

Spectrofluorimetric Method for the Simultaneous Determination of Zinc and Cadmium

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The simultaneous determination of zinc and cadmium using derivative synchronous and multi-wavelength synchronous spectrofluorimetry is described. Effects of experimental variables on the fluorescence intensities and on the spectral resolution of the fluorescent chelates of these metals with 8-(*p*-toluenesulfonamido)-quinoline (TSQ) in a sodium lauryl sulfate micellar medium have been studied. Mixtures containing 10–200 $\mu\text{g L}^{-1}$ Zn and 15–250 $\mu\text{g L}^{-1}$ Cd have been analyzed with good results. The proposed method has been applied to a biological sample.

KEY WORDS: Spectrofluorimetry; zinc; cadmium; *p*-(toluenesulfonamido)-quinoline.

INTRODUCTION

Trace amounts of zinc and cadmium can be determined fluorimetrically via the formation of fluorescent complexes with organic ligands [1], but most of the chelating agents used for cadmium also form fluorescent complexes with zinc. Even if recent studies show important differences in the emission spectra of the complexes of zinc and cadmium with (anthryl-methyl)pentacyclen [2], generally the overlap of the band spectra makes impossible to determine these metal ions in their mixture by single-wavelength measurements.

Nowadays different approaches have been developed to exploit the information available in luminescence measurements for multicomponent analysis. Synchronous and derivative synchronous fluorescence spectroscopy [3–5], which can be easily performed with commercially available modern instrumentation, are popular means to improve spectral resolution of overlapping peaks. Another usual approach involves the use of computer-based mathematical techniques [6,7].

Multidimensional luminescence measurements, such as excitation emission matrix and time-resolved and phase-resolved fluorescence spectroscopy [8–10], are other techniques that have proved to be useful for the analysis of complicated multicomponent samples, but they require more specialized instrumentation.

In this paper, multiwavelength synchronous and first-derivative synchronous spectrofluorimetry has been used for the determination of two-component mixtures of zinc and cadmium using 8-(*p*-toluenesulfonamido)quinoline (TSQ) as chelating agent, in an anionic micellar medium.

Sulfonamido derivatives of 8-aminoquinoline have been investigated as photometric and fluorimetric reagents for metal ions [11], and TSQ has been described for the determination of cadmium and zinc after extraction in chloroform [12,13], but they mutually interfere. According to Serebryakova *et al.* [13], TSQ (Fig. 1a) forms basically a M(II)-TSQ complex with 1:2 stoichiometry (Fig. 1b), but these authors also indicate that other, less stable complexes may exist in solution. The proposed method makes use of an aqueous micellar medium that enhances the fluorescence of these metal complexes and avoids the extraction into organic solvents.

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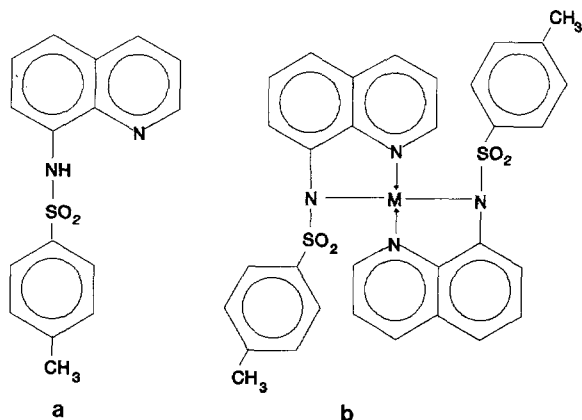


Fig. 1. Structures of (a) TSQ and (b) complexes of TSQ with Zn(II) and Cd(II).

The method is sensitive and selective and has been proved to be suitable for determining low levels of zinc and cadmium in biological samples.

EXPERIMENTAL

Reagents

Standard Metal Solutions. Stock solutions of Zn(II) and Cd(II) nitrates (Merck) containing 1.000 g L^{-1} of metal ion were used. Working solutions were prepared daily by diluting these stock solutions to appropriate concentrations.

