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RAPID COMMUNICATION

An Enhancement of Europium-/Gadolinium- Diphacinone - *DL*-Histidine- Cetylpyridine Bromide System for the Determination of Diphacinone in Serum

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Abstract As one of the first generation anticoagulant rodenticide, diphacinone (DPN) was extensively used in China, and often occurred accidental and intentional intoxications. To meet the needs of clinical emergency rescue, a simple, rapid and sensitive method was necessary for the determination of DPN in serum. In this paper, a fluorimetric method using a ternary europium-gadolinium-DPN-*DL*-histidine-cetylpyridine bromide system was developed. The fluorescence intensity was measured with a 1 cm quartz cell at 335 nm excitation wavelength and 612 nm emission wavelength. The fluorescence intensity of the europium-DPN- *DL*- histidine complex system was further enhanced by using gadolinium ion (Gd^{3+}) in a cetylpyridine bromide (CPB) colloid-type suspension. By adding Gd^{3+} at pH 8.5 to 9.5 in the presence of an ammonia-ammonium chloride buffer solution, about a 100-fold enhancement of the fluorescence intensity was achieved. The enhanced fluorescence intensity showed a good linear relationship with the DPN concentration from 0.01 mg/L to 5.0 mg/L. The limit of detection was 0.001 mg/L in serum. The established method was used to determine DPN in real human serum in clinical specimens. The luminescence mechanism was discussed in detail. In the fluorescence system of the europium-gadolinium-DPN-*DL*-histidine-CPB, the CPB not only acted as the surfactant but also acted as the energy donor.

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Introduction

Diphacinone (2-diphenylacetyl-1,3-indandione, DPN) (Fig. 1) is one of the first generation anticoagulant rodenticides. Like other rodenticides, DPN was used to control the mice and rat populations in China for several decades. Due to the extensive use, both accidental and intentional DPN intoxications in both domestic animals and humans have been often occurred, however, most of these poisoning cases were happened in rural areas where advanced diagnostic instruments were not available. For rapid clinical diagnosis and appropriate continuous treatment, it is necessary to establish a simple, fast and low-cost method for the determination of DPN in serum in clinical emergency rescue.

Different methods had been reported for determining trace DPN in various biological samples, such as thin layer chromatography [1], ultraviolet spectrophotometry [2], gas chromatography (GC) [3, 4], liquid chromatography (LC) [5–12] and LC coupled with mass spectrometry (LC-MS) [13–16]. The thin layer chromatography and ultraviolet spectrophotometry methods lack sufficient sensitivity and selectivity. The GC, LC and LC-MS methods are difficult to carry out at rural hospitals due to the lack of expensive apparatus and professional operators. As rare earth ions have luminescence characteristics such as narrow spectral width, long luminescence lifetime, large Stokes shift and strong binding with biological molecules, they are commonly used as fluorescence probes to study the fluoroquinolone antibiotics [17–21] containing β-diketone ligand. The co-luminescence effect of rare earths, acting as a

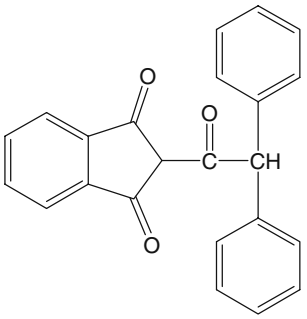

Fig. 1 Chemical structure of DPN

fluorescence enhancement effect, was first found and studied by Jinghe Yang and Guiyun Zhu in 1986 [22]. This effect has become an important way to improve the sensitivity of rare earth ions by fluorimetry [23–28].

The rare earth Eu^{3+} is often used as the fluorescence probes for determining some substances because of the high fluorescence quantum efficiency of the Eu^{3+} chelates [29–34]. Although two literatures had been reported [35, 36] for the detection of DPN in water using Eu^{3+} as a fluorescence probe, the limits of detection for both methods were 0.027 mg/L, these sensitivities required to be further improved to meet the detection requirements of poisoning samples in emergency. Luckily, our latest experiments indicated that the fluorescence of the Eu^{3+}-DPN- *DL*-histidine-NH_3 complex was greatly enhanced by the addition of gadolinium ion (Gd^{3+}) and cetylpyridine bromide (CPB). The results showed that the fluorescence intensity of this method could enhance about 100 times compared with the system of no addition Gd^{3+}.

