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Improved photometric detection of metal ions by capillary zone electrophoresis after precapillary complexation¹

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Abstract

Precapillary reactions using complexing reagents are increasingly being used to carry out separations of metal ions by capillary zone electrophoresis. To compensate for the sensitivity limitations of on-column photometric detection, several approaches were evaluated. Parameters affecting the sensitivity response of a direct detection system, such as the detection wavelength, sample size, applied voltage, and electrolyte concentration, were examined to produce the best possible sensitivity for metal ions complexed with cyclohexane-1,2-diaminetetraacetic acid. Using injection in the electrokinetic mode, a 2-fold increase in detectability over hydrodynamic (gravity) injection was obtained for certain metal analytes that corresponds to the detection limits in the sub- $\mu\text{g}/\text{l}$ range. A characterization and optimization of photometric detection in an indirect format have been conducted. An organic dye, combined with the carrier electrolyte to impart an absorbance background in the visible range and selected with due account for its spectral and electrophoretic characteristics, was found to provide micromolar-level sensitivities. Feasibility of on-line (stacking) sample concentration for metal complexes has also been demonstrated. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Since the advent of capillary zone electrophoresis (CE) for metal ion separations, one of the main trends in its development has been towards increased sensitivity. The necessity to lower the detection limits arises both due to the trace concentration levels at which many metal species exist in environmental and biological samples and also to the

modest detectability attainable with photometric detectors most commonly implemented in commercial CE instruments. Another reason that necessitates sensitivity enhancement in CE is due to the fact that the technique's predecessor and main competitor in metal ion separation and speciation, modern liquid chromatography, is considerably more sensitive than CE. These circumstances by no means impede wide acceptance of CE for use in routine laboratories.

There is much that can be done to improve sensitivity in CE. An examination of reported work dealing with the CE determination of metal ions shows that approaches to help address this problem

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can be divided into three groups: alternative detection modes, pre- or on-capillary derivatization chemistry, and sample concentration strategies. Continuing efforts on developing alternative detection schemes as a more revolutionary way, promising substantial gains in detectability, led recently to a number of interesting modes. Of these, one must first look to inductively coupled plasma mass spectrometry (ICP-MS) [1,2], ion spray MS [3,4], laser-induced fluorescence [5–7] and end-column conductometry [8–10] for the most efficient solution to the problem. Other but seemingly less versatile newcomers in the area are pulse amperometry [11,12], chemiluminescence [13], proton-induced X-ray emission [14,15] and radioactivity detection [16].

However, only a few of the newly emerging detection modes are already realized for available commercial CE systems. This stimulated us to explore the enhancement of sensitivity for metal ions with absorbance spectroscopic detection as based on the chemical means and on-column sample concentration. Within the frames of the present study we give the prevalence of sample ions precapillary complexed into strong metal complexes since, in the first place, such a derivatization makes possible direct optical measurements for the separated metal species.

2. Experimental

A Waters Quanta 4000 CE system (Millipore Waters, Milford, MA, USA) and a model 270A CE system (Applied Biosystems, Forster City, CA, USA), both equipped with a negative power supply were employed throughout the experiments. Polyimide-coated, fused-silica capillaries (Polymicro Technology, Phoenix, AZ, USA), were 50 cm in length with an I.D. of 75 μm and a distance of 42.5 cm from the point of injection to the window for on column detection. Direct UV detection was carried out at 185, 214 or 254 nm as provided the standard lamp set of Quanta instrument, while the absorbance detector of variable wavelength capacity of ABI 270A instrument was used in indirect detection mode. A voltage of 15 kV was applied for separations, unless stated otherwise. The time of hydrodynamic or vacuum injections and applied voltage

value and duration for electrokinetic injections were varied as specified below. Electropherograms were recorded and processed with a Hewlett-Packard 3359 data acquisition system.

All solutions, electrolytes and standards were prepared with analytical-reagent grade chemicals and doubly distilled water. The main electrolyte buffer was composed of sodium tetraborate (Merck, Darmstadt, Germany) and 1×10^{-3} M cyclohexane-1,2-diaminetetraacetic acid (CDTA) (St. Louis, MO, USA). Bromxylenol blue, eosin yellow, nitroso-R-salt, and other organic dyes, providing the absorbance background, were purchased from different suppliers and were used as received. Metal ion standard solutions were prepared as described elsewhere [17]. To prepare metal complexes, sufficient reagent, as a 5×10^{-3} M solution in 0.01 M sodium tetraborate, was added to metal stock solution to give a 2.5-fold molar excess in the final sample.

