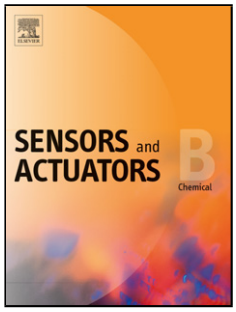

Title: Design and synthesis of a novel lanthanide fluorescent probe (Eu^{III}-dtpa-(bis)melamine) and application in melamine detection in milk products

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Articles

Design and synthesis of a novel lanthanide fluorescent probe (Eu^{III}-dtpa-(bis)melamine) and application in melamine detection in milk products

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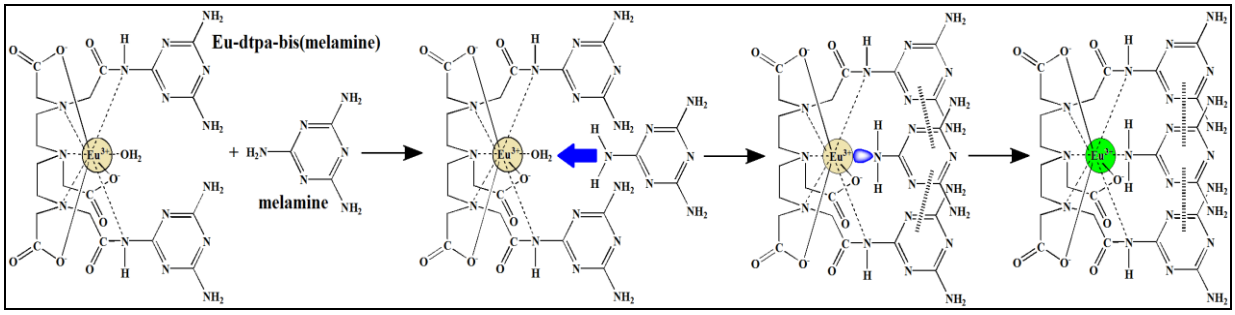
Eu^{III}-dtpa-bis(mel) was designed and synthesized as a novel targeting fluorescence probe for melamine detection. The ligand field change around Eu^{III} caused by melamine leads to a highly selective fluorescence "turn-on" response. The competitive assay showed that the presence of other analogues (cyanuric acid, cyanuric

chloride, trichloroisocyanuric acid and acetoguanamine) rarely affected the fluorescence intensity in probing melamine. The results displayed a linear correlation beteewn melamine concentration and fluorescence intensity. The linear equation was $y = 38.68x + 568.32$ ($R^2 = 0.9972$) with melamine concentration ranged in 5-100 μmol/L. When melamine concentration reaches 100 μmol/L, the fluorescence intensity becomes the maximum. The formed Eu^{III}-dtpa-bis(mel)-melamine complex was extremly stable with the stability constant (*k*) of 9.90×10^6 and the ratio was 1:1. The detection limit (LOD) was 0.3743 μmol/L. The recoveries ranged from 99.80 to 100.50 %, when melamine was detected in some milk products. It can be confirmed that Eu^{III}-dtpa-bis(mel) shows high selectivity, sensitivity and recognition ability in melamine detection.



Research Highlights

1 Eu^{III}-dtpa-bis(mel) complex was synthesized as fluorescene probe to detect melamine.

2 Melamine was used to improve recognition ability of Eu^{III}-dtpa for melamine.

3 Eu^{III}-dtpa-bis(mel) as fluorescence probe can detect melamine in milk products.

A B S T R A C T

Eu^{III}-dtpa-bis(melamine) was designed and synthesized as a novel targeting fluorescence probe for melamine detection in milk products. The ligand field change around Eu^{III} caused by melamine leads to a highly selective fluorescence “turn-on” response. The competitive assay showed that the presence of other analogues (cyanuric acid, cyanuric chloride, trichloroisocyanuric acid and acetoguanamine) rarely affected the turn-on fluorescence intensity in probing melamine. The obtained results displayed a linear correlation beteewn melamine concentration and fluorescence intensity. The linear equation was $y = 38.68x + 568.32$ ($R^2 = 0.9972$) with melamine concentration ranged from 5-100 μmol/L. When melamine concentration reaches 100 μmol/L, the fluorescence intensity becomes the maximum. The formed Eu^{III}-dtpa-bis(melamine)-melamine complex was extremly stable with the stability constant (k) of 9.90×10^6 and the complexation ratio was 1:1. The detection limit (LOD) was 0.3743 μmol/L. The recoveries ranged from 99.80 to 100.50 %, when melamine was detected in some milk products. It can be confirmed that Eu^{III}-dtpa-bis(melamine) shows high selectivity, sensitivity, targeting and recognition ability in melamine detection.

1. Introduction

Melamine (Mel, 1,3,5-triazine-2,4,6-triamine), a kind of triazine analogue with three amino groups, is an industrial chemical used in the production of melamine-formaldehyde resins. It has been used as a filler for protein-rich diets by unethical manufacturers, because of substantial amount of nitrogen (66 % by mass) [1-3]. This was mirrored in the pet food incident in early 2007 and the milk products scandal recently when melamine (Mel) was added to raw materials to obtain high protein contents. Melamine has a low oral acute toxicity, but the chronic administration of high concentrations can induce renal pathology and even death, especially in babies and children. Melamine in combination with cyanuric acid is able to form insoluble melamine cyanurate crystals in the kidney, which causes renal failure [4]. Therefore, the selective, sensitive and convenient detection method for low concentrations of melamine would urgently needed in food, medical, biological and other fields.

