

# The fluorescence enhancement effect of Tb–Gd–adenosine triphosphate–phen system and its analytical application

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## Abstract

It is found that the fluorescence of Tb–adenosine triphosphate (ATP)–phenanthroline (phen) system can be enhanced by Gd<sup>3+</sup>. The fluorescence enhancement of the Tb–Gd–ATP–phen system is considered to originate from intramolecular and intermolecular energy transfers, and the energy-insulating sheath effect of Gd–ATP–phen complex. In addition, a new energy transfer pathway in Tb–ATP–phen system is proposed. As a mediator, phen can transfer the energy absorbed by ATP to Tb<sup>3+</sup> through the stacking action between aromatic ring of phen and purine ring of ATP. The proposed method has been used to determine trace amount of ATP. The detection limit is  $5.4 \times 10^{-9}$  mol/l, which is about 40 times lower than that of the Tb–ATP–phen system. The proposed method is one of the most sensitive fluoremetries of ATP.

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**Keywords:** Co-luminescence effect; ATP; Fluorescence

## 1. Introduction

The energy cycle of all living organisms involves ATP, which captures the chemical energy released by the metabolism of nutrients and makes it available for cellular functions such as muscle contraction and transmission of nerve messages. So it is significant to develop a simple, fast, sensitive and reliable method for determination of ATP. Fluorometry is a common method for the determination, the principle of which was that ATP reacts with chloroethanal [1,2], glyoxal [3], bromoethanal [4], glucose and NADP [5] and fluoresceinase [6], to form fluorescent compounds. But most of these methods are complicated and time-consuming.

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 used as fluorescence probes to study biological molecules.

While studying the Eu<sup>3+</sup> multi-complex system, a fluorescence enhancement phenomenon was found when some of rare earth ions were added in 1986. This enhancement

effect was named the co-luminescence effect. This method has been used for the characterization of rare earth elements [7–16] and nucleic acids [17].

In present paper, a new co-luminescence system of terbium–gadolinium–ATP–phen was found and studied. Under the optimum condition, we investigated the fluorescence enhancement of Tb–Gd–ATP–phen system and their interaction mechanism in detail, and used this probe for the determination of ATP.

## 2. Experimental

### 2.1. Chemicals

Stock standard solutions (0.01 mol/l) of rare earth ions were prepared by dissolving the corresponding oxides (99.9%) in hydrochloric acid and diluting with deionized water.

Phen solution ( $1.00 \times 10^{-3}$  mol/l) is prepared by dissolving 0.0198 g phenanthroline in 100 ml volumetric flask with deionized distilled water.

The hexamethylenetetramine (HMTA) solution (10%) was made by dissolving 10 g of HMTA in 100 ml distilled water. The pH was adjusted to 7.0 with 6 mol/l HCl.

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The ATP solution ( $1.00 \times 10^{-3}$  mol/l) was prepared by dissolving 0.0603 g ATP in 100 ml volumetric flask with deionized distilled water. This solution needed to be stored at 0–4 °C.

## 2.2. Apparatus

All fluorescence measurements were made with Hitachi 850 spectrofluorimeter. All absorption spectra were recorded with an UV-240 spectrophotometer (Shimadzu). All pH measurements were made with a Delta 320-S pH meter (Mettler Toledo).

## 2.3. Procedure

To a 25 ml test tube, solutions were added in the following order:  $\text{Tb}^{3+}$ , ATP, phen,  $\text{Gd}^{3+}$ , HMTA. The mixture was diluted to 10 ml with water. The fluorescence intensity was measured in a 1 cm quartz cell; the excitation and emission slits were both 10 nm.

