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ORIGINAL PAPER

Study on the New Fluorescence Enhancement System of Tb –N -(2 - Pyridinyl) Ketoacetamide-Et_3N-Zn and its Application

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Abstract A sensitive fluorescence enhancement system was developed for the determination of zinc (II). The fluorescence intensity of the Tb- N- (2 - Pyridinyl) ketoacetamide (PKA) system was greatly enhanced by the addition of triethylamine (Et_3N) and zinc nitrate in the methanol solution. The excitation and emission wavelengths were 329 nm and 546 nm, respectively. Under optimal conditions, the fluorescence intensities varied linearly with the concentration of Zn^{2+} in the range of $8.0 \times 10^{-7} - 5.0 \times 10^{-6}$ M with a detection limit of 9.9×10^{-8} M. The interferences of some substances were described. This method was applied to the determination of amounts of Zn^{2+} in soybean, rice, and wheat, respectively. The results showed that the proposed procedure is a high selective, simple, and rapid method to the determination of Zn^{2+} ion. The mechanism of fluorescence enhancement was also studied.

Keywords N- (2 - Pyridinyl) ketoacetamide · Triethylamine · Terbium (III) · Zinc (II) · Fluorescence enhancement · Methanol

Introduction

In recent years, there has been a growing need for developing highly sensitive and selective probes for the detection of metal ions in biological and environmental samples. A variety of divalent metal ions are known to be involved in the structural, catalytic, and regulatory aspects of the biological system, and some such metal ions serve as prognostics of certain human diseases [1]. For example, Cu^{2+}, Zn^{2+}, and Fe^{2+} have been found to be involved in aggregating β-amyloid peptides during the onset of Alzheimer's disease [2]. However, due to the lack of metal ion specific probes, the relative contribution of one type of metal ion versus the other in causing the disease is not clearly understood. The inability to differentiate among different types of divalent metal ions in biological samples has been one of the major impediments in the area of bio-analytical chemistry. Although there has been some success in detection of biologically significant metal ions by developing fluorescence probes (e.g., fura-2 for Ca^{2+}), most of the probes exhibit cross-reactivities for other metal ions [3]. This is not surprising since both physical and electronic properties of these metal ions are not too disparate, and they tend to exhibit comparable binding affinities with their cognate chelating agents.

Due to diversity in functional roles of Zn^{2+} in biological system (viz., DNA synthesis, apoptosis, structural motifs in proteins, enzyme co-factors, etc.), we became interested in the determination of Zn^{2+}. In this endeavor, we noted that several methods have been described for the determination of Zn^{2+} using detection techniques such as molecular spectrophotometry [4–7], atomic absorption spectrometry [8–10], and chemiluminescence's analysis [11]. However, most of these methods are lacking in sensitivity and require pretreatment of the samples and sometimes, a long incubation period. To date, there has been no report on using the β – diketonate ligands-terbium complex as a fluorescence probe for the determination of Zn^{2+}. These lanthanide chelates have characteristics of the large Stokes shift, narrow emission bands and long lifetime hence to avoid potential background fluorescent emission interferences from the biological matrix. Presently, we have designed a series of

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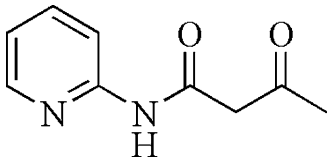

Fig. 1 Structure of N - (2-Pyridinyl) ketoacetamide (PKA)

