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ORIGINAL PAPER

Spectrofluorimetric Determination of Dopamine Using Chlorosulfonylthenoyltrifluoroacetone–Europium Probe

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Abstract A new spectrofluorimetric method was developed for the determination of trace amounts of dopamine (DA). Using chlorosulfonylthenoyltrifluoroacetone (CTTA)–europium ion (Eu^{3+}) as a fluorescent probe, in a buffer solution at pH=10.0, DA can remarkably enhance the fluorescence intensity of the CTTA-Eu^{3+} complex at λ= 612 nm; the enhanced fluorescence intensity of Eu^{3+} is proportional to the concentration of DA. Optimum conditions for the determination of DA were also investigated. The linear range and detection limit for the determination of DA were 5.0×10^{-8}~1.6×10^{-5} mol/l and 3.2×10^{-8} mol/l. This method is simple, practical and relatively free of interference from coexisting substances, and can be applied to assess DA in injection and human serum samples with good precision and accuracy.

Keywords Dopamine · Chlorosulfonylthenoyltrifluoroacetone · Europium · Spectrofluorimetry

Introduction

Dopamine (DA) is a typical neurotransmitter that plays an important role in brain functions. Extreme abnormalities in DA levels may result in some serious diseases such as Parkinsonism. The hydrochloride salt of dopamine is used in the treatment of shock, which may be caused by trauma, hearth attack, open heart surgery, heart failure, kidney failure and severe bacterial infections of the blood [1, 2]. Thus, a simple and exact method for the determination of DA is very important in pharmaceutical injections and clinical analysis. In recent years, development of new methods for determination of DA has received great interests by many researchers. Several methods, such as spectrophotometry [3, 4], spectrofluorimetry [2, 5–7], capillary electrophoresis [8], liquid chromatography [9], chemiluminescence [10] and electrochemical [1, 11–15] methods have been developed.

Chlorosulfonylthenoyltrifluoroacetone (CTTA), containing the β-diketonate configuration and sulfonyl chloride group, was a successful bifunctional ligand, which has been used as a fluorescent label for immunoassays [16, 17]. β-diketonate configuration is the ideal ligand for Eu^{3+}. A literature survey showed that β-diketonate configuration allows efficient energy transfer from ligand to Eu^{3+} with a high fluorescence quantum yield, large stokes shift, narrow emission bonds and a large fluorescence lifetime; hence, it avoids potential background fluorescent emission interferences from the biological background [18]. In our group, a lot of researches about using this kind of europium probes had been done, tetracycline analogues (the typical substances containing β-diketonate configuration, include tetracycline, doxycycline and oxytetracyline)–europium probes were mainly used for the determination of some bimolecular [19–22]. CTTA is a better ligand. The active chloride in sulfonyl chloride group can be covalent combine with the amino group in DA, in order to form the Eu^{3+}–CTTA–DA ternary complex. The possible reaction mechanism is shown in Fig. 1. Thus, we choose CTTA as the ligand of Eu^{3+} and also the effective ligand for DA in this work. Experimental results showed that the characteristic peak of Eu^{3+} at 612 nm could be greatly enhanced, and that the enhancement in fluorescence intensity was

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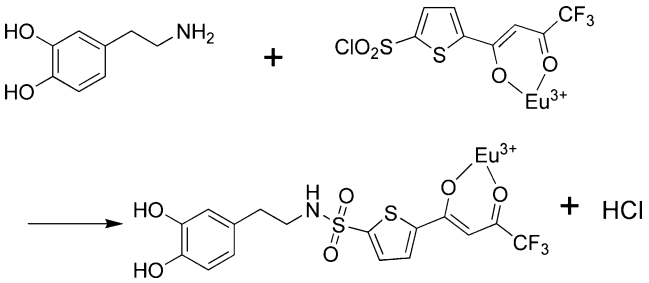

Fig. 1 A scheme of the reaction process

proportional to the concentration of DA. We also find that Triton X-100, a surfactant, can enhance the fluorescence intensity further. Therefore, a new method, with high sensitivity, for the spectrofluorimetric determination of DA has been established. This method is simple, relatively free of interference from coexisting substances and can be successfully applied to the determination of DA in injection and human serum samples.