Conventionally, some routine methods for the determination of melamine in feed and food are chromatography, ultraviolet spectroscopy, Raman spectroscopy, mass spectrometry coupled with other techniques and chemiluminescence [5-9]. However, most of these techniques are high cost and need complicated experimental conditions and can hardly be used in routing detection. A currently used conventional method is fluorescence probe analysis. A lanthanide complex probe consists of two parts, an antenna moiety and a chelator moiety [10-12]. To achieve sensitive detection, we must control the luminescence properties of the used probe, which should change the luminescence obviously, when it reacts with target molecule. The Eu^{III}-dtpa (dtpa = diethylenetriaminepentaacetic acid) complex has been widely used as chelator moiety in various fields at present, because it has good coordination

ability to form a stable complex and long luminescence lifetimes to emit stabilized characteristic luminescence [13-16]. Besides, it has high solubility in aqueous solution [17,18]. Although this methd has several advantages over routine methods, such as low costs, simple operation, high sensitivity and high accuracy, but the selectivity is still low, because the antenna moiety can interaction with the analyte analogues, without targeting and recognition. So it is necessary to synthesize a novel lanthanide fluorescent probe based on low concentration analyte. We have searched some special compounds, which can recognize melamine, to modify the Eu^{III}-dtpa complex. As we know, the recognition capability between molecule themselves should be strongest, so we choose melamine as the antenna moiety to synthesis Eu^{III}-dtpa-bis(melamine) [19-21]. The interaction of π-π* conjugation between melamine molecules was strongest, so the melamine could be catched by the Eu^{III}-dtpa-bis(melamine) complex more easily [22,23], in contrast to other analogues, such as cyanuric acid (Cya), cyanuric chloride (Cyach), trichloro isocyanuric acid (Isocya) and acetoguanamine (Methyla). As the coordination bond of the complex changed, the ligand field around the Eu^{III} ion also changed, and then leads to the fluorescence of the probe changed. It provides better selectivity and sensitivity, especially strong targeting property and high recognition ability. The probe was composed of melamine and Eu^{III}-dtpa and designed according to Scheme 1.

In this work, we detect melamine and its analogues using this novel lanthanide fluorescent probe, Eu^{III}-dtpa-bis(melamine). Concentration and competitive assays were also conductd to demonstrate the characteristic of Eu^{III}-dtpa-bis(melamine). The results show that this fluorescent probe has good selection and recognition abilities, and displays a linear response beteewn the fluorescence intensity of Eu^{III}-dtpa-bis(melamine) and melamine concentration at 562 nm in the range of 5-100 μmol/L, while other analogues have not any interference. Moreover, Eu^{III}-dtpa-bis(melamine) could further be

applied for detection of melamine in milk products, and the obtained result was satisfactory.

2. Experimental

2.1. Material

Diethylenetriaminepentaacetic acid (dtpa) and melamine (Mel) (A.R., Beijing SHLHT Science & Trade Co., Ltd, China) were purchased and used to synthetize dtpa-bis(melamine) ligand. Cyanuric acid (Cya), cyanuric chloride (Cyach), trichloroisocyanuric acid (Isocya) and acetoguanamine (Methyla) (A.R., Beijing SHLHT Science & Trade Co., Ltd, China) were purchased and used as distractions. $Eu(NO_3)_3 \cdot 6H_2O$ (99.99 %, Yuelong Rare Earth Co., Ltd, China) was obtained and used to prepare the Eu^{III}-dtpa-bis(melamine) as fluorescence probe. Anhydrous acetic anhydride and dimethyl formamide (DMF) (analytical purity, Shenyang Chemical Reagent Plant, China) were purchased and used as solvent. Pyridine (analytical purity, Shenyang Chemical Reagent Plant, China) was obtained and used as acid-binding-agent. Tris (hydroxyl-methyl) aminomethane (Tris) and HCl (analytical purity, Shenyang Chemical Reagent Plant, China) were used to prepare Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol/L) buffer solution in order to maintain the ionic strength and solution acidity.

2.2. Apparatus

Fourier Transform-Infrared (FT-IR) spectra were taken in KBr disks on a FTIR spectrometer (Nicolet 5700, Nicolet Company, USA). Fluorescence enhancing experiments were carried out by fluorophotometer (Cary 300, Varian Company, USA). NMR spectra were conducted with an spectrometer (Plus-400MR, Agilent Company, USA) with DMSO-*d*6, D_2O and NaOH-*d1* as the solvent and tetramethysilane (TMS) as internal standard. Elemental analysis of sample was carried out

by organic elemental analyzer (Perkin Elmer 2400, Perkin Elmer Company, USA). Magnetic stirring oil bath pot was used for synthesising Eu-dtpa-bis(melamine).