polyfunctional ligands having both selective ability to coordinate transition metal ions and good luminescence properties with lanthanide ions, by providing proper conjugate absorption groups suitable for energy transfer, which could be used as a luminescent device. In the present work, we synthesized N – (2 Pyridinyl) ketoacetamide (PKA, Fig. 1) containing a β-diketonate configuration. As elaborated below, its Tb^{3+} complex emits intrinsic fluorescence of Tb^{3+} when excited at 329 nm in methanol solution. After Zn^{2+} was introduced into the above solution, the emission peak at 546 nm was enhanced strongly. We investigated the possibility of the enhancement of the Tb^{3+} sensitized fluorescence by Zn^{2+}. Experimental results indicate that the enhancement comes from the intermolecular energy transfer in ternary complex. The other reagents such as La^{3+}, Lu^{3+}, Gd^{3+}, Y^{3+}, Al^{3+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Na^{+}, and K^{+} had not the same effect as Zn^{2+} had and some of them even quenched the fluorescence of Tb^{3+}. According to this character, a new fluorescence method with high sensitivity and selectivity is established for the determination of Zn^{2+} using PKA-Tb^{3+} as a fluorescent probe. This method is easily carried out, affords good precision and accuracy and has been successfully applied to the determination of Zn^{2+} in food samples.

Experimental section

Apparatus

Elemental analyses were determined on an Elementar Vario EL analyzer. IR-spectra were measured on Nicolet Nexus 670 FT-IR using KBr pellets in the range of 400–4000 cm^{-1}. The ^1H NMR spectra were recorded on a Bruker AM 200 spectrometer using $CDCl_3$ as solvent and Me_4Si as internal reference. Fluorescence measurements were performed on a Hitachi F-4500 spectrophotometer equipped with quartz curettes of 1 cm path length. The excitation slit width was 2.5 nm and the emission one was 2.5 nm.

Reagents

Analytical-reagent grade chemicals were used without further purification. A stock solution of Tb^{3+} (1×10^{-4} M) was prepared by dissolving the desired amount of Tb_4O_7 (99.99% purity) in concentrated nitric acid, evaporating to near dryness and diluting with relevant organic solvents. A stock PKA solution (5×10^{-4} M) was prepared by adding the appropriate amount of PKA to 50 mL relevant organic solvents. The Et_3N stock solution (1×10^{-3} M) and Zn^{2+} stock solution ($5 \times 10^{-4+}$M) were prepared by dissolving the desired amount of Et_3N or zinc nitrate in relevant organic solvents.

Synthesis of ligand PKA

The ligand was prepared by the reaction of 2-aminopyridine with ethyl acetoacetate, as described previously [12]. Anal. Calcd. for $C_9H_{10}N_2O_2$ (%): C, 60.67; H, 5.61; N, 15.73. Found: C, 60.58; H, 5.62; N, 15.71.^1H NMR (ppm), δ: 2.31 (s, 3H), 3.61 (s, 2H, exchangeable), 7.07 (dt, 1H), 7.71 (dt, 1H), 8.21 (bd, 1H), 8.32 (dt, 1H), 9.68 (bs, 1H). IR (cm^{-1}) in KBr pellet: υ (CO) = 1720 and υ (NH) = 1685. M.p. = 109–110°C.

General procedure

To a 10 mL test tube, 1.4 mL of 1×10^{-4} M Tb^{3+}, 0.3 mL of 5×10^{-4} M PKA, 0.6 mL of 1×10^{-3} M Et_3N, and an appropriate amount of Zn^{2+} solution were added in that order. Then the mixture was diluted to 5 mL with relevant organic solvents. The fluorescence intensity of blank and sample solution, F_0 and F_1, were measured at 546 nm keeping the excitation wavelength at 329 nm. The ΔF values ($=F_1–F_0$) were obtained.

Results and discussion

Spectral characteristics

The fluorescence spectra of Tb-PKA, Tb-PKA-Et_3N and Tb-PKA-Et_3N-Zn systems in methanol solutions were systematically studied (Fig. 2). After Et_3N and Zn^{2+} was introduced in the Tb-PKA system, the fluorescence intensity of Tb-PKA system (Fig. 2, curve 1) was successively enhanced from more than 2-fold (Fig. 2, curve 2) to 6-fold (Fig. 2, curve 3). Figure 2 showed the fluorescence intensity enhancement at 546 nm was stronger than that at 490 nm, so the peak at 546 nm was chosen to detect the fluorescence intensity throughout all the experiments. At the same time, the emission spectra of other different systems were also systematically studied. Under the same experiment conditions, the Et_3N-Zn, Tb -Et_3N, Tb -Et_3N-Zn systems had no emission peak at 546 nm. It indicated that increasing fluorescence of the Tb-PKA system took place only in the presence of Et_3N and Zn^{2+}.