Experimental

Apparatus

All fluorescence measurements were carried out on an RF-540 recording spectrofluorimeter (Shimadzu, Kyoto, Japan). A constant temperature apparatus (Shandong Yongxing Device Works, China) was used to make the temperature at 40.0±0.1°C. All pH measurements were made with a pHs-3C digital pH meter (Shanghai Leici Device works, China).

Materials

Dopamine hydrochloride (1.0×10^{-3} mol/l, Alfa Aesar) was used as a stock standard reagent for the determination of DA. The stock solution of Eu^{3+} (1.0×10^{-3} mol/l) was prepared by dissolving Eu_2O_3 (Shanghai Yuelong Chemical Plant, China) with 0.1 mol/l hydrochloric acid. The working standard solutions (1.0×10^{-5} mol/l for both DA and Eu^{3+}) were freshly prepared by appropriate dilution with doubly distilled demineralized water. 0.5% (*v*/*v*) Triton X-100 (Rohm and Haas Company) was used.

Tris–(hydroxymethyl) aminomethane (Tris) was purchased from Shanghai Chemical Reagent Company (China). 0.1 mol/l Tris–HCl (pH=10.0) buffer solution was used for this system.

Chlorosulfonylthenoyltrifluoroacetone (CTTA) was synthesized from 2-thenoyltrifluoroacetone (TTA, the Third Reagent Plant of Shanghai, China) according to the method reported by Ci et al. [17] and a solution of CTTA (1.0×10^{-3} mol/l) was prepared in dry acetone.

All chemicals used were of analytical-reagent grade or higher. Doubly distilled demineralized water was used for the preparation of the above solutions and for all determinations.

General procedure

Solutions were added to 10 ml color comparison tubes in the following order: 1.0 ml 0.1 mol/l Tris–HCl (pH=10.0) buffer solution, 0.8 ml 1.0×10^{-3} mol/l CTTA solution, 1.0 ml 0.5% (*v*/*v*) Triton X-100 solution, 1.5 ml 1.0×10^{-5} mol/l Eu^{3+} solution, and 1.5 ml 1.0×10^{-5} mol/l DA solution. The mixture was diluted to the mark with doubly distilled demineralized water and incubated for 40 min at 40.0°C. The fluorescence intensity was measured at λex/λem=385 nm/612 nm. The enhanced fluorescence intensity of CTTA-Eu^{3+} caused by DA was represented as $\Delta F=F-F_0$. Here F and F_0 are the fluorescence intensities of the systems with and without DA, respectively.

Results and discussion

Fluorescence spectra

The fluorescence excitation spectrum and emission spectrum of Eu^{3+}, Eu^{3+}–CTTA, Eu^{3+}–DA, Eu^{3+}–CTTA–DA, Eu^{3+}–CTTA–DA–TritonX-100 and Eu^{3+}–CTTA–TritonX-100 are shown in Fig. 2. The Eu^{3+}, Eu^{3+}–CTTA, Eu^{3+}–DA and Eu^{3+}–CTTA–TritonX-100 solutions showed little or no peak at 612 nm. The system of Eu^{3+}–CTTA–DA (curve 4) appears two characteristic peaks of Eu^{3+} at 590 and 612 nm, and they are the $^5D_0-^7F_1$ transition and $^5D_0-^7F_2$ transition of Eu^{3+}, respectively. Thus, the characteristic peaks of Eu^{3+} can be effectively excited by the coeffect on CTTA and DA. From curve 5 in Fig. 2, the fluorescence intensity of Eu^{3+}–CTTA–DA–TritonX-100 at 612 nm is much larger than that of Eu^{3+}–CTTA–DA system. Here TritonX-100 (a surfactant) offered the hydrophobic atmosphere, so that the microenvironment was changed in the fluorescent system and the non-radiative energy lost through O-H vibration of H_2O in the original Eu^{3+} complex and the collision of H_2O and O_2 in the solvent could be decreased greatly, which was more propitious to the coordination of Eu^{3+}–CTTA–DA system. Thus, the fluorescence intensity of Eu^{3+} at 612 nm can be effectively enhanced by the interactions of CTTA, DA and Triton X-100.

