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ORIGINAL PAPER

# Silver Nanoparticle-Enhanced Chemiluminescence Method for Determining Naproxen Based on Europium(III)-Sensitized Ce(IV)-$Na_2S_2O_4$ Reaction

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**Abstract** A simple and sensitive chemiluminescence (CL) method coupled with flow-injection technique is proposed to determine naproxen (NAP). The method is based upon the enhancement of the weak CL signal arising from the reaction of Ce(IV) and $Na_2S_2O_4$ with $Eu^{3+}$ to form the $Eu^{3+}$-Ce(IV)-$Na_2S_2O_4$ system. The CL intensity was significantly increased by the introduction of NAP into this system in the presence of silver nanoparticles (Ag NPs). Examination of the recorded UV–vis spectra and fluorescence spectra indicated that the energy of the intermediate $SO_2$*, which originated from the redox reaction of Ce(IV) and $Na_2S_2O_4$, was transferred to $Eu^{3+}$ via NAP and that the process was accelerated by Ag NPs due to their catalytic activity. Under the optimum conditions, the CL intensity was increased with increasing NAP concentration and the correlation was linear ($r$=0.9992) over the NAP concentration range of 1–420 ng $mL^{-1}$. The limit of detection (LOD) was 0.11 ng $mL^{-1}$ with a relative standard deviation (RSD) of 1.15% for 5 replicate determinations of 200 ng $mL^{-1}$ NAP. The method was successfully applied to determine NAP in pharmaceutical and biological samples.

**Keywords** Naproxen · Cerium(IV) · Sodium hyposulphite · Europium(III) · Silver nanoparticles

## Introduction

Naproxen (NAP), (*S*)-6-methoxy-α-methyl-2-naphtheneacetic acid, is a non-steroidal anti-inflammatory drug. Due to an aryl acetic structure, NAP exhibits analgesic and antipyretic properties and has been widely used to reduce pain, fever, inflammation and stiffness. It is commonly used in the treatment of several diseases including rheumatoid arthritis, osteoarthritis, degenerative joint disease, ankylosing spondylitis, acute gout and primary dismenorrea [1]. NAP is associated with some serious side effects such as gastrointestinal complaints, kidney failure and with minor side effects like headache, drowsiness, vomiting, diarrhea, constipation, decreased appetite, rash, and dizziness. It is therefore very important to develop a simple, effective and sensitive method to determine NAP in pharmaceutical and biological purposes.

Several analytical methods have been reported for determining NAP including spectrophotometry [2, 3], fluorometry [4, 5], synchronous spectrometry [6], phosphorimetry [7], voltammetry [8, 9], liquid chromatography [10, 11], capillary electrophoresis [12], high performance liquid chromatography (HPLC) [13–15], and chemiluminescence (CL) [16–20]. Among the above methods, CL coupled with flow injection offers the advantages of equipment simplicity, high detection sensitivity, low background noise and good reproducibility. This method has therefore been applied in various fields.

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Enhancing the CL intensity has become critical in order to increase the sensitivity and expand the range of applications. Nanoparticles (NPs) have recently attracted strong research attention because of their unique physical and chemical properties. Moreover, NPs are used as nanocatalysts to enhance a variety of CL reactions [21–26]. With the development of nanotechnology, researchers demonstrated that the NPs-catalyzed CL system offered potential applications in analytical chemistry. Among the metal NPs, silver NPs (Ag NPs) have attracted particular attention because of their excellent catalytic and electrocatalytic activities: they are more sensitive to electrons and photons than gold or platinum NPs and exhibit better catalytic activities which enhance the CL intensity [25, 26].

The objective of this study is development of a novel Ag NPs-catalyzed and europium(III)-sensitized CL system for determining NAP. This system was chosen for investigation because trivalent lanthanide ions, especially europium(III) and terbium(III) ions have recently been used as powerful CL sensitizers which offer high sensitivity, wide dynamic range, large Stocks shift, narrow emission bands and long luminescence lifetime. Li et al. [27, 28] reported that europium(III) sensitized $KMnO_4$-sulfite CL system for determining ibuprofen and atenolol. Wang et al. [29] determined ulifloxacin and prulifloxacin using the terbium(III)-sensitized $KMnO_4$-$Na_2S_2O_4$ CL system. The NAP molecule has an α,β-diketonates group that can form a complex with trivalent europium ions [20]. In this study, we report a Eu(III)-sensitized Ce(IV)-$Na_2S_2O_4$ CL system catalyzed by Ag NPs to determine NAP. The formation of the Eu(III)-NAP complex enhanced the weak CL of the Ce(IV)-$Na_2S_2O_4$ reaction. The introduction of Ag NPs into the Eu(III)-NAP-Ce(IV)-$Na_2S_2O_4$ system greatly enhanced the CL intensity. Based on the above phenomenon, a sensitive and rapid chemiluminescence method coupled with flow-injection is developed to determine NAP in pharmaceutical and biological samples. The possible CL reaction mechanism of the presented method is also investigated.