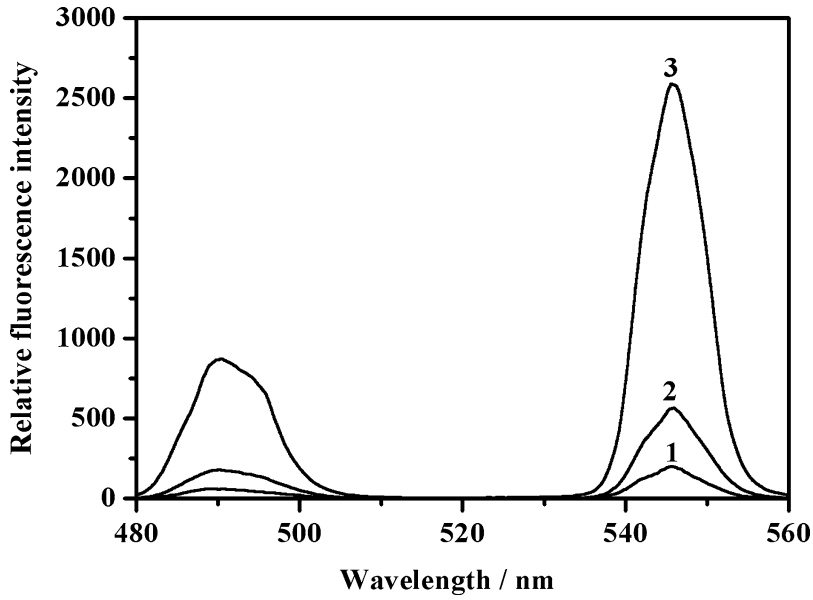

Fig. 2 The emission spectra of (1) Tb-PKA, (2) Tb-PKA-Et_3N and (3) Tb-PKA-Et_3N-Zn systems in methanol solutions. Conditions: Tb^{3+}, 2.8×10^{-5} M; PKA, 3.0×10^{-5} M; Et_3N, 1.2×10^{-4} M; Zn^{2+}, 1.2×10^{-5} M

Effect of solvents

The influence of the solvents on fluorescence intensity was investigated on the above experiment conditions. The results were shown in Fig. 3. It can be seen that the enhancing effect of organic solvents in the Tb-PKA-Et_3N-Zn system is arranged in the order $CH_3OH > CH_3CN > C_2H_5OH >$ THF. The permittivity (ε) values of these solvents are 37.5 (CH_3CN), 32.6 (CH_3OH), 24.3 (C_2H_5OH), and 7.0 (THF), respectively. The order of ΔF in the system is in agreement with the ε values of these solvents that contain oxygen atoms except for methanol. We consider the reason that methanol can sensitize the fluorescence of Tb-PKA-Et_3N-Zn system is mainly its coordination ability with Tb^{3+}. This is the coordinating effect of solvents, which is solvate effect [13]. The present ligand containing O, O and N, O donor sets forms a caverned conformation suitable for the coordination with lanthanide ions, but this ajar cavity could not prevent absolutely the solvent molecules from entering. Together with the raising coordination abilities of CH_3OH, CH_3CN, C_2H_5OH, THF for the lanthanide ions in this system, the oscillatory motions of the entering molecules consume more energy which the ligand triple level transfer to the emitting level of the lanthanide ion. It is inferred that coordination ability of the solvent besides polarity is also an important factor in this system. Consequently, methanol was selected as an appropriate solvent.