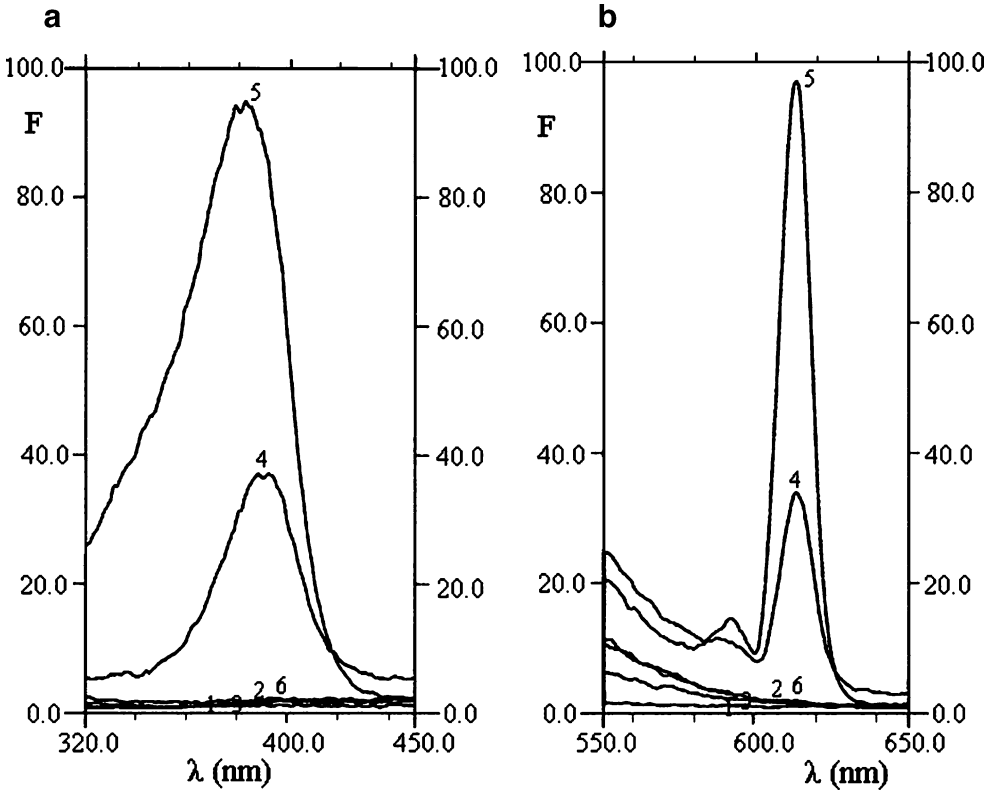

Fig. 2 Fluorescence excitation (**a**) and emission (**b**) spectra of 1:Eu^{3+} 2:Eu^{3+}–CTTA 3:Eu^{3+}–DA 4:Eu^{3+}–CTTA–DA 5:Eu^{3+}–CTTA–DA–TritonX-100 6:Eu^{3+}–CTTA–TritonX-100. A 10.0 ml solution contains CTTA (2.0×10^{-4} mol/l), Eu^{3+} (5.0×10^{-6} mol/l), DA (2.0×10^{-5} mol/l), TritonX-100 (0.1%) and Tris–HCl buffer solution (pH 10.0). The results were obtained after incubated 40 min at 40.0°C

Optimization of experimental conditions

Effect of pH

A series of 0.10 mol/l Tris–HCl buffer solutions with pH from 8.0 to 12.0 were measured at λex/λem=385 nm/612 nm. The pH of the medium had a significant effect on the fluorescence intensity of the system, as shown in Fig. 3. The experimental results showed that the ΔF reached a maximum and remained constant from pH=9.8 to pH=10.2. Therefore pH=10.0 with the using of 0.10 mol/l Tris–HCl buffer solution was selected for further study. As the volume of buffer solution added 0.8~1.2 ml Δ*F* reached a maximum and remained constant, then 1.0 ml buffer solution was added in the following experiments.