8-(p-Toluenesulfonamido)-quinoline (TSQ) Solution. A fresh 10^{-2} M ethanolic solution was prepared daily.

Surfactant Solutions. Stock solutions of 0.1 M Brij-35 (Scharlau), $4 \cdot 10^{-2} \text{ M}$ cetyltrimethylammonium bromide (CTAB) (Merck), and 0.9 M sodium lauryl sulfate (SLS) (Merck) in water were prepared.

Buffer Solutions. Buffer solutions of 0.2 M acetic acid–sodium hydroxide (pH 4.0–5.5), 0.2 M succinic acid–sodium hydroxide (pH 5.5–6.8), and 0.2 M Tris-hydrochloric acid (pH 7.7–9.0) were used.

All reagents were of analytical grade or Suprapur quality. Doubly deionized water (Culligan Ultrapure GS, $18.3\text{-M}\Omega \text{ cm}^{-1}$ resistivity) was used. All glassware was previously soaked in 10% nitric acid for 24 h and rinsed with doubly deionized water.

Apparatus

Fluorescence measurements were performed on a Perkin–Elmer MPF 66 spectrofluorimeter equipped with a 150-W xenon arc lamp and 10-mm silica cells. The slit widths were adjusted to 3 nm in both monochromators unless otherwise stated. The spectrofluorimeter was calibrated daily using a Perkin–Elmer fluorescence intensity sample of ovalene. The fluorescence data are given without spectral correction. The spectrometer was connected to a Perkin–Elmer 7500 data station provided with PECLS III applications software. This data station was also connected to a PC-compatible computer via the RS-232 serial interface to send the spectral data in ASCII code for further treatment.

For pH measurements, a Radiometer PHM 64 pH-meter equipped with a combined glass–calomel electrode was used.

Procedure

Transfer up to 10 ml of slightly acidic sample solution, containing 0.25–6 μg of zinc and 0.35–6 μg of cadmium, into a 25-ml volumetric flask. Add 2 ml of Tris buffer to adjust the pH to ca. 8.5, 10 ml of SLS solution, and 1 ml of TSQ solution and dilute to volume. Record the synchronous spectrum using the following instrumental parameters: $\Delta\lambda = 135 \text{ nm}$; scan speed, 240 nm min^{-1} ; and spectrofluorimeter response time, 0.5 s. After correction for blank signal, derive the synchronous spectrum using 49 data points. Store both spectra on a disk file. Use standard solutions of zinc and cadmium prepared in the same way to obtain calibration graphs.

The determination from the synchronous spectra was carried out by univariate [14] and multivariate calibration by classical least squares [15]. Measurements were carried out every 5 nm between 320 and 425 nm.

In addition, first-derivative spectra were used to determine Zn(II) and Cd(II) concentrations at two fixed wavelengths. The derivative signal at the zero-crossing of zinc spectra (374.2 nm) was used to measure the Cd signal, and vice versa for the Zn signal (364.2 nm). This method involves the construction of independent calibration curves for each component.

RESULTS AND DISCUSSION

The fluorescent properties of zinc–TSQ and cadmium–TSQ complexes in different media were investigated. Although the excitation and emission maxima, as

well as the fluorescence intensities, are solvent dependent, the observed spectra are broad, featureless, and very similar for both metals in each tested media. Free TSQ fluoresced at similar wavelengths but with a very low intensity. When a close-shell diamagnetic metal ion is complexed with a fluorogenic ligand, the complex is likely to fluoresce. Because emission is localized mainly in the ligand, the fluorescence spectrum of the complex tends to resemble that of the free ligand [1,16]. The large changes in fluorescence intensity upon complexation are also observed for other ligands involving a quinoline or other N-aromatic heterocycles. Studies on the luminescence of quinoline derivatives [17–19] show that 8-aminoquinoline (TSQ is a substituted 8-aminoquinoline), as well as 8-hydroxyquinoline, fluoresces weakly in fluid media. Quenching of the fluorescence molecules is believed to be due primarily to a hydrogen bonding effect in both ground and excited states [19]. Complexation by a metal involves both hydrogen atoms and prevents hydrogen bond formation.