## 3. Result and discussion

### 3.1. Fluorescence spectra

Excitation and emission spectra of Tb (1), Tb-ATP (2), Tb-phen (3), Tb-ATP-phen (4) and Tb-Gd-ATP-phen (5) systems are shown in Fig. 1. From this figure, it can be seen that the systems of (3)–(5) have the same excitation peak at 300 nm and two emission peaks at 490 and 545 nm of  $\text{Tb}^{3+}$ , corresponding to the  $^5\text{D}_4\text{--}^7\text{F}_6$  and  $^5\text{D}_4\text{--}^7\text{F}_5$  transitions of  $\text{Tb}^{3+}$ , respectively. The fluorescence intensity at 545 nm is the strongest. The fluorescence spectrum of Tb-ATP-phen system is similar to that of Tb-Gd-ATP-phen system, but the fluorescence intensity of the system is much enhanced when  $\text{Gd}^{3+}$  is added to the Tb-ATP-phen system; this is a newly found co-luminescence system.

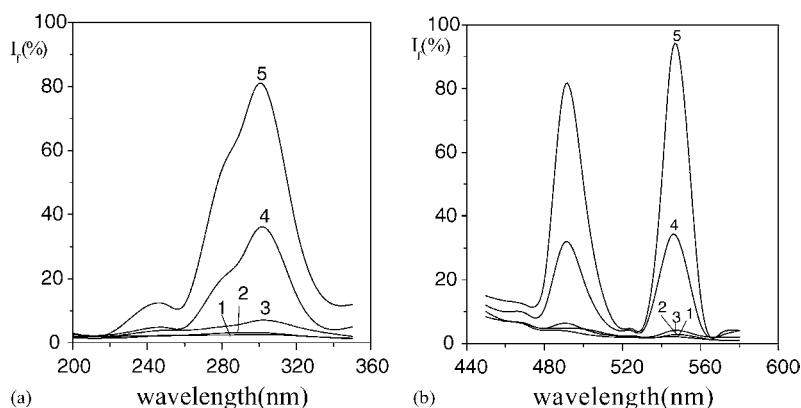


Fig. 1. Excitation (a) and emission (b) spectra: (1) Tb; (2) Tb-ATP; (3) Tb-phen; (4) Tb-ATP-phen; (5) Tb-Gd-ATP-phen. Conditions— $\text{Tb}^{3+}$ :  $2.0 \times 10^{-5}$  mol/l;  $\text{Gd}^{3+}$ :  $1.0 \times 10^{-4}$  mol/l; phen:  $2.0 \times 10^{-4}$  mol/l; ATP:  $1.0 \times 10^{-5}$  mol/l; HMTA: pH 7.0.

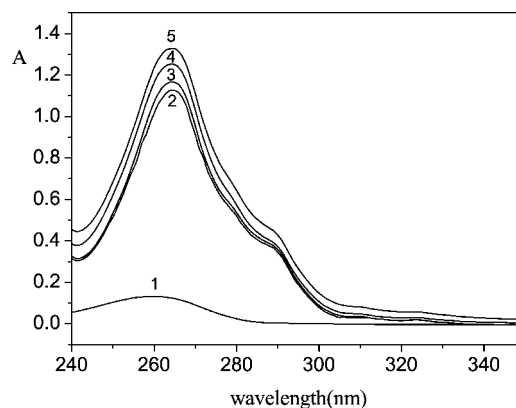


Fig. 2. Absorption spectra: (1) ATP; (2) phen; (3) ATP-phen; (4) Tb-ATP-phen; (5) Tb-Gd-ATP-phen. Conditions— $\text{Tb}^{3+}$ :  $2.0 \times 10^{-5}$  mol/l;  $\text{Gd}^{3+}$ :  $1.0 \times 10^{-4}$  mol/l; phen:  $2.0 \times 10^{-4}$  mol/l; ATP:  $1.0 \times 10^{-5}$  mol/l; HMTA: pH 7.0.

### 3.2. The formation of complexes and luminescence mechanism

#### 3.2.1. The formation and composition of the ternary complex

From Fig. 2, it can be seen that the absorption spectra of phen, Tb-ATP-phen and Tb-Gd-ATP-phen are similar. And the absorption intensity of ATP is very weak. Therefore, this indicated that the ultraviolet absorption of the Tb-ATP-phen and Tb-Gd-ATP-phen systems mainly came from that of phen. After adding  $\text{Tb}^{3+}$  and ATP, the absorption intensity of phen can be increased. It can prove the formation of Tb-ATP-phen ternary complex.