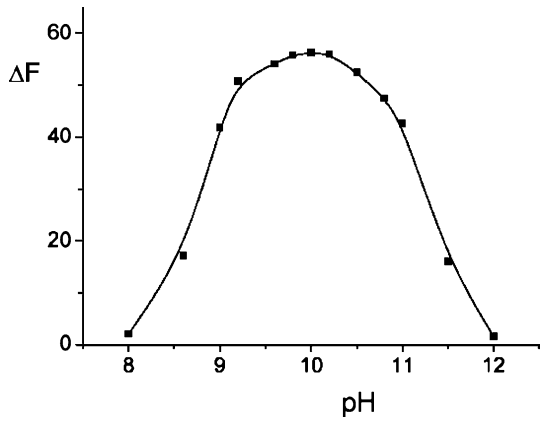

Fig. 3 Effect of pH on the enhanced fluorescence intensity (measured at λex/λem=385 nm/612 nm). A 10.0 ml solution contains CTTA (1.0×10^{-4} mol/l), Eu^{3+} (1.0×10^{-5} mol/l), DA (1.0×10^{-5} mol/l), TritonX-100 (0.05%) and Tris–HCl buffer solution (pH from 8.0 to 12.0). The results were obtained after incubated 30 min at 40.0°C

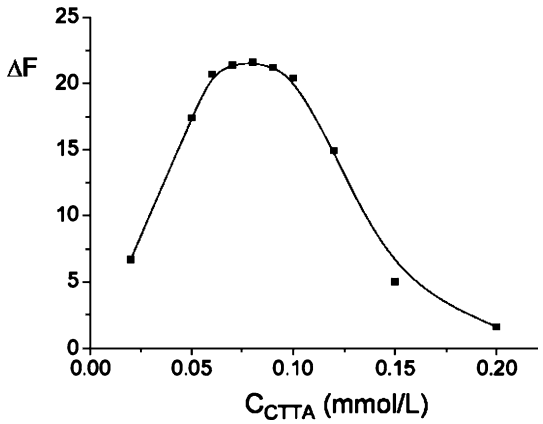

Fig. 4 Effect of the concentration of CTTA on the enhanced fluorescence intensity (measured at λex/λem=385 nm/612 nm). A 10.0 ml solution contains CTTA (0.02~0.2 mmol/l), Eu^{3+} (1.0×10^{-6} mol/l), DA (1.5×10^{-6} mol/l), TritonX-100 (0.05%) and Tris–HCl buffer solution (pH 10.0). The results were obtained after incubated 30 min at 40.0°C

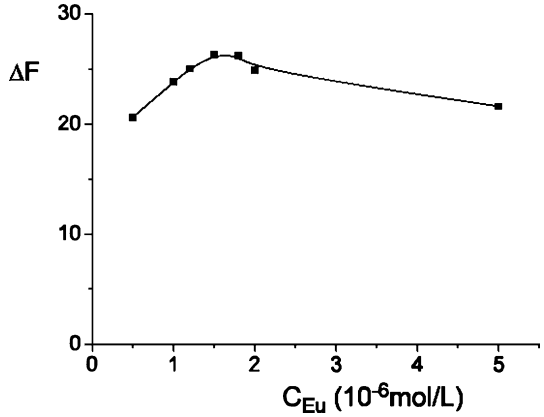

Fig. 5 Effect of the concentration of Eu^{3+} on the enhanced fluorescence intensity (measured at λex/λem=385 nm/612 nm). A 10.0 ml solution contains CTTA (8.0×10^{-5} mol/l), Eu^{3+} ($0.5\sim5.0\times10^{-6}$ mol/l), DA (1.5×10^{-6} mol/l), TritonX-100 (0.05%) and Tris–HCl buffer solution (pH 10.0). The results were obtained after incubated 30 min at 40.0°C