The detection limit was defined as three times the signal-to-noise ratio. The linearity of calibration curves was evaluated from plots of sensitivity vs. concentration [18].

3. Results and discussion

In the past years many research groups have conducted work on the development of a variety of metal-complexing systems for CE. A recent review [19] has summarized and commented strategies for achieving multi-element separations using precapillary-formed metal complexes. The resolution of as many as 23 metal ions complexed with CDTA has been reported in the first part of this study (Ref. [20]), that is well comparable with the best separation accounts on the base of partial in-capillary complexation chemistry [21,22]. However, modest concentration limits of detection of this and many other complexing systems (typically on the order of 10^{-6} M) continue to be a challenging problem for trace level applications. To our mind, before deciding whether or not to implement an alternative detection system of high sensitivity, the practitioners of CE have to examine critically a range of approaches described below that can improve the detectability of an existing CE system without superfluous investment costs.

3.1. Optimization of system CE parameters

3.1.1. Detection wavelength

Detection performed in the farther UV range was expected to result in a general increase in sensitivity for metal–CDTA complexes. Indeed, setting the detection wavelength at 185 nm gave rise to increased peak intensities at comparable background signal and thereby to substantially better detection performance than UV detection carried out at 214 or 254 nm. It can be seen in Table 1 that the sensitivity was increased by a factor ranging between 1.4 and 15, compared with another conventional wavelength of 214 nm used in most previous work on this complexing system [20,23,24]. Turning to the detection limits, the strong UV absorbance exhibited by metal–CDTA complexes at 185 nm allows the detection of 2.3×10^{-8} M Ca(II) and Cu(II), 1.8×10^{-8} M Hg(II), and 6×10^{-9} M Fe(III), chosen here and hereafter as the test metal ions.

3.1.2. Sample size

Another straightforward approach is to enhance the loadability of the system. Within a single capillary setup, extension of the loading capacity can be most easily achieved by injecting greater sample volumes until an increase in sample plug length comes into contradiction to the separation performance. The sample size was varied by varying sampling time from 10 to 90 s with hydrostatic injection at 100 mm. The effect was examined using the test four-component mixture containing 2×10^{-5} M of each metal. Fig. 1 presents the relationships between the injection time and the peak height and area. As can be seen, both responses, although differently, increase with the injection time. For peak area plots, this increase has a virtually linear character up to about 50 s. Beyond this value, a loss of linearity was

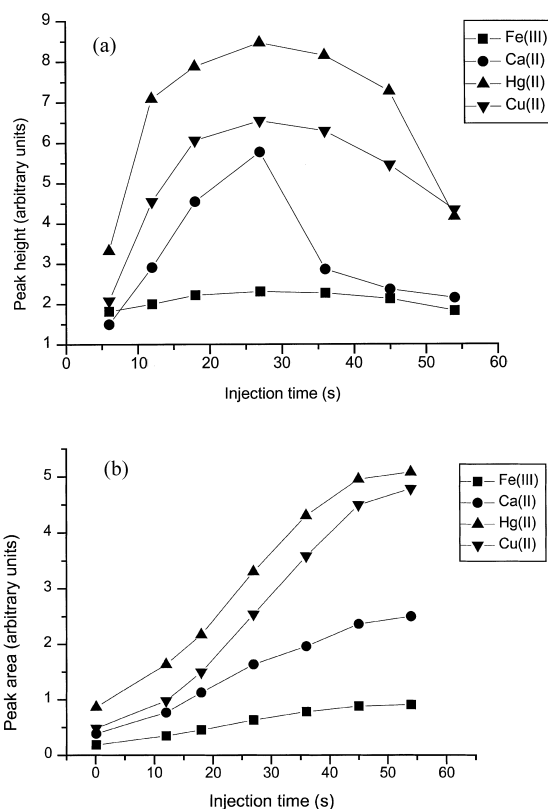


Fig. 1. Effect of sample size on peak height and area. Electrolyte, 10 mM sodium tetraborate buffer, 1 mM CDTA; injection, gravity, at 10 cm; detection at 185 nm; metal ion concentration, 2×10^{-5} M. Other conditions as for Table 1.

observed due to the decreased peak efficiency and limited column capacity. The same appearances likely became the factors responsible for reduced peak heights at larger injection times. Similar dependences have been observed by Motomizu and co-workers [23,25,26], and Baraj et al. [27], who used a vacuum injection system. The appropriate injection

Table 1

Relative sensitivities of metal–CDTA complexes at different wavelengths determined by comparison of peak areas for 3×10^{-4} M per complex

Detection wavelength (nm)	Cr(III)	Cu(II)	Fe(III)	Ni(II)	Zn(II)
185	8.5	15.7	600	4.5	7.7
214	1.5	2.8	40	3.2	3.7
254	1	1	1	1	1

CE conditions: capillary, fused-silica, 50 cm \times 75 μ m I.D.; electrolyte, 10 mM sodium tetraborate, 1 mM CDTA (pH 9.0); applied voltage, 15 kV; injection, 10 s at 10 cm; detection, direct UV.

time of 40 s, then, was chosen because longer injections may lead to uncertainty in measuring the broader peaks near by the detection limit.

