High-Performance Liquid Chromatographic Separation of Metal Ions with 2-Hydroxy-5-sulfoaniline-*N*-salicylidene

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High-performance liquid chromatography (HPLC) for the separation and determination of metal ions as their chelates with organic reagents has received increasing attention in recent years.¹⁻³ An attractive approach is the precolumn method, in which the metal chelate formation is completed prior to the separation and determination by HPLC. In most cases, a reagent forms less soluble chelates in water, and the chelates and the reagent itself are held strongly on the analytical column. Thus an organic modifier such as methanol, acetonitrile or a surfactant is added to the mobile phase in order to keep the chelates in solution or to elute them from the column. Organic solvents for HPLC purposes are, however, expensive and must be treated after use. Moreover, organic modifiers have different absorbances and refractive indexes from water, and these make baseline noises and troublesome system peaks.^{4,5} The ideal mobile phase for the reversed-phase HPLC should be an aqueous solution. In this paper, a sulfonated derivative of salicylidene *o*-aminophenol⁶, 2-hydroxy-5-sulfoaniline-*N*-salicylidene (HSS) has been investigated as a precolumn chelating reagent for the reversed-phase HPLC with spectrophotometric detection. Neat aqueous solutions (0.05 M acetate buffer, pH 5.0) have been used in both complexation and chromatographic elution.

Experimental

Apparatus

The chromatographic system consisted of a Shimadzu LC-6A pump unit, an SPD-6A spectrophotometric detector, a Rheodyne 7125 sample injector with a 100- μ l loop and a Tosoh CO-8011 column oven. A LiChrosorb RP-18 column (150 $\times$ 4 mm i.d., 5- μ m spherical octadecyl silica) was used. The pH measurements were carried out with a Horiba E-11 pH meter.

Reagents

HSS was synthesized and purified as described previously.⁷ Elementary analysis of the resulting

compound gave the following results: Calcd. for $C_{13}H_{10}O_5NSK \cdot H_2O$: C, 44.7; H, 3.44; N, 4.01%. Found: C, 45.0; H, 4.26; N, 4.01%. Solutions of 2.5×10^{-3} M ($M = \text{mol dm}^{-3}$) HSS in distilled water were prepared daily. Standard solutions of metal were prepared by dilution of commercially available standards (1000 $\mu\text{g/cm}^3$, Wako Pure Chemicals) for AAS. The mobile phase was prepared by mixing 0.05 M sodium acetate and acetic acid solution, this was adjusted to pH 5.0. The mobile phase was filtered through a 0.45- μ m cellulose acetate membrane filter and degassed by ultrasonication before use. All aqueous solutions were prepared with distilled and deionized water, purified with a Millipore Milli-Q system.

Procedure

An aqueous solution containing Al^{3+} , Cr^{6+} , Cu^{2+} , Ga^{3+} and V^{5+} was taken in a 50 ml plastic volumetric flask. To this solution, 10 ml of 2.5×10^{-3} M HSS and 5 ml of 0.5 M acetate buffer solution (pH 5.0) were added, and this mixture was diluted up to 50 ml with water. After 30 min standing, a 100- μ l aliquot of the solution was injected into the HPLC column through a 100- μ l sample loop. HSS metal chelates were eluted with 0.05 M acetate solution (pH 5.0) at a flow rate of 1.0 ml/min and detected at 405 nm. By measuring the peak height, the amounts of each metal were determined.

Results and Discussion

Spectrophotometric properties of HSS-metal chelates

HSS reacts with several metal ions to produce yellow anionic chelates. The optimum pH range for the chelate formation lay around 4–7, and color development proceeds immediately. The absorption maxima and molar absorption coefficients (ϵ) of HSS-metal chelates at pH 5.0 were 405 nm for Al^{3+} ($\epsilon=19000$), 420 nm for Cr^{6+} ($\epsilon=12000$), 405 nm for Cu^{2+} ($\epsilon=16000$), 415 nm for Ga^{3+} ($\epsilon=32000$) and 420 nm for V^{5+} ($\epsilon=14000 \text{ l mol}^{-1} \text{ cm}^{-1}$). A detection wavelength between 400 and 430 nm