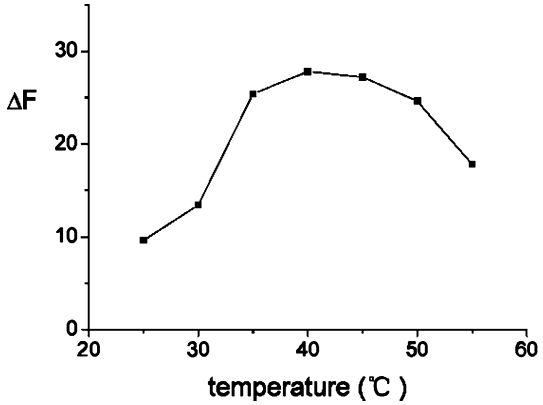

Fig. 6 Effect of reaction temperature on the enhanced fluorescence intensity (measured at λex/λem=385 nm/612 nm). A 10.0 ml solution contains CTTA (8.0×10^{-5} mol/l), Eu^{3+} (1.5×10^{-6} mol/l), DA (1.5×10^{-6} mol/l), TritonX-100 (0.05%) and Tris–HCl buffer solution (pH 10.0). The results were obtained after incubated 30 min at 25.0~55.0°C, respectively

Effect of the concentration of CTTA

The influence of the concentration of CTTA on the fluorescence intensities of the solutions is shown in Fig. 4. The results showed that ΔF reached a maximum and remained constant when the concentration of CTTA was 7.0×10^{-5} mol/l to 9.0×10^{-5} mol/l. Thus 8.0×10^{-5} mol/l CTTA was selected for further study.

Effect of the concentration of Eu3+

The amount of Eu^{3+} was also optimized, as shown in Fig. 5. The enhanced fluorescent intensity ΔF increased with the increasing amount of Eu^{3+} up to 1.5×10^{-6}mol/l and then decreased. Thus 1.5×10^{-6} mol/l Eu^{3+} was selected for further study.

Effect of the addition order of reagents

Adding the reagents in different orders had an influence on the F, F_0 and ΔF values. The experimental results showed that the following order was optimal: buffer, CTTA, Triton X-100, Eu^{3+} and DA. So this order was chosen in the following experiments.

Effect of reaction temperature

A series of the same solutions were made to react at the temperature ranging from 25.0 to 55.0°C, as shown in Fig. 6. The results showed that ΔF reached maximum when the solutions was incubated at 40.0, so 40.0°C was fixed as the reaction temperature in this study.

Effect of reaction time

The effect of reaction time was experimented. To complete the chelation reaction of the CTTA–Eu^{3+}–DA–TritonX-100 system at 40.0°C needed 30 min at least. The fluorescence intensity then remained constant within 1 h. Therefore the reaction was processed for 40 min at 40.0°C for further study.

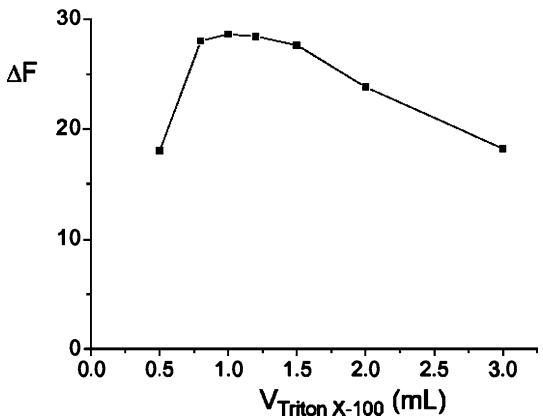

Fig. 7 Effect of TritonX-100 on the enhanced fluorescence intensity (measured at λex/λem=385 nm/612 nm). A 10.0 ml solution contains CTTA (8.0×10^{-5} mol/l), Eu^{3+} (1.5×10^{-6} mol/l), DA (1.5×10^{-6} mol/l), TritonX-100 (0.025~0.15%) and Tris–HCl buffer solution (pH 10.0). The results were obtained after incubated 40 min at 40.0°C

Table 1 Effect of coexisting substances

Coexisting substances	Concentration (mol/l)	*F*%	Coexisting substances	Concentration (mol/L)	*F*%
$Na^+(Cl^-)$	5.0×10^{-3}	1.85	Cytosine	2.2×10^{-6}	3.19
$K^+(Cl^-)$	1.0×10^{-4}	4.03	L-Histidine	1.0×10^{-4}	−0.67
$Cu^{2+}(SO_4^{2-})$	5.0×10^{-6}	−2.01	L-Cystine	4.0×10^{-6}	−1.38
$Cd^{2+}(Cl^-)$	1.0×10^{-6}	−2.35	L-Lysine	1.9×10^{-5}	3.11
Mn^{2+}	2.5×10^{-6}	−1.21	L-Tyrosine	1.0×10^{-4}	0.67
Cr^{3+}	3.8×10^{-8}	4.32	L-Leucine	1.0×10^{-4}	−3.36
Guanine	1.6×10^{-7}	−4.66	Glycin	1.0×10^{-4}	3.70
Adenine	3.6×10^{-6}	−3.70	Tryptophan	2.5×10^{-5}	4.87
Thymine	3.7×10^{-5}	−4.49	Methionine	2.0×10^{-4}	2.69

