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NEWS

Trace Measurement of Phenothiazine Drugs in Tablets by Micellar-Enhanced Fluorophotometric Method

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Abstract It was found that in buffer solution of pH 7.0, the addition of sodium dodecyl sulfate (SDS) to the solution of phenothiazine drugs, such as chlorpromazine, promethazine and trifluoperazine, showed a remarkable enhancement of their fluorescence intensity. A further study proved that the phenothiazine drugs can be determined by fluorophotometric method in micellar system. Under optimal conditions, there was a good linear relationship between fluorescence intensity and phenothiazine compounds concentration, and the detection limit of 3.0×10^{-8} M chlorpromazine, 3.0×10^{-8} M promethazine and 1.5×10^{-8} M trifluoperazine ($S/N = 3$) were also obtained. This method has been used to determine phenothiazine drugs in tablets with satisfactory results.

Keywords Phenothiazine drugs · Micellar-enhanced fluorophotometric method · Trace measurement

Introduction

Antipsychotic drugs are widely used as therapeutic agents for treating various mental and personality disorders. Phenothiazines are a group of basic drugs including a phenothiazine ring with different substituents attached at the 2- and 10-position, which are used as antipsychotics, neuroleptics and antihistamines. Chlorpromazine hydrochloride, promethazine hyrdochloride and trifluoperazine hyrdochloride are belong to the phenothiazine neuroleptic group.Their chemical structures are shown in Scheme 1.

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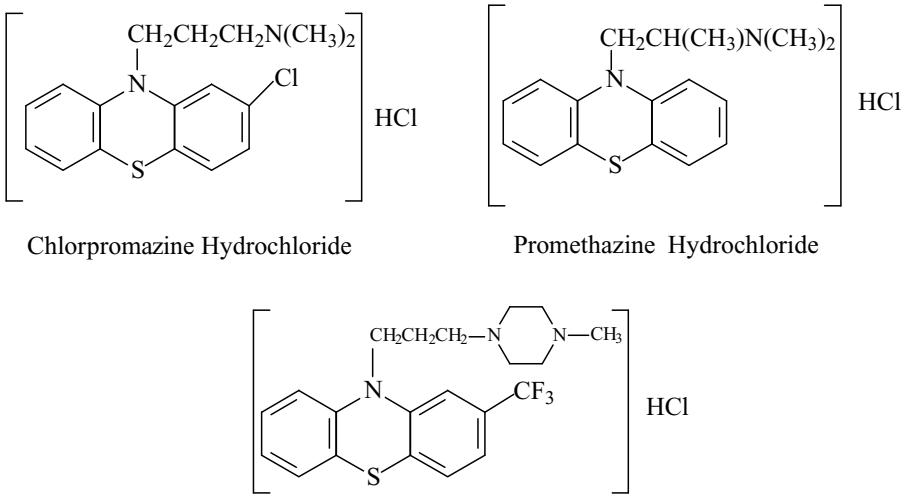
Some reports on determination of phenothiazine drugs have appeared in the literatures. Spectrophotometric method has been used for the determination of trifluoperazine [1], chlorpromazine [2] and promethazine [3] in commercial tablets and in pharmaceutical formulations. Electrochemical methods [4–6] have been applied to the determination of phenothiazine drugs in pharmaceuticals and biological fluids. High performance liquid chromatography (HPLC) was also used to determine trifluoperazine [7], promethazine[8] and chlorpromazine [9] in tablets and biological fluids. In addition, some other methods, such as electrochemiluminescent [10], capillary zone electrophoresis [11], flow-injection spectroelectroanalytical method [12] and nephelometric titration [13], are also used to determine some phenothiazine drugs. In addition, the interaction of phenothiazine drugs with different organic and micellear solutions was also investigated. For examples, Tabak M et al. [14, 15] reported that the characteristics of binding of two phenothiazine antipsychotic drugs, namely, chlorpromazine and trifluoperazine, to cationic cetyltrimethylammonium chloride, zwitterionic *N*-hexadecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate, neutral *t*-octylphenoxypolyethoxyethanol and polyoxyethylene dodecyl ether, anionic sodium dodecyl sulfate micelles were investigated by using electronic absorption spectroscopy, and the binding constants K_b and pK_a values of drugs in micelles were estimated by using the red shifts of the maximum absorption upon alkalization or in the presence of detergents. Sapre V A et al [16, 17] studied the interaction of phenothiazine and 10-methylphenothiazine in organic and micellar solution by spectrophotometric and fluorimetric techniques.