2.3. Synthesis of probe Eu-dtpa-bis(melamine)

2.3.1. Synthesis of diethylenetriamine pentaacetic acid dianhydride (dtpaa)

The diethylenetriamine pentaacetic acid dianhydride (dtpaa) was synthesized from diethylenetriaminepentaacetic acid by using the esterification according to the reported method [24-26]. Its synthesis procedure is described in Scheme 1. Diethylenetriaminepentaacetic acid (dtpa) (7.80 g, 0.02 mmol) was dissolved in acetic anhydride (8.00 mL, 0.08 mmol) and pyridine (100 mL, 0.12 mmol) as acid-binding agent under anhydrous condition. The mixed solution was stirred for one day under heat-refluxing at 65 °C. Afterwards, the reaction mixture was cooled to room temperature (25.00 ± 1.00°C), the solvent was removed by reduced pressure filter. The residue was washed twice by acetic anhydride and anhydrous diethyl ether. Finally, the residue was dried to give 5.94 g white powder under vacuum (52 kpa) at 80 °C with yield of 83.11 %. FT-IR (KBr, cm^{-1}): 1642, 1772, 1821, 2341, 2820 and 2979. ^{1}H NMR (500 MHz, DMSO): d = 2.593 (t, 4H), 2.748 (t, 4H), 3.300 (s, 2H), 3.705 (s, 8H) and 11.013 (s, 1H).

Scheme 1.

2.3.2. Synthesis of dtpa-bis(melamine)

The dtpa-bis(melamine) was synthesized from dtpaa and melamine by a new improving synthetic method [27-31]. Its synthesis procedure is shown in scheme 1. Dtpaa (3.57 g, 10 mmol) was dissolved in 50 mL DMF and 30 mL pyridine as base under anhydrous condition. Subsequently, melamine was added to the mixed solution. The mixed solution was stirred 24 h at 80 °C. The mixture was then

cooled to room temperature. After the solvent was removed by vacuum filter, a white solid was obtained. The white solid was evaporated to dryness under vacuum (52 kpa) at 50 ℃ to give 4.70 g white powder solid with yield of 77.18 %. FT-IR (KBr, cm^{-1}): 814, 1387, 1437, 1551, 1654, 1709, 3127, 3340, 3317 and 3342. Elemental analysis (calcd. %) for dtpa-bis(melamine) ($C_{20}H_{31}N_{15}O_8$): C 39.41, N 34.48 and H 5.09. Found (%): C 39.82, N 34.92 and H 5.16.

2.3.3. Synthesis of the Eu(III) complex with dtpa-bis(melamine) ligand

0.1523 g of dtpa-bis(melamine) (0.25 mmol) and 0.1115 g of $Eu(NO_3)_3 \cdot 6H_2O$ (0.25 mmol) were mixed in 50 mL Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) buffer solution, following by refluxing and stirring for 1.0 h. And then it was added into a constant 500 mL volumetric flask and kept in refrigerator 4.0 ℃ as stock solution to be used.

2.4. Preparation of the solution of various bases

0.0315 g melamine, 0.0323 g cyanuric acid (cya), 0.0461 g cyanuric chloride (cyach), 0.0581 g trichloroisocyanuric acid (isocya) and 0.0313 g acetoguanamine (methyla) were dissolved in Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) buffer solution and constant in 500 mL volumetric flask to obtained 5.00×10^{-4} mol/L solutions, respectively. The stock soultions were kept in refrigerator 4.0 ℃. The stock solutions were diluted to required concentrations with Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) buffer solution when needed in experiments. The structures of the used melamine, cyanuric acid (Cya), cyanuric chloride (Cyach), trichloroisocyanuric acid (Isocya) and acetoguanamine (Methyla) were shown in Fig. 1.

Fig. 1.

2.5. Spectrum experiments

The dilute solutions (1.00×10^{-4} mol/L) of Eu^{III}-dtpa-bis(melamine), dtpa-bis(melamine), Eu^{3+}, melamine, cyanuric acid (Cya), cyanuric chloride (Cyach), trichloroisocyanuric acid (Isocya) and acetoguanamine (Methyla) were prepared in Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) buffer solution, respectively. Blank solution of Eu^{III}-dtpa-bis(melamine) without other interfering compounds was prepared by the same procedure. The fluorescence emission spectra were recorded in the wavelength range from 500 to 600 nm with excitation wavelength at 282 nm. Both the excitation and emission slits were set at 10 nm. All experiments were carried out at room temperature (20.00 ± 1.00 °C).

2.6. Sample pretreatment

Because melamine-contaminated milk cannot be purchased from the market anywhere, the milk was spiked with appropriate amounts of melamine standard solution directly to obtain the determined samples. The milk products were pretreated according to the following procedure. 1.00 mL milk sample and appropriate amounts of melamine powder were transferred to a 500 mL flsk, and then diluted with Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) buffer solution to the volume scale to obtain the sample for detection. Besides, using the same method 1.00 mL milk sample without adding melamine powder was transferred another 500 mL flsk and the diluted milk sample was used as the blank standard sample. The detection methd was as the same as spectrum experiment part.