The composition of the complex has been examined by the molar ratio method and the results are that the ratio of Tb:ATP:phen is 2:1:6. We also compared the fluorescent intensities of the Tb-phen systems with AMP, ADP or ATP. It can be found that their fluorescent intensities are  $\text{ATP} \gg \text{ADP} > \text{AMP}$ . So we consider that the binding site of ATP with Tb is mainly on the third  $\text{PO}_4^{3-}$  of ATP.

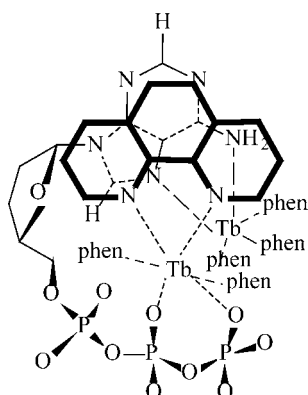


Fig. 3. The structure of Tb-ATP-phen complex.

Yamauchi et al. has done many researches on the stacking action of chelators in metal ion's ternary complex [18,19]. Results showed that aromatic ring stacking action is a special action form between the chelators in the complex. The bigger aromatic ring of stacking chelator is, the stronger the stacking action is, and the higher is the stability of formed complex. Up to now, there has study a series of aromatic carboxylic acid, such as nucleoside, amino acids. In this work, the  $^1\text{H}$  NMR spectra are measured and the results show that the signal peak of ATP moves to the upfield after the addition of phen, indicating that there exist the stacking action between aromatic ring of phen and purine ring of ATP in Tb-ATP-phen complex. The frame of this complex is shown in Fig. 3.

For the same reason, the Gd-ATP-phen complex can also form in the Tb-Gd-ATP-phen system. From Fig. 4, it can be seen that the resonance light scattering intensity of Tb-Gd-ATP-phen system is considerably stronger than that of Tb-ATP-phen system, which indicates that

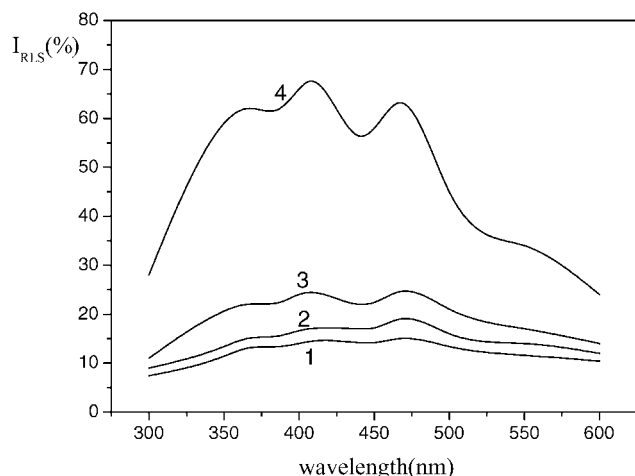
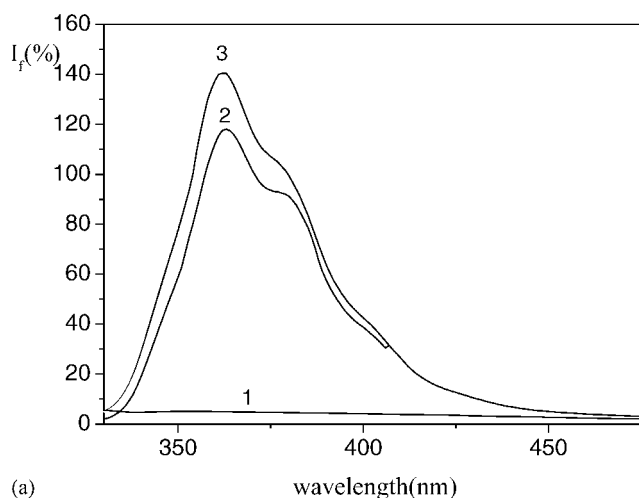