Effect of Different Surfactants

The nonionic surfactant Brij-35 and the anionic sodium lauryl sulfate increased the fluorescence intensity of both complexes, whereas the presence of the cationic surfactant CTAB caused little increase with respect to that found in hydroorganic media. Fluorescence intensities in a Brij-35 micellar medium were higher than in a SLS medium. In a Brij-35 micellar medium, the spectra of zinc and cadmium complexes completely overlap, with emission maxima at 515 nm for both complexes and excitation maxima at 364 nm for cadmium and 367 nm for Zn. Although synchronous scanning improves spectral resolution, the extent of this overlap makes it difficult to solve the mixed spectrum, either by the derivative signal at fixed wavelengths on the derivative spectrum or by a numerical multicomponent analysis program. In SLS micellar media, a blue-shift of λ_{em} for cadmium (507 nm) and a red-shift of λ_{em} for zinc (523 nm) were observed. This provides sufficient discrimination between the synchronous and the derivative synchronous spectra to allow the simultaneous determination of both compounds in mixtures. Therefore, although some intensity was lost, SLS was chosen for this study.

Selection of Experimental Variables

The chemical variables that affect the fluorescence signal were also optimised. The variables tested and the optimum values found are shown in Table I. Although fluorescence intensity is maximum and constant over the

Table I. Selection of Chemical Variables^a

| Variable | Range tested | Optimum value | Selected value |
|----------|--------------|---------------|----------------|
| pH | | | |
| Zn(II) | 5.0–11.0 | 8.2–10.0 | 8.5 |
| Cd(II) | 5.0–11.0 | 8.0–9.5 | |
| [SLS] | | | |
| Zn(II) | 0.008–0.24 M | 0.08–0.24 M | 0.16 M |
| Cd(II) | 0.008–0.24 M | 0.04–0.24 M | |
| [TSQ] | | | |
| Zn(II) | 0.01–0.5 mM | 0.08–0.5 mM | 0.4 mM |
| Cd(II) | 0.01–0.5 mM | 0.04–0.5 mM | |

^a The order of the addition of reagents has no influence on the fluorescence intensity. The emission intensity remains constant for at least 3 h.

pH range 8.2–9.5, a value of 8.5 was chosen to improve selectivity. A TSQ concentration of 4×10^{-4} M was chosen to ensure an excess of ligand in two-component mixtures.

The order of the addition of reagents has no influence on the fluorescence intensity. At room temperature, the fluorescence of the complexes remains constant for at least 3 h.

The fluorescence of both complexes increased with decreasing temperature, but the variation is small in the temperature range 15–30°C.

The $\Delta\lambda$ for recording the synchronous spectra was chosen to combine both maximum difference between the spectra and high emission intensities. Wavelength differences of 100–175 nm were tested and a value of 135 nm was selected.

To obtain the derivative spectra by the Savitzky–Golay [20] method, the speed of the monochromators, the response time, and the band width (number of data points) were optimized. The scan speed did not appreciably affect the shape of the spectra but did affect the analytical signal. As the peak height increased with increasing speed, a value of 240 nm min⁻¹ was selected. The response time and the number of data points had no significant effect on the derivative spectra. The selected values were 0.5 s and 49, respectively.

The synchronous spectra and the synchronous derivative spectra of solutions of Zn(II) complex, Cd(II) complex, and a mixture of the two, obtained with the optimized instrumental parameters, are given in Figs. 1 and 2.