In the present paper, the fluorescence characteristics of phenothiazine drugs, such as chlorpromazine, promethazine and trifluoperazine, are investigated in micellar media. Based on obtained results, sensitive micelle-enhanced

Scheme 1 Chemical structure of the three phenothiazine drugs



Chlorpromazine Hydrochloride

Promethazine Hydrochloride

Trifluoperazine Hydrochloride

spectrofluorimetric method has been firstly developed for the determination of phenothiazine drugs in tablet samples. Our studies show that the micellar-enhanced fluorophotometric method will provide a simple and rapid approach for analysis of commercial tablets.

Experimental

Reagents

Phenothiazine compounds, such as chlorpromazine hyrdochloride, promethazine hyrdochloride and trifluoperazine hyrdochloride, kindly provided by Yangzhou Institute of Drug Control, were used without further purification. A stock solutions of 1.0×10^{-2} M phenothiazine compounds were prepared by bidistilled water. Working standard solutions were prepared by suitable dilution of stock solution with distilled water. (n-hexadecyl)triethylammonium bromide(CTAB) and sodium dodecyl sulfate (SDS) were purchased from Alfa-Aesar (Johnson Matthey Company). A 0.10 M stock solutions of SDS and CTAB were prepared by bidistilled water, respectively. Tris-(hydroxymethyl)aminomethane was obtained from Sigma. 0.01 M Tris-HCl buffer solution was prepared, and its pH values were adjusted by addition of NaOH and HCl until the target pH value was reached. Other regents were of analytical grade. All solutions were prepared with bidistilled water.

Apparatus

Fluorescence spectra and intensity measurements were taken on a Shimadzu Spectrofluorimeter Model RF-5301 equipped with xenon lamp and 1-cm quartz cells. Slit widths of both monochromator were set at 5 nm. All measurements were performed at $25 \pm 0.5^{\circ}$C. A model pHS-25 pH meter (Shanghai REX Instrument Factory) was used for pH measurements.

General procedure

0.5 mL 0.01 M Tris-HCl buffer solution (pH 7.0), 1.0 mL 0.1 M SDS solution and a suitable volume of phenothiazine compounds standard solution were transferred into a set of 10 mL volumetric flasks, and diluted to the volume with bidistilled water. The experiments were conducted at $25 \pm 0.5^{\circ}$C after the solutions were placed in a thermostat at $25 \pm 0.5^{\circ}$C more than 20 minutes.

Preparation of samples

Twenty tablets, each containing 12.5 mg/tablet chlorpromazine, 12.5 mg/tablet promethazine and 5 mg/tablet trifluoperazine, were accurately weighed and finely powdered, respectively. To accurately weighed amount of the powder equivalent to approximately 20 mg of chlorpromazine, 30 mL of bidistilled water was added. The mixture was shaken for 20 min and filtered into a 100 mL volumetric flask. The residue was washed several times with bidistilled water and the solution was diluted to the mark. After 0.2 mL sample solution, as well as 0.5 mL 0.01 M Tris-HCl buffer solution and 1.0 mL 0.1 M SDS solution, was added to 10 mL volumetric flasks and then diluted to the volume by bidistilled water, the solution was taken for micelle-enhanced spectrofluorimetric determination of chlorpromazine.

As to promethazine and trifluoperazine tablet samples, the pretreated procedure and method were the same for chlorpromazine tablet sample.