Effect of Tb^{3+} concentration

Figure 4 showed the influence of Tb^{3+} concentration on the fluorescence intensity. As the concentration of Tb^{3+} increased up to 2.4×10^{-5} to 3.2×10^{-5} M, maximum and constant ΔF was observed. Above 3.2×10^{-5} M of Tb^{3+} concentration, ΔF was decreased. Hence, a 2.8×10^{-5} M of Tb^{3+} solution was used for the subsequent work.

Effect of PKA concentration

The effect of PKA concentration on the fluorescence intensity of the system was also examined (Fig. 5). When the concentration of PKA was less than 3.0×10^{-5} M, the fluorescence intensity increased with its concentration. When the concentration of PKA reached 3.0×10^{-5} M, the maximum ΔF value was gained and then the excess of PKA made the complex fluorescence quench slightly owing to the reagent self-absorption. Thus, the optimum PKA concentration was kept at 3.0×10^{-5} M in all further experiments.

Effect of Et_3N concentration

The influence of the amount of Et_3N in the range of 0 to 1.8×10^{-4} M on the fluorescence intensity was studied. The results were shown in Fig. 6. When the concentration of Et_3N was controlled between 1.0×10^{-4} and 1.6×10^{-4} M, ΔF reached a maximum. Therefore, 1.2×10^{-4} M was employed as the final Et_3N concentration.

Effect of reaction time

The effect of time on the fluorescence intensity was investigated. The results had shown that the solutions could be

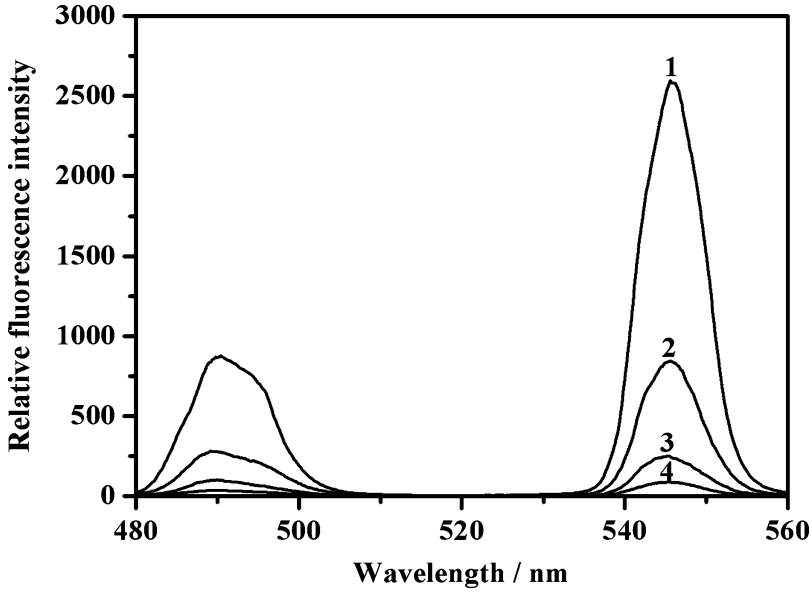

Fig. 3 The influence of solvents on the relative fluorescence intensity of the Tb-PKA-Et_3N-Zn system. 1 CH_3OH, 2 CH_3CN, 3 C_2H_5OH, 4 THF. Conditions: Tb^{3+}, 2.8×10^{-5} M; PKA, 3.0×10^{-5} M; Et_3N, 1.2×10^{-4} M; Zn^{2+}, 1.2×10^{-5} M

allowed to stand for at least 2 h under normal laboratory conditions. The reactions occurred rapidly at room temperature (< 1 min). So this assay did not require crucial timing.

