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Determination of Norfloxacin in Tablets and Biological Fluids Using Terbium-Caffeine Coordination Polymer Luminescence

Shaima Al-Salhi, Saleh Al-Busafi, Beena Verghese, Salma M. Al-Kindy*, FakhrEldin O. Suliman and Rengaraj Selvaraj

Department of Chemistry, College of Science, Sultan Qaboos University, P.O. Box 36, Al-Khod, PC 123, Muscat, Sultanate of Oman

*Email address: alkindy@squ.edu.om

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ABSTRACT

The usefulness of caffeine (CF) as a chelating agent to increase the luminescence intensity of terbium (Tb^{3+}) was studied. The efficiency of the developed Tb^{3+}/CF- coordination nanoparticles was evaluated as a sensing platform for the detection of fluoroquinolone antibiotics, using norfloxacin (NF) as a model analyte. The luminescence conditions of Tb^{3+}/CF-NF were investigated and optimized. Under the optimized experimental conditions, the linearity of the method was established with a limit of detection (LOD) of 5.8 nM, and the effect of some expected interferes was examined. The developed method was successfully applied for the detection of norfloxacin in tablet and urine samples with recoveries between 88.9-96.0% and 90.7-96.3%, respectively.

Keywords: Luminescence; Sensitization; Chemo-sensors; Caffeine; Norfloxacin.

تحديد النورفلوكساسين في الأقراص والسوائل البيولوجية بإستخدام تلألؤ معقدات التيربيوم- كافيين البوليمرية

شيماء الصالحي، صالح اليوصافي، بينا فيرجيس، سلمى الكندي، فخر الدين سليمان و رنجراج سلفراج

الملخص: في هذا البحث تمت دراسة الكافيين (CF) كعامل ربط كيميائي ومنشط ضوئي لعنصر التيربيوم (Tb). كما تم دراسة وتقييم فعالية الجزئيات النانوية من مركب التيربيوم مع الكافيين كمادة اساسية للكشف عن المضادات الحيوية الفلوروكوينولينية باستخدام النورفلوكساسين (NF) كنموذج. أيضًا تمت دراسة و تحديد الظروف الضوئية المناسبة للمعقد النتائج (Tb^{3+}/CF). في ظل الظروف التجريبية المحسنة، تم إنشاء الحالة الخطية لنظام الكشف بحد اكتشاف (LOD) قدره 5.8 نانومتر، وتم فحص تأثير بعض التداخلات المتوقعة. لقد تم تطبيق الطريقة المطورة بنجاح للكشف عن النورفلوكساسين في عينات الأقراص والبول حيث تراوحت نسبة استعاده تساوي 88.9-96.0% و 90.7-96.3% على التوالي.

الكلمات المفتاحية: كاشف ضوئي، تحسس، حساس كيميائي، كافيين، نورفلوكساسين.

1. Introduction

Coordination polymer nanoparticles from lanthanide ions with multifunctional organic ligands have been recognised as the most efficient luminescent chemo-sensors in recent years. One of the initial challenges of lanthanides (Ln) to display specific photophysical properties is its Laporte forbidden f-f transitions and thereby weak luminescence [1]. One way researchers have managed to bypass these drawbacks is by binding the matched organic ligands, which function as antenna molecules and facilitate the efficient energy transfer from ligand to Ln(III) ions. Known for possessing astounding luminescence properties, such as long lifetimes, unique narrow emission bands, large Stokes shifts, and high resistance to photo-bleaching, these hybrid nanomaterials have captured the attention of analytical chemists due to their potential applications in food, biological and pharmaceutical analysis [2-5].

Notably, Tb^{3+} and Eu^{3+} provide a comprehensive framework to design and fabricate lanthanide coordination polymer nanoparticles (CPNPs) with specific sensing function for a target analyte at trace levels even in highly complex matrices. For instance, highly luminescent Tb^{3+} complexes are adopted for fluoroquinolone antibiotics (FQs) determination in different media and several strategies have been introduced for enabling such a remarkable performance [6-9]. The bridging aromatic organic ligands, amongst all seem to be the key element of enhanced lanthanide luminescence because of their unique optical and magnetic properties arising from their 4f electron structure [10-11]. While using adenine as a bridging ligand, it has been reported that Tb^{3+} based CPNPs served as an excellent fluorescent probe for the detection of ciprofloxacin in tablets and biological fluids [12]. However, sensitization of Tb^{3+} by caffeine in lanthanide coordination polymer nanoparticles and its application in FQs determination has not yet been reported.