3. Results and discussion

3.1. FT-IR spectrum of Eu-dtpa-bis(melamine)

The synthetic procedure of Eu^{III}-dtpa-bis(melamine) as fluorescence probe was shown in Scheme

1. The dtpa-bis(melamine) was synthesized by the acylation action between dtpaa and melamine. All compounds were fully characterized by fourier transform infrared (FT-IR) spectra shown in Fig. 2. It can be found that the ν_s(C-O) and ν_{as}(C=O) of dtpa-bis(melamine) ligand appear at 1437 cm^{-1} and 1709 cm^{-1}, respectively. Furthermore, the ν_{as}(CONH) and ν_{as}(NH_2) of dtpa-bis(melamine) also appears at 1654 cm^{-1} and 3340 cm^{-1}, respectively. The characteristic broad absorption peaks of hydroxy group can be found around 3317 cm^{-1}. These shifts confirme that the dtpa-bis(melamine) ligand has been synthesized by the acylation reaction between dtpaa and melamine.

Fig. 2.

3.2. Fluorescence spectrum of Eu-dtpa-bis(melamine)

The fluorescence spectrum of Eu^{III}-dtpa-bis(melamine) in Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) solution and the corresponding fluorescence intensity are shown in Fig. 3. In Fig. 3(a), the Eu^{III}-dtpa-bis(melamine) exhibits certain fluorescence emission under the excitation of 280 nm wavelength light owing to the presence of Eu^{III} ion and large conjugated system as well as flat rigid structure. In order to evaluate the selectivity of the Eu^{III}-dtpa-bis(melamine) as fluorescence probe toward melamine against other analogues, five types of triazine analogues at the concentration level of 1.00×10^{-4} mol/L, including melamine (Mel), cyanuric acid (Cya), cyanuric chloride (Cyach), trichloro isocyanuric acid (Isocya) and acetoguanamine (Methyla) were tested, rsepectively. As illustrated in Fig. 3(b), the addition of only melamine significantly enhanced the fluorescence intensity of Eu^{III}-dtpa-bis(melamine) in aqueous solution at 562 nm, due to the change of ligand field around Eu^{3+} ion induced by the coordination of melamine to Eu^{III}-dtpa-bis(melamine) [32]. However, the others can slightly quench or hardly change the fluorescence of Eu^{III}-dtpa-bis(melamine) in aqueous

solution. The results indicated that the Eu^{III}-dtpa-bis(melamine) was suitable for selectively probing melamine even in the presence of a range of melamine analogues.

Fig. 3.

The fluorescence emission spectra of Eu^{III}-dtpa-bis(melamine) in Tris-HCl ([Tris-HCl] = 50 mmol/L and pH = 7.40) solution along with the increase of melamine concentrations and the corresponding fluorescence intensities are shown in Fig. 4. From Fig. 4(a) it can be found that the fluorescence emission intensity of Eu^{III}-dtpa-bis(melamine) gradually becomes strong with the addition of melamine. It indicated again that the presence of melamine can increase the fluorescence emission of Eu^{III}-dtpa-bis(melamine) and that the fluorescence intensity can further be enhanced with the increase of melamine concentration. Fig. 4(b) demonstrated the gradient enhancements of fluorescence emission intensities of Eu^{III}-dtpa-bis(melamine) at 562 nm with the increase of melamine concentrations (0-1000 μmol/L). It predicted that the Eu^{III}-dtpa-bis(melamine) might be used for the detection of melamine [33]. Firstly, a linear response of fluorescence emission intensities at 562 nm as a function of melamine concentration can be observed in the range of 5-100 μmol/L. The linear equation is $y = 38.68x + 568.32$ ($R^2 = 0.9972$), where y is the fluorescence intensity at 562 nm measured for given melamine concentration and x represents the concentration of melamine. When the concentration of melamine reached 100 μmol/L, the fluorescence intensity of Eu^{III}-dtpa-bis(melamine) solution became maximum. Along with the further increase of the melamine concentration ranged from 100-1000 μmol/L, the fluorescence intensity increases only slightly and then until it does not change. The linear equation was $y = 0.04x + 998.82$ ($R^2 = 0.9928$). The slope was very small and the straight line was almost parallel to x-axis. The stability constant (k) was 9.90×10^6 according to the

formula $k = (1-\alpha)/[\text{complex}]\cdot\alpha^2$, where $\alpha = (A' - A)/A'$, A' is the fluorescence intensity value corresponding the point of the tangents intersection and A represents the fluorescence intensity value corresponding curve intersection, as shown in Fig. 4(b). It can be found that the Eu^{III}-dtpa-mel-melamine complex is extremely stable because of the larger k value. And the complexation ratio is 1:1.

Fig. 4.