The aim of this work was to develop a sensitive spectrofluorimetric method for the determination of DPN in serum. The formation condition and the factors affecting the Eu-Gd-DPN-CPB-*DL*-histidine system were studied. This is a newly found fluorescence enhancement system of rare earth ion and can be used in the determination of trace DPN. The luminescence mechanism of the system was discussed.

Experimental

Apparatus

The fluorescence spectra and measurements were performed on a F96 spectrofluorimeter (Shanghai Lengguang Technology, Shanghai China) with a quartz cell (1.0× 1.0 cm^2 cross-section) equipped with a xenon lamp (100 W). All absorption spectra were recorded on a UV-2401PC spectrophotometer (Shimadzu, Japan). All pH measurements were measured with a MP 220 pH acidity meter (Mettler-Toledo Ltd., Shanghai, China). A Legend RT centrifuge (Heraeus Ltd., Hanau, Frankfurt, Germany) was used to centrifugate blood samples.

Reagents

All chemicals and reagents were of analytical grade, and deionized water was obtained from a Millipore Milli-Q water purification system (Molsheim, France). Diphacinone (>99.5%, DPN) was purchased from Sigma (St. Louis, MO, USA). Stock solution of DPN (1.0 mg/mL) was prepared by dissolving 100.0 mg DPN in 10 mL ethanol and then diluted to 100 mL with water. Gadolinium (III) oxide and europium (III) oxide were obtained from Shanghai Reagent Factory (Shanghai, China) with purities of at least 99.9%. *DL*-Histidine monohydrochloride monohydrate and cetylpyridine bromide (CPB) were of analytical grade and purchased from Shanghai Reagent Factory (Shanghai, China). Stock solutions of the gadolinium (Gd^{3+}) and europium (Eu^{3+}) ions (1.0 mg/mL) were prepared by dissolving a known amount of the appropriate gadolinium (III) oxide and europium (III) oxide in hydrochloric acid. A solution of surfactant (CPB, 0.5%) was prepared by dissolving 0.5 g CPB in 100 mL water. A stock solution of *DL*-Histidine (1.0%) was prepared by dissolving 1.35 g *DL*-Histidine monohydrochloride monohydrate in 100 mL water. The NH_3-NH_4Cl buffer solution was prepared with 1.0 mol/L ammonium chloride adjusted pH with strong aqua ammonia to 9.0.

The blank blood samples were collected from healthy, drug-free volunteers (three women and three men), aged from 21 to 25 years, from Ningbo University (Ningbo, China). Suspected blood samples were provided by Ningbo Public Security Bureau (Ningbo, Zhejiang, China) and the Ningbo Second People's Hospital (Ningbo, Zhejiang, China), as shown in Table 1. All blood samples were immediately centrifuged in 5 mL polypropylene centrifuge tubes for 5 min at 7,800 rpm and the serum specimens were kept in a freezer at −20°C until processing. A series of spiked samples (0.05, 1.0 and 4.0 mg/L) were prepared by blank serum samples.

General Procedure

The frozen serum samples (1.0 mL) were firstly thawed to room temperature in 5 mL polypropylene centrifuge tubes before analysis, and then were vortex-mixed and extracted using 1.0 mL of 10% (*v*/*v*) methanol in acetonitrile for 5 min. After centrifuging the samples for 5 min at 7,800 rpm, the upper organic layer was transferred to a disposable glass tube. The samples were then re-extracted with 10% (*v*/*v*) methanol in acetonitrile (1.0 mL) as mentioned above. The organic layers were combined and evaporated to dryness in a disposable tube under a stream of nitrogen on a heating block at 50°C. The evaporated samples were dissolved with 2.0 mL water, immersed into a

Table 1 Sample information and the diphacinone concentration using different methods

Sample	Gender	Age (year)	Concentration (mg/L)	
			Fluorescence method	LC-MS/MS method
A	woman	15	0.13	0.12
B	man	12	4.42	4.23
C	man	23	n.d.[a]	n.d.[b]
D	man	45	3.21	3.11
E	man	11	n.d.	n.d.
F	woman	7	2.49	2.67