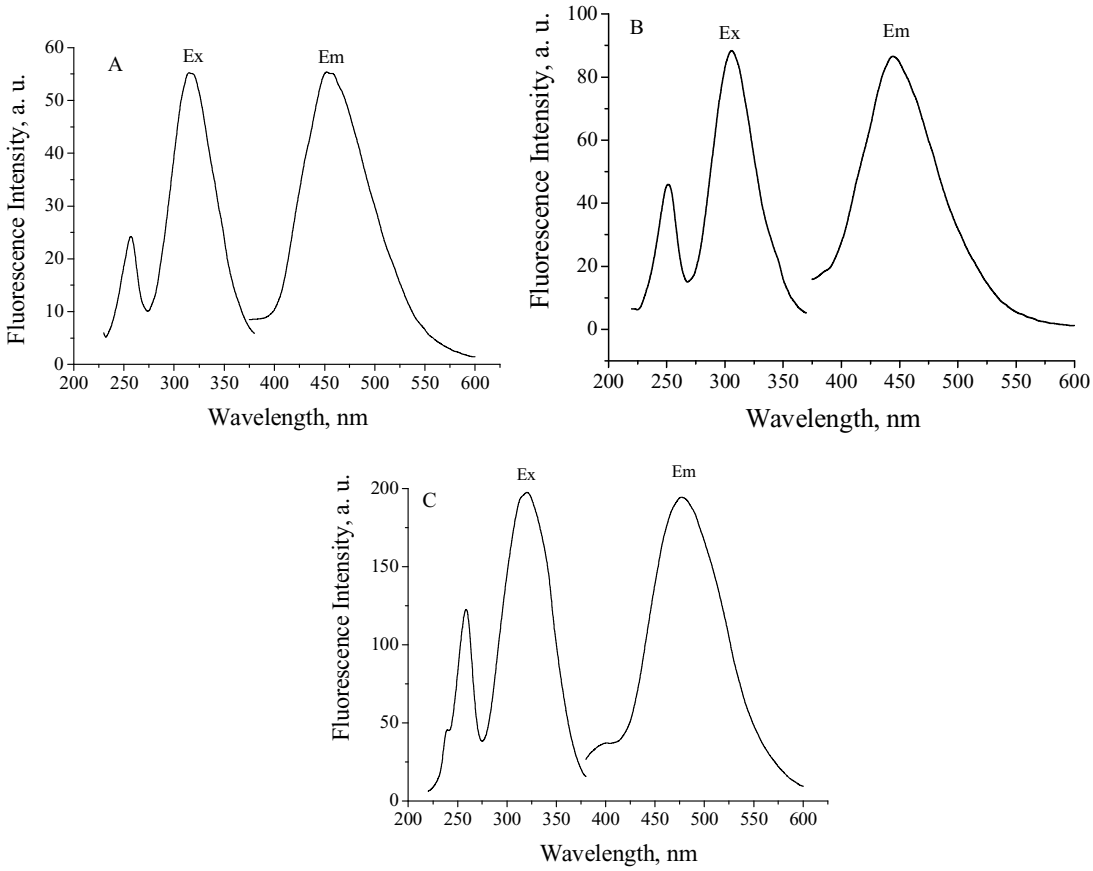


Fig. 1 Excitation and emission spectra of 1.0×10^{-5} M chlorpromazine (A), promethazine (B) and trifluoperazine (C) in water

Results and discussion

Excitation spectra and emission spectra of phenothiazine compounds in aqueous solution

Phenothiazine compounds showed the strong native fluorescence signals in aqueous solution. Figure 1 showed the excitation and emission spectra of phenothiazine compounds obtained in water, and the maximum excitation wavelength and the maximum emission wavelength was listed in Table 1.

Effect of different buffer solutions on the fluorescence intensity of phenothiazine compounds.

The effect of different buffer solutions on the fluorescence intensity of phenothiazine compounds was investigated, and the results were shown in Table 2. It can be seen that the fluorescence intensity changed slightly in weak acid, neutral and weak base buffer solutions. At the same time, the effect of pH on fluorescence intensity was also studied (shown in Fig. 2). As can be seen, the fluorescence intensity of phenothiazine compounds hardly changed in the pH range of 5.0 ~ 8.5. Considering the environment and action of drug in the human biological conditions, the Tris-HCl (pH 7.0) buffer solution was selected in this work.

