mn, Cd, Cr, Ni, Zn, Pb, and Cu) in solution by

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Chlorella vulgaris was studied. The quantitation of the metals bound by the algae was indirectly determined compared with a single metal solution. Detection of the metal ions was accomplished by indirect UV absorbance measurement.

Experimental

Reagents and standards

Water used for the preparation of all solutions was obtained from a Milli-Q water purification system (Millipore, Bedford, MA) and contained no detectable analyte cations. Standards containing Mn, Cd, Cr, Ni, Zn, Pb, and Cu were prepared from the different 1000 g/mL Titrisol stock solutions of these elements (Merck, Darmstadt, Germany). All metal ion solutions were prepared from their chloride salts, except Pb(II) (nitrate salt). Standard solutions (10 mg/L) were prepared, mixed, and diluted to the specified concentrations used in the different CE runs. BGE containing 6mM CIA-Pak HIBA α-hydroxyisobutiric acid (Waters, Milford, MA) was prepared by dissolving 0.068 g of HIBA in a 100-mL plastic volumetric flask and adding 64 µL of UV CAT-1 reagent (CIA-Pak, Waters). The pH was adjusted by titration with acetic acid to an adequate pH (4.4). The resolution for the seven peaks was enhanced when the pH of the BGE was adjusted with acetic acid.