was suitable, whereas the absorbance of HSS itself was small ($\epsilon < 100 \text{ l mol}^{-1} \text{ cm}^{-1}$, at 405 nm). The absorption coefficients of all metal chelates $> 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$, are sufficient to ensure the ng/cm^3 level detection of metal ions. On the other hand, HSS chelates with Al^{3+} and Ga^{3+} show intense greenish fluorescence.⁸ The simultaneous determination of Al^{3+} and Ga^{3+} with fluorometric detection by precolumn reversed-phase HPLC is also possible.⁹

Mobile phase

Solutions of acetate, phosphate, perchlorate, sulfate, tartrate, malate, borate, oxalate, malonate, formate, propionate, butyrate, citrate (0.01, 0.05 and 0.1 M) were investigated as both the buffer solution of complexation and the mobile phase under pH 5.0. All solutions except borate showed elution behavior, but gave small, broad and tailed peaks of HSS-metal chelates. In the case of using tartrate, oxalate or propionate solution, the peaks of chelates were overlapped (Al^{3+} and Ga^{3+} , Cr^{6+} and V^{5+} , and Ga^{3+} and V^{5+} , respectively), and did not resolve successfully. Acetate, malate and formate were found to be suitable with respect to the resolution and the detection sensitivity of the metal chelates, and acetate was used throughout in this work. The elution order of HSS chelates was Cr^{6+} , Al^{3+} , Ga^{3+} , V^{5+} and Cu^{2+} and did not change with the difference of mobile phase used except for butyrate solution (where the order is Cr, Al, Ga, Cu and V).

Chromatogram

A typical chromatogram obtained for a solution containing the Al^{3+} , Cr^{6+} , Cu^{2+} , Ga^{3+} and V^{5+} chelates is shown in Fig. 1. All five metal chelates were well resolved. The detection was done at 405 nm, where the peak of reagent (HSS) could not be observed in the chromatogram. This is due to the low absorptivity of the reagent⁷ and no appearance of the reagent peak is advantageous for measurement of metal chelates.

HSS reacts with other metal ions^{7,10} such as In^{3+} , Mo^{6+} , W^{6+} and Zr^{4+} , but these metal chelates are probably decomposed in the HPLC column owing to their labile nature. The additions of HSS to the mobile phase may permit the chromatographic separation of these chelates, but this complicates the detection of the chelates in the elution.

Effect of pH

The pH of solutions for both complexation and elution were adjusted in the range 3.0–6.0; then the effect of pH on the resolution of HSS chelates was investigated (Fig. 2). The capacity factor (k') of Al^{3+} and Ga^{3+} chelates increased gradually with increasing the pH, but the other chelates were unchanged. On the other hand, the peak height of Al^{3+} and Ga^{3+} chelates decreased with increasing pH due to the hydrolysis, whereas the peak height of V^{5+} , and Cu^{2+} chelates increased in the pH range 4–6. The peak height of Cr^{6+} was independent of pH. The peak of Al^{3+} and Ga^{3+} chelates could not be observed

Fig. 1 Chromatogram of HSS metal chelates. Column: LiChrosorb RP-18 (150×4 mm i.d.). Mobile phase: 0.05 M acetate (pH 5.0). Flow rate: 1.0 ml/min. Detection: at 405 nm. Metal ion: 40 ng (Al^{3+} , Cr^{6+}), 100 ng (Cu^{2+} , Ga^{3+} , V^{5+}).

Fig. 2 Effect of pH on capacity factor (k'). Chromatographic conditions were the same as given in Fig. 1.

below pH 4.0 and 3.0, respectively, because the protonation of the HSS was predominant. It is evident that highly selective determinations are possible by choosing the pH of the eluent and the buffer solution of complexation. In this work, the pH of both solutions were adjusted to 5.0 using acetate, so five metal chelates were observed in the chromatogram and could be separated successfully.

