

Sensitivity Determinations for Capillary Electrophoresis Coupled with X-ray Fluorescence

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Introduction

Capillary electrophoresis (CE) is a method for separating molecules. The basis for the way it works is that different species have different mobility in an applied electric field and that they consequently elute at the detection window at different times. In previous work at the National Synchrotron Light Source (NSLS), we showed that CE can be coupled to x-ray fluorescence (XRF) to give a valuable new method for determining the metal concentration in metalloproteins. One limitation of our work to date has been relatively poor detection limits. We used MHATT-CAT beamline 7-ID at the APS to perform a CE experiment coupled with fluorescence (UV) and XRF using a Si-drift detector. Results show a significantly improved detection limit.

Methods and Materials

We separated mixtures by using the CE-XRF technique. The energy of the incident beam was fixed at 10 keV with a photon flux of 2.2×10^{11} photons/second.

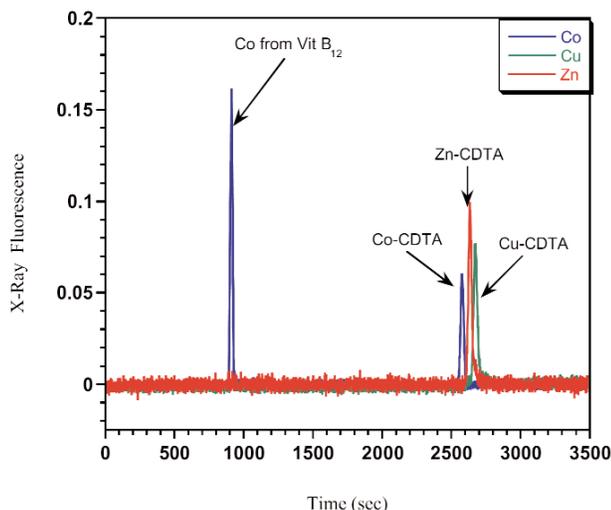


FIG. 1. Electropherogram for separation of a mixture of 1-mM samples. The K-alpha fluorescence intensities for Co, Cu, and Zn were measured by a Si-drift detector. Separations were performed by using a 20-mM sodium borate and 1-mM CDTA buffer (pH 9.0), with 12 kV of applied electric field in a capillary with a 100- μ m inner diameter.

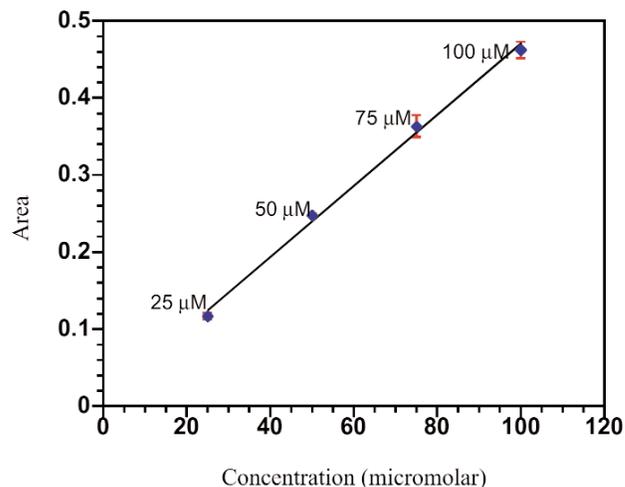


FIG. 2. The area under the peak obtained from electropherograms versus concentrations of Zn. The error bars from using different fitting techniques in Matlab are included. Separations were performed by using 20-mM sodium borate and 1-mM CDTA buffer (pH 9.0), with 12 kV of applied electric field in a capillary with a 100- μ m inner diameter.

The slit size was $10 \times 100 \mu\text{m}$. Vitamin B₁₂ was used as a neutral component, and metal-CDTA (metal = Fe, Co, Cu, Zn) complexes were used as negatively charged species (Fe-CDTA has a net -1 charge, and Co/Cu/Zn-CDTA have a net -2 charge). Depending on their mobility, which is a function of their charge, the complexes eluted at different times, as shown in Fig. 1.

In order to determine the detection limits, metal-CDTA solutions of different concentrations from 25 μM to 1 mM were injected in the capillary, and fluorescence was measured as a function of time for a fixed excitation energy of 10 keV. The area under the peak obtained from electropherograms for each different concentration is plotted as a function of concentration in Fig. 2.

Results

From the area under the peak for different concentrations of zinc and from the noise level for each separation, the lowest concentration that could be detected (signal-to-noise ratio of at least 3) under these

conditions was about 8 μM . The primary limitation on this detection limit was the relatively slow counting rate of the silicon-drift detector. With an optimized detector, it should be possible to decrease our detection limit by at least another order of magnitude. This would be sufficiently low to permit study of biologically important samples.

Acknowledgment

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