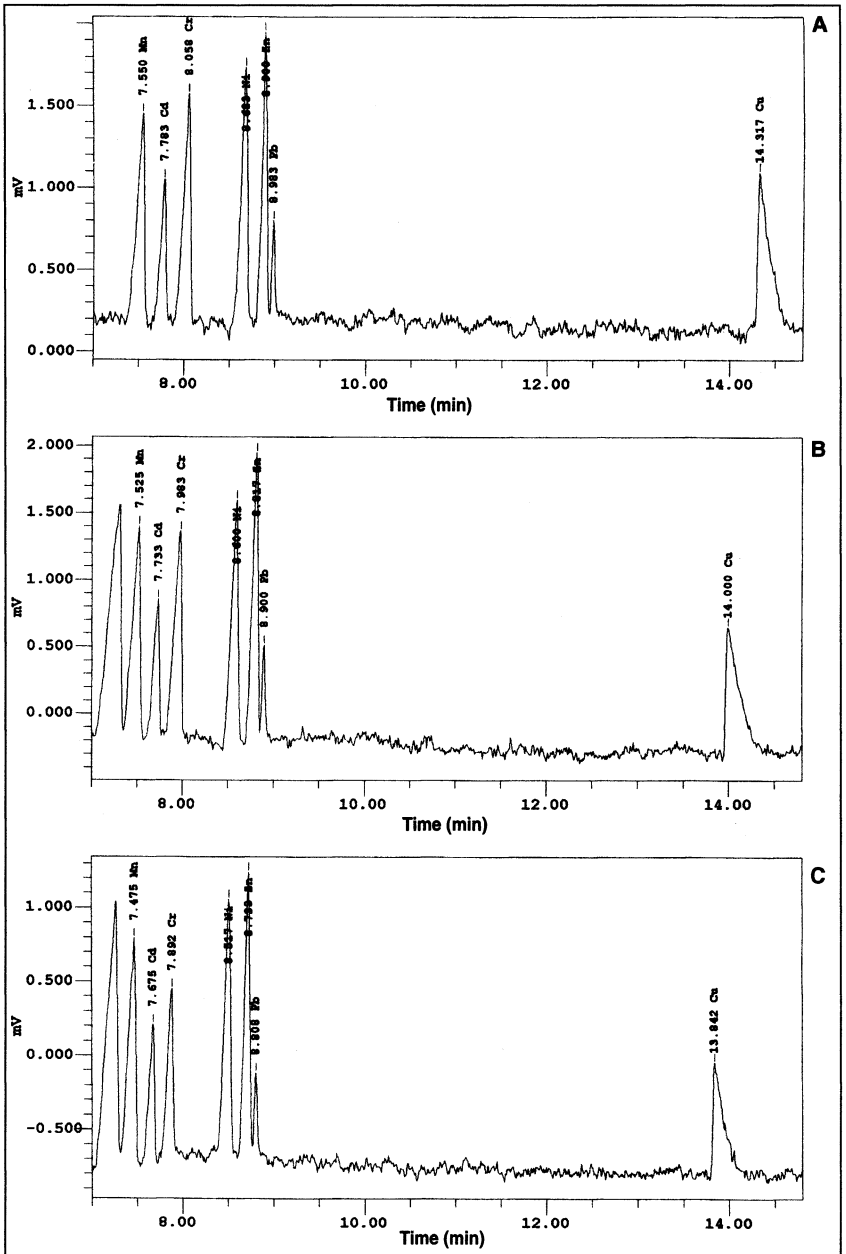

Figure 1. Electropherograms obtained for metal separations: A, pH 4.0; B, pH 5.0; and C, pH 6.0. Electrophoretic conditions: BGE, UV CAT-1 HIBA; applied voltage, +15 kV; electromigration injection, 7 s at +15 kV.

Instrumentation

A Waters Quanta 4000 capillary electrophoresis system with a 20-sample carousel, a positive power supply, and a mercury lamp detector (185 nm) was used. Conventional 60 cm × 75-µm i.d. (363-µm o.d.) fused-silica capillaries obtained from Supelco (Chromatography Products, Barcelona, Spain) were used in all analyses. A positive voltage of 15 kV was applied. The detector time constant was 0.3 s. The sample injections were carried out in electromigration mode at +15 kV for 7 s. The pH was measured and adjusted with a Crison pH-meter model Micro pH 2000 (Barcelona, Spain) calibrated immediately prior to use. A 466 NEC personal computer (Boxborough, MA) installed with a Millenium Data Station and version 2.15 Millenium Chromatography Manager (Waters) was used to control the instrument settings, data acquisition, and analysis. Absolute peak areas were used in all calculations.

Capillary preparation and cleaning

Every morning the capillary was cleaned and prepared according to the following procedure: a 1-min wash with Milli-Q purified water, a 5-min wash with 0.1M KOH, and a 2-min wash with Milli-Q water. The capillary was also conditioned with BGE for at least 10 min. The whole cleaning procedure was repeated at the end of the day, and the capillary was rinsed and conditioned with 10% methanol–H_2O overnight.

Samples

Aliquots (10-mL) of prepared solution with 1 mg/L each of Mn, Cd, Cr, Ni, Zn, Pb, and Cu were added to the samples of algae and then mixed for 30 s. The pH of each sample was modified adding 0.2M NaOH; the pH of the solution did not change after adding the algae. The samples were subsequently centrifuged at 4000 rpm for 15 min. The supernatant was decanted from the algae pellet and simultaneously analyzed by

CE for the seven elements. The fraction of the metal bound to the algae was indirectly determined by comparing the electropherograms performed with and without the algae. The decanted algae was subjected to biomass monitoring. Sedimented cells were then washed three times in distilled water containing 15 mg $NaHCO_3$ per liter, transferred to tared porcelain cups, dried overnight in a hot-air oven at 105°C, and weighed (13).

Results and discussion

Cation analysis

The standard solutions, as prepared in the Experimental section, were adjusted to different pH values (4.0, 5.0, and 6.0). Figure 1 shows the electropherograms for Mn, Cd, Cr, Ni, Zn, Pb, and Cu (1 µg/mL each). It has been found that an increase in the pH of the solution results in a decrease in the quantitation of the metals by CE because the cations combine with hydroxyl ions, forming precipitates (14). Higher precipitation was found for Cr and Cu at pH 6.0. The fraction of metals precipitated was evaluated in advance to calculate the amount of metals bound by the algae.

Analysis of metal ions in algal solution samples

The residence time of the algae in each solution was held constant (20 min) at 21°C, keeping the biomass of algae at 9 mg/sample. Figure 2 shows the electropherograms obtained after adding a biomass of algae to the standard solutions at three different pH values. The fraction of metals precipitated at pH 4.0, 5.0, and 6.0 was evaluated prior to the quantitation of the amount of metals bound by the algae. An increase in the pH value resulted in an enhancement of the fraction of the metal bound to the algae.

Comparing the electropherograms with and without the algae, the fraction of metal bound by the algae can be indirectly determined. Table I shows the pH dependence on the simultaneous binding of seven metals to *Chlorella vulgaris*. The maximum binding of these metals to *Chlorella vulgaris* rose at a pH of approximately 6.0. This seemed to indicate that competition among the metals for binding sites in the algae was occurring. Cr and Cu seemed to predominate the process.