3.2. On-column preconcentration by electrostacking

In order to achieve an optimum sensitivity, the operational parameters of CE system were adjusted on the basis of a wider range of analytes. Increasing the concentration of sodium tetraborate in an electrophoretic solution has a beneficial effect on the detection signal for the majority of metal–CDTA complexes as shown in Table 2. Also, an increased applied voltage produced enlarged peak heights (Fig. 2). The reason why alterations of these CE conditions caused different sensitivity can be explained in view of electrostacking. The higher the conductivity of the electrolyte solution and voltage applied, the greater the effect of stacking (or ‘stacking force’) becomes, as was also studied for metal complexes by Zhang et al. [28]. Simultaneously, the increased baseline noise as a result of excessive Joule heating limited the detectability at too high electrolyte concentrations and electric fields (see, e.g., Fig. 2). From these experiments, the sodium tetraborate concentration and operational voltage chosen as the optimum were 20 mM and 12.5 kV.

Under the above sampling and electrophoretic conditions, the quantitative performance of the system was evaluated by constructing sensitivity vs. concentration plots [18]. Measured for the range 3×10^{-4} to 4×10^{-7} M, these plots indicate that the lower boundary of the apparently linear dynamic range corresponds to ca. 5×10^{-6} M. This means that beyond this value, the concentration points should be

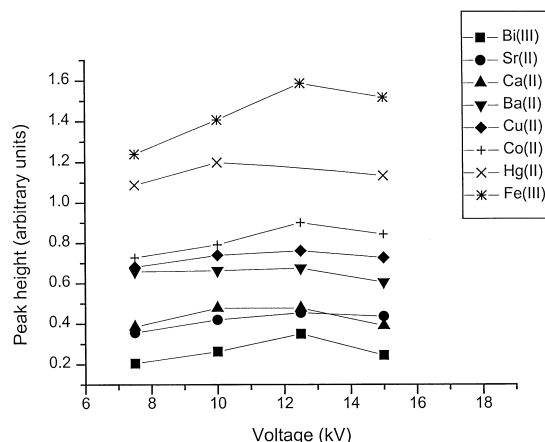


Fig. 2. Peak height as a function of operating voltage. Electrolyte, 10 mM sodium tetraborate buffer, 1 mM CDTA. Other conditions as for Table 2.

taken with care while treated using conventional, peak area–concentration calibration curves.

The quantitative performance of the method was further tested by evaluating the short-term repeatability with seven consecutive injections of a 5.6×10^{-5} M standard solution. The data were gathered using peak area measurements. Variations do not exceed 3.5% R.S.D., which is the proof of reasonable precision of the method.

3.3. Comparison of hydrodynamic and electrokinetic injection

The method of injection has a profound effect on the detection limits in CE. For instance, in a work by Xu and Ma [29], electrokinetic injections produced about 40% higher sensitivity for the iron(III)–1,10-phenanthroline complex than that from hydrodynamic injection. Therefore, electrokinetic injection

Table 2
Peak intensities for metal–CDTA complexes at varied sodium tetraborate concentration

Electrolyte buffer concentration (mM)	Peak height, arbitrary units								
	Complex:	Ba(II)	Bi(III)	Co(II)	Cu(II)	Fe(II)	Fe(III)	Hg(II)	Ni(II)
10		0.44	0.25	0.36	0.44	0.89	1.64	1.07	0.71
15		0.50	1.13	0.48	0.53	1.21	1.93	1.25	0.75
20		1.00	2.11	0.89	1.63	1.95	3.68	2.37	0.83

CE conditions: electrolytes, sodium tetraborate buffer, 1 mM CDTA; metal ion concentration, 5×10^{-5} M Fe(II) and 1×10^{-4} M other metals; other CE conditions as for Table 1.

mode was applied for metal–CDTA complexes in an attempt to improve the detectability further.