[a] less than the LOD (0.001 mg/L) for the fluorescence method

[b] less than the LOD (0.0005 mg/L) for the LC-MS/MS method

KQ 500DB ultrasonic cleaning bath (Kunshan Ultrasonic, Jiangsu, China) and ultrasonicated for 5 min to facilitate dissolution. Prior to the fluorescence reaction, the extract was filtered though a 0.45 μm nylon syringe filter (Agilent Technologies, Waldbronn, Germany) into a 10 mL glass test tube. The other each solution was added 1.0 mL according to the following order: 10.0 mg/L europium, 25.0 mg/L gadolinium, 0.01% *DL*-histidine, 0.05% CPB and NH_3-NH_4Cl buffer solution. The mixture was diluted to 10 mL with distilled water. After 10 min, the fluorescence intensity was measured at the excitation and emission wavelengths of 335 nm and 612 nm, respectively.

Results and Discussion

Fluorescence Spectra

Figure 2 showed the emission spectra of the Eu^{3+}-DPN-*DL*-histidine-CPB composite at pH 9.0, with and without Gd^{3+} in the solution. It can be seen that the intrinsic Eu^{3+} emission of the Eu^{3+}-DPN-*DL*-histidine-CPB composite was very weak, but an approximate 100-fold increase of the fluorescence intensity was observed in the case of Gd^{3+} addition at the excitation and emission wavelengths of 330 nm and 612 nm, respectively.. Emission lines appeared at 585 nm, 595 nm, 612 nm and 652 nm, corresponding to the $^5D_0 \rightarrow {}^7F_0$, $^5D_0 \rightarrow {}^7F_1$, $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_3$ transitions of Eu^{3+} [35, 37, 38], respectively. The peak at 612 nm was more intense, so this wavelength was used for subsequent experiments. In contrast, under the described experimental conditions, the Gd^{3+}-DPN-*DL*-histidine-CPB composite system did not exhibit fluorescence.

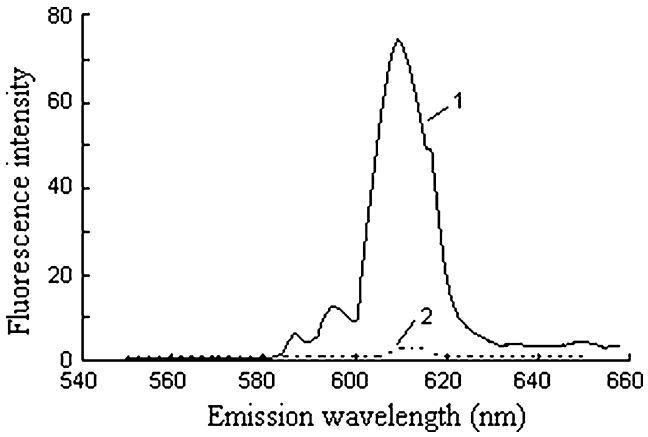

Fig. 2 Fluorescence emission spectra. 1. Eu^{3+}-DPN-*DL*-histidine-CPB, Conditions: Eu^{3+} 10.0 mg/L, Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, *DL*-histidine 0.01%, CPB 0.05%, pH 9.0; 2. Eu^{3+}-DPN- *DL*-histidine-CPB, Conditions: Eu^{3+} 10.0 mg/L, DPN 2.0 mg/L, *DL*-histidine 0.01%, CPB 0.05%, pH 9.0

Effect of pH and the Buffers

The effect of pH, between 5.5 and 10.5, on the fluorescence intensity of the Eu^{3+}-DPN-*DL*-histidine-CPB system was studied, the results were shown in Fig. 3. The NH_3-NH_4Cl buffer solution was used and the pH adjusted with hydrochloric acid or ammonia solution. The results indicated that the fluorescence intensity increased with increasing pH up to 8.5, and remained maximum fluorescence intensity between pH 8.5–9.5, and then decreased when the pH was above 9.5. As a result, pH=9.0 was selected for further research.