In another set of experiments, the effect of the concentration of acetate on the elution behavior of the

metal chelates was investigated at the constant pH of 5.0. The k' increased with increasing concentration of acetate up to 0.1 M, then decreased gradually. For example, the k' of Cu^{2+} chelate increased from 9.0 to 11.5 when the concentration of acetate changed from 0.01 to 0.1 M. On the other hand, the peak height of metal chelates decreased with increasing the concentration of acetate (20% decreased for Cu^{2+} as before); therefore, 0.05 M acetate adjusted to pH 5.0 was used.

Effect of HSS concentration

The effect of HSS concentration in sample solutions was investigated. The retention times of all chelates tested were independent of the concentration of HSS in the sample solution. The peak heights of Cr^{6+} , Ga^{3+} , V^{5+} and Cu^{2+} chelates were independent on HSS concentration, but that of Al^{3+} increased markedly in the concentration range 1.0×10^{-4} – 5.0×10^{-4} M. Sometimes the color development of Al^{3+} with organic reagents is very slow; then heating of sample solution to 70–90°C is required.^{11,12} Therefore, the effects of temperature and standing time on the formation of the HSS chelates were investigated. It was found that if a higher concentration of HSS ($>3 \times 10^{-4}$ M) was added to the sample solution, the formation of Al-chelate, as well as those of the other chelates, proceed fast enough to produce sufficient stable complexes, so that it was not necessary for the sample solution to maintain a evaluated temperature nor to stand for a long time. The concentration of HSS in sample solution was decided to be 5.0×10^{-4} M; the solution was kept for 30 min before injection onto the column.

Interferences

The effect of possible interferences was studied by adding various foreign ions to the sample solution before chromatographic separation. Alkali metals, alkaline earth metals, As^{3+} , Be^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Cu^+ , Fe^{2+} , Fe^{3+} , La^{3+} , Mn^{2+} , Mo^{6+} , Ni^{2+} , Pb^{2+} , Sb^{3+} , Sc^{3+} , Se^{4+} , Sn^{2+} , Ti^{4+} , Y^{3+} and Zn^{2+} ions were tested. A 10-fold excess of these ions showed no peaks and gave no interference ($<5\%$ change in peak height) with the separation and determination of Al^{3+} , Cr^{6+} , Cu^{2+} , Ga^{3+} and V^{5+} . Only Fe^{3+} showed an elution peak which was overlapped with the peak of V^{5+} chelate. It was not possible to improve the resolution of iron chelate by adding a suitable masking agent, such as triethanolamine, nitriloacetate

and Tiron. Large amounts (100-fold) of common anions such as Cl^- , Br^- , NO_3^- , HCO_3^- and SO_4^{2-} could be tolerated, but F^- and H_2PO_4^- reduced the peak height of Al^{3+} by 80 and 60%, respectively. But a 10-fold excess of H_2PO_4^- and the same amount of F^- caused no interference.

Calibration

In order to examine the potential of the technique for the determination of metal ions, calibration curves were prepared for the five tested ions by measuring the peak height. Good linear calibration curves were obtained over the concentration range 0.1–1.0 $\mu\text{g}/\text{cm}^3$ for Al^{3+} and 0.2–3.0 $\mu\text{g}/\text{cm}^3$ for Cr^{6+} , Cu^{2+} , Ga^{3+} and V^{5+} . The proposed method is highly sensitive to Al^{3+} whose detection limit is 40 ng/cm³. The detection limit for V^{5+} is 60 ng/cm³ and 100 ng/cm³ for Cr^{6+} , Cu^{2+} and Ga^{3+} at a signal-to-noise ratio of 3. The reproducibility of the present method was investigated for 0.4 $\mu\text{g}/\text{cm}^3$ of Al^{3+} and 1 $\mu\text{g}/\text{cm}^3$ of Cu^{2+} and V^{5+} . The relative standard deviations were 2.1% for Al^{3+} , 5.4% for Cu^{2+} and 3.8% for V^{5+} in five replicate determinations.

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