In order to optimize the signal response the effects of injection time and voltage on peak height and plate number were investigated. Injection times increased up to 20 s induced a pronounced improvement in the detection sensitivity (data not shown). Beyond this period, however, an increase in peak height took place at the expense of separation efficiency and resolution. The injection voltage also has an influence on peak height response since the higher the voltage the greater the amount of metal complexes introduced into the capillary. As can be seen in Fig. 3 for the detection of iron(III), a maximum was reached at approx. 20 kV. Above this value, peaks tend to be broadened that results in a noticeable loss of efficiency.

The detection limits obtained under these conditions, i.e., at a 20-kV electrokinetic injection for 20 s, are reported in Table 3 in comparison with hydrodynamic detection thresholds. Among the metal complexes examined, the electrokinetic injection appears to be more sensitive for calcium and copper, whereas complexes of Fe and Hg show similar or poorer detectability. These differences are apparently related to the fact that the amount of material loaded electrokinetically in a counter-electrosmotic mode varies as a function of the electrophoretic mobility of each sample component. Unlike electrokinetic injections, hydrodynamic injections exhibit no discrimination among different mobility

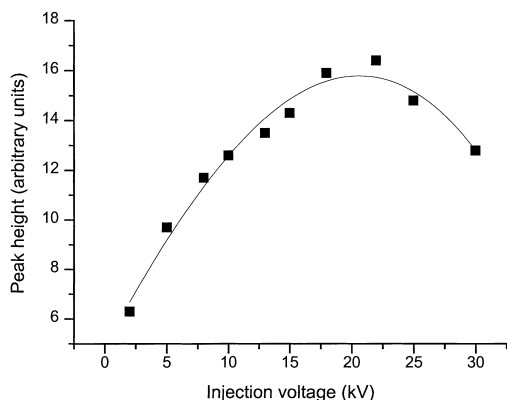


Fig. 3. Peak height vs. injection voltage for iron(III)–CDTA complex. Injection, electrokinetic, at 15 s. Detection at 185 nm; iron concentration, 1×10^{-3} M. Other conditions as for Table 2.

Table 3

Detection thresholds obtained using the hydrodynamic and electrokinetic injection modes

Complex	Detection limit (M)	
	Hydrodynamic injection ^a	Electrokinetic injection ^b
Ca(II)	2.3×10^{-8}	1.2×10^{-8}
Cu(II)	2.3×10^{-8}	1.2×10^{-8}
Fe(III)	6×10^{-9}	5.1×10^{-8}
Hg(II)	1.8×10^{-8}	6.0×10^{-8}

^aInjection height, 10 cm; time, 20 s.

^bInjection voltage, 20 kV; time, 20 s.

species. Another bias in quantitative CE measurements caused by electrokinetic sample injection is brought about by the different amounts of the same ions injected with different sample electrolyte concentrations. Alternatively, this bias can be avoided entirely by using hydrodynamic injection mode.

3.4. Indirect detection in the visible range

Indirect detection performed in the UV region is commonly adopted in CE for monitoring free or partially complexed metal cations that otherwise are not detectable by photometric techniques. A few studies dealt with indirect UV detection of metal complexes of EDTA [30] (J.-F. Jen, M.-H. Wu, unpublished data), however, metal-complexed species are detected with lower sensitivity due to their slight low-energy UV absorption. One more feature of metal–ligand systems necessary to consider while optimizing detection strategies is due to the inclination of the less stable complexes to partial dissociation during the CE run. Incorporation of a certain concentration of the complexing reagent into the running buffer, which is the most universal way to prevent this undesirable phenomenon, ultimately adversely affects the detection sensitivity, whatever mode of photometric detection is utilized. As a promising solution to the above problems, the use of the visible light range of wavelengths where both analytes and buffer components have negligible absorption was therefore tried.

According to the principle of indirect photometric detection, the light-absorbing ion to be selected should feature: (i) unchangeable anionic function in the pH range optimal for the separation; (ii) a strong absorption at the detection wavelength; and (iii) an

