Effects of Surfactants on the Fluorescence of Beryllium-Morin Complex

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The effects of surfactants, CTAB, SDS, and TX-100, on the fluorescence emission intensity of Be-morin complex was studied in terms of pH, temperature, added electrolyte, and concentration of surfactants. The ionic surfactants decreased the fluorescence intensity of the complex. However, the addition of TX-100 caused a substantial increase in the fluorescence intensity without spectral shift, by which means the detection limit of Be was obtained to be 0.3 ppb. Optimum pH for Be determination was near 9.6 and the fluorescence intensity showed a continuous decline with the increase of temperature. Nitrate ion lowered the fluorescence intensity considerably. Mechanism of the interference remains to be elucidated.

Introduction

Beryllium has been of interest in nuclear technology because it is the best moderator of all metals and has the lowest absorption cross section for thermal neutrons among other favorable properties.[1] Unfortunately, extreme toxicity of beryllium to lung and body tissues requires a method of high sensitivity for its detection.

Morin is probably one of the most sensitive chelating agents[2] for the fluorimetric determination of Be and several sensitive fluorimetric methods have been published.[3-7] Another potential reagent for the analysis of Be is Nuclear Fast Red.[8] Morin also forms fluorescent complexes with a large number of metal ions including Al, Zr, and Th.[9]

Escriche and coworkers[10] reported the fluorimetric determination of Al with morin enhanced by the addition of a short-tailed nonionic surfactant, Genapol PF-20. However, TX-100 led to quenching of the fluorescence of Al-morin Complex. In general, micellar media show improvements in detection limits of spectroscopic[11] and fluorimetric[12] methods for metal ions. However, micellar systems for the fluorimetric analysis of Be have not been applied, possibly because the mechanisms which are operating in these systems are not well understood.[12]

Therefore it has been attempted to study the effects of surfactants for the purpose of increasing the sensitivity of the fluorimetric determination of Be in terms of type and concentration of surfactants, pH, temperature, and electrolyte.

Experimental

All fluorimetric measurements were made with a Hitachi 650-50 spectrofluorimeter one hour after preparation. A Julabo S thermostat with temperature control to ± 0.2°C was used to hold the sample compartment temperature constant. Absorbance was measured on a Varian Cary 17D spectrophotometer. A Chemtrix 60A was used to measure pH. Borax (Kants, reagent grade) was used to set pH 9.6.

Morin (3,5,7,2′,4′-pentahydroxyflavone) solution was prepared by dissolving 0.10g (Merck, analytical reagent) in 100 m*l* of ethanol solution containing 9% methanol and 5% water and stored in a refrigerator. Surfactants used were TX-100 (Shingo Pure Chemicals), sodium dodecylsulfate (SDS, Tae Dong Chemicals), and cetyltrimethylammonium bromide (CTAB, BDH Chemicals) of reagent grade. A stock solution of beryllium was prepared from analytical reagent grade of $Be(NO_3)_2 4H_2O$ (Merck) in distilled water.

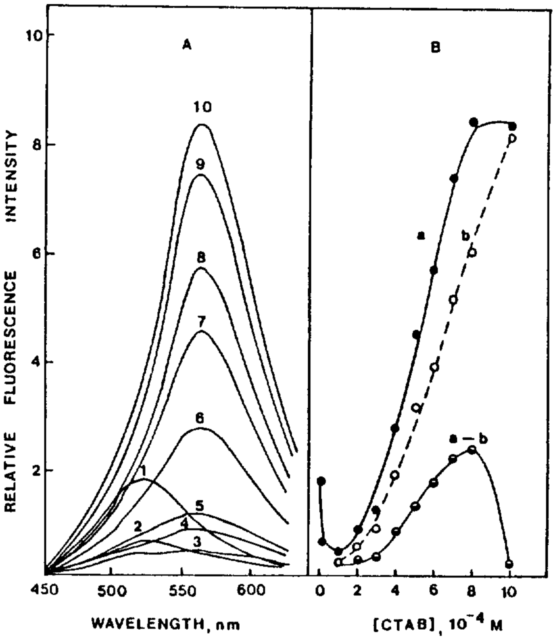

Figure 1. Dependence of fluorescence emission spectra(A) of a Be^{2+} and morin solution and of maximum emission intensities(B) at 565 nm of morin with (a) and without (b) Be^{2+} on CTAB concentration. Concentrations: Be^{2+}, 5.5×10^{-6}M; morin, 3.0×10^{-5}; CTAB (10^{-4}M), 0(1), 0.1(2), 1.0(3), 2.0(4), 3.0(5), 4.0(6), 5.0(7), 6.0(8), 7.0(9), 8.0(10), 10.0(11). Curve a-b is the difference between curves a and b.