Caffeine (3,7-dihydro-1,3,7-trimethyl-1,4-purine-2,6-di-one) has leapt to relative fame as the most popular stimulant for centuries and ranks as one of the topmost commonly consumed dietary ingredients throughout the world. But the use of this molecule is not only limited to the science of cooking, but possess a wide range of diverse biological functions, including antitumor, antioxidant, and antidiabetic activities, as well as playing a role in the regulation of lipid metabolism [13-14]. This work recognizes that caffeine can easily coordinate to Ln^{3+} ions and the developed Tb^{3+}/caffeine (metal node/bridging ligand) coordination polymer nanoparticles has been performed as a luminescent sensor for the detection of norfloxacin. By the virtue of its high luminescence intensity and the longer wavelength of emission, this sensor is anticipated to exhibit high selectivity and low detection limit.

2. Experimental

2.1 Reagents and Instruments

All the chemicals and solvents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Company (Sigma-Aldrich, USA). Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA, USA). A stock solution of NF (1.00×10^{-5} mol L^{-1}) was prepared in ultrapure water and the working solutions of desired concentrations were freshly prepared by appropriate dilution of each stock solution with water. The selectivity of the proposed method was investigated in the presence of $NaNO_3$, KNO_3, Mg $(NO_3)_2$, Ca $(NO_3)_2$, NaCl, KCl, $MgCl_2$, $CaCl_2$, fructose, ascorbic acid, glucose, starch, sucrose, and lactose.

Luminescence measurements were carried out on Perkin Elmer LS-55 luminescence spectrometer (Perkin Elmer, Beaconsfield UK) using a 1 cm quartz cell. The luminescence mode is phosphorescence with 0.05 ms delay time and 1.00 ms gate time. The emission spectra were collected at an excitation wavelength of 276 nm and the excitation spectra were recorded at an emission wavelength of 543 nm. All measurements were done repeatedly and reproducible results were obtained. The pH measurements were done using JENWAY 3015 pH meter (UK). IR spectra were determined by using Bruker Alpha II FT-IR Spectrometer (Bruker, USA). Crystallographic studies were done in X Pert PRO (PANalytical, USA) X-ray diffractometer using Cu Kα (λ = 1.54 Å) on powder sample. The Surface morphology of Tb/CF CPNPs were characterized by JOELJSM-7800 F field emission scanning electron microscope (FESEM, Japan) working at 30 kV.

2.2 Sample Preparation

2.2.1 Preparation of Tb^{3+}/caffeine CPNPs

An aqueous solution of $TbCl_3$ (80 mM, 2 ml) was dripped into a vigorously shaking mixture of an aqueous solution of caffeine (80 mM, 2 ml) and N, N-dimethylformamide (DMF) (16 ml). The final volume was adjusted by adding ultrapure water. The mixture was first incubated for 30 min at room temperature in a Teflon-lined stainless-steel vessel and then transferred to an oven at 150°C for 4h. After cooling to room temperature, the products were collected by centrifugation (10,000 rpm, 10 min) and washed three times with absolute ethanol to remove the unreacted residue. Finally, a CPNP suspension was prepared by dispersing the prepared precipitate in ultrapure water (2 mL).

2.2.2 Determination of NF in tablet and urine Samples

The norfloxacin tablets known to contain 616 mg of active ingredient were purchased from a medical shop (Pegasus Farmaco, India). Firstly, five weighed tablets were finely powdered and a portion of the powder known to contain an equivalent amount of one tablet was accurately weighed and dissolved in ultrapure water. Appropriate aliquots were taken to give final concentrations of NF ranging from 0.4 to 2 µM tablet samples.