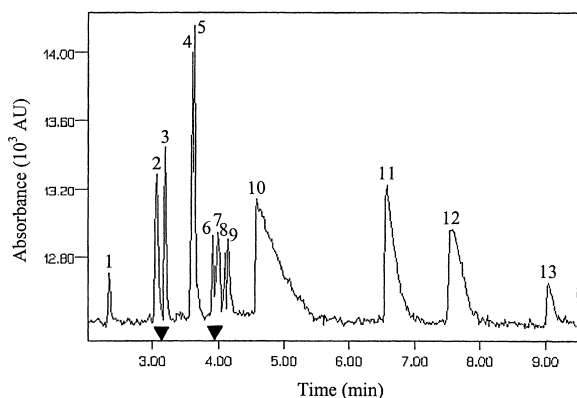


Fig. 4. Electropherogram of a mixture of metallochromic ligands tested as visualizing co-ions. Electrolyte, 5 mM sodium tetraborate (pH 9.2); injection, 15 s at 10 cm; detection at 214 nm. Peak identities: 1, thymol blue; 2, methyl red; 3, bromocresol green; 4, eosin yellow; 5, bromphenol blue; 6, brompyrogallol red; 7, indigo carmin; 8, alizarinsulfonic acid; 9, eriochrom cyanin R; 10, naphthol green B; 11, bromxylenol blue; 12, chromotropic acid; 13, nitroso-R-salt. Arrows indicate the range of effective mobilities for four metal-CDTA complexes tested.

ionic mobility similar to that of tested metal complexes. A set of charged organic dyes listed in the caption of Fig. 4 meet these requirements. To shorten this list to the most promising visualizing dye anions, the absorbance spectra were recorded and relative mobilities were evaluated as shown in Fig. 4. Both experimental series were conducted using 5 mM sodium tetraborate in solution (running buffer), which was considered to be a good compromise between detection sensitivity and attainment of the sufficient buffering capacity. Higher concentrations of a nonabsorbing electrolyte constituent yield an increase in the minimum detectable concentration of the analyte [31]. Finally, eosin yellow, bromxylenol blue, and nitroso-R-salt were chosen as high molar-absorptivity representatives of different λ_{\max} and μ_{ep} .

Indirect response signals were obtained with both three visualizing co-ions. In all cases, however, the electropherograms contained a system (positive) peak with the migration time corresponding to that of a given dye (Fig. 5). In terms of separation, this finding is unfavorable because the system peak poses potential interference for the detection of metal

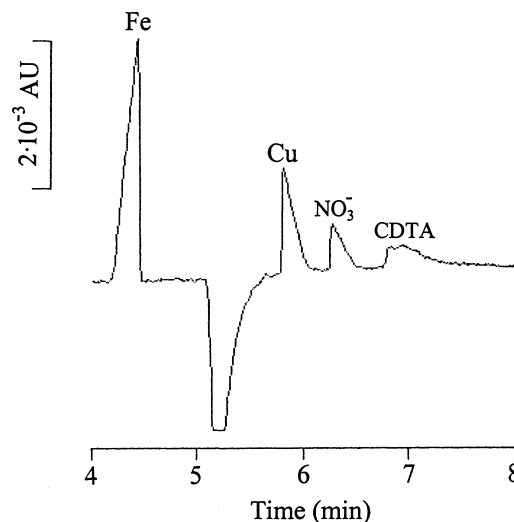


Fig. 5. Indirect visible absorbance detection of iron(III) and copper(II) complexes with CDTA using bromxylenol as the visualizing co-ion. Capillary, fused-silica, 60 cm \times 75 μ m I.D.; electrolyte, 0.5 mM bromxylenol blue, 7.5 mM sodium tetraborate, 1 mM CDTA; injection, 2 s (vacuum); detection at 614 nm; metal concentration, 3×10^{-4} M Fe(III) and 6×10^{-4} M Cu(II).

analytes. The problem was not investigated in further detail, but we did not overlook the fact that similar metallochromic ligands have a large sorption on the untreated capillary walls [32].

In order to maximize the detectability, a series of electrolytes comprising the dye and sodium tetraborate concentrations in the range from 0.1 to 1 mM and from 1 to 10 mM, respectively, were prepared. Best results, regarding sensitivity and resolution, were obtained with an electrolyte buffer containing 0.5 mM eosin yellow and 7.5 mM sodium borate. The minimum detection limit of 2×10^{-6} M was found for iron(III). Bromxylenol electrolytes exhibited slightly poorer sensitivity than eosin, but gave also acceptable separation from the system peak, whereas nitroso-R-salt was the least sensitive reagent.

In summary, although indirect detection has no decided advantages over direct detection mode in terms of sensitivity, it offers certain advantages of versatility and a higher degree of matrix indepen-

dence. Anticipated area of application might be simultaneous CE determination of anions and complexed metal cations.

4. Conclusions

The use of CE for metal ion analysis is an expanding area, and the future progress in the area will ultimately take place as following an advance in detection methodology. Several ways of compensating for the sensitivity limitations of existing commercial UV–VIS systems regarding metal–CDTA complexes are evaluated above. Given advantages of this complexing system in terms of metal separation selectivity, the minimum detectable concentrations at the level of 10^{-8} M and lower which were attained are promising for applications in fields such as environmental analysis and medicine.

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