Results and Discussion

Morin reacts with Be^{2+} to give fluorescent complexes. The fluorescence emission spectrum of Be-morin complex in aqueous solutions near pH6 shows a broad maximum at 525 nm with a full width at half maximum of 70 nm. Approximate

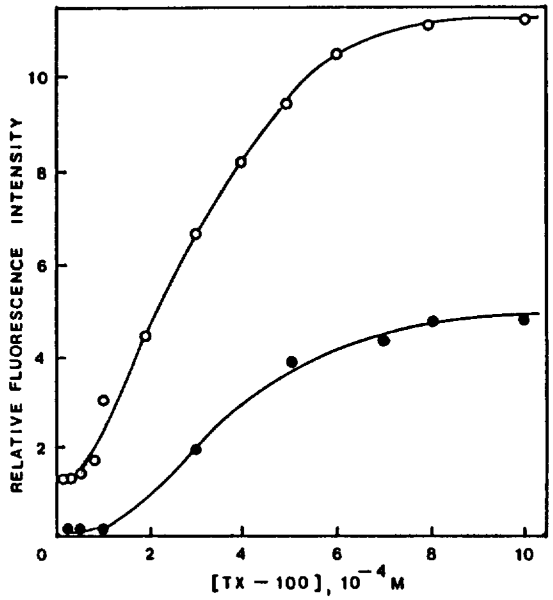

Figure 2. Fluorescence intensities of Be-morin complex with TX-100(○) and morin with TX-100(●) at 523 nm vs. TX-100 concentration. [Be^{2+}] = 5.5 × 10^{-6}M, [morin] = 3.0 × 10^{-5}M.

pK values for ionization constants (pK_1...pK_5)[3] for morin determined from curves of absorbance vs. pH are –1,4,8,7, 9,13. The predominant form of the complex near pH6 was identified as 1:2 complex[9] by the method of continuous variation.

Figure 1 presents the variation of fluorescence emission spectra and of respective maximum intensity of 5.5 × 10^{-6}M Be^{2+}(50 ppb) and 3.0 × 10^{-5}M morin at various analytical concentrations of CTAB. The cationic surfactant decreases the fluorescence intensity of Be-morin complex up to 1.0 × 10^{-4}M CTAB, beyond which a new emission band peaked at 565 nm is growing rapidly. Above 2.0 × 10^{-4}M CTAB, the fluorescence emission spectrum became similar to that of reagent blank, i.e., the solution containing no Be^{2+}.

The pH of the Be^{2+} and morin solution was measured 5.6, in which morin exists as negatively charged species. In the presence of CTA^+, Be^{2+} may compete with CTA^+ for morin species. Apparently the association between CTA^+ and morin under the experimental condition is dominant by concurrent hydrophobic and electrostatic interaction over the reaction between Be^{2+} and morin or betwen Be-morin complex and CTA^+. The fluorescence emission at 565 nm thus appears to be responsible for morin-CTA^+ association. The net enhancement in fluorescence intensity caused by the addition of high concentration of CTAB was largely due to the increased association of morin-CTA^+. The additional enhancement due to Be-morin complexation was considered not to be significant. Since CTAB not only shifted the fluorescence emission spectrum by probably the formation of morin-CTA^+ association in addition to Be-morin complex in high concentration region but reduced the fluorescence intensity of Be-morin complex in low concentration region, no additional investigation was pursued with the cationic surfactant.

Upon the addition of anionic surfactant, SDS, instead of CTAB to Be^{2+} and morin solutions of otherwise identical concentrations the fluorescence intensity remained essentially the same up to 2.3 × 10^{-4}M SDS and then declined continuously. No shift was observed in the excitation and emission wavelengths. SDS presumably impedes the formation of Be-morin complex above 2.3 × 10^{-4}M and is unable to solubilize morin.