Characteristics of the Analytical Method

The additivity of the fluorescent emission spectra at different concentration levels was confirmed by in-

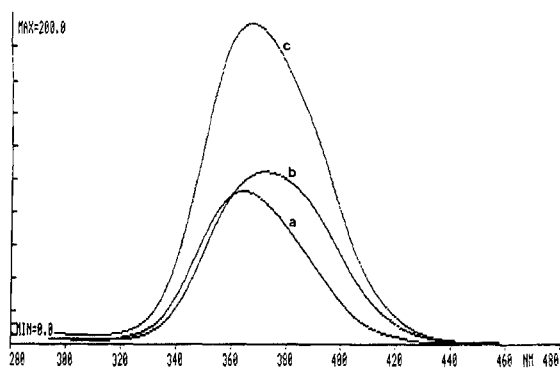


Fig. 2. Synchronous spectra of (a) Cd(II) complex, (b) Zn(II) complex, and (c) a mixture of the two complexes. $c_{Cd} = 68 \mu\text{g L}^{-1}$; $c_{Zn} = 47 \mu\text{g L}^{-1}$.

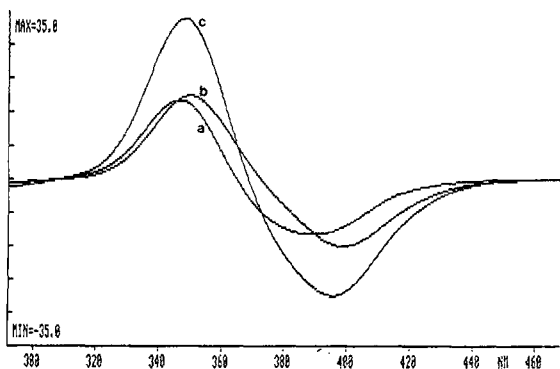


Fig. 3. Derivative synchronous spectra of (a) Cd(II) complex, (b) Zn(II) complex, and (c) a mixture of the two complexes. $c_{Cd} = 68 \mu\text{g L}^{-1}$; $c_{Zn} = 47 \mu\text{g L}^{-1}$.

strumentally adding individual spectra of cadmium and zinc and comparing them with those obtained from synthetic mixtures of the two components.

The mutual independence of the analytical signal for zinc and cadmium was verified by plotting calibration graphs obtained from two series of standard solutions containing $10\text{--}200 \mu\text{g L}^{-1}$ of zinc, with and without $200 \mu\text{g L}^{-1}$ of cadmium, and two series containing $15\text{--}250 \mu\text{g L}^{-1}$ of cadmium, with and without $200 \mu\text{g L}^{-1}$ of zinc. Figures 3 and 4 show the derivative synchronous spectra obtained.

The calibration graphs, obtained both from multi-wavelength measurements on the synchronous spectra and from single-wavelength measurements on the first-derivative spectra are linear over the concentration range $10\text{--}250 \mu\text{g L}^{-1}$ for both ions. The precision was evaluated for two series of 15 solutions containing $37 \mu\text{g}$

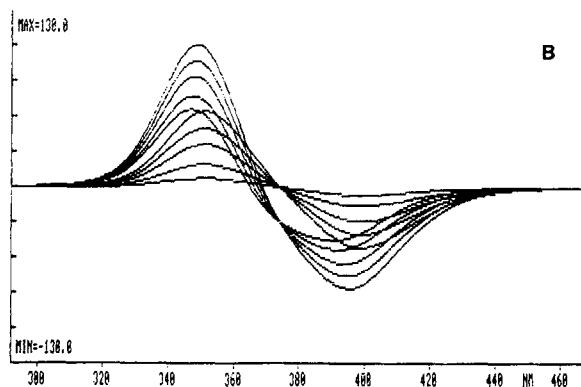
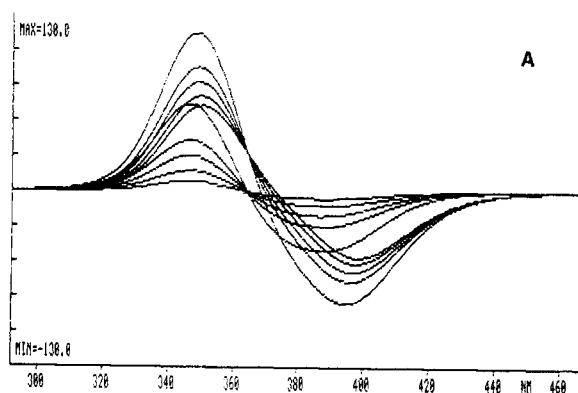


Fig. 4. (A) Synchronous derivative spectra of solutions with variable amounts of Cd(II) ($29\text{--}246 \mu\text{g L}^{-1}$) and their mixtures with a fixed content of Zn(II) ($200 \mu\text{g L}^{-1}$). (B) Synchronous derivative spectra of solutions with variable amounts of Zn(II) ($24\text{--}200 \mu\text{g L}^{-1}$) and their mixtures with a fixed content of Cd(II) ($204 \mu\text{g L}^{-1}$).