Competitive assay also displayed the selective response of Eu^{III}-dtpa-bis(melamine) toward melamine. From Fig. 5(a) it can be seen that the Eu^{III}-dtpa-bis(melamine) in aqueous solution gives a moderate intensity of fluorescence emission at 562 nm under excitation of 280 nm wavelength light. However, after adding melamine the fluorescence emission intensity of Eu^{III}-dtpa-bis(melamine) was obviously enhanced. It indicates that the presence of melamine can strengthen the fluorescence emission of Eu^{III}-dtpa-bis(melamine). Nevertheless, the further addition of some analogues (cyanuric acid (Cya), cyanuric chloride (Cyach), trichloro isocyanuric acid (Isocya) and acetoguanamine (Methyla)) hardly change the fluorescence emission intensity of Eu^{III}-dtpa-bis(melamine). Noticeably, the analogues rarely affect the turn-on fluorescence intensity of probing melamine due to their different interaction mode with Eu^{III}-dtpa-bis(melamine). More specifically, from Fig. 5(a) it can be found that these analogues (cyanuric acid (Cya), cyanuric chloride (Cyach), trichloro isocyanuric acid (Isocya) and acetoguanamine (Methyla)) do not change the fluorescence emission spectrum of Eu^{III}-dtpa-bis(melamine), and also do not affect the fluorescence intensity of Eu^{III}-dtpa-bis(melamine) in the presence of melamine. It indicated that the Eu^{III}-dtpa-bis(melamine) exhibitd high sensitivity and selectivity to melamine because of the specific binding. Besides, two melamine molecules as arms

in Eu^{III}-dtpa-bis(melamine) further enhanced the recognition and selection ability of the fluorescence probe for melamine detection [34].

Fig. 5.

3.3. Detection mechanism and process of Eu^{III}-dtpa-bis(melamine) to melamine

As shown in Scheme 2, the possible binding mechanism of Eu^{III}-dtpa-bis(melamine) with melamine was proposed. It is well know that the Eu^{3+} ion can form nine-coordinate complexes with various aminopolycarboxylic acid ligands [35-37]. For this novely synthesized dtpa-bis(melamine) ligand, it was modified by two melamine at its two ends (up and down), respectively, and also an eight-dentate ligand [38,39]. Therefore, in Eu^{III}-dtpa-bis(melamine), one water (H_2O) molecule should be coordinate with Eu^{3+} ion as second ligand (ninth coordination atom). The Eu^{3+} is coordinated with the three nitrogen atoms and five oxygen atoms from one dtpa-bis(melamine) ligand, besides, one oxygen atom from coordinate H_2O molecule. Upon addition of the melamine, because of the flat molecular structure, the π-π* stacking binding will be formed between melamine in Eu^{III}-dtpa-bis(melamine) due to the same molecular structure and chemical compositions [40,41].

Figuratively, like two arms two melamine molecules can capture third melamine molecule tightly and then three melamine molecules form a tripolymer layer structure. Moreover, the formation of π-π* stacking binding makes the nitrogen atom of melamine further to be close to the Eu^{3+} ion until forming a stable and complete coordination bond [42,43]. At the same time, the original coordinate water molecule will be replaced by the closer melamine. Under normal conditions, the nitrogen atom is difficult to replace the oxygen atom. Nevertheless, due to the π-π* stacking bonding, the melamine is forced to be close to the Eu^{3+} ion in Eu^{III}-dtpa-bis(melamine) forming a new coordination bond. As the

ligand field around the Eu^{3+} ion was changed, the fluorscence intensity of the Eu^{III}-dtpa-bis(melamine) changed obviously [44-47]. That is, the fluorescence intensity was distinctly enhanced when adding the melamine. However, other melamine analogues, such as cyanuric acid (Cya), cyanuric chloride (Cyach), trichloro isocyanuric acid (Isocya) and acetoguanamine (Methyla), only slightly quench or no change the fluorescence of Eu^{III}-dtpa-bis(melamine). Thus, it was confirmed that the sensitivity and specificity of Eu^{III}-dtpa-bis(melamine) as fluorescence probe could be used to detect melamine.

3.4. Determination of melamine in actual milk products

The calibration curve for the determination of melamine was constructed by plotting the fluorescence emission intensity at 562 nm versus the melamine concentrations of Fig. 4(b). It has been found that the calibration curve is linear in the concentration range from 5-100 μmol/L. And the correlation coefficient R^2 is 0.9972, suggesting that the linearity was satisfactory for the determination of melamine. The limit of detection (LOD) of melamine was 0.3743 μmol/L (LOD = 3σ/s), which is far lower than the standard in food set by the EPA (U.S. Environmental Protection Agency) and WHO (World Health Organization), and also lower than previous reported works. Therefore, the Eu^{III}-dtpa-bis(melamine) as fluorescence probe could be applied to the determination of melamine in milk products. Because the existing milk in the market is free of melamine, the samples were spiked with certain amounts of melamine standard solution directly. The recovery acquired by the proposed sensor ranged from 99.80 % to 100.50 % (Table 1), suggesting an acceptable detection result. Therefore, the developed lanthanide fluorescent probe could be reliable and effective for the determination of melamine in real samples.

Table 1.

Table 2 gives the comparison of the results of melamine determination obtained by several methods. Compared with several current techniques for the assay of melamine, the Eu^{III}-dtpa-bis(melamine) as fluorescence probe has some advantages. The LOD of this method is lower than that using other detection measures. The method had the advantage of cheap reagents and easy to fabricate. It should be noted that the recovery obtained meets the requirements for determining melamine in real samples.

Table 2.