Table 1 The exciation and emmsion wavelength of phenothiazine compounds

Compounds	Excitation wavelength/nm	Emission wavelength/nm
Chlorpromazine	316.0	455.0
Promethazine	305.0	444.0
Trifluoperazine	320.0	475.0

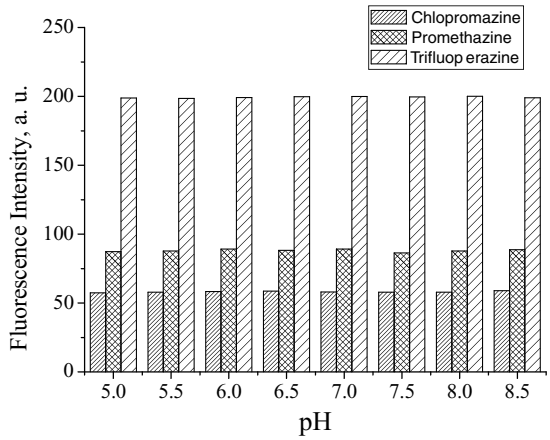


Fig. 2 Effect of pH on the fluorescence intensity of phenothiazine compounds (1.0×10^{-5} M) in 5 mM Tris-HCl buffer solution

Table 2 The effect of different buffer solutions on fluorescence intensity of phenothiazine compounds[a]

Buffer solutions	Fluorescence Intensity of Emission spectrum		
	Chlorpromazine	Promethazine	Trifluoperazine
In water	55.51	86.67	193.09
In 5 mM HAc-NaAc (pH 4.5)	56.09	86.01	197.84
In 5 mM Tris-HCl (pH 7.0)	57.08	88.99	199.92
In 5 mM NH_3-NH_4Cl (pH 9.6)	56.45	87.79	193.45

[a]Concentration: 1.0×10^{-5} M.

At the same time, the effect of the volume of Tris-HCl buffer solution on fluorescence intensity of phenothiazine compounds was also studied. The results showed that the fluorescence intensity of phenothiazine compounds hardly changed in different Tris-HCl concentration from 1 to 10 mM (data not shown). So the 5 mM Tris-HCl was selected in the work.

Effect of different surfactants on fluorescence intensity of phenothiazine compounds.

It is well known that addition of a surfactant at a concentration above its critical micellar concentration (*CMC*) to a given fluorophore solution increases the molar absoptivity and/or fluorescence quantum yield of fluorophore in many cases [18]. This fact has been used to improve the performance of spectrofluorimetric methods for the determination of various analytes [19, 20].

The fluorescence properties of phenothiazine compounds in different micellar media were studied using anionic (SDS) and cationic (CTAB) surfactants (shown in Fig. 3). There was an obvious enhancement of fluorescence intensity in the presence of SDS and CTAB compared with aqueous solution.This phenomenon can be explained by protection of lowest excited state of fluorophore in SDS or CTAB micellar microenvironment from non-radiative or possible quenching processes that readily occurred in bulk aqueous solutions. At the same time, a slight blue shift in the emission maximum was observed in the medium. Due to obvious higher enhanced-fluorescence intensity in SDS micellar system than that in CTAB micellar system, so the anionic surfactant of SDS was employed in this work.

Figure 4 showed the effect of different SDS concentration on the fluorescence of phenothiazine compounds. When the SDS concentration was less than 8 mM, the fluorescence intensity increased rapidly with the increase of SDS concentration. And when the SDS concentration was higher than 8 mM, the fluorescence intensity hardly changed. So a 0.01 M SDS medium was employed in further studies.