A urine sample from a healthy adult volunteer was collected and was filtered through a 0.45 µm filter membrane, before being diluted 100-folds with water. The diluted urine sample was spiked with various concentrations of NF solution ranging from 0.4-2 µM. The concentration of NF in both samples were determined using the optimized procedure developed in this study. Finally, the percentage recovery was calculated by comparing the luminescence intensity of equal concentrations of the pure standard solutions with those of the spiked samples.

3. Results and discussion

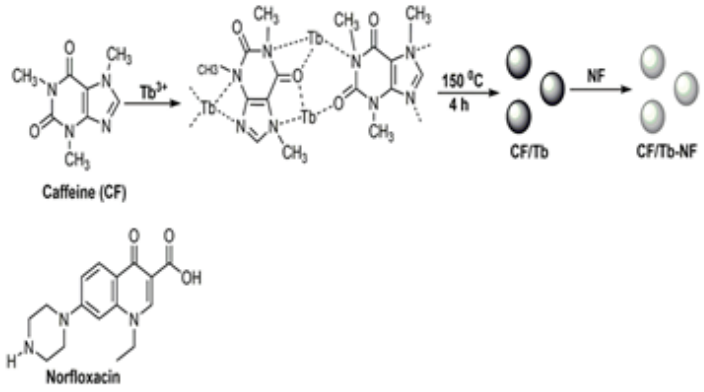
3.1 Design and characterization of Tb^{3+}/CF NPs

As illustrated in Scheme 1, the proposed Tb^{3+}/CF NPs-based sensor is designed in such a way that the caffeine (CF) has three acceptor sites for hydrogen bonding with Tb^{3+}, which are two oxygen atoms of the carbonyl groups and the imidazole nitrogen. This type of chemical coordination is expected to sensitize the emission of Tb^{3+} via an intramolecular energy transfer process. In a preliminary study, the luminescence properties of Tb/CF NPs were evaluated in the presence and absence of the model analyte, NF. As Figure 1 shows, the spectrum of NPs alone demonstrates a very weak luminescence due to the low Tb^{3+} molar absorption coefficient and forbidden f-f transition. On the other hand, the appearance of well-shaped and highly intense similar bands on the addition of NF (2 µM) provides a clear indication of complexation. Both solutions demonstrate the excitation wavelengths at 276 nm and 324 nm, on scanning from 250 nm to 400 nm. However, the peak at 276 nm showed an increase in luminescence intensity (~two times) than that of the other and was fixed as excitation wavelength for collecting the emission spectra. The emission spectra depicted the presence of characteristic four peaks of terbium ion based on $^5D_4$–$^7F_6$ (489 nm), $^5D_4$ – $^7F_5$ (543 nm), $^5D_4$ – $^7F_4$ (585nm) and $^5D_4$ – $^7F_3$ (620 nm) transitions. The respective luminescence intensities of the four peaks are distinctly different and the peak at 543 nm was further considered for monitoring the Tb/CF-NF complex, owing to the increased intensity.

3.1.1 Structural confirmation of Tb^{3+}/CF NPs

The FT-IR was used to confirm the coordination between caffeine and terbium chloride. In the IR spectrum of caffeine, the C = O, and C = N stretching bands appear, as expected, at 1696 and 1645 cm^{-1}, respectively. When caffeine is coordinated with terbium, the two peaks merge as one strong peak, and appear at a lower frequency, 1574 cm^{-1} (Figure 2). The coordination of caffeine with terbium occurs through C = O and C = N as shown in Scheme 1. Such coordination weakens C=O and C=N bonds because of the single-bond character which leads to lower IR frequency. In addition, the characteristic $TbCl_3$ frequencies, 1066 and 745 cm^{-1} also appear in the IR spectrum of the CF-Tb complex.

