

## Polymer-Mediated Extraction of Porphyrinato Chelates for a Spectrophotometric Approach to Sub-ppt Level Detection of Metal Ions

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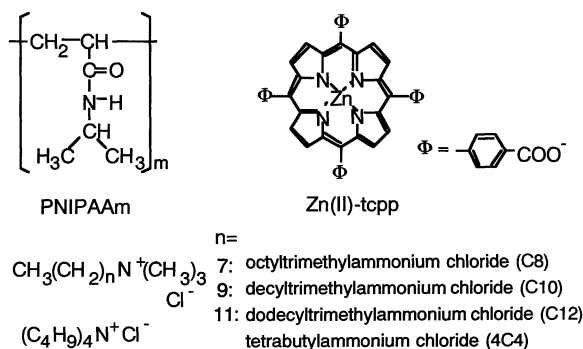
Negatively charged copper(II) and zinc(II) chelates with 5, 10, 15, 20-tetrakis(4-carboxyphenyl)-21*H*, 23*H*-porphine were incorporated into the precipitates formed from the aqueous solutions of thermo-responsive polymer; poly(*N*-isopropylacrylamide). In the presence of an appropriate kind and amount of quaternary ammonium ion, the recovery of the chelate was quantitatively. Sub-ppt level zinc(II) in water by the photometric detection in HPLC was successfully achieved, because of the highly efficient concentration.

A kinetically discriminative mode (KD-mode) of high performance liquid chromatography (HPLC) with the photometric detection has been accepted as a powerful method for the detection of sub-ppb level metal ions.<sup>1</sup> With the aim of highly sensitive detection of desired metal ions, a number of chelating reagents have been developed and tested as the pre-column reagents for KD-mode HPLC. Among them, a water-soluble porphine; 5, 10, 15, 20-tetrakis(4-carboxyphenyl)-21*H*, 23*H*-porphine (tcpp) seems to be excellent chromogenic reagents for metal ions because of their extremely high molar absorption coefficients ( $\epsilon > 5 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$ ).<sup>2</sup> Thus, the efficient concentration of metal-tcpp chelate prior to HPLC would provide an extremely sensitive method for detecting metal ions.

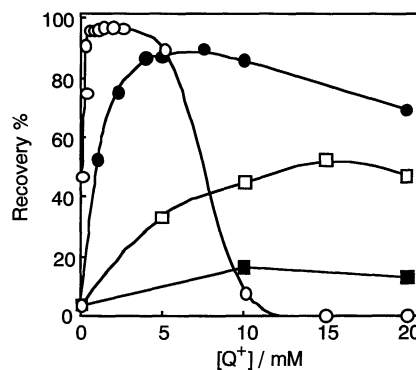
Recently, we proposed a kind of rapid and simple extraction system for concentrating hydrophobic compounds in the aqueous solutions in the basis of the thermo-responsive precipitation phenomena of the water-soluble polymers (polymer-mediated extraction).<sup>3</sup> That is, in aqueous polymer solution, any hydrophobic materials present will be solubilized and incorporated into the thermo-responsive polymer assembly, which becomes water-insoluble above the lower critical solution temperature, ca. 32 °C. As the polymer precipitated are condensed to an aggregates (polymer phase) having extremely small volume, the hydrophobic compounds in the aqueous solution are highly concentrated into the phase. Thus, extremely sensitive detection system will be obtained by the combination of this method with the KD-mode HPLC using highly sensitive chromogenic reagents.

In this study, we examined the possibility in the application of the polymer-mediated extraction to the concentration of charged metal chelates with tcpp. The sub-ppt detection of the metal chelates was successfully achieved by spectrophotometrically.

A thermo-responsive polymer used was PNIPAAm (Figure 1) (average molecular weight: 125000 by GPC (polystyrene standard)), which was synthesized by aqueous redox polymerization of *N*-isopropylacrylamide (Aldrich) with ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine.<sup>4</sup> The formation of the metal chelates with tcpp (Tokyo Kasei Co. Ltd.) was performed by the method reported



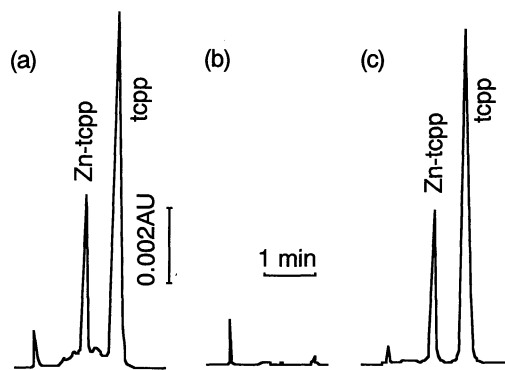
**Figure 1.** Structure of PNIPAAm, zinc(II)-tcpp chelate and quaternary ammonium salts.