Fig. 4. Resonance light scattering spectra: (1) Tb-ATP; (2) Tb-phen; (3) Tb-phen-ATP; (4) Tb-Gd-phen-ATP. Conditions— $\text{Tb}^{3+}$ :  $2.0 \times 10^{-5}$  mol/l;  $\text{Gd}^{3+}$ :  $1.0 \times 10^{-4}$  mol/l; phen:  $2.0 \times 10^{-4}$  mol/l; ATP:  $1.0 \times 10^{-5}$  mol/l; HMTA: pH 7.0.

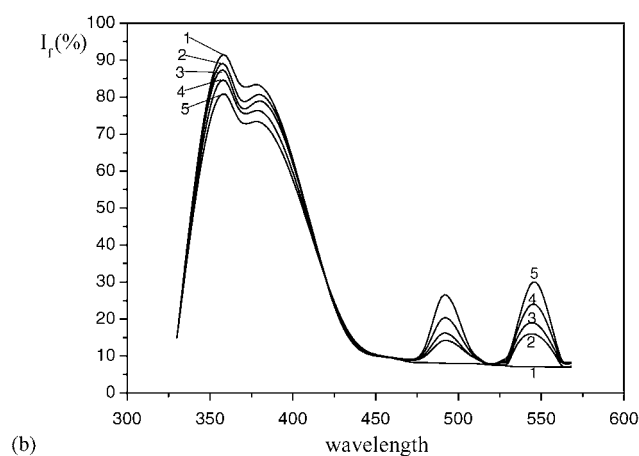
two complexes aggregate together and form a large congeries. In the congeries, each of Tb-ATP-phen complex molecules is surrounded by many Gd-ATP-phen complex molecules.

### 3.2.2. The energy transfer in the Tb-Gd-ATP-phen system

In Tb-ATP-phen system, the ultraviolet absorption of the Tb-ATP-phen systems mainly came from that of phen. And the absorption intensity of ATP is very weak, but it can enhance the absorption of phen. This indicated that there was the interaction between ATP and phen. From Fig. 5(a), it can be seen that ATP itself has no fluorescence, and phen has fluorescence at 362 nm. Whereas the fluorescent intensity of phen can be enhanced by ATP. This indicated that there is the energy transfer between ATP and phen, i.e. the energy absorbed by ATP can be transferred to phen, and thus enhanced the fluorescent intensity of phen. This conclusion is



(a)



(b)

Fig. 5. (a) The comparison of fluorescence spectra: (1) ATP; (2) phen; (3) ATP-phen. Conditions—phen:  $2.0 \times 10^{-4}$  mol/l; ATP:  $1.0 \times 10^{-5}$  mol/l; HMTA: pH 7.0. (b) The effect of  $\text{Tb}^{3+}$  concentration on the fluorescence intensity of Tb-ATP-phen system, (1) 0.0 mol/l  $\text{Tb}^{3+}$ ; (2)  $5 \times 10^{-6}$  mol/l  $\text{Tb}^{3+}$ ; (3)  $1.0 \times 10^{-5}$  mol/l  $\text{Tb}^{3+}$ ; (4)  $1.5 \times 10^{-5}$  mol/l  $\text{Tb}^{3+}$ ; (5)  $2 \times 10^{-5}$  mol/l  $\text{Tb}^{3+}$ . Conditions— $\text{Tb}^{3+}$ :  $2.0 \times 10^{-5}$  mol/l; phen:  $2.0 \times 10^{-4}$  mol/l; ATP:  $1.0 \times 10^{-5}$  mol/l; HMTA: pH 7.0.

