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Study on the fluorescence system of chlortetracycline-Eu-TOPO-sodium dodecyl sulfonate and the determination of chlortetracycline[1]

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Abstract

The fluorescence system of Eu-chlortetracycline-TOPO-sodium dodecyl sulfonate was studied. It was found that chlortetracycline formed a complex with Eu(III) at pH 8.0–9.0 and then emitted the characteristic fluorescence of Eu(III). TOPO and sodium dodecyl sulfonate greatly enhanced the fluorescence intensity of the system. The experiments indicated that under the optimum determining conditions a linear relationship was obtained between the fluorescence intensity and chlortetracycline concentration in the range of 2.0×10^{-8}–1.0×10^{-5} M. The detection limit was 6.0×10^{-9} M. In addition, the luminescence mechanism of the complex system has been discussed. © 1997 Elsevier Science B.V.

Keywords: Europium; Spectrofluorimetry; Chlortetracycline determination

1. Introduction

Chlortetracycline (CTC) has been widely used as a broad-spectrum antibiotic mainly in clinical medicine and food science. Therefore, it is necessary to develop an assay method with high sensitivity, accuracy and selectivity.

Many methods for the determination of CTC have been reported, mainly based upon chromatography [1], spectrophotometry [2] or voltammetry [3]; few fluorimetric methods [4–6] have been reported. Experiments showed that CTC could form a multibasic complex with Eu(III) and trioctyl phosphine oxide (TOPO), which emitted a strong characteristic fluorescence of Eu^{3+}; it was also shown that sodium dodecyl sulfonate (SDS) greatly increased the sensitivity of the system. For the above reason, a simple and sensitive fluorimetric method of determining CTC was put forward. The proposed method was applied to determine CTC in serum and urine and the results were satisfactory.

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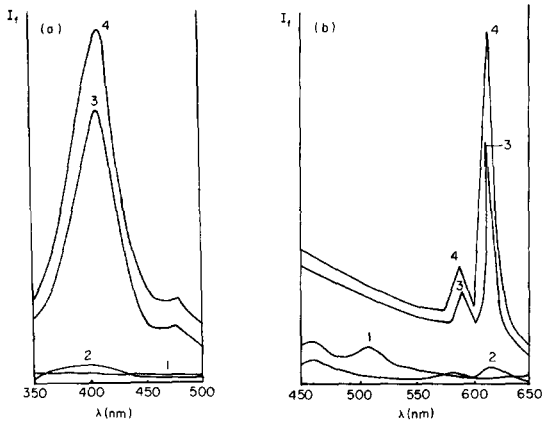

Fig. 1. Flourescence spectra (a) excitation spectra ((1) λ_{em} = 510 nm, (2), (3), (4), λ_{em} = 615 nm); (b) emission spectra ((1) λ_{ex} = 396 nm, (2), (3), (4), λ_{ex} = 405 nm). (1) CTC-borac, (2) Eu-CTC-borax, (3) Eu-CTC-TOPO-borax, (4) Eu-CTC-TOPO-SDS-borax. Conditions: Eu: 1.0×10^{-5} M; CTC: 1.0×10^{-5} M; SDS: 1.0×10^{-3} M; TOPO: 1.0×10^{-3} M; Borax: 0.48% 2 ml.

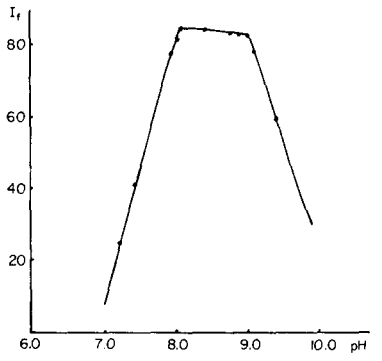

Fig. 2. Effect of pH. Conditions: Eu^{3+}: 1.0×10^{-5} M; CTC: 1.0×10^{-5} M; TOPO: 1.0×10^{-3} M; SDS: 1.0×10^{-3} M.

2. Experimental

2.1. Apparatus

All fluorescence measurements were made on an RF-540 spectrofluorimeter (Shimadzu, Japan) equipped with a 150 W Xe arc lamp.

2.2. Reagents

2.2.1. Chlortetracycline solution

Chlortetracycline solution was prepared by dissolving 0.0479 g CTC (master standard, Chinese Institute for the control of pharmaceutical and biological products Beijing) in 100 ml of distilled water. The stock solution was 1.00×10^{-3} M.