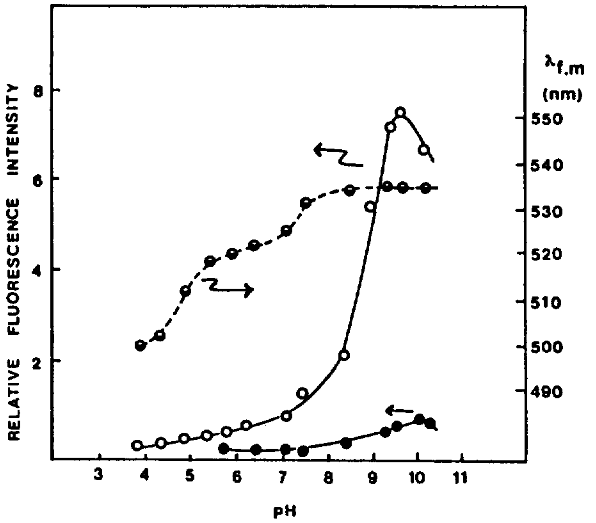

Figure 3. Fluorescence intensities of Be-morin with TX-100(○) and morin with TX-100(●) at 536 nm and $\lambda_{f,m}$(⊖) vs.pH: Be^{2+}, 5.5 × 10^{-6}M; morin, 3.0 × 10^{-5}M; TX-100, 8.0 × 10^{-4}M.

When the apparent electrostatic interaction between surfactant and reagents was excluded, the spectral behavior became desirable from analytical point of view. That is, it was found that the addition of TX-100 yielded a substantial hyperchromic shift analogous to that obtained by Escriche *et al.* for Al^{3+}-morin system.[10] No spectral shift was accompanied, implying that the dipole interaction between TX-100 and Be-morin complex is not strong enough to alter the ground and excited states of the complex. The increase in fluorescence intensity may be attributed to the suppression of non-radiative decay processes due to the achievement of more rigid structure of the complex on the surface of the surfactant.

The effect of TX-100 concentration is shown in Figure 2. It compares the maximum fluorescence intensities at 523 nm of mixed solutions containing Be^{2+}, morin, and various concentrations of TX-100 concentration. Beyond 1.0 × 10^{-4}M TX-100 the fluorescence intensities of both Be-morin complex and morin itself are enhanced considerably. This reflects that the solubilization and subsequent immobilization of morin is increased and that the fluorescence intensity is further enhanced by forming Be-morin complexes on the surfactant. The latter is consistent with the fact that by complexing Be^{2+} with morin a net increase in fluorescence intensity is assured.

The effect of temperature on the fluorescence intensity of 5.5 × 10^{-6}M Be^{2+}, 3.0 × 10^{-5}M morin, and 8.0 × 10^{-4}M TX-100 solution and of reagent blanks was studied between 15°C and 55°C and it was found that the fluorescence intensity showed a monotonic decline for all solutions with increasing temperature in agreement with the result by Laitinen and Kivalo.[5] Identical solutions with 5.5 × 10^{-5}M Be^{2+} yielded a similar trend. With the increase of temperature the adsorption of Be-morin complex onto the micelle becomes less favorable and the collisional deactivation becomes more