Effect of other substances

The interference from foreign ions on the fluorescence intensity of the Tb -PKA- Et_3N-Zn was tested by analyzing a standard solution containing Tb^{3+} 2.8×10^{-5} M, PKA 3.0×10^{-5} M, Et_3N 1.2×10^{-4} M, Zn^{2+} 1.2×10^{-5} M, to which interfering species were added. The tolerance allowed in the variation of the fluorescence intensity was $\pm 10\%$. The results showed that 100 fold molar excess of Cd^{2+}, 50 fold molar excess Na^+, Ca^{2+}, NO_3^-, Cl^-, 30 fold molar excess Ac^-, 20 fold K^+, I^-, 10 fold molar excess Ba^{2+}, Br^-, 5 fold molar excess Mn^{2+}, Ni^{2+}.

Analytical characters

The calibration graph for the determination of zinc (II) was constructed under the optimal conditions. Excellent linearity $\Delta F = 128.35c–28.82$ (where c is in 10^{-6} M and represents the concentration of Zn^{2+} with a linear correlation coefficient (r) of 0.997) was obtained in the range $8.0 \times 10^{-7} - 5.0 \times 10^{-6}$ M. The limitation of detection (LOD) was calculated by multiplying the standard

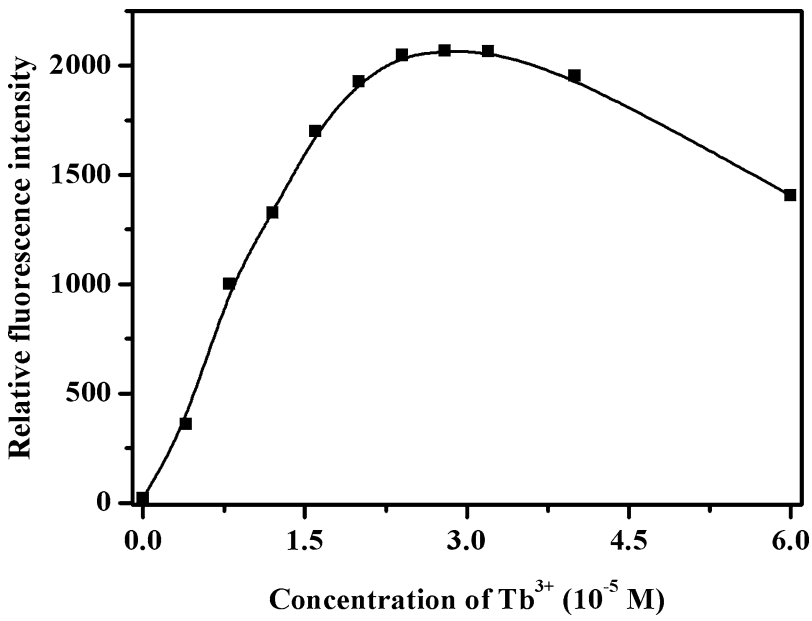

Fig. 4 Effect of the concentration of Tb^{3+} on relative fluorescence intensity. Conditions: PKA, 3.0×10^{-5} M; Et_3N, 1.2×10^{-4} M; Zn^{2+}, 1.2×10^{-5} M