The experiments showed that the buffers also had a large effect on the fluorescence intensity. The following buffers (1.0 mol/L) were tested at fixed pH 9.0: Tris–HCl,

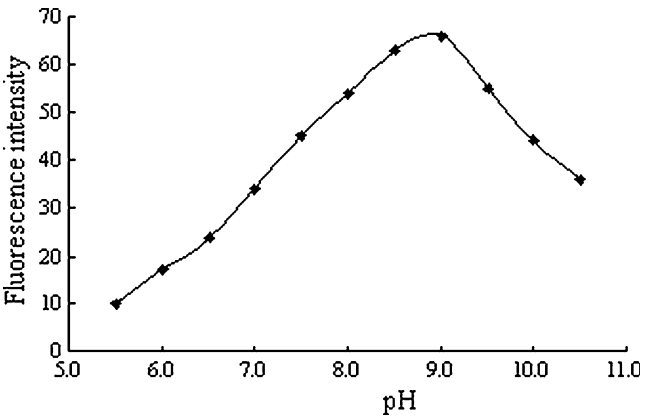

Fig. 3 Effect of pH on fluorescence. Conditions: Eu^{3+} 10.0 mg/L, Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, *DL*-histidine 0.01%, CPB 0.05%

$Na_2B_4O_7$-HCl, NH_3-NH_4Cl, and CH_3COONa-CH_3COOH. The results indicated that NH_3-NH_4Cl was the most suitable buffer, as shown in Table 2. The NH_3-NH_4Cl buffer at pH 9 was selected for further research.

The effect of the NH_3-NH_4Cl buffer concentration on the fluorescence intensity at fixed concentrations of Eu^{3+} (10.0 mg/L), Gd^{3+} (25.0 mg/L) and DPN (2.0 mg/L) was studied. The fluorescence intensity also increased with increasing ammonium chloride concentration up to 1.0 mol/L and remained constant over the concentration ranging from 1.0 mol/L to 2.0 mol/L. The experiments indicated that the buffers also had a large effect on the fluorescence intensity of the system. In this study, we selected a NH_3-NH_4Cl buffer solution prepared with 1.0 mol/L ammonium chloride adjusted pH with strong aqua ammonia to 9.0.

Effect of Eu^{3+} Concentration

The effect of the Eu^{3+} concentration on fluorescence intensity of the system was studied at the fixed concentrations of DPN (2.0 mg/L) and Gd^{3+} (25.0 mg/L). A maximum and constant fluorescence intensity was observed when the Eu^{3+} concentration was greater than 10.0 mg/L, as shown in Fig. 4. Contrastively, no intrinsic europium emission was observed in the absence of DPN. The excited singlet states of DPN in both the Eu^{3+}-DPN-*DL*-histidine and Gd^{3+}-DPN-*DL*-histidine complexes underwent a radiationless transition to the triplet states. Eu^{3+} could be excited by both the intramolecular energy transfer from the excited triplet state of DPN in the Eu^{3+}-DPN-*DL*-histidine complex and by the intermolecular energy transfer from the excited triplet state of DPN in the Gd^{3+}-DPN-*DL*-histidine complex. Because the concentration of the Gd^{3+} complex was greater than that of the Eu^{3+} complex in the CPB solution, each Eu^{3+}-DPN-*DL*-histidine molecule was surrounded by Gd^{3+}-DPN-*DL*-histidine molecules, and the fluorescence of the Eu^{3+} complex was considerably enhanced. In this paper, Eu^{3+} concentration of 10.0 mg/L was selected for further research.

Effect of Gd^{3+} Concentration

Figure 5 showed the fluorescence intensity variation with the concentration of Gd^{3+} in the presence of a fixed amount of Eu^{3+} (10.0 mg/L). The results indicated that the fluorescence intensity of the system increased with increasing Gd^{3+} concentrations in the rang of 2.5 to 25.0 mg/L and a maximum intensity occured at 25.0 mg/L of Gd^{3+}. From Fig. 5, it can be seen that fluorescence enhancement by Gd^{3+} occurred at concentrations in the range of 2.5 to 25.0 mg/L. The fluorescence was constant for Gd^{3+} concentrations above 25.0 mg/L. We hypothesized that there is an intermolecular energy transfer between the Eu^{3+} and Gd^{3+} complexes. The energy absorbed by the Gd^{3+} complex could be transferred to the Eu^{3+} in the Eu^{3+} complex and, therefore, enhances the fluorescence intensity of Eu^{3+}. In this paper, Gd^{3+} (25.0 mg/L) was selected for further research.