2.2.2. Eu(III) standard solution

This was prepared by dissolving Eu_2O_3 (99.99%, Yuelong chemical plant, Shanghai, China) in dilute HCl, then diluting the solution to a concentration of 1.00×10^{-2} M with distilled water. Working solutions were prepared by appropriate dilution with distilled water.

2.2.3. TOPO solution

TOPO solution was prepared by dissolving 0.3867 g TOPO (95%, Tianjin Chemical Reagent Plant, China) in 80 ml of alcohol, then diluting the solution to a concentration of 1.00×10^{-2} M with distilled water.

2.2.4. SDS solution

It was prepared by dissolving 0.2734 g SDS in 100 ml of distilled water, then diluting the solution to a concentration of 1.00×10^{-2} M.

2.2.5. Buffer solution

Borax-HCl buffer solution was prepared by dissolving 0.48 g borax in 100 ml of distilled water and then adjusting the pH to 9.0 with 0.5 M HCl.

Table 1
Effect of synergistic ligands[a]

Synergistic ligands (1.0×10^{-3} M)	None	DPG	BP	TTA	Phen	BA	TOPO
I_f(%)	23	10	18	20	25	31	100

[a]DPG, diphenyl guanidine; TTA, thenoyltrifluoroacetone; BP, α,α-bipyridine; BA, benzoylacetone; Phen, phenanthroline; TOPO, trioctylphosphine oxide.

Table 2
Effect of surfactants[a]

Surfactants (1.0×10^{-3} M)	None	DPB	CTMAB	GA	TX-100	SDBS	SDS
I_f(%)	38	34	40	41	65	85	100

[a]DPB, dodecylpyridinium bromide; GA, gum acacia; SDBS, sodium dodecylbenzene sulfonate; SDS, sodium dodecylsulfonate.

2.3. Procedure

To a 25 ml test tube, solutions were added in the following order: 1.0 ml of CTC solution, 1.0 ml of Eu(III) solution, 1.0 ml of TOPO solution, 2.0 ml of 0.48% borax HCl buffer solution and 1.0 ml of 10^{-2} M SDS solution. The mixture was diluted to 10 ml with distilled water, thoroughly mixed by shaking and then allowed to stand for 20 min. The fluorescence intensity was measured in a 1-cm quartz cell with excitation and emission wavelengths of 405 and 615 nm, respectively.

3. Results and discussion

3.1. Fluorescence spectra

The excitation and emission spectra of CTC-borax, Eu-CTC-borax, Eu-CTC-TOPO-borax, Eu-CTC-TOPO-SDS-borax are shown in Fig. 1.

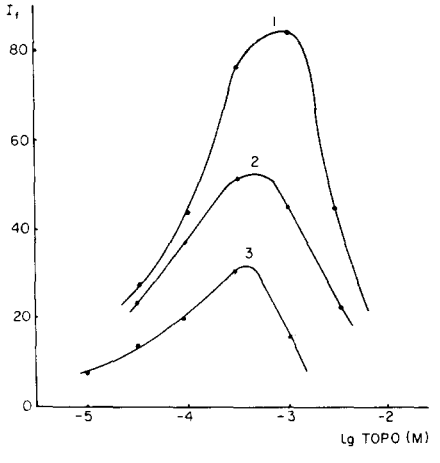

Fig. 3. Effect of synergistic ligands. Conditions: (1) CTC 1.0×10^{-5} M; (2) CTC 1.0×10^{-6} M; (3) CTC 1.0×10^{-7} M. Eu^{3+}: 1.0×10^{-5} M; SDS: 1.0×10^{-3} M; Borax: 0.48% 2 ml.

From Fig. 1, it can be seen that the excitation and emission wavelengths of system 1 are 396 and 510 nm, respectively. This is the intrinsic fluorescence of CTC. In contrast, excitation and emission wavelengths of systems 2, 3, 4 are 405 and 615 nm, respectively; this is the characteristic fluorescence of Eu(III), which corresponds to the transitions from $^5D_0$ level of Eu^{3+} to the $^7F_1$ and $^7F_2$ level, respectively. In addition, in systems 2, 3, 4, the broad peak of CTC disappears; this also shows that CTC forms a complex with Eu^{3+}.