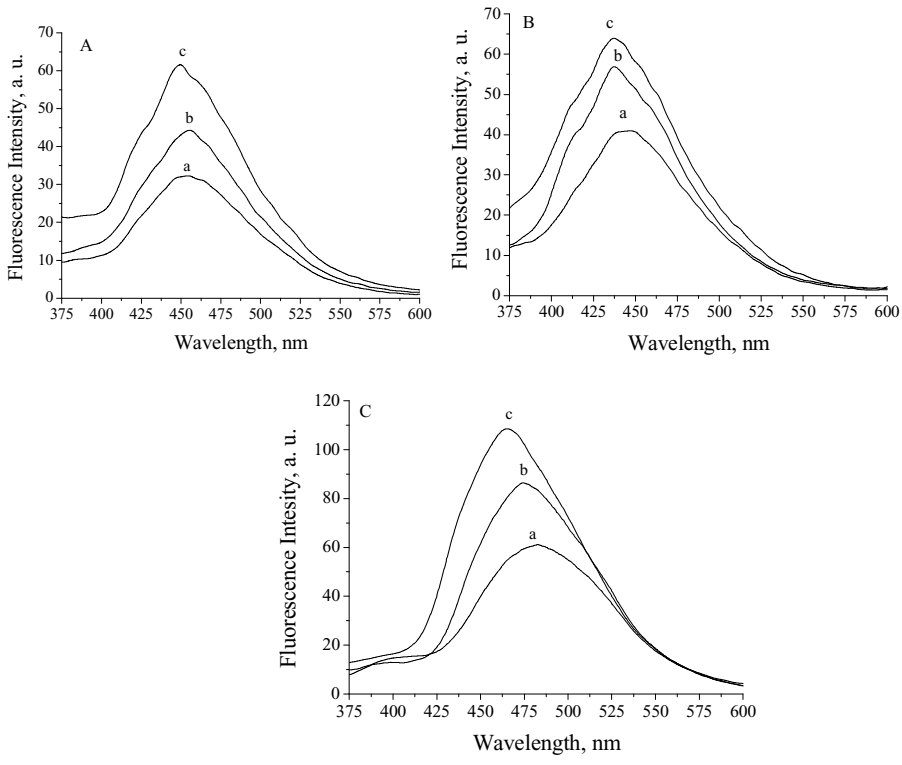


Fig. 3 Effect of different surfactants on emission spectra of 4.0×10^{-6} M chlorpromazine (A), promethazine (B) and trifluoperazine (C) in 5 mM Tris-HCl buffer solution (pH 7.0). Curve a: Without surfactant; Curve b: curve a + 0.01 M CTAB; Curve c: curve a + 0.01 M SDS