Table 1  
Interference from foreign substances

Foreign substance	Concentration coexisting ( $\times 10^{-4}$ mol/l)	Change of $I_f$ (%)
Mg <sup>2+</sup> , SO <sub>4</sub> <sup>2-</sup>	0.4	-4.96
Al <sup>3+</sup> , NO <sub>3</sub> <sup>-</sup>	1.0	3.8
K <sup>+</sup> , Cl <sup>-</sup>	1.0	-2.5
NH <sub>4</sub> <sup>+</sup> , Cl <sup>-</sup>	3.5	2.34
Mn <sup>2+</sup> , SO <sub>4</sub> <sup>2-</sup>	0.2	-4.0
Na <sup>+</sup> , SO <sub>4</sub> <sup>2-</sup>	6.8	4.8
Ba <sup>2+</sup> , Cl <sup>-</sup>	0.8	4.78
Al <sup>3+</sup> , Cl <sup>-</sup>	4.0	3.33
Fe <sup>3+</sup> , Cl <sup>-</sup>	0.2	1.6
Fe <sup>3+</sup> , SO <sub>4</sub> <sup>2-</sup>	0.2	4.9
Na <sup>+</sup> , Cl <sup>-</sup>	6.0	-4.48
Na <sup>+</sup> , CO <sub>3</sub> <sup>2-</sup>	13.0	3.47
Zn <sup>2+</sup> , Cl <sup>-</sup>	1.0	-4.05
Ca <sup>2+</sup> , Cl <sup>-</sup>	6.3	2.34
L-Ala	0.9	4.31
L-Asp	0.1	3.37
L-Arg	2.35	3.9
DL-Thr	0.65	4.55
L-Tyr	0.1	4.89
DL-Tyr	0.3	3.45
L-Phe	2.05	3.32
L-His	0.7	4.33
L-Cys	0.2	4.76
HCl-Lys	0.5	4.88
BSA	0.5	3.83
HSA	0.7	3.98

Conditions—Tb<sup>3+</sup>:  $2.0 \times 10^{-5}$  mol/l; Gd<sup>3+</sup>:  $1.0 \times 10^{-4}$  mol/l; phen:  $1.5 \times 10^{-5}$  mol/l; ATP:  $1.0 \times 10^{-6}$  mol/l; HMTA: pH 7.0.

the same to that of absorption spectra. We thought that this transfer is due to the stacking action between aromatic ring of phen and purine ring of ATP. Therefore, in Tb-ATP-phen system, phen can transfer the energy absorbed by both phen and ATP to Tb<sup>3+</sup>.

From Fig. 5(b), it can be seen that Gd-ATP-phen complex emits ultraviolet fluorescence at 360 and 380 nm. After continuously adding Tb<sup>3+</sup> to Gd-ATP-phen complex, the fluorescence of Gd complex is gradually weakened and that of Tb<sup>3+</sup> is gradually enhanced. It is considered that Gd<sup>3+</sup> possesses a relatively stable half-full 4f shell and the luminescence level of Gd<sup>3+</sup> is higher than the triplet state of ATP-phen in the gadolinium complex, so the energy of ATP-phen cannot be transferred to Gd<sup>3+</sup> by the intramolecular energy transfer. Because the distance between Gd-ATP-phen and Tb-ATP-phen complexes is very small, thus the energy of ATP-phen in the Gd-ATP-phen complex can be transferred to Tb<sup>3+</sup> in the Tb-ATP-phen

system through intermolecular energy transfer. As the concentration of the gadolinium complex is much greater than that of the terbium complex, the fluorescence intensity of Tb<sup>3+</sup> is considerably enhanced. In addition, the surrounding gadolinium complex can act as an energy-insulating sheath, which can prevent collision with water molecules and decrease the energy loss of terbium complex, thus increasing the fluorescence quantum yield of terbium complex considerably.

### 3.3. Optimization of analytical procedure and its application

#### 3.3.1. Optimum conditions for the reaction

The experiments show that the optimum conditions for the determination of ATP are as follows: 1.0 ml of 10% HMTA buffer (pH 7.0),  $2.0 \times 10^{-5}$  mol/l Tb<sup>3+</sup>,  $1.0 \times 10^{-4}$  mol/l Gd<sup>3+</sup> and  $2.0 \times 10^{-4}$  mol/l phen.