Validation of the method

In order to characterize the quality of the results obtained with this method, the method had to be validated.

Limit of detection

In the presence of solvent stacking, the limit of detection (LOD) is quite difficult to estimate. In fact, injection yield increases when the concentration decreases (3). For this reason, we decided to use the limit of quantitation (LOQ), which is more appropriate for a quantitative method. The LOQ for different metals was calculated using the equation (15):

$$LOQ = x + 10s \qquad \text{Eq 1}$$

where x is the mean value of 10 analyses of the baseline noise (blank signal) and s is the standard deviation of the mean noise level. The values obtained for the different cations were approximately 0.6 µmol/L, which corresponded to 50 ppb (Table II). Several investigations have shown that the electrokinetic (EK) mode provides better sensitivity than the HD mode. This is attributed to the "stacking" effect (6,11) that occurs when the ion concentration in the sample plug is considerably lower than the background electrolyte in the separation buffer. This phenomenon leads to a concentration of the analyte ions. The low values obtained for the different cations in the LOQ (about 50 ppb) could be a result of the stacking effect.

Linearity

The linearity of the calibration curves, expressed as peak area versus metal ion concentration, was evaluated in the concentration range where the highest and lowest standard differed by a factor of 16. A good linearity (correlation coefficient r^2 = 0.9933–0.9984) was obtained for most ions. In the EK mode, the sensitivity is much better in low concentrations (16). Typical calibration curves for the seven metal ions are shown in Table III.

Reproducibility

Reproducibility was studied by making ten consecutive runs of a sample. The results obtained were expressed in terms of relative standard deviation. Excellent precision was obtained for the migration time for all ions (1.2% for Mn; 1.3% for Cr; 1.4% for

Table I. Percentage of Metals Bound to *Chlorella vulgaris*

Metal	pH 4.0	pH 5.0	pH 6.0
Mn	26.70	49.73	60.91
Cd	49.00	64.22	70.93
Cr	87.50	89.32	87.00
Ni	38.43	51.74	60.00
Zn	16.35	55.00	65.80
Pb	100.0	100.0	100.0
Cu	83.23	100.0	100.0

Table II. Detection Limits of Metal Ions*

Metal	LOQ (µM)	LOQ (ppb)
Mn	0.23	13
Cd	0.62	70
Cr	0.57	30
Ni	0.41	24
Zn	0.65	43
Pb	0.49	102
Cu	1.01	64

* The minimum detectable concentration of EK injection at +15 kV for 7 s.

Table III. Linearity

Metal	Linearity range	r^2
Mn	20–320 µg/L	0.995087
Cd	70 µg/L–1.12 mg/L	0.988380
Cr	30–480 µg/L	0.998047
Ni	30–480 µg/L	0.993366
Zn	40–640 ug/L	0.998445
Pb	0.1–1.6 mg/L	0.992034
Cu	60–960 µg/L	0.996636