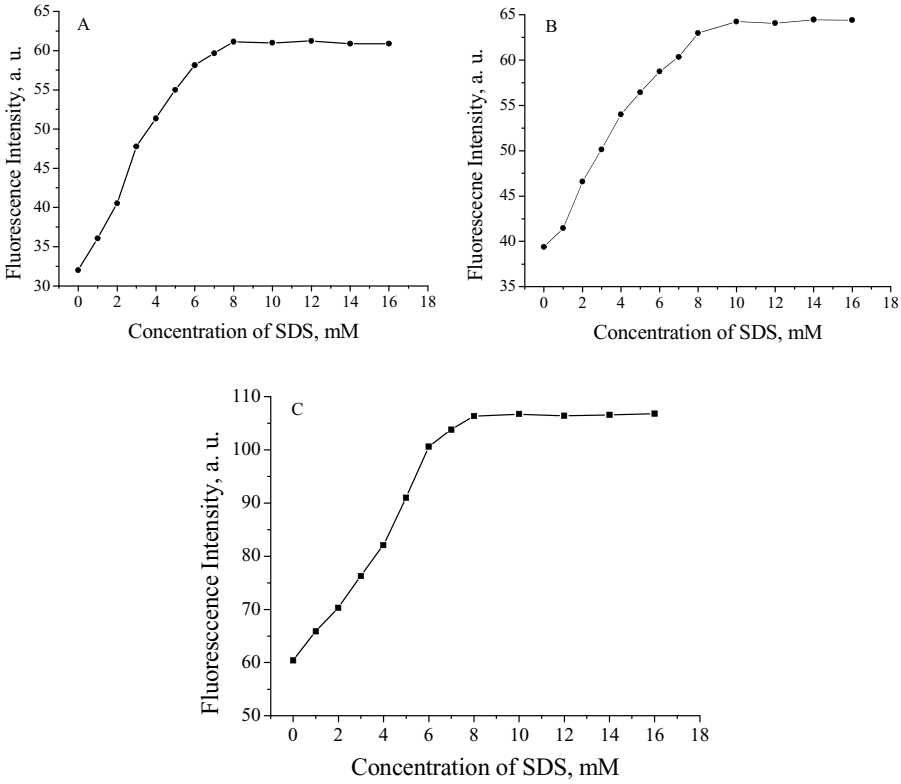


Fig. 4 Effect of SDS concentration on the fluorescence intensity of 4.0×10^{-6} M chlorpromazine (A), promethazine (B) and trifluoperazine (C) in 5 mM Tris-HCl buffer solution (pH 7.0)

Effect of equilibrium time

In order to mainly obtain the stable fluorescence intensity of phenothiazine compounds and better reproducibility of micellar-nehanced spectrofluorimetric method, the effect of the equilibrium time of phenothiazine compounds in the micellar system was investigated (shown in Fig. 5). It can be seen that the micellar-enhanced fluorescence

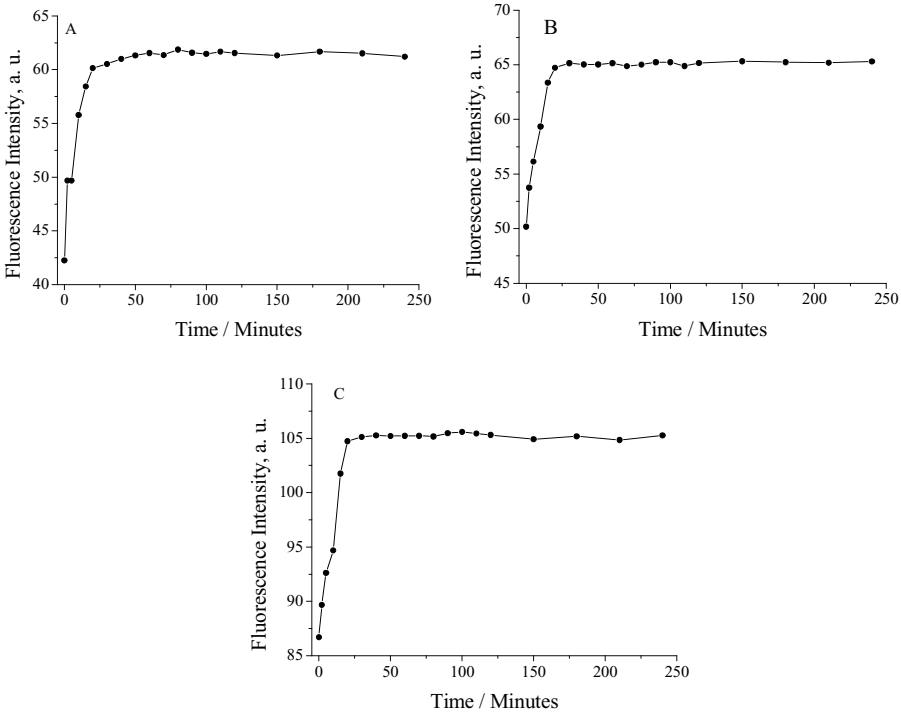


Fig. 5 Effect of equilibrium time on the fluorescence intensity of of 4.0×10^{-6} M chlorpromazine (A), promethazine (B) and trifluoperazine (C) in 5 mM Tris-HCl buffer solution (pH 7.0) containing 0.01 M SDS