4. Conclusion

In this work, the melamine fluorescence probe (Eu^{III}-dtpa-bis(melamine)) was designed and synthesized. In the Eu^{III}-dtpa-bis(melamine), two melamine molecules located at two ends (up and down) can combine with third melamine exclusively with the help of π-π* stacking interaction. Due to the formation of coordination bond between melamine and Eu^{III} ion in Eu^{III}-dtpa-bis(melamine) complex, the fluorescence intensity was enhanced clearly. Other some analogues, such as cyanuric acid (Cya), cyanuric chloride (Cyach), trichloroisocyanuric acid (Isocya) and acetoguanamine (Methyla), slightly quench or almost no change the fluorescence intensity. Therefore, it can be confirmed that the Eu^{III}-dtpa-bis(melamine) as a fluorescence probe displays the sensitivity and specificity in the detection of melamine. The Eu^{III}-dtpa-bis(melamine)-melamine complex was found to be quite stable with the stability constant (k) of 9.90×10^6. Furthermore, a linear response equation $y = 38.68x + 568.32$ ($R^2 = 0.9972$) on the fluorescence intensity at 562 nm as a function of melamine concentration was observed in the range of 5-100 μmol/L. The complexation ratio was 1:1 and the detection limit is 0.3743 μmol/L.

Acknowledgments

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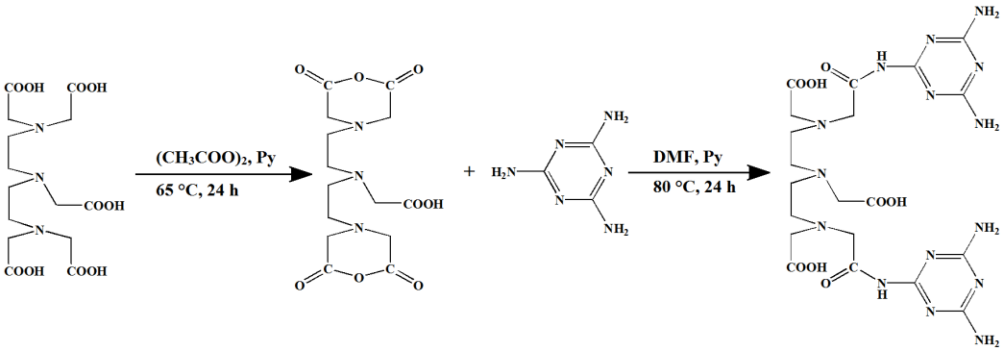
Content of Schemes, Figures and Tables:

melamine (Mel) as well as melamine (Mel)+cyanuric acid (Cya), melamine (Mel)+cyanuric chloride (Cyach), melamine (Mel)+trichloroisocyanuric acid (Isocya), melamine (Mel)+acetoguanamine (Methyla) and the corresponding fluorescence intensities (b). ([Eu-dtpa-mel] = [Mel] = [Cya] = [Cyach] = [Isocya] = [Methyla] = 1.00×10^{-4} mol/L, [Tris-HCl] = 50 mmol/L, pH = 7.40, T_{solu} = 20.00 ± 0.02 °C.)

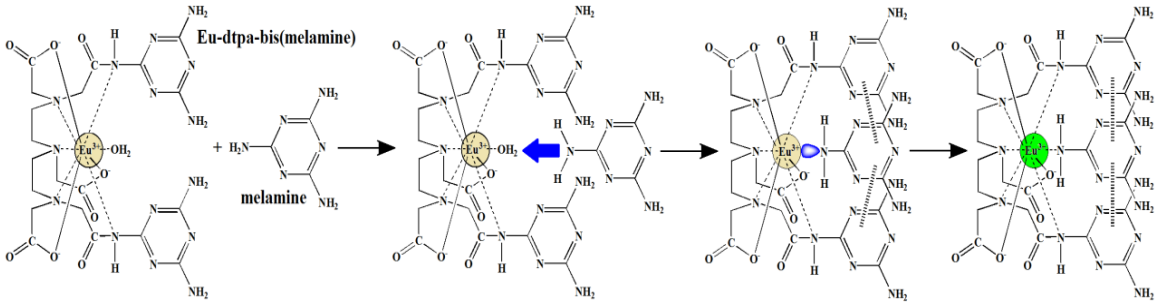
Table 1. Determination results of melamine in spiked milk products (n = 3).

Table 2. Comparison of the proposed sensor for melamine detection with other methods.

Schemes:



Scheme. 1. The structure and synthetic route of the dtpa-bis(melamine) ligand.



Scheme. 2. The reaction mechanism of Eu-dtpa-bis(melamine).

Figures:

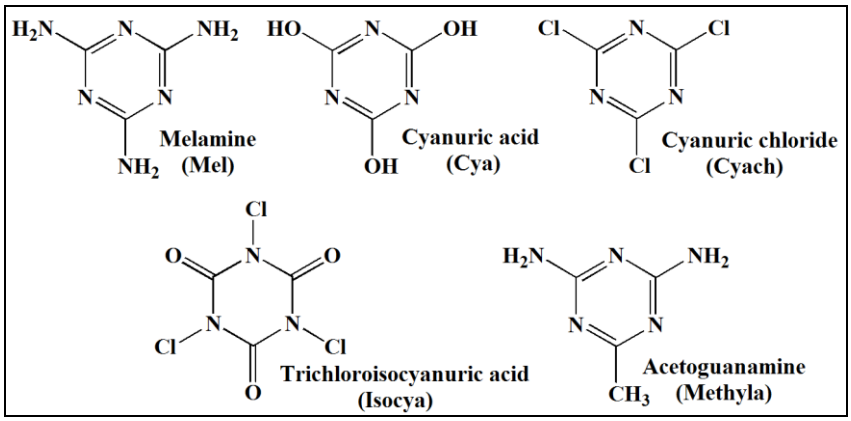


Fig. 1. The structure of melamine and analogues as possible co-existing compounds.