Effect of CPB Concentration

CPB is known to enhance the lanthanide luminescence in lanthanide-β-diketone complexes. Since CPB is insoluble in water, a micellar media is necessary to solubilize CPB in the aqueous medium. The experiments indicated that the surfactants had a large effect on the fluorescence intensity of the system. In this paper, we selected CPB as a synergic fluorescence enhancement reagent. The luminescent properties of the fluorescent europium complexes were obviously enhanced in the CPB matrix and by the interactions between the fluorescent complexes and the CPB molecules. The effect of CPB concentration on the fluorescence intensity was investigated. From Fig. 6, it is clear that the luminescence intensity increased with increasing CPB concentration ranging from 0.005% and 0.05% and approached a maximum value at 0.05%. CPB aids in the dissolution of both the Eu^{3+}-DPN-*DL*-histidine and Gd^{3+}-DPN-*DL*-histidine complexes and provides protection against solvent (water) molecule collisions, which would result in a loss of energy. The optimum concentration of CPB used in this study was fixed at 0.05%.

Effect of *DL*-Histidine Concentration

The effect of *DL*-histidine concentration on the fluorescence intensity was shown in Fig. 7. *DL*-histidine played a synergistic ligand role in the fluorescence system and combined with europium and DPN to form a steady ternary complex of Eu^{3+}-DPN-*DL*-histidine. The results indicated that the fluorescence intensity increased when the *DL*-

Table 2 Buffer comparison

Buffer	Tris–HCl	$Na_2B_4O_7$-HCl	NH_4Cl-NH_3	CH_3COONa-CH_3COOH
Fluorescence intensity	25.6	6.7	76.1	22.5

[a] Conditions: Eu^{3+} 10.0 mg/L, Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, *DL*-histidine 0.01%, CPB 0.05%, Tris–HCl 1.0 mol/L, $Na_2B_4O_7$-HCl 1.0 mol/L, NH_4Cl-NH_3 1.0 mol/L, CH_3COONa-CH_3COOH 1.0 mol/L, the pH values for all buffers were fixed at 9.0

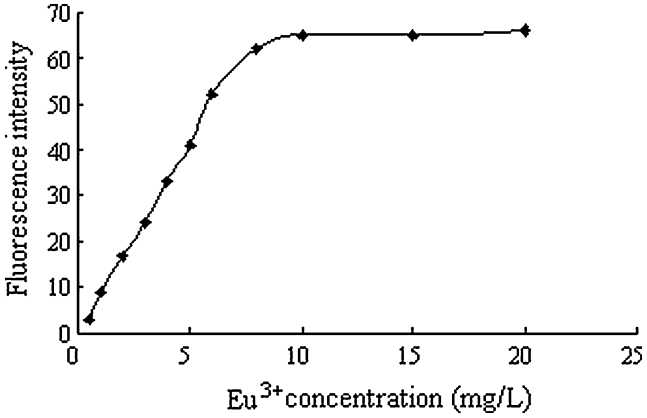

Fig. 4 Effect of Eu^{3+} concentration on fluorescence. Conditions: Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, pH=9.0, *DL*-histidine 0.01%, CPB 0.05%

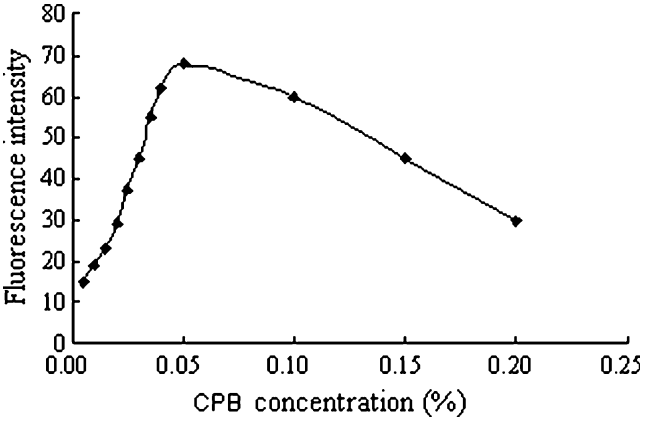

Fig. 6 Effect of CPB concentration on fluorescence. Conditions: Eu^{3+} 10.0 mg/L, Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, pH=9.0, *DL*-histidine 0.01%