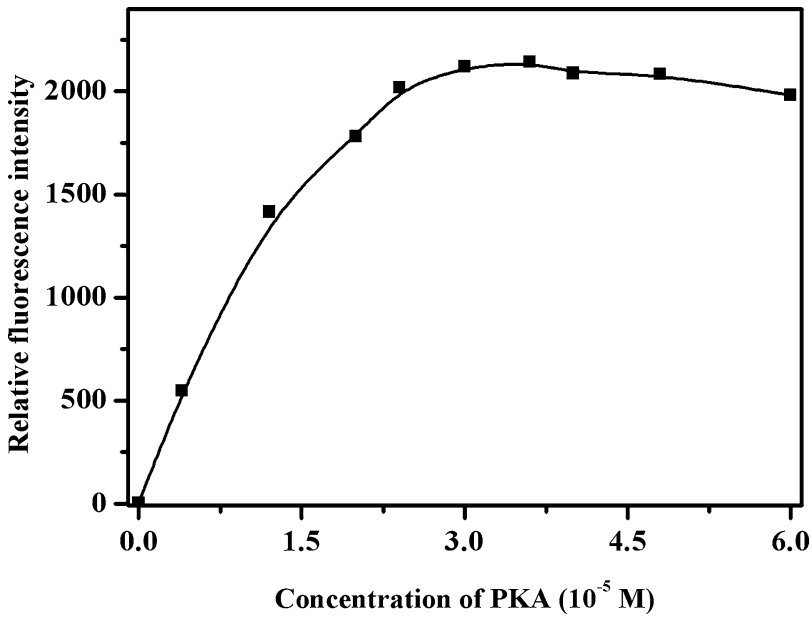

Fig. 5 Effect of the concentration of PKA on relative fluorescence intensity. Conditions: Tb^{3+}, 2.8×10^{-5} M; Et_3N, 1.2×10^{-4} M; Zn^{2+}, 1.2×10^{-5} M

derivation of 10 blank measurements by three and dividing by the slope of the linear calibration curve as 9.9×10^{-8} M.

Recovery study and sample analysis

The procedure was used for the determination of amounts of Zn^{2+} in soybean, rice, and wheat, respectively. The samples were pretreated for the determination of Zn^{2+} according to literature [14]. The results were shown in Table 1. From the results presented it can be concluded that the proposed procedure is a high selective, simple, and rapid method to the determination of Zn^{2+} ion.

Mechanism for the fluorescence enhancement

PKA possesses an equilibrium between keto-type and enol-type as a β-diketonate derivative, and base such as Et_3N makes it easier enolization, which can lead to more coordination ability with metal ion [15–17], thus the characteristic fluorescence of Tb^{3+} is enhanced in the Tb-PKA system by Et_3N.

When Zn^{2+} was added to the Tb-PKA + Et_3N system, the fluorescence of Tb^{3+} is more greatly enhanced. In the Tb–PKA–Et_3N–Zn system, it is considered that there existed simultaneously two complexes: Tb–PKA–Et_3N and

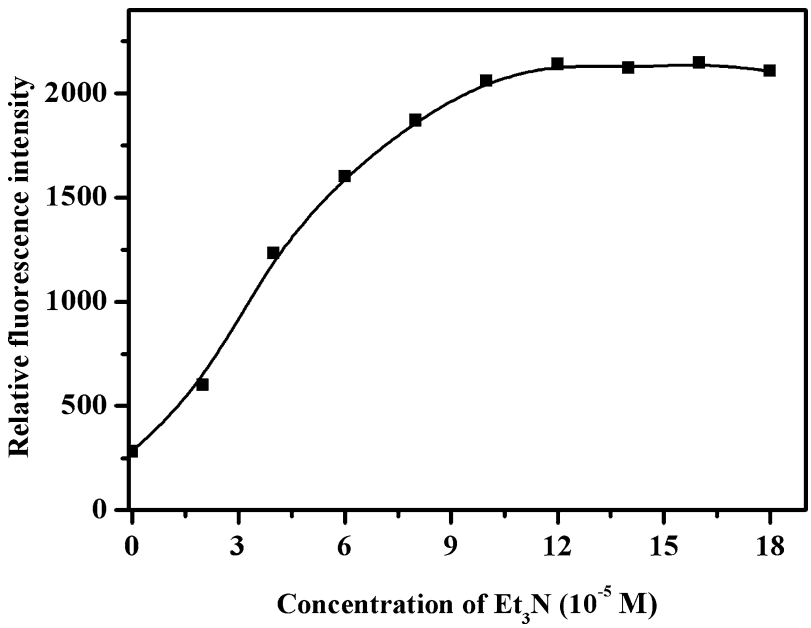

Fig. 6 Effect of the concentration of Et_3N on relative fluorescence intensity. Conditions: Tb^{3+}, 2.8×10^{-5} M; PKA, 3.0×10^{-5} M; Zn^{2+}, 1.2×10^{-5} M