#### 3.3.2. Tolerance of foreign substances

The interference of foreign substances was tested and shown in Table 1. It was found that these foreign substances had little effect on the determination of ATP under the permission of 5% error.

#### 3.3.3. Calibration curve and detection limit

Under the optimum condition defined, the calibration graphs for ATP was obtained and showed in Table 2. It can be seen that there is a linear relationship between the fluorescence intensity of the system and the concentration of ATP. The limit of detection is  $5.4 \times 10^{-9}$  mol/l, which is about 40 times lower than that of the Tb-ATP-phen system [20]. The proposed method is one of the most sensitive fluoremetries of ATP. In addition, the dynamic range of this method is over one order of magnitude larger than that of the latter.

#### 3.3.4. Sample determination

In recovery tests, the standard addition method was used. The results are shown in Table 3. It can be seen that the method is satisfactory.

Otherwise, actual sample of ATP was also tested by standard addition method. Its process was as follows: take four slices of ATP-disodium salt tablets (about 20 mg ATP-disodium salt per tablet), grind into fine and even powder, then weigh 0.0880 g and dissolve in 500 ml of distilled water. Shake and allow standing for a few minutes, then taking the upper clear solution for analysis. Determination

Table 2  
Analytical parameters of this method

Sample	Linear range (mol/l)	Linear regression equation (mol/l)	$r^a$	LOD <sup>b</sup> (mol/l)
ATP	$1 \times 10^{-7}$ to $5 \times 10^{-5}$	$I_f = 15.6 + 1.07 \times 10^7 C$	0.9997	$5.4 \times 10^{-9}$

<sup>a</sup> Correlation coefficient ( $n = 11$ ).

<sup>b</sup> Limit of detection ( $S/N = 3$ ).

Table 3  
Recoveries of ATP from synthetic samples

Sample	ATP added ( $\times 10^{-6}$ mol/l)	ATP found ( $\times 10^{-6}$ mol/l)	Average ( $\times 10^{-6}$ mol/l)	R.S.D. (%)	Recovery (%)
1	0.5	0.502, 0.502, 0.507, 0.506, 0.506	0.504	0.24	100.9
2	5.0	5.04, 5.09, 5.04, 5.06, 5.02	5.05	2.6	101

Table 4  
Determination of ATP in the tablet

ATP (ultraviolet method) (%)	ATP (this method) (%)	Average (%)	R.S.D. (%)	Error (%)
7.88	7.82, 8.01, 8.12, 7.95, 7.78	7.94	0.14	−0.76

result of ATP using this method was shown in Table 4. Compared to the result of ultraviolet spectrophotometry, the result of this method was satisfactory.

#### 4. Conclusion

It is found that the fluorescence of Tb–ATP–phen system can be enhanced by  $Gd^{3+}$ . This is a new co-luminescence system. This system is studied in detail and is applied to determine ATP. The detection limit is  $5.4 \times 10^{-9}$  mol/l, which is about 40 times lower than that of the Tb–Gd–ATP–phen system. The proposed method is one of the most sensitive fluorimetries of ATP. In addition, the dynamic range of this method is over one order of magnitude larger than that of the Tb–ATP–phen system. The studies indicated that in the Tb–Gd–ATP–phen system, there is both Tb–ATP–phen and Gd–ATP–phen complexes, they aggregate together and form a large congeries. For Tb–ATP–phen complexes, phen can transfer the energy absorbed by both phen and ATP to  $Tb^{3+}$ . The energy absorbed by Gd–ATP–phen complex can be transferred to  $Tb^{3+}$  in Tb–ATP–phen complex by inter-molecular energy transfers, and the energy-insulating sheath effect of Gd–ATP–phen complex can reduce the energy loss for collision with water molecule. Therefore the fluorescence intensity can be obviously enhanced.

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