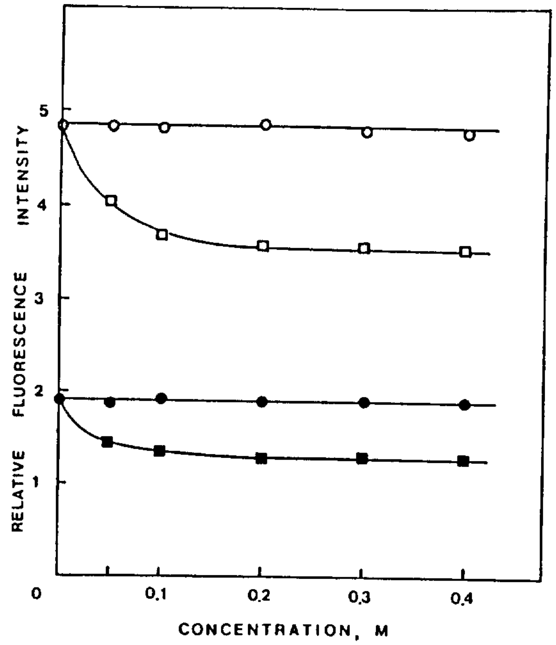

Figure 4. Effects of NaCl (circles) and $NaNO_3$ (rectangles) on the fluorescence intensities of Be-morin complex with (open points) and without (closed points) TX-100 vs. concentration of electrolyte at 25°C: Be^{2+}, $5.5 \times 10^{-6}M$; morin, $3.0 \times 10^{-5}M$; TX-100, $8.0 \times 10^{-4}M$.

probable, both of which lead the reduction of fluorescence emission. However, Al-morin complex in the presence of a nonionic surfactant, Genapol PF-20 exhibited a maximum fluorescence intensity at 25°C.[10]

The effect of pH on Be-morin complex with TX-100 was investigated over a pH range of 3.0-10.2 pH was adjusted with HCl and NaOH. Figure 3 plots the wavelengths of maximum fluorescence emission, $\lambda_{f,m}$. Above pH 8.0 and below 3.5 $\lambda_{f,m}$ remains at 536 nm and 494 nm, respectively, and between the two limits $\lambda_{f,m}$ shifts toward red with increasing pH. A highly deprotonated morin and a least deprotonated morin species, respectively, are probably responsible for the $\lambda_{f,m}$'s at 536 and 494 nm while between the limits several Be-morin complexes contribute to the measured fluorescence spectra. Also in Figure 3 included are the fluorescence intensities at 536 nm with the corresponding reagent blanks. The net enhancement of intensity progressively increases with the increase of pH up to pH 9.6 beyond which a slight decrease is noted. It follows from these results that Be^{2+} may be best determined in alkaline media as far as sensitivity is concerned although three principal species,[3] BeL, Be_2L_2, and BeL_2 formed in basic solutions may complicate the analysis, where L stands for morin species. In addition, morin is oxidatively decomposed more rapidly in basic than in acidic or neutral solutions.[9]

In practice, diverse ionic species are inevitably present in Be^{2+} solutions. The concentration and kinds of such electrolytes would influence the ion association between Be^{2+} and morin. Laitinen and Kivalo observed that 1.5M NaCl gave a high fluorescence intensity.[5] Recently an intensive study was conducted on the effect of salts to the absorbance of a dye, rhodamine 6G.[13] Among a large number of anions, only nitrate ion lowered the absorbance. No mechanism for such interference has been proposed yet. The effect of salts was tested to Be^{2+}-morin system. Figure 4 shows the effect of chloride and nitrate ions on the fluorescence intensities at 523 nm of $5.5 \times 10^{-6}M$ Be^{2+}, $3.0 \times 10^{-5}M$ morin, and $8.0 \times 10^{-4}M$ TX-100. The electrolytes did not cause any spectral shift. The effect of chloride ion is clearly negligible up to 0.4M. However, nitrate ion especially in the lower concentration region decreases the fluorescence intensity. Above 0.2M NO_3^-, fluorescence intensity was cut down by 33%. The addition of nitrate ion caused essentially no absorbance change in the region. Similar reduction albeit different extent was observed with borate and carbonate ions. Mechanism of such interferences remains to be elucidated. It is speculated, however, that Be-morin complex might be competed with nitrate ion for TX-100.

The enhancement of fluorescence intensity caused by TX-100 could result in lowering the detection limit of Be. The detection limit was estimated for a value of k = 3 recommended by Long and Weinfordner[14,15] from 20 replica data using blank, 6 ppb, and 9 ppb Be^{2+} samples keeping morin and TX-100 concentrations at $3.0 \times 10^{-5}M$ and $8.0 \times 10^{-4}M$, respectively. The detection limit was obtained 0.3 ppb for Be^{2+} at a precision to 1.0% on 6 ppb Be^{2+}. The calibration graph was linear up to 66 ppb Be^{2+} with the correlation coefficient of 0.995. The value is better than the lowest detection limit reported, 0.4 ppb, by Sill and Willis under a alkaline solution without surfactant.[6]

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