histidine concentration was in the range of 0.0005% to 0.01% and a maximum intensity was reached at about 0.01% of *DL*-histidine. In this paper, we selected 0.01% *DL*-histidine for the further research. The composition of the complex in the Eu^{3+}-DPN-*DL*-histidine system was speculated on the literature's reports [38–41] of the general coordination number 6–12. Figure 8 showed the possible complex structures before and after the addition of *DL*-histidine. This putative conclusion could be supported with the excess of Eu^{3+} concentration and the lack of DPN content in the solution, which lead to a partial coordination (6) between DPN and Eu^{3+}. When *DL*-histidine was added, we thought that this amino acid acted as a synergistic ligand in the complex, and the absence of coordination was fully available (12).

Analytical Characteristics

Under the optimum conditions, the calibration curve for the determination of DPN was constructed by a series of DPN standard solutions (0.01, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0 and 5.0 mg/L) which were diluted from the stock solution (1.0 mg/mL) with water. The fluorescence intensity showed a good linear relationship with the DPN concentration ranging from 0.01 to 5.0 mg/L with a coefficient of determination (r^2) of 0.994. The regression equation was $C=0.02435F+0.0426$ and the limit of detection (signal-to-noise ratio of 3) for DPN was 0.001 mg/L in serum. A comparison with the most reported methods [31, 32], it was about 30 times more sensitive than that of the literature reports (0.027 mg/L).

A spiked serum sample at a concentration of 0.01 mg/L was prepared as the sample preparation process for the determination of LOQ. For quantification purposes, the signal to noise ratio (S/N) should set to be more than 10. The LOQ was 0.003 mg/L for DPN in serum.

The recovery of the method was examined by a series of spiked serum samples (0.05, 1.0, 4.0 mg/L) with the same prepared extraction procedures as the serum samples. Intra-day precision was evaluated by performing continuously six replicates within a day for determining the spiked serum samples. Inter-day precision was evaluated by performing the determination of the spiked serum samples each day on five consecutive days. The summary of results is shown in Table 3.

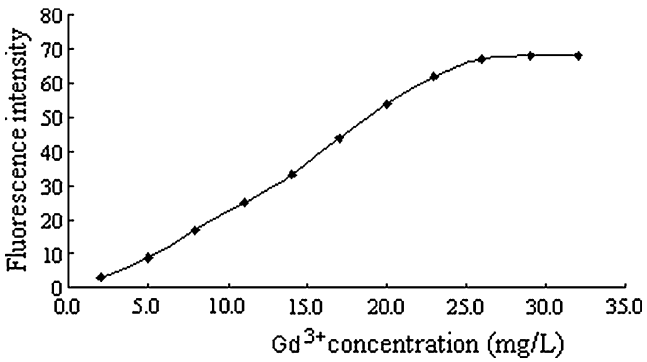

Fig. 5 Effect of Gd^{3+} concentration on fluorescence. Conditions: Eu^{3+} 10.0 mg/L, DPN 2.0 mg/L, pH=9.0, *DL*-histidine 0.01%, CPB 0.05%

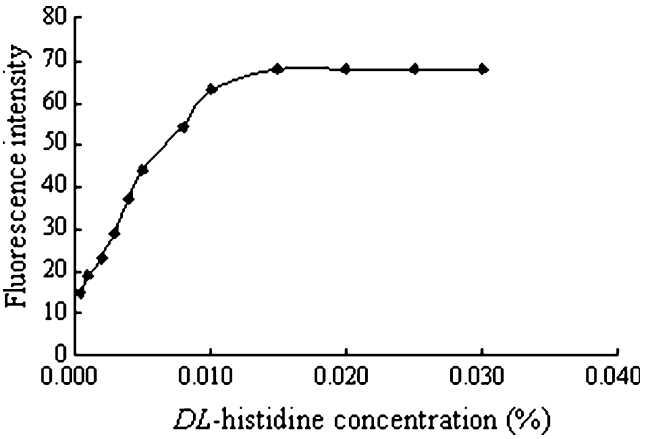

Fig. 7 Effect of *DL*-histidine concentration on fluorescence. Conditions: Eu^{3+} 10.0 mg/L, Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, pH=9.0, CPB 0.05%