The notable changes observed in the Powdered X ray Diffraction (PXRD) patterns of Tb^{3+}/CF NPs compared to that of its individual components confirm the formation of a new crystalline material (Figure 3). The diffractogram of $TbCl_3.6H_2O$ exhibits sharp and highly intense peaks, whereas the CF shows patterns with a significant reduction in peak intensity. On the contrary, Tb^{3+}/CF NPs show an X-ray diffraction pattern of moderately intense peaks and it combines some peaks from CF and Tb^{3+}. For example, the peak of $TbCl_3$ at 2θ values 17.34 and 43.35° and the peaks of CF at 24.34, 30.63, and 53.84° were found in the diffraction pattern of Tb^{3+}/CF NPs. The appearance of new peaks with different 2θ° values than that of pure CF and Tb^{3+} suggests the formation of the new material.



Scheme 1: An illustration of Tb/CF NPs for the detection of NF.

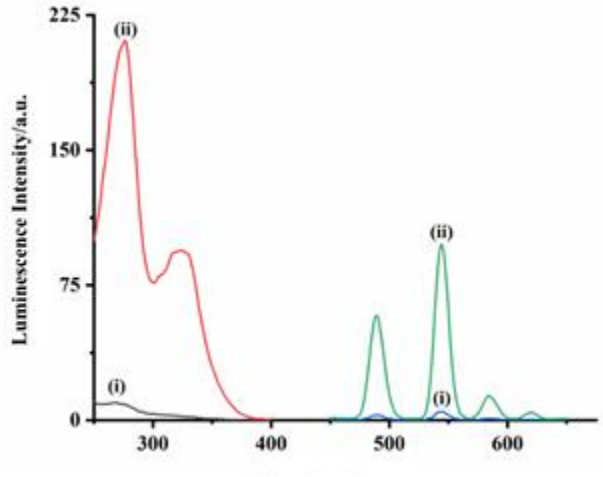


Figure 1. Excitation and emission spectra of (i) Tb/CF NPs and (ii) Tb/CF-NF complex.

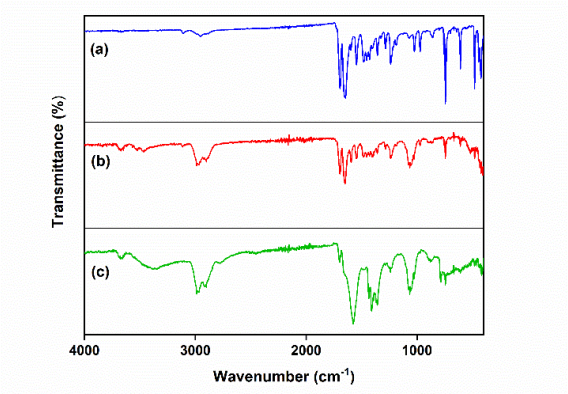


Figure 2. FTIR spectra of (a) pure CF, (b) pure $TbCl_3.6\ H_2O$ and (c) Tb/CF NPs).

The SEM microphotographs (Figure 4) of Tb^{3+}, CF, and Tb^{3+}/CF NPs clearly elucidate the difference in their surface morphology. As seen from the SEM pictures, (a) pure caffeine appears as rod-like particles with different dimensions (b) pure Tb^{3+} reveal as a compact and homogeneous sphere and (c) Tb^{3+}/CF NPs visualize rough surfaces of irregular size and shape, in which the original morphology of Tb^{3+} and CF vanished.

3.2 Optimization of reaction conditions for NF detection

Further, to promote the complexation efficiency of Tb^{3+}/CF NPs, various parameters were evaluated because the luminescence-intensity of Tb^{3+} is directly proportional to the degree of complexation between Tb^{3+}/CF NPs and NF. Since the complexation is favoured in optimal Tb^{3+}/CF NPs concentration, the influence of different NPs volumes has been studied. As shown in Figure 5, the luminescence intensity kept increasing from 40 µl to 80 µl and further a decline in intensity was found. Therefore, 80 µl was selected as the optimized Tb^{3+}/CF NPs volume in this study. As stated in the literature, HEPES buffer is always the best choice for bioanalytical applications and it was noticed that there is a significant enhancement in luminescence intensity in the presence of HEPES buffer at pH 4 [15]. Hence by keeping the same buffer, the complexation between Tb^{3+}/CF NPs and NF was conducted under pH values 2 to 10.