Effect of the surfactant

The influence of the surfactant was experimented (including TritonX-100, sodium dodecyl sulfate, and hexadecyl trimethyl ammonium bromide), and TritonX-100 were the best one to enhance the fluorescence intensities of the system. The influence of the amount of TritonX-100 on the enhanced fluorescence intensities is shown in Fig. 7. The experimental results showed that ΔF reached a maximum and remained constant when TritonX-100 [0.5% (*v*/*v*)] was added from 0.8 to 1.2 ml. Thus 1.0 ml 0.5% (*v*/*v*) TritonX-100 was used for further study.

Effect of coexisting substances

A systematic study of the effects of various coexisting substances on the fluorescence in the determination of DA (1.5×10^{-6} mol/l) was carried out under the optimal conditions. The criterion for interference was fixed at a±

Table 2 Comparison of some methods for the determination of DA

Method	Linear range	Detection limit	References
Spectrophotometry	0.2~48.0 μg/ml[a]		[3]
Spectrophotometry	2.0×10^{-4}~6.0×10^{-3} mol/l	3.0×10^{-5} mol/l	[4]
Spectrofluorimetry	1.0×10^{-5}~1.0×10^{-4} mol/l	3.7×10^{-6} mol/l	[2]
Spectrofluorimetry	0.05~0.6 μg/ml[b]	0.04 μg/ml[c]	[5]
Spectrofluorimetry	0.02~0.06 μg/ml[d]	18 ng/ml[e]	[6]
Spectrofluorimetry	0.10~3.50 μg/ml[f]	0.082 μg/ml[g]	[7]
Capillary electrophoresis	10^{-9}~10^{-6} mol/l		[8]
Liquid chromatography	5×10^{-10}~5×10^{-7} mol/l	1.95×10^{-11} mol/l	[9]
Chemiluminescence	30~100 μg/l and 400~3,000 μg/l[h]	5 μg/l[i]	[10]
Electrochemical method	5.0×10^{-8}~1.0×10^{-5} mol/l	1.0×10^{-8} mol/l	[1]
Electrochemical method	1.6×10^{-8}~6×10^{-4} mol/l	8×10^{-9} mol/l	[11]
Electrochemical method	1.0×10^{-5}~1.0×10^{-4} mol/l	2.0×10^{-6} mol/l	[12]
Electrochemical method	2×10^{-4}~9.5×10^{-4} mol/l	1.51×10^{-5} mol/l	[13]
Electrochemical method	6.60×10^{-5}~3.70×10^{-2} mol/l		[14]
Electrochemical method	6.6×10^{-6}~2.41×10^{-5} mol/l	6.4×10^{-8} mol/l	[15]
This method	5.0×10^{-8}~1.6×10^{-5} mol/l	3.2×10^{-8} mol/l	

[a] 0.2~48.0 μg/ml is equivalent to 1.05×10^{-6}~2.53×10^{-4} mol/l.
[b] 0.05~0.6 μg/ml is equivalent to 2.64×10^{-7}~3.16×10^{-6} mol/l.
[c] 0.04 μg/ml is equivalent to 2.11×10^{-7} mol/l.
[d] 0.02~0.06 μg/ml is equivalent to 1.05×10^{-7}~3.16×10^{-7} mol/l.
[e] 18 ng/ml is equivalent to 9.49×10^{-8} mol/l.
[f] 0.10~3.50 μg/ml is equivalent to 5.27×10^{-7}~1.85×10^{-5} mol/l.
[g] 0.082 μg/ml is equivalent to 4.32×10^{-7} mol/l.
[h] 30~100 μg/l and 400~3000 μg/l is equivalent to 1.58×10^{-7}~5.27×10^{-7} mol/l and 2.11×10^{-6}~1.58×10^{-5} mol/l.
[i] 5 μg/l is equivalent to 2.64×10^{-8} mol/l.