3.2. Factors affecting the fluorescence intensity

3.2.1. Effects of pH

The effect of pH on the fluorescence intensity of system is shown in Fig. 2. It can be seen that the fluorescence intensity is strongest in the range of pH 8.0–9.0.

In this work, the following buffers were examined: borax-HCl; boric acid-NaOH; NH_4Cl-NH_3, ethanolamine-HCl; and glycine-HCl. The results showed that borax-HCl buffer solution was the most suitable.

3.2.2. Effect of Eu(III) ion concentration

The results show that when the concentration of CTC is 1.0×10^{-5} M the most suitable concentration of Eu^{3+} is 1.0×10^{-5} M; when the concentration of CTC is 1.0×10^{-6} M or 1.0×10^{-7} M, the most suitable concentration of Eu^{3+} is 1.0×10^{-6} M. An excess of Eu^{3+} can quench the fluorescence of the system.

3.2.3. Effect of synergistic ligands

The effect of different synergistic ligands on the fluorescence intensity of the system are shown in Table 1. It can be seen that TOPO is the most suitable synergistic ligand.

The effect of different synergistic ligands on the fluorescence intensity of the system are shown in Table 1. It can be seen that TOPO is the most suitable synergistic ligand.

The effect of the concentration of TOPO on fluorescence intensity of the system was examined. The results are shown in Fig. 3. This shows that when the concentration of CTC is 1.0×10^{-5} M, the most suitable concentration of TOPO is 1.0×10^{-3} M; when the concentration of CTC is 1.0×10^{-6} M or 1.0×10^{-7} M, the most suitable concentration of TOPO is 5.0×10^{-4} M.

3.2.4. *Effect of surfactants*

The effects of different kinds of surfactants on the fluorescence intensity are shown in Table 2. Some cationic surfactants decrease the fluorescence intensity while anionic and nonionic surfactants increase the fluorescence intensity to different extents. The most effective surfactant is SDS.

The effects of the concentration of SDS on both the fluorescence intensity and the surface tension are shown in Fig. 4. It can be seen that the fluorescence intensity of the system increases with the concentration of SDS and reaches its maximum intensity at 1.0×10^{-3} M SDS. From the effect of the concentration of surfactant on the surface tension of the system, it can be seen that at the CMC (2.0×10^{-5} M) the change of the fluorescence intensity is the biggest, this

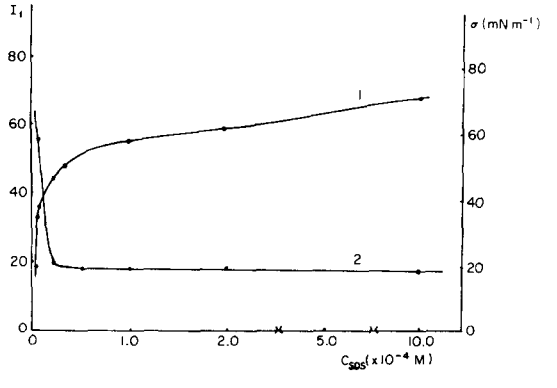

Fig. 4. Effect of surfactant. (1) Curve of fluorescence intensity; (2) curve of surface tension. Conditions: CTC: 2.0×10^{-6} M; Eu^{3+}: 1.0×10^{-6} M; TOPO: 5.0×10^{-4} M; Borax: 0.48% 2 ml.

Table 3
Effect of body elements

Elements	K^+	Na^+	Ca^{2+}	Ba^{2+}	Mg^{2+}	Al^{3+}	Zn^{2+}	Fe^{3+}	Mo^{2+}	Cu^{2+}
Highest permissible concentraion (mg ml^{-1})	5.0×10^{-3}	2.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	5.0×10^{-4}	5.0×10^{-4}	2.0×10^{-4}	2.5×10^{-6}	3.2×10^{-6}	6.4×10^{-8}

Table 4
Determination of CTC in serum and urine[a] samples (standard addition method)

Added CTC ($\times 10^{-6}$ M)	CTC found ($\times 10^{-6}$ M) X	$\bar{X} \pm s$	Recovery (%)
Serum 1.0	0.94, 0.91, 0.97, 0.95, 0.95	0.944 ± 0.022	94.4
Serum 0.20	0.210, 0.194, 0.206, 0.196, 0.212	0.204 ± 0.0082	102
Urine 1.0	1.03, 0.98, 0.93, 1.00, 1.05	0.998 ± 0.047	99.8
Urine 0.20	0.218, 0.206, 0.200, 0.214, 0.198	0.207 ± 0.0087	103.5

[a]Serum and urine were diluted to 100-fold.

proves that the formation of the micelle is important in increasing the fluorescence intensity.