As can be seen in Figure 6, the intensive luminescence behavior at pH 4.5 may indicate the formation of ionized forms of CF and NF and availability for complexation. Beyond this point and when the pH approaches basicity, a sudden fall in luminescence intensity was observed. This is presumably due to the formation of terbium hydroxide and eventually leads to the disassembly of Tb^{3+}/CF NPs. Thus, pH 4.5 was determined as the optimal pH of the reaction medium. According to this pH dependence, further optimization was performed using sodium acetate, TRIS, and HEPES buffer type. As expected, HEPES buffer performed well and was selected (Figure 7). In order to probe that luminescence sensitization is brought up by the presence of target analyte NF, the luminescence intensities in various concentrations of NF (0.7 to 2 µM) were monitored at optimized conditions. It is confirmed that there is an increase in the luminescence intensity along with an increase in the concentration of NF (Figure 8).

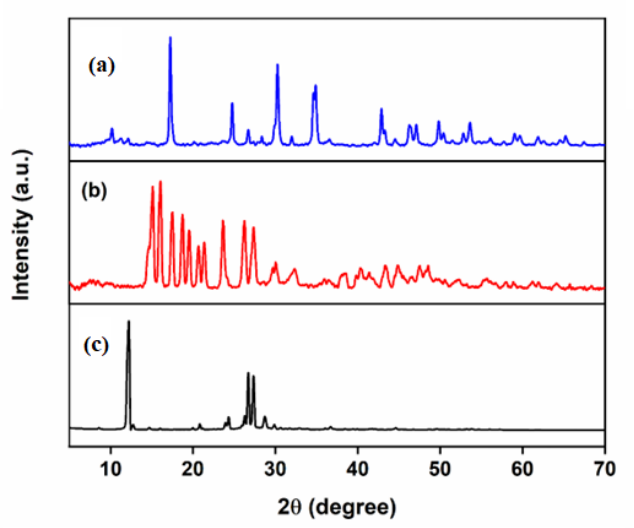


Figure 3. XRD spectra of (a) pure CF, (b) pure $TbCl_3.6\ H_2O$ and (c) Tb/CF NPs).

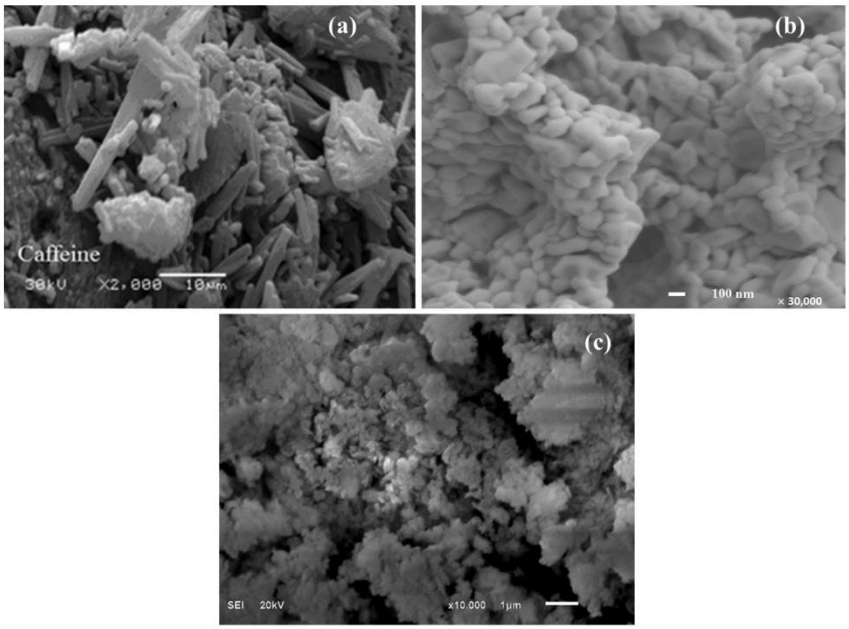


Figure 4. SEM images of (a) pure CF (b) pure $TbCl_3.6\ H_2O$ (c) Tb/CF NPs.