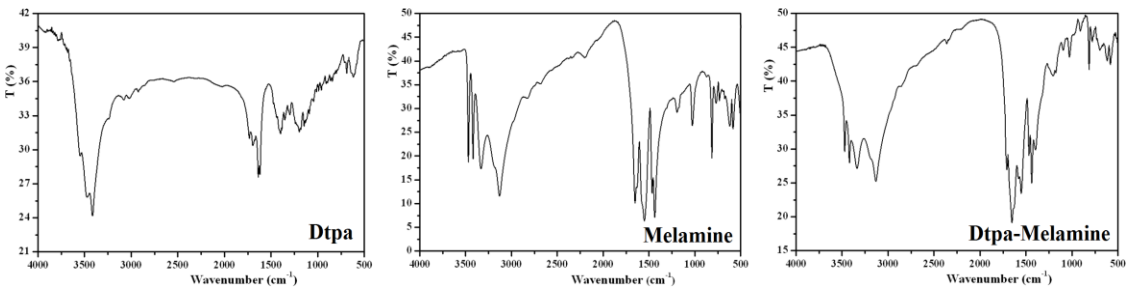


Fig. 2. Infrared spectra of melamine, diethylenetrinmine pentaacetic acid (dtpa) and dtpa-bis(melamine).

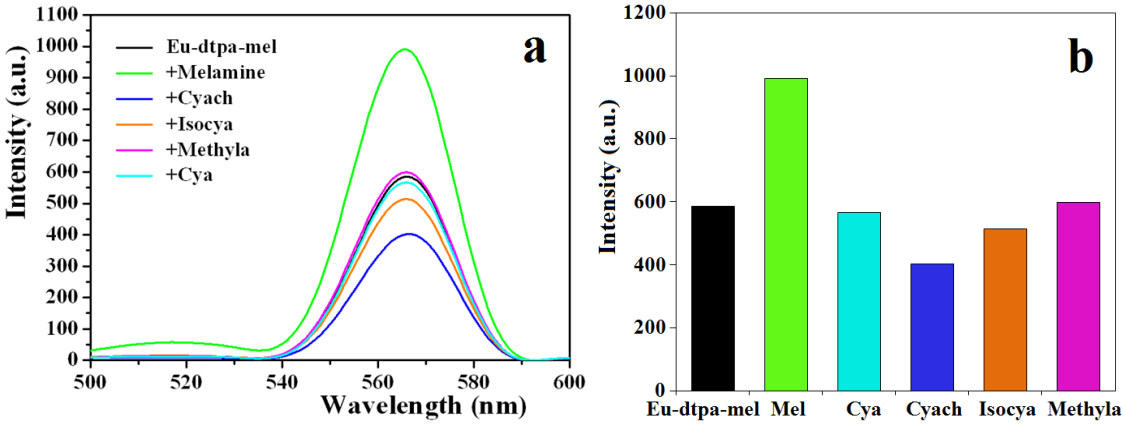


Fig. 3. Fluorescence spectra (λ_{ex} = 280 nm) of Eu-dtpa-mel in Tris-HCl buffer solution upon the addition of melamine (Mel), cyanuric acid (Cya), cyanuric chloride (Cyach), trichloroisocyanuric acid (Isocya), acetoguanamine (Methyla). ([Eu-dtpa-mel] = [Mel] = [Cya] = [Cyach] = [Isocya] = [Methyla] = 1.00×10^{-4} mol/L, [Tris-HCl] = 50 mmol/L, pH = 7.40, T_{solu} = 20.00 ± 0.02 °C.)