Table 1 Analytical results of samples

Samples	Analytical results (μg g^{-1})	Average (μg g^{-1})	R.S.D. (%)	Added (μg[a])	Found (μg g^{-1})	Recovery (%)
Soybean	18.5, 18.7, 19.4, 18.3, 19.2	18.8	2.5	1.00	0.975	97.5
Rice	12.8, 13.5, 12.6, 12.4, 13.1	12.9	3.4	1.00	1.033	103.3
Wheat	37.0, 37.8, 36.6, 36.9, 37.2	37.1	1.2	1.00	0.986	98.6

[a]The amount of Zn^{2+} in standard solution in 5 mL comparison tubes.

Zn–PKA–Et_3N. In order to know more details of this transfer, according to Förster theory [18, 19]:

$$E_a = 1 - \frac{I_{da}}{I_d}$$

the energy transfer efficiency E_a is calculated, where I_{da} and I_d are the fluorescence intensities of the donor in presence of acceptor and donor in absence of acceptor, respectively. The values of E_a are listed in Table 2. From Table 2, the E_a from the complex of Zn^{2+} to Tb^{3+} is 0.71, and E_a from PKA + Et_3N to Tb^{3+} is raised to 0.84 in the system of Tb–PKA–Et_3N–Zn. This indicates that there is intermolecular energy transfer between these two complexes. Since the concentration of the zinc complex is much greater than that of the terbium complex and the distance between Zn –PKA–Et_3N and Tb–PKA–Et_3N complexes is short enough, Tb^{3+} in the Tb–PKA–Et_3N can accept the energy from Zn –PKA–Et_3N through intermolecular energy transfer in this system, resulting in the enhanced fluorescence of Tb^{3+} compared to the system in the absence of Zn^{2+}.

In addition, it is found that the fluorescence intensity of the Tb–PKA–Et_3N–Zn system is considerably stronger than that of the Tb–PKA–Et_3N system. In the latter system the concentration of Tb^{3+} equals the sum of the concentrations of Tb^{3+} and Zn^{2+} in the former system. This indicates that besides the intermolecular energy transfer mentioned above, the increasing of the fluorescence quantum yield is another reason for the fluorescence enhancement of the Tb–PKA–Et_3N–Zn system. Because the concentration of the Zn^{2+}complex is much higher than that of the Tb^{3+} complex, each of Tb–PKA–Et_3N complex molecules is surrounded by many Zn–PKA–Et_3N complex molecules. These surrounding complexes could act as an energy-insulating sheath, which could prevent collision with solvent molecules and decrease the energy loss of Tb–PKA–Et_3N complex, thus improving the fluorescence quantum yield.

Table 2 The efficiency of energy transfer (E_a)

System	Donor	Acceptor	E_a
Tb–PKA–Et_3N–Zn	PKA–Et_3N–Zn	Tb	0.71
	PKA–Et_3N	Tb	0.84

Conclusion

In this work, it is found that the complex of N- (2-Pyridinyl) ketoacetamide (PKA) with Tb^{3+} in methanol solution can emit the intrinsic fluorescence of Tb^{3+}. When Et_3N and Zn^{2+} are added to the above system, the fluorescence is significantly enhanced. Based on it, a method is established to determine zinc ions selectively and its detection limit reaches as low as 9.9×10^{-8} M. Its reliability is validated by detecting the amounts of zinc (II) in the food samples and its result is satisfactory. The mechanism of fluorescence enhancement is in detail studied. In the Tb–PKA–Et_3N–Zn system, it is considered that there existed simultaneously two complexes: Tb–PKA–Et_3N and Zn–PKA–Et_3N. The complexes of Zn^{2+} not only transfer energy to the Tb^{3+} complexes but also increase the fluorescence quantum yield. Hence, the fluorescence of Tb^{3+} is significantly enhanced.

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