L^{-1} Zn and $48 \mu\text{g L}^{-1}$ Cd and $160 \mu\text{g L}^{-1}$ Zn and $16 \mu\text{g L}^{-1}$ Cd, respectively. The calibration parameters are summarized in Table II.

The effects of various ions were studied by adding known amounts of foreign ions to solutions containing equal amounts of zinc and cadmium at three concentrations (20 , 80 , and $160 \mu\text{g L}^{-1}$), and it was found that most ions do not interfere. Cu(II), Ni(II), and Co(II), which quench the fluorescence of Zn(II) and Cd(II) complexes, caused no interference when present at concentrations up to four times the zinc or cadmium concentration, whereas Fe(III) can be tolerated at concentrations up to 15-fold. Calcium and magnesium ions, which fluoresce slightly in the working conditions, had no effect when present at concentrations below 10 and 5mg L^{-1} , respectively. The addition of phosphate (0.04M) increased the tolerance level for Mg(II) to 10mg L^{-1} .

Table II. Calibration and Precision Parameters of Zn and Cd Solutions

| Method | Zn | Cd |
|-----------------------------|---------|---------|
| Derivative method | | |
| Number of standards | 17 | 17 |
| Analytical wavelength (nm) | 364.2 | 374.2 |
| Slope | 0.1546 | 0.1328 |
| Intercept | 0.4306 | 0.0545 |
| Correlation coefficient (R) | 0.9994 | 0.9992 |
| Precision (as rsd %) | | |
| A ^a | 4.1 | 3.8 |
| B ^b | 1.1 | 9.2 |
| Univariate calibration | | |
| Wavelength range (nm) | 320-425 | 320-425 |
| Wavelength increment (nm) | 5 | 5 |
| Number of spectra | 17 | 17 |
| sdr ^c | 1.37 | 1.92 |
| Precision (as rsd %) | | |
| A | 3.9 | 1.7 |
| B | 0.9 | 8.8 |
| Multivariate calibration | | |
| Wavelength range (nm) | 320-425 | |
| Wavelength increment (nm) | 5 | |
| Number of spectra | 14 | |
| sdr | 2.81 | |
| Precision (as rsd %) | | |
| A | 4.1 | 1.7 |
| B | 1.0 | 8.8 |

^a At 37 $\mu\text{g}\cdot\text{L}^{-1}$ Zn and 48 $\mu\text{g}\cdot\text{L}^{-1}$ Cd.

^b At 160 $\mu\text{g}\cdot\text{L}^{-1}$ Zn and 16 $\mu\text{g}\cdot\text{L}^{-1}$ Cd.

^c Standard deviation of residuals, in emission intensity units.

Analysis of Synthetic Mixtures

Synthetic mixtures with a Zn/Cd ratio from 13/1 to 1/15, and at several concentration levels, were prepared, and Zn and Cd determined by the derivative method and the synchronous method. In results obtained by the synchronous mode there were no significant differences between multivariate and univariate calibration.

As shown in Table III, there is a good agreement between the results and the theoretical values for mixtures with moderate Zn/Cd ratios. Results for mixtures with a low Zn/Cd ratio are very good for Cd and quite good for Zn. In mixtures with a high Zn/Cd ratio, results are very good for Zn, but only acceptable for Cd. This is logical considering that, for a given concentration of metal ion, the Zn-TSQ complex has a higher fluorescence intensity than the Cd-TSQ complex.