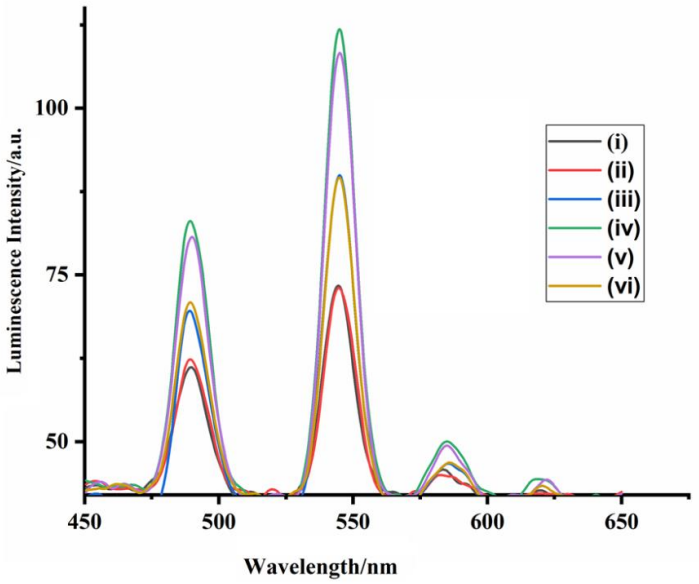


Figure 5. Effect of Tb3+/CF NPs concentration with 10 µM NF solution and HEPS buffer of pH 6.9: (i) 5 (ii) 40 (iii) 60 (iv) 80 (v) 90 and (vi) 100 µl.

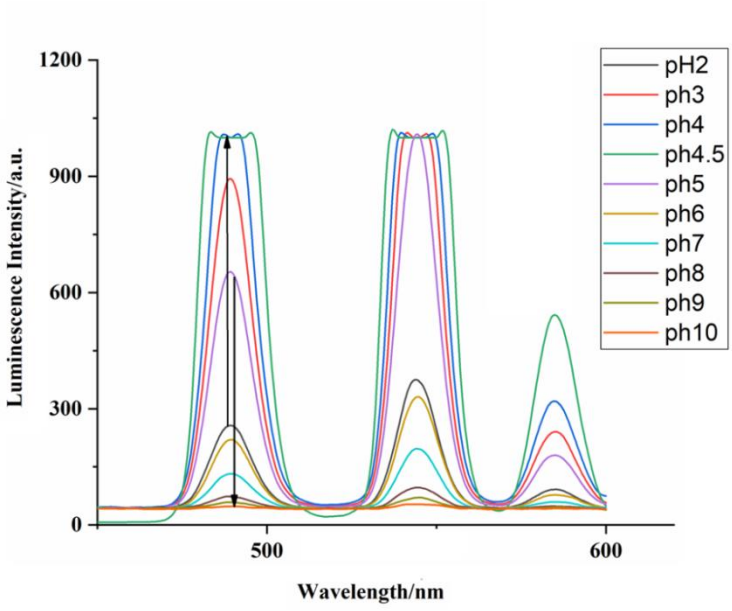


Figure 6. Effect of different pH of HEPES buffer with 80 µL of Tb^{3+}/CF NPs and 10 µM NF solution: The upwards and down arrows represent pH 2-4.5 and 5 -10, respectively.