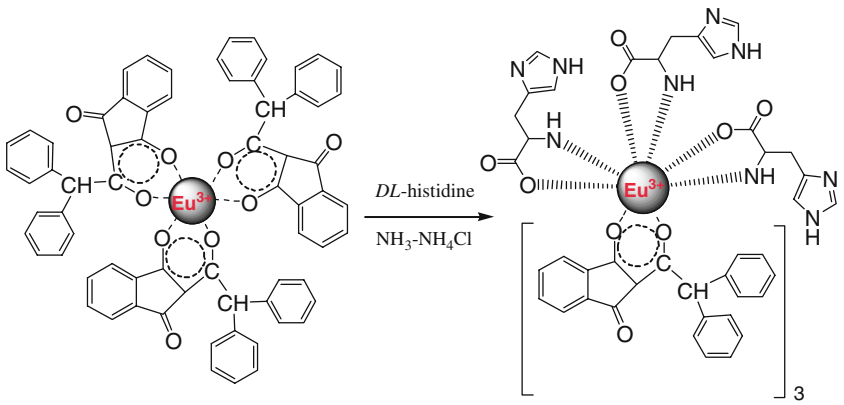

Fig. 8 Possible complex structures of Eu^{3+}-DPN complex to Eu^{3+}-DPN-*DL*-histidine complex in the prescence of DL-histidine and NH_3-NH_4Cl buffer. Conditions: Eu^{3+} 10.0 mg/L, DPN 2.0 mg/L, pH=9.0, *DL*-histidine 0.01%, CPB 0.05%

It showed that the recoveries were between 87.0% and 93.3%, the intra-day RSDs were between 4.0% and 5.7%, and the inter-day RSDs were between 6.7% and 8.9% for DPN.

Stability Test

The results showed that the fluorescence intensity of the Eu^{3+}-DPN-*DL*-histidine complex system at room temperature reached a maximum in 10 min after all the reagents were added. The fluorescence intensity remained stable for at least 24 h.

Application to Real Samples

Samples A-F were analyzed using the established fluorescence method. The results indicated that the DPN concentrations were 0.13 mg/L, 4.42 mg/L, 3.21 mg/L and 2.49 mg/L for samples A, B, D and F, respectively. For samples C and F, the DPN concentration was less than the LOD. To evaluate the accuracy of the established fluorescence method, we used our previously developed LC-MS/MS method [16] to analyze the DPN content in samples A-F, as shown in Table 1. It can be seen that the DPN concentrations in samples A, B, C, D, E and F were in good agreement between the two methods, however, DPN was not detected in samples C and E using both methods.

Luminescence Mechanism

It is apparent that the intrinsic emission peaks of Eu^{3+} were greatly enhanced when Gd^{3+} and CPB are added to Eu^{3+}-DPN-*DL*-histidine fluorescence system, as shown in Figs. 2 and 9. We thought there are several possible reasons for these fluorescence enhancement effects.

Firstly, from Fig. 9, it can be seen that the Eu^{3+}-DPN-*DL*-histidine system has an obvious absorption peak at about 335 nm, which is consistent with the excitation peak of the system. Fig. 9 also showed that when DPN- *DL*-histidine or DPN- *DL*-histidine-CPB were added to Eu^{3+} ion solution, the absorption of the Eu^{3+}-DPN-*DL*-histidine-CPB system was significantly increased, which indicated that a coordination compound between DPN-*DL*-histidine and Eu^{3+} ions was formed. Moreover, these results indicated that after the Eu^{3+}-DPN-*DL*-histidine complex absorbs light energy, Eu^{3+} can be excited to the $^5D_0$ level by intramolecular energy transfer from DPN to Eu^{3+}.

Secondly, the fluorescence intensity difference between spectra 1 and spectra 4 (Fig. 9) showed that the absorption can been intensified by added CPB into the solution. The result indicated that CPB might interact with Eu^{3+}, and lead to the energy transfer from CPB to Eu^{3+}. It may be explained why CPB can enhance the characteristic fluorescence of Eu^{3+} in Eu^{3+}-DPN-*DL*-histidine system (Fig. 2). Compared the absorption intensity of spectra 2 with spectra 4 in Fig. 9, it can be seen that the absorption intensity of DPN-*DL*-histidine-CPB system was increased appreciably after addition Eu^{3+} into the solution. The increasing adsorption was more increased greatly when Eu^{3+} together with CPB were added, which was in accordance with the fluorescence enhancement of the excitation spectra in the DPN- Eu^{3+}-CPB-*DL*-histidine system (Fig. 2), and the maximum absorption wavelength underwent a red shift from 320 nm to 335 nm.