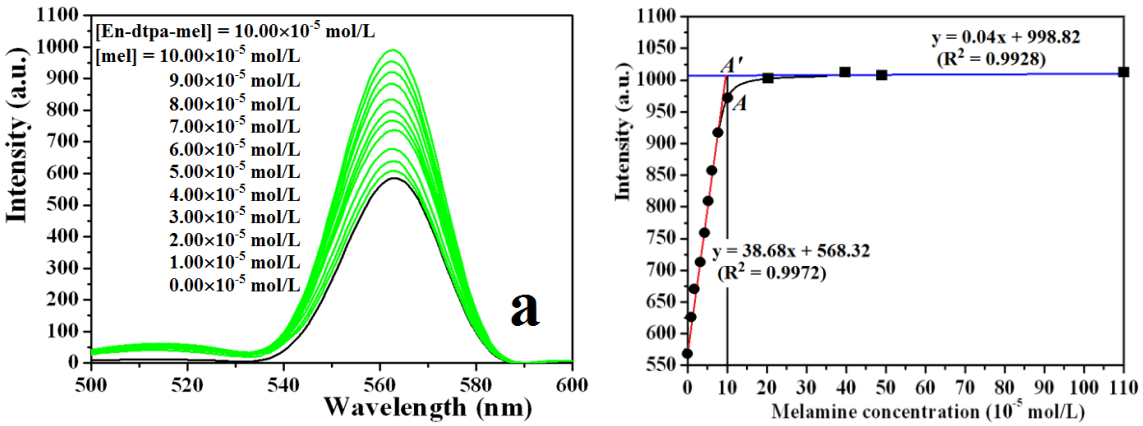


Fig. 4. Fluorescence spectra (λ_{ex} = 280 nm) (a) of Eu-dtpa-mel in Tris-HCl buffer solution upon the addition of melamine (Mel) with different concentrations (melamine concentrations from 0.00 × 10^{-5} mol/L - 10.00 × 10^{-5} mol/L) and linear responses (b) of Eu-dtpa-mel as a function of melamine concentration (0.00 - 110.00 × 10^{-5} mol/L). ([Eu-dtpa-mel] = 1.00 × 10^{-4} mol/L, [Tris-HCl] = 50 mmol/L, pH = 7.40, T_{solu} = 20.00 ± 0.02 °C.)

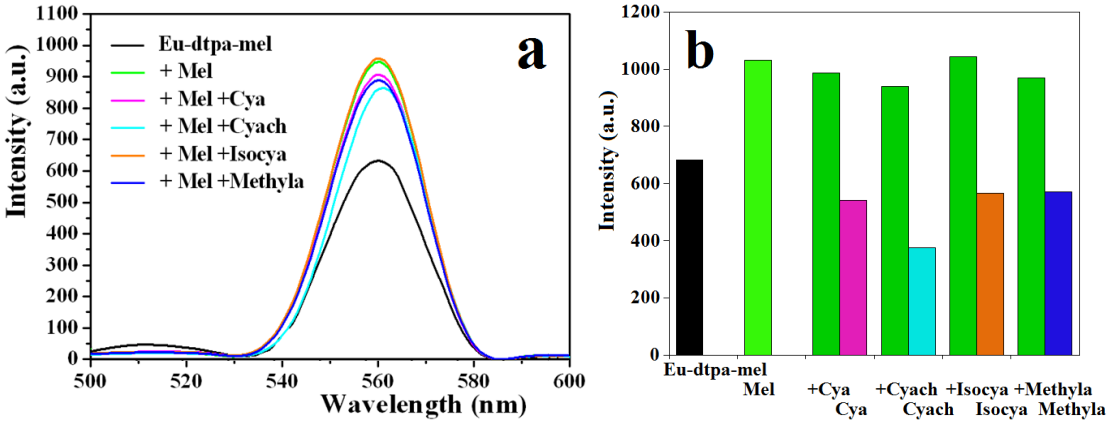


Fig. 5. Fluorescence spectra (λ_{ex} = 280 nm) (a) of Eu-dtpa-mel in Tris-HCl buffer solution upon the addition of melamine (Mel) as well as melamine (Mel)+cyanuric acid (Cya), melamine (Mel)+cyanuric chloride (Cyach), melamine (Mel)+trichloroisocyanuric acid (Isocya), melamine (Mel)+acetoguanamine (Methyla) and the corresponding fluorescence intensities (b). ([Eu-dtpa-mel] = [Mel] = [Cya] = [Cyach] = [Isocya] = [Methyla] = 1.00×10^{-4} mol/L, [Tris-HCl] = 50 mmol/L, pH = 7.40, T_{solu} = 20.00 ± 0.02 °C.)

Tables:

Table 1. Determination results of melamine in spiked milk products (n = 3).

Sample	Added/mol/L	Found/mol/L	Recovery/%
Milk	1.00×10^{-4}	1.00×10^{-4}	100.00
Yoghourt	1.00×10^{-4}	1.00×10^{-4}	100.00
Milk powder	1.00×10^{-4}	0.99×10^{-4}	99.00

Table 2. Comparison of the proposed sensor for melamine detection with other methods.

Methods	Linear range	Detection limit	Recovery/%	References
Electrochemistry(oligonucleotides/Au)	0.039-3.3 μM	9.6×10^{-9} M	95.0	[5]
MIP/CL	0.1-50 μg/ml	0.02 μg/ml	102.3-104.0	[8]
Electrochemistry(MIP/potentiometric sensor)	5.0 μM-10 mM	1.6 μM	95.0-110.0	
LC	1-400 μg/ml	65 μg/g	93-107	[7]
HPLC–MS/MS	20-500 ng/ml	5.6 ng/ml	66.2-95.5	[6]
GC–MS/MS	0.004-1.6 mg/kg	0.002 mg/kg	61.4-117.2	
UV	1.26-10 μg/ml	0.2 μg/ml	97.0	[15]
CZE	-	0.5 mg/kg	-	
Electrochemistry(acoustic sensor)	5 nM-1 mM	5 nM	-	
Probe	5-100 μmol/L	0.3743 μmol/L	99.80-100.50	This methd