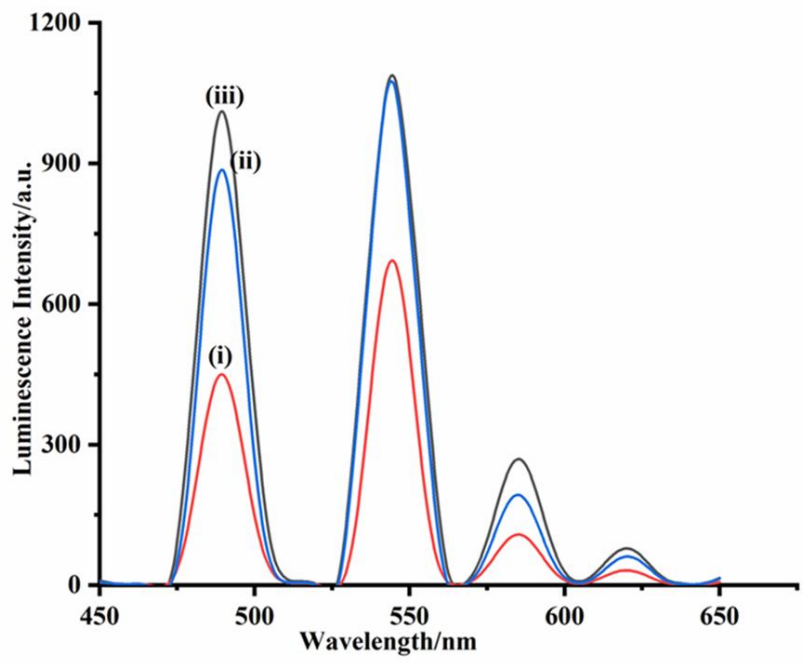


Figure 7. Effect of different type of buffer of pH 4.5 with 80 µL Tb^{3+}/CF NPs and 10 µM NF solution: (i) TRIS buffer (ii) Sodium acetate (iii) HEPES.

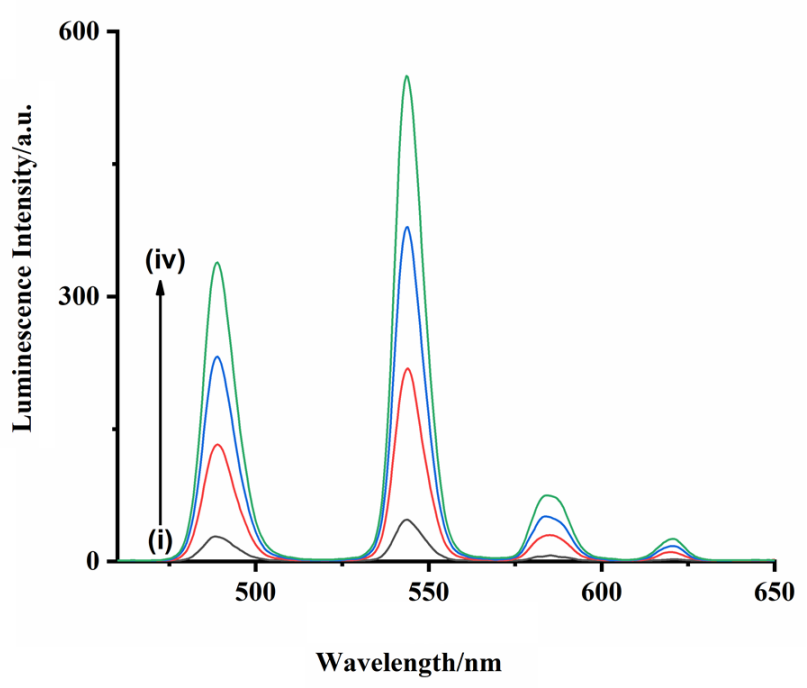


Figure 8. Effect of different concentrations of NF solution with 80 µL Tb^{3+}/CF NPs and 0.1 M HEPES buffer of pH 4.5: (i) 0 (ii) 0.7 (iii) 1.4 and (iv) 2 µm.