Table 3 The recovery and precision of the method

Analyte	Added (mg/L)	Found (mg/L)[a]	Recovery (%)	RSD (%)	
				Intra-day[a]	Inter-day[b]
DPN	0.05	0.044±0.002	88.0	4.5	6.7
	1.0	0.87±0.05	87.0	5.7	8.9
	4.0	3.73±0.15	93.3	4.0	7.8

[a] Determined in 1 day, n=6

[b] n=3 replicates × 5 days

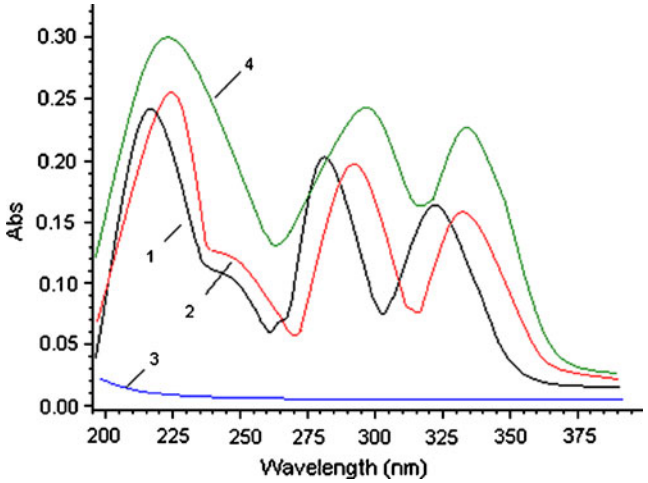

Fig. 9 Absorption spectra of the Eu^{3+}-DPN-*DL*-histidine system. *1*. DPN-*DL*-histidine; *2*. DPN-*DL*-histidine- CPB; *3*. Eu^{3+}; *4*. Eu^{3+}-DPN-*DL*-histidine-CPB. Conditions: Eu^{3+} 10.0 mg/L, Gd^{3+} 25.0 mg/L, DPN 2.0 mg/L, pH=9.0, *DL*-histidine 0.01%, CPB 0.05%

Thirdly, in Gd^{3+}-DPN- *DL*-histidine-CPB system, the characteristic emission of Gd^{3+} was not observed, as shown in Fig. 2. This is probably due to the fact that Gd^{3+} possesses a relatively stable half full shell and the luminescence level of Gd^{3+} is higher than the triplet state of DPN- *DL*-histidine-CPB in the Gd^{3+} complex. Therefore, the energy of DPN- *DL*-histidine-CPB cannot be transferred to Gd^{3+} by the intramolecular energy transfer. However, the addition of Gd^{3+} to Eu^{3+}- DPN- *DL*-histidine-CPB system, the intrinsic fluorescence of Eu^{3+} is enhanced, as shown in Fig. 2. We considered that there is the intermolecular energy transfer between Eu^{3+} and Gd^{3+} complexes. The energy absorbed by Gd^{3+} complex can be transferred to Eu^{3+} in Eu^{3+} complex, and enhances the fluorescence intensity of Eu^{3+}.

Conclusions

An accurate, simple, and sensitive method for the determinationn of DPN in human serum was developed using a Eu^{3+}-DPN-*DL*-histidine-NH_3-CPB fluorescence complex system. The fluorescence intensity was greatly enhanced by the addition of Gd^{3+} and CPB, which formed a Gd^{3+}-DPN-*DL*-histidine-NH_3-CPB complex system. The results showed that this improved method had a good limit of detection for DPN of 0.001 mg/L. This method can be applied to clinical and toxicological investigations. Compared with LC-MS/MS, the proposed method is cost effective, short analysis time and not using expensive instruments. Furthermore, the approach is a potentially useful analytical means for analysis trace DPN in various mixtures.

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