Table 3 Determination of DA in injection samples (the labeled value of each ampoule is 20 mg/2 ml)

Samples	Developed method (mg/ampoule)			Pharmacopoeia method [23] (mg/ampoule)	Relative error (%)
	Found	Average	RSD (%)		
Injection 1	21.97, 21.61, 21.69	21.76	0.87	20.92	104.0
Injection 2	20.74, 20.26, 20.38	20.46	1.22	20.75	98.6
Injection 3	21.69, 21.17, 22.45	21.77	2.96	21.00	103.7

Injection 1: Guangzhou Mingxing Pharmaceutical Co. Ltd. of China (060607)
Injection 2: Shanghai Harvest Pharmaceutical Co. Ltd. of China (980504)
Injection 3: Manufacturing Branch of Jiangsu Yabang Pharm Group Co. Ltd. of China (050127)

5% variation in the average fluorescence intensity calculated for the established level of DA. The experimental results are shown in Table 1. F% in Table 1 was the result of $(F'-F)/F$. Here F' and F are the fluorescence intensities of the systems with and without the coexisting substance, respectively. Most of the coexisting substances were found to have no influence.

Analytical application

Linear range and limit of detection

Under the optimal experimental conditions, there is a linear relationship between fluorescence intensity and DA concentration in the range of 5.0×10^{-8}~5.0×10^{-6} mol/l and 5.0×10^{-6}~1.6×10^{-5} mol/l with the correlation coefficient of 0.998 and 0.999. The regression equation are $\Delta F=19.5\times C+1.35$ and $\Delta F=7.47\times C+59.9$, respectively. The limit of detection was determined to be 3.2×10^{-8} mol/l when the standard deviation was 0.207 as obtained from a series of 10 reagent blanks. In Table 2, the characteristics of the developed method are compared with those of other different methods reported previously for the determination of DA. The sensitivity of the proposed method is better than that of the spectrophotometry and spectrofluorimetry.

Determination of dopamine in injection samples

The method developed here was used to determine DA in injection samples. To perform an assay on DA in injection samples, they must be diluted appropriately within the linear range of the determination of DA, and then the sample solutions were analyzed by the method developed above, using the standard calibration method. The same samples were also determined by the China pharmacopoeia method [23]. The results after conversion from each sample by two different analysis techniques are given in Table 3. From Table 3, it can be seen that the two methods give similar results. Thus, the method developed here can be successfully applied to assess DA in injection samples.

Determination of dopamine in human serum samples

The method developed here was used to determine DA in human serum samples. 0.1 ml human serum (from a normal person offered from the attaching hospital of our school) was directly used as a background, then mixed with 1.00×10^{-5} mol/l of the standard DA solutions for DA determination as the method developed above. The recoveries of DA were from 97.1 to 102.9%, as shown in Table 4. Thus, the developed method can be applied to assess DA in human serum samples.

Table 4 Determination of DA in human serum samples

Samples	Added (10^{-5} mol/l)	Found (10^{-5} mol/l)	Average (10^{-5} mol/l)	RSD (%)	Recovery (%)
Human serum 1	1.00	1.000, 0.992, 0.963	0.985	1.98	98.5
Human serum 2	1.00	1.007, 1.044, 1.036	1.029	1.89	102.9
Human serum 3	1.00	0.947, 0.976, 0.990	0.971	2.26	97.1

Conclusion

In this work, a new spectrofluorimetric method, using CTTA–Eu^{3+} as a fluorescent probe, was developed for the determination of DA. Spectrophotometric and spectrofluorimetric method are the common methods used in pharmaceutical and clinical analysis and easy to carry out automatic analysis. The sensitivity of the spectrofluorimetric method developed here is better than that of the other spectrophotometic and spectrofluorimetric methods. This method is simple, relatively free of interference from coexisting substances and can be successfully used to determine DA in injection and human serum samples with good precision and accuracy. Thus, it can be used as an alternative to assess DA in pharmaceutical and clinical analysis.

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