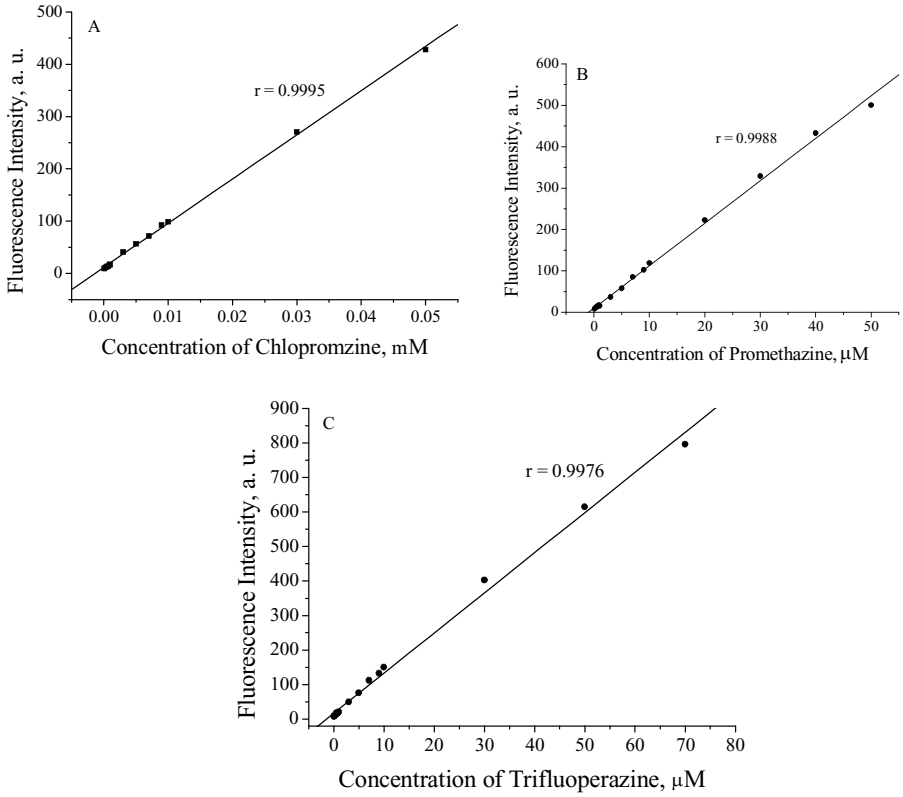


Fig. 6 Dependence of micelle-enhanced fluorescence intensity on the concentration of chlorpromazine (A), promethazine (B) and trifluoperazine (C)

intensity increased with the equilibrium time below 20 minutes. And when the equilibrium time was in the range of 20 to 240 minutes, the micellar-enhanced fluorescence intensity hardly changed. So the spectrofluorimetric experimentations were performed after the equilibrium time of 20 minutes, which obtained the phase equilibrium between SDS micelle-system and phenothiazine compounds [21]. In addition, the whole system could be stable at least 3 h at 25°C within a change of 2%, which was long enough to complete the determination of phenothiazine compounds.

Calibration curve for phenothiazine compounds

Under the selected conditions given, the dependence of the relative fluorescence intensity on the concentration of phenothiazine compounds was shown in Fig. 6. The linear range and detection limit ($S/N = 3$) of phenothiazine compounds were shown in Table 3. A comparison of the performances between the proposed method and those of literature methods for phenothiazine compounds determination is summarized in Table 3. It can be seen that the detection limit of this work is lower than that found in the previous reports.

Table 3 Comparison of linear range and detection limits for trazodone hydrochloride by different methods