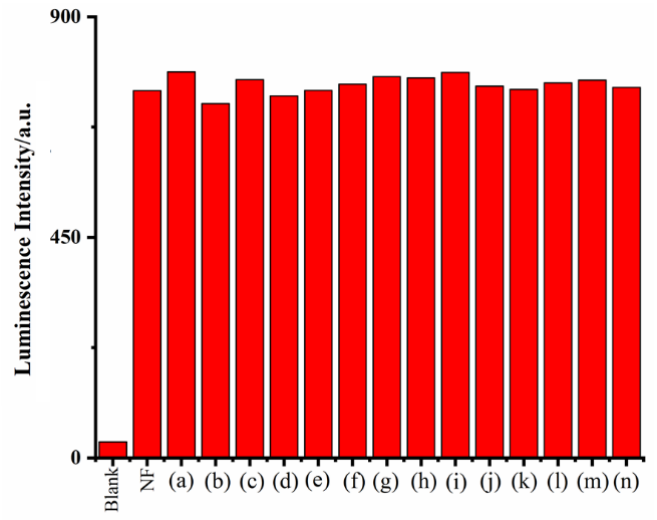


Figure 9. Luminescence intensity of Tb^{3+}/CF NPs and NF (2 µM) in the presence of interferes (1 mM): (a) NF + $NaNO_3$, (b) NF + KNO_3, (c) NF + Mg $(NO_3)_2$, (d) NF + Ca $(NO_3)_2$, (e) NF + NaCl, (f) NF + KCl, (g) NF+$MgCl_2$, (h) NF + $CaCl_2$, (i) NF + fructose, (j) NF + ascorbic acid, (k) NF + glucose, (l) NF + starch, (m) NF + sucrose, and (n) NF + lactose.

3.3 Analytical applications

3.3.1 Linearity and Limit of Detection

The analytical performance of this method shows a linear relationship between luminescence intensity and NF concentration under the optimum conditions in the range of 0.1 to 0.64 ppm. The calibration equation is given by I = 774.08C + 45.067 (where I is the luminescence intensity and C is the concentration of NF in ppm). The displayed correlation coefficient (R^2) of 0.998 (n=5) reflects the linear nature of the calibration curve. The detection limit according to signal-to-noise ratio ($3S_b/m$) is found to be 5.8 $\times 10^{-9}$ mol L^{-1}, Where S_b is the standard deviation of the blank and m is the slope. The superiority of the proposed method is highlighted by comparing the detection limit with those previously reported in the literature, using photoinduced fluorometric (PIF) detection – multi-emission scanning ($1.9x10^{-8}$ mol L^{-1}) [16], porous β-cyclodextrin polymer-based solid phase extraction coupled with HPLC, (1.72×10^{-8} mol L^{-1}) [17], capillary electrolysis with micellar electrokinetic chromatography (6.70×10^{-5} mol L^{-1}) [18]. However, lower detection limits were reported when capillary electrolysis with chemiluminescence detection was used for the determination of norfloxacin (2.9×10^{-10} mol L^{-1}) [19]. This limitation in sensitivity is addressed by the stability, selectivity, easy sample preparation as well as direct and fast detection procedure by Tb^{3+}/CF NPs.

3.3.2 Effect of Interference

The selectivity of Tb^{3+}/CF NPs for NF detection was studied under the above optimum conditions by investigating the luminescence of caffeine/Tb-NF complex in the presence of different expected interference molecules in drug and biological fluids. These molecules are being sought to lower the luminescence intensity by formation complex with NF or Tb (III). In this regard, 1 mM of interference molecule solutions including metal ions (Na^+, K^+, Ca^{2+}, Mg^{2+}), anions (NO_3^-, Cl^-), fructose, ascorbic acid, glucose, starch, sucrose, and lactose were added to solutions contained 80uL of Tb^{3+}/CF NPs, 2µM NF. Figure 9 shows that there is no obvious change in the luminescence intensity by the addition of expected interference.

3.3.3 Recovery experiments

Since caffeine/Tb CPNPs appears as a unique elective sensor for NF, the proposed method was used to measure the NF level in tablet and urine samples. The obtained recoveries of NF in this application ranged from 88.9 % to 96.0 % and 90.7 % to 96.3 % in tablet and urine respectively. The relative standard deviation (RSD, n = 5) in both applications is all less than 0.88 %. The good recoveries and the precision show promising potential for caffeine/Tb CPNPs for the detection of NF. The proposed strategy might provide a new platform for the design and application of fluorescent sensors based on lanthanide coordination polymers.

4. Conclusion

A sensitive, simple, and responsive photoluminescence method has been proposed based on the sensitization of the characteristic PL intensity of Tb^{3+} by caffeine as a coordinate ligand for the first time. The fabricated CPNPs can be successfully applied for the determination of NF tablets and urine samples. The developed method shows acceptable recoveries and precision and the detection of NF at a lower concentration level of 5.8×10^{-9} mol/L, reflects the equivalency of the developed method with other previously described work.

Conflict of interest

The authors declare no conflict of interest.

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