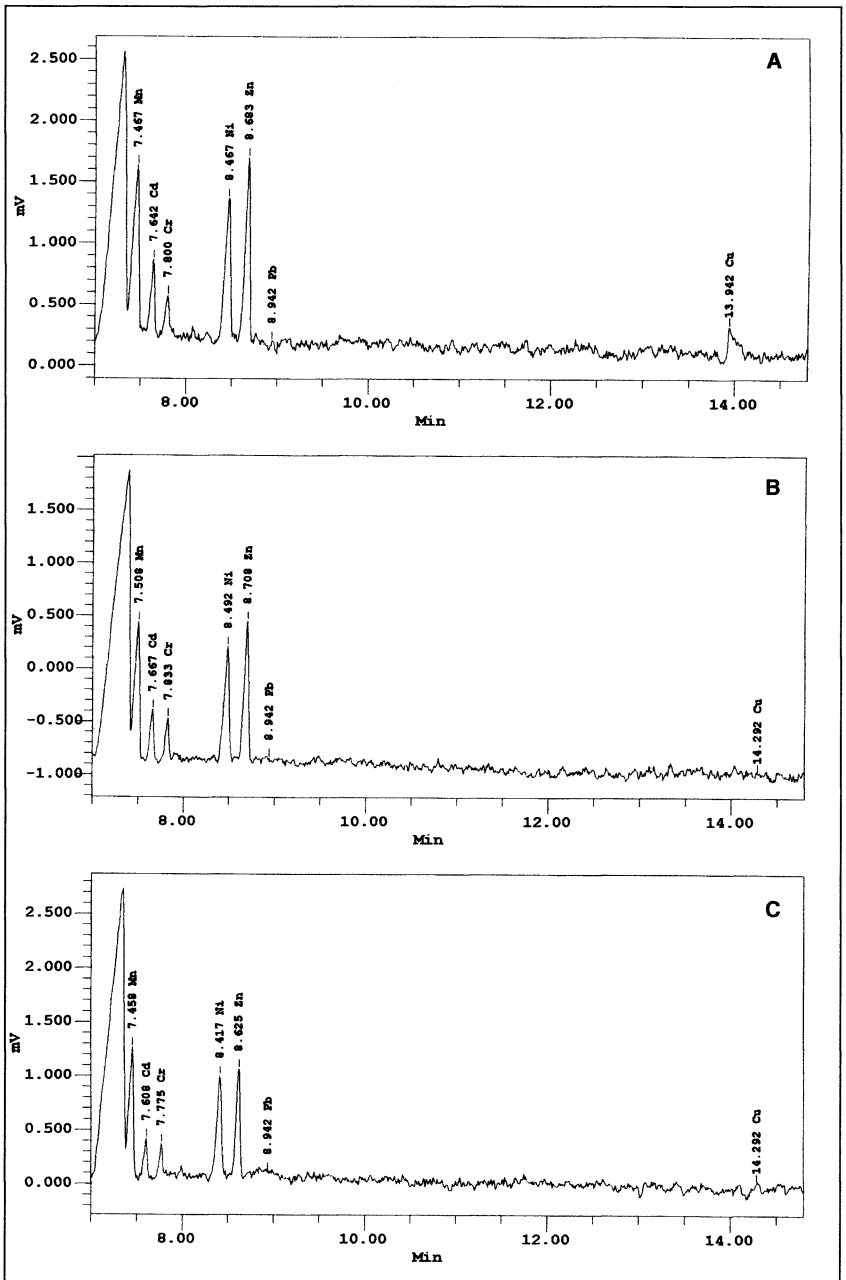

Figure 2. Electropherograms obtained after adding the biomass of algae to the standard solutions. A, pH 4.0; B, pH 5.0; and C, pH 6.0. Electrophoretic conditions: BGE, UV CAT-1 HIBA; applied voltage, +15 kV; electromigration injection, 7 s at +15 kV.

Cd, Ni, Zn, and Pb; and 2.0% for Cu) and good precision for peak area (4.4% for Pb, 4.8% for Ni, 4.9% for Zn, 5.0% for Cr, 6.7% for Mn, and 7.0% for Cd and Cu). Peak area was used to plot calibration graphs because it has better reproducibility and provides a larger linear scaling.

Conclusion

A rapid, reliable CE method for determining metal ions in algal solution samples was developed. This method presented some advantages over the techniques usually employed, such as ion chromatography and spectroscopy. CE allows the simultaneous detection of different elements in very short times, and the operating costs are very low.

Chlorella vulgaris is able to strongly bind to the seven metals studied, with particularly strong affinity for Cu and Cr. The pH that allows maximum binding is approximately 6.0. The removal of more than one element in solution by this algal species has been shown to be a competitive process (12).The actual mechanism of this competition for binding sites is dependent upon the properties of each individual metal. In other words, all the metals in solution compete for a limited number of binding sites or functional groups within the algae cells. The accumulation of metals by *Chlorella vulgaris* can take place by entrapment using cellular components or active transport across the cell membrane (both processes associated with living cells). Harris and Ramelow (17) investigated the metal-binding properties to *C. vulgaris* and *S. quadricauda*. The binding of Cu, Cd, and Zn was pH-dependent, the effect being most pronounced for Cu.

The use of microorganisms for the removal of toxic metals from natural waters or wastewaters appears to be very promising. The most general technique used for removing metals from waste streams is treatment by precipitation as hydroxides. An alternative treatment system that uses inexpensive materials to remove and reclaim metals could be of technical and comercial interest (14). Another possible application is to concentrate metals from environmental samples to increase the sensitivity of existing analytical techniques (17).

Acknowledgments

This work has been supported by Xunta de Galicia, XUGA 17202A95.

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Manuscript accepted April 2, 1998.