Compounds	Methods	Linear range	Detection limits	Refs.
Chlorpromazine	Micellar-enhanced spectrofluorimetric method	$9.0 \times 10^{-8} \sim 5.0 \times 10^{-5}$ M	3.0×10^{-8} M	This work
	Spectrophotometric method	$5.6 \times 10^{-6} \sim 1.4 \times 10^{-5}$ M	/	2
	Electrochemical method	$1.4 \times 10^{-7} \sim 2.8 \times 10^{-6}$ M	1.3×10^{-7} M	4
	HPLC method	$2.8 \times 10^{-8} \sim 8.4 \times 10^{-7}$ M	/	9
Promethazine	Micellar-enhanced spectrofluorimetric method	$1.0 \times 10^{-7} \sim 5.0 \times 10^{-5}$ M	2.5×10^{-8} M	This work
	Spectrophotometric method	$1.6 \times 10^{-6} \sim 7.8 \times 10^{-5}$ M	/	3
	Electrochemical method	$3.1 \times 10^{-7} \sim 3.1 \times 10^{-6}$ M	1.3×10^{-7} M	4
	HPLC method	$8.3 \times 10^{-5} \sim 8.3 \times 10^{-4}$ M	1.1×10^{-5} M	8
Trifluoperazine	Micellar-enhanced spectrofluorimetric method	$5.0 \times 10^{-8} \sim 7.0 \times 10^{-5}$ M	1.5×10^{-8} M	This work
	Spectrophotometric method	$4.2 \times 10^{-6} \sim 2.1 \times 10^{-5}$ M	/	1
	HPLC method	/	3.1×10^{-8} M	7

Table 4 Determination of Chlorpromazine, promethazine and trifluoperazine in tablets of phenothiazine compounds

Compounds	Pharacopoeia method ($n=4$)	This method ($n=5$)
Chlorpromazine	12.8 mg/tablet	12.9 mg/tablet
Promethazine	12.3 mg/tablet	12.6 mg/tablet
Trifluoperazine	4.83 mg/tables	4.87 mg/tablet

Effect of potential interferences

The effect of foreign substances was tested by analyzing a standard solution of phenothiazine compounds (1.0×10^{-6} M) to which increasing amounts of interfering species were added, using an error less than 5% as the criterion [22]. The fluorescence intensity of phenothiazine compounds was also measured in the presence of some foreign species. There was no interference for Na^+, K^+, Mg^{2+} and Zn^{2+} at molar ratios of cations/phenothiazines for 1000. When the molar ratios of organic compounds/ phenothiazines were less than 300, the organic compounds, such as uric acid, urea, ascorbic acid and glucose, did not interfere with the fluorescence intensity. And Co^{2+} (molar ratio $<$ 150), Ni^{2+} (molar ratio $<$ 40) and Cu^{2+} (molar ratio $<$ 30) appeared no obvious interference for measuring the fluorescence intensity.

Determination of phenothiazine drugs in tablets

The above present method was applied to the determination of phenothiazine drugs in tablets, which were prepared as described in the experimental section. The accuracy of this method was determined by comparing to UV-vis method of pharmacopoeia [23] and its recovery. Table 4 showed that there was a good agreement between two methods. Based on the above present method of measuring phenothiazine drugs by micellar-enhanced fluorescence, the recovery of phenothiazine drugs for tablets was listed in Table 5. The satisfactory results showed that the above present method of micellar-enhanced fluorescence can be used to determine concentration of phenothiazine compounds in commercial tablets.

Table 5 Recovery test of phenothiazine compounds in tablet[a]

Compounds	Added ($\times 10^{-6}$M)	Found ($\times 10^{-6}$M)	Recovery (%)	RSD (%)
Chlorpromazine	0.00	1.16	/	2.1
	2.00	3.07	95.50	1.9
	4.00	5.22	101.50	1.7
	6.00	7.39	103.83	1.4
	8.00	8.98	97.75	1.6
Promethazine	0.00	1.25	/	1.9
	2.00	3.34	104.50	1.7
	4.00	5.17	98.00	1.3
	6.00	7.46	103.50	1.5
	8.00	9.18	99.12	1.2
Trifluoperazine	0.00	1.19	/	2.0
	2.00	3.14	97.50	1.5
	4.00	5.27	102.00	1.1
	6.00	6.98	96.50	1.4
	8.00	9.24	100.62	1.7

[a]number of samples assayed: 6.

Conclusion

The SDS micellar system provides a simple means to enhance the fluorescence of phenothiazine compounds. This work shows that micellar-enhanced fluorimetric proposed method is simple, accurate enough and can be recommended for drugs determination in quality control and routine pharmaceutical analysis.

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