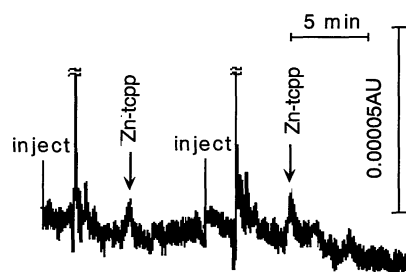
**Figure 2.** Recoveries of copper(II)-tcpp chelate in the polymer phase as a function of the concentration of quaternary alkylammonium salt. Counter ion; (■) 4C4, (□) C8, (●) C10, (○) C12. 0.10% (w/v) PNIPAAm, 0.1 M Tris-HCl (pH 8.0).

by Ishii et al.<sup>2a</sup> To a 100 mL of aqueous buffer solution of metal-tcpp chelates, a 1 mL of 2.00% (w/v) PNIPAAm and the prescribed amounts of aqueous solutions of quaternary alkylammonium salts were added. The salts examined as the counter ions were summarized in Figure 1. After the solution was incubated at 40 °C for 5 min, it was shaken for inducing the formation of condensed polymer phase. The polymer phase was taken from the solution and, then, was dissolved into 200- $\mu\text{L}$  portion of acetonitrile. The resulting solution (20  $\mu\text{L}$ ) was directly injected to a Jasco (PU-980/UV-970) HPLC. The chromatographic conditions was the same as those reported by Igarashi et al.<sup>2b</sup>

In the absence of counter ions, the recovery of copper(II)-tcpp chelate was very low, probably due to the water-solubility of the negatively charged chelate. In contrast,



**Figure 3.** Chromatograms obtained by the injection of  $1.0 \times 10^{-6}$  M Zn(II)-tcpp (a), the aqueous solution after the polymer-mediated extraction, and the polymer phase dissolved by acetonitrile to equi-volume to the original solution. Mobile phase: 40% (v/v) aqueous acetonitrile (pH 3.5).



**Figure 4.** Chromatographic peak of  $1.0 \times 10^{-12}$  M Zn(II)-tcpp after the polymer-mediated extraction. Mobile phase: 40% (v/v) aqueous acetonitrile,  $1 \times 10^{-3}$  M tetrabutylammonium bromide, 0.01 M Tris-HCl (pH 7.0). Chelate formation was performed in the presence of excess copper(II) ion for diminishing the effect of contaminations.

the chelate was well incorporated in the presence of quaternary alkylammonium ion (Figure 2). Among them, C12 having a longest alkyl-substituent was the most effective for heightening the recovery of the chelate. The extractability was almost quantitative in the C12 concentration range from 1 to 2.5 mM. However, it decreased with the further increase in the C12 concentration, probably due to the formation of the micelle-like aggregates in the bulk aqueous solution. In this study, the concentration of C12 was settled to 2 mM.

After the extraction of the chelate, the polymer phase could be dissolved small amount of polar organic solvents. Among the solvents examined, acetonitrile was the best choice, because the sample solutions, which are compatible to

aqueous-acetonitrile mobile phase, were obtained by the addition of the least amounts of the solvent.<sup>3e</sup> Figure 3 illustrates the chromatograms obtained by the injection of  $1.0 \times 10^{-6}$  M Zn(II)-tcpp (a), the aqueous phase (b), and the polymer phase redissolved by acetonitrile to original volume (c). These figures also shows the predominant extraction of the chelate to the polymer phase. The chromatographic signals ( $S/N = 2$ ) of  $2.0 \times 10^{-12}$  M (ca. 0.1 ppt) Zn(II)-tcpp after the polymer-mediated extraction was obtained as shown in Figure 4. This indicates the great potential of the present method to achieve the extreme enhancement of the signal intensity of the metal chelates in the chromatogram. Since the injection of the concentrated polymer solution hardly interfere with the chromatogram, this method will be also applied in the simultaneous detection system for all metal ions which can complex with tcpp.

In conclusion, the combination of a highly sensitive detection system with a simple and rapid concentrating method having high concentration factor would provide us an ultra-sensitive manner, which is comparable to highly sensitive instrumental methodologies. The application to the concentration of other metal chelates will be fruitful to extend the feasibilities of this method for the highly sensitive detection of varieties of metal ions.

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