3.2.5. Effect of time

The tests showed that the fluorescence intensity reached a maximum in 20 min after all the reagents had been added and remained stable for about 40 min.

3.2.6. Effect of body elements

The effects of body elements were studied for 1.0×10^{-5} M Eu^{3+} and 2.0×10^{-6} M CTC. The highest permissible concentrations causing a variation of $\pm 10\%$ in the fluorescence intensity are shown in Table 3. It can be seen that most of the body elements (except Cu, Mo, Fe) do not interfere with the determination of CTC.

3.3. Analytical application

Under the optimum conditions, a linear relationship is obtained between the fluorescence intensity and CTC concentration in the range of 2.0×10^{-8}–1.0×10^{-5} M. The detection limit (signal-to-noise ratio of 2) is 6.0×10^{-9} M.

The standard addition method was used for the determination of CTC in serum and urine. The results are shown in Table 4. It can be seen that the proposed method was satisfactory.

3.4. Composition of the complex

The excitation (Fig. 1) and absorption spectra showed that Eu^{3+} was able to combine with CTC, TOPO and SDS in the system studied and formed an ion association complex expressed as $[Eu(CTC)_n(TOPO)_m][SDS]_p$. The coordination numbers were fluorimetrically examined using the molar method; the results indicated that n and p were equal to 2 and 1, respectively, whereas m, which increased with increasing concentration of TOPO, was not a constant. Therefore, the ion association complex was expressed as $[Eu^{3+}(CTC)_2(TOPO)_m]^+[SDS]_1^-$.

3.5. Luminescence mechanism

From Fig. 1, it was shown that in the Eu-CTC-TOPO-SDS-borax system, the intrinsic emission peak of CTC disappeared whereas the characteristic emission of Eu^{3+} was observed. It seems logical to conclude that the fluorescence of the system is transferred from CTC to Eu^{3+} in the $[Eu^{3+}(CTC)_2(TOPO)_m]^+[SDS]^-$ molecule by intramolecular energy transfer [7,8]. In the $[Eu^{3+}(CTC)_2(TOPO)_m]^+[SDS]$ molecule, CTC is excited to its excited singlet state after absorbing light energy; it then changes to its triplet state through an intersystem crossing owing to the strong effect of Eu^{3+}. Because the luminescence level $^5D_0$ of Eu^{3+} is lower than that of the excited triplet state of CTC and because Eu^{3+} has few energy levels, it appears that the excited CTC can transfer its energy to the level $^5D_0$ of Eu^{3+} through intramolecular energy transfer so that the characteristic emission of Eu^{3+} is observed. Therefore, the luminescence of the complex is attributed to the M*–M luminescence mechanism.

After adding TOPO and SDS to the Eu-CTC-Borax system, the fluorescence intensity of Eu^{3+} was considerably enhanced. The experimental

data show that the fluorescence intensity of the Eu-CTC-TOPO-borax and the Eu-CTC-TOPO-SDS-borax system increased by 30 and 50 times in comparison with the Eu-CTC-borax system. This evidence shows that fluorescence enhancement after adding TOPO and SDS to the system is attributed to the increase of the fluorescence efficiency. It is thought that in the binary complex Eu-CTC, the coordination number of Eu^{3+} is unsaturated, and the water molecules which occupy the coordination sites of Eu^{3+}, can waste part of the energy of excited state of the complex and decrease the effective energy transferred to Eu^{3+}. As a result, the luminescence is weak. After adding TOPO, it replaces the coordinated water molecules and forms a coordination saturated complex; thus it decreases the radiationless deactivation of the excited state of the complex; which is caused by the coordinated water molecules, and greatly increases the fluorescence efficiency. The added SDS can neutralize the electric charge of $[Eu^{3+}(CTC)_2(TOPO)_m]^+$ and form an ion association complex which is dissolved in the micelles of surfactant. This can not only increase the stability of the complex but also change its micro-environment. In addition, the formation of the micelles not only lowers the mobility of the complex molecules dissolved in them but also prevents the complex molecules from colliding with the solvent molecules; this acts as the insulating shell of energy so that the fluorescence efficiency is greatly increased.

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