Results obtained by the synchronous method are usually more accurate than those obtained by the derivative method, but the differences are really important at

Table III. Analysis of Synthetic Mixtures

| Ratio, Zn/Cd | Theoretical ($\mu\text{g}/\text{L}$) | | Synchronous ($\mu\text{g}/\text{L}$) | | Derivative ($\mu\text{g}/\text{L}$) | |
|--------------|--|-----|--|-----|---------------------------------------|-----|
| | Zn | Cd | Zn | Cd | Zn | Cd |
| 13/1 | 185 | 15 | 184 | 12 | 181 | 21 |
| 8/1 | 166 | 19 | 168 | 20 | 163 | 27 |
| 6/1 | 92 | 15 | 94 | 13 | 94 | 17 |
| 3/1 | 48 | 15 | 49 | 15 | 49 | 16 |
| 2/1 | 185 | 97 | 182 | 98 | 179 | 102 |
| 2/1 | 85 | 49 | 84 | 48 | 85 | 54 |
| 3/2 | 166 | 107 | 166 | 101 | 167 | 109 |
| 1/1 | 92 | 97 | 91 | 98 | 90 | 104 |
| 1/1 | 185 | 194 | 181 | 193 | 172 | 193 |
| 4/5 | 85 | 107 | 83 | 105 | 84 | 111 |
| 3/4 | 11 | 15 | 12 | 14 | 10 | 16 |
| 3/4 | 166 | 218 | 174 | 224 | 160 | 223 |
| 1/2 | 48 | 97 | 48 | 95 | 46 | 97 |
| 2/5 | 92 | 243 | 91 | 245 | 82 | 237 |
| 2/5 | 85 | 218 | 84 | 210 | 87 | 222 |
| 1/3 | 15 | 49 | 14 | 53 | 12 | 55 |
| 1/5 | 48 | 242 | 49 | 247 | 43 | 241 |
| 1/6 | 18 | 107 | 20 | 109 | 20 | 117 |
| 1/9 | 11 | 97 | 11 | 99 | 10 | 99 |
| 1/15 | 15 | 218 | 16 | 223 | 17 | 218 |

very high or very low Zn/Cd ratios. At moderate ratios both methods are quite comparable.

Determination of Zinc and Cadmium in Real Samples

The proposed multiwavelength synchronous method was applied to the determination of zinc and cadmium in a biological reference material (BCR, CRM No. 186) consisting of lyophilized pig kidney. As the certified content of cadmium in the available reference materials was very low, the sample was spiked with two different amounts of this element. The derivative method was not used because its results are usually worse.

The sample was dried to constant weight at 103°C, as recommended by BCR. The required amount (0.3 g) was digested with concentrated nitric acid (15 ml) in a PTFE bomb at 140°C for 8 h. The resulting solution was transferred to a PTFE beaker, followed by the addition of 1 ml of perchloric acid and evaporation to dryness on a sandbath. The residue was dissolved with a few drops of nitric acid and diluted to 50 ml in a volumetric flask. A 5-ml aliquot of this solution was transferred to a 25-ml volumetric flask to determine zinc and cadmium as described previously. A blank was run through the entire procedure.

Table IV. Determination of Zinc and Cadmium in Real Samples

| Sample | Certified ($\mu\text{g/g}$) | Added ($\mu\text{g/g}$) | Found ($\mu\text{g/g}$) | Recovery (%) |
|--------|-------------------------------|---------------------------|---------------------------|--------------|
| A | | | | |
| Zn | 128 \pm 3 | — | 128 \pm 2 | 100.4 |
| Cd | 2.7 \pm 0.15 | 42.5 | 44.6 \pm 1.3 | 98.7 |
| B | | | | |
| Zn | 128 \pm 3 | — | 130 \pm 3 | 101.5 |
| Cd | 2.7 \pm 0.15 | 15.5 | 14.8 \pm 1.8 | 81.3 |

The results are given in Table IV. It can be seen that the method gives accurate values for samples with similar zinc and cadmium concentrations. At low ratios of cadmium to zinc the results can still be considered acceptable, although they are less accurate.

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