## Experimental

### Reagents and Sample Solutions

All reagents were of analytical reagent grade and were used without further purification. Double deionized (DI) water (Millipore, MilliQ Water System, USA) was used throughout the experiment. NAP, silver nitrate, and $Ce(SO_4)_2\ 4H_2O$, were purchased from Sigma-Aldrich (St. Louis, USA). Sodium borohydride ($NaBH_4$) was obtained from Merck (Germany). NAP stock solution (2 mg $mL^{-1}$) was prepared by dissolving an appropriate amount of NAP in DI water and was stored in a refrigerator. The Ce(IV) solution (5 mmol $L^{-1}$) was prepared by dissolving the $Ce(SO_4)_2{\cdot}4H_2O$ in 40 mmol $L^{-1}$ sulphuric acid. $AgNO_3$ (1 mmol $L^{-1}$) and $NaBH_4$ (2 mmol $L^{-1}$) solutions were prepared by dissolving in DI water. The sodium hyposulphite solution (1.5 mmol $L^{-1}$) obtained from Fluka Chemical Co. (Switzerland) was freshly prepared by dissolving sodium hyposulphite in DI water. Working solutions of desired concentrations were freshly prepared by appropriate dilution of each stock solution with DI water before use.

### Apparatus

A schematic diagram of the flow system used in this study is shown in Fig. 1. Two peristaltic pumps ($P_1$, $P_2$) (Model 404, Ismatec, Zurich, Switzerland) were used to deliver all solutions. One pump conveyed $Eu^{3+}$ and $Na_2S_2O_4$ solutions while the other delivered NAP, Ce(IV) and colloidal Ag NP solutions. The $Eu^{3+}$ and $Na_2S_2O_4$ solutions were injected by a Rheodyne (Model 7125, Cotati, CA, USA) six-way injection valve with a loop which was mixed with Ce(IV), NAP and the colloidal Ag NPs mixture solution streams at the T-piece. Polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system to carry all solutions. An F-4500 spectrofluorimeter (Hitachi, Japan) equipped with a coiled glass flow cell (1.0 mm i.d., 20 mm

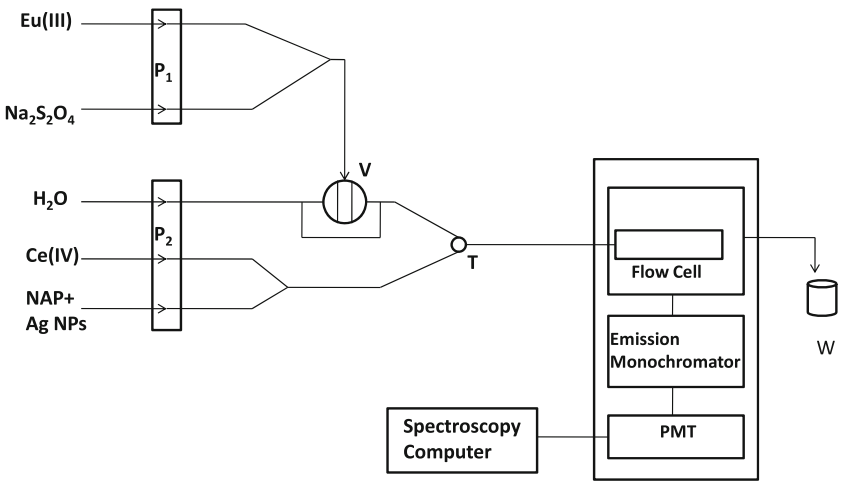



**Fig. 1** Schematic diagram of the FIA-CL manifold used for determining naproxen (NAP). $P_1$, $P_2$, peristaltic pumps; V, injection valve; T, Y-pieces; W, waste

total diameter) was used for detecting and recording the CL intensity of the reaction product. For the CL measurement, the light source of the spectrofluorimeter was switched off. The high voltage for the photomultiplier tube (PMT) (Model R 928, Hamamatsu, Japan) was set to 950 V. The UV-1800 (Shimadzu, Japan) spectrophotometer was used to record the absorption spectrum.

### Synthesis of Silver Nanoparticles (Ag NPs)

Ag NPs were prepared by chemical reduction of silver nitrate using sodium borohydride as a reducing agent in aqueous solution according to the procedure described in the literature [30] with slight modification. Briefly, 25 mL of 1 mmol $L^{-1}$ $AgNO_3$ aqueous solution was added to 75 mL of 2 mmol $L^{-1}$ $NaBH_4$ aqueous solution dropwise with vigorous stirring. During the mixing, the mixture was turned to bright yellow, and this color change indicated that the reduction of silver ions was completed. After 10 min, 5 mL aqueous solution of sodium citrate (1% w/w) was added to the resultant solution to stabilize the Ag NPs. The colloidal solution of Ag NPs was stirred for another 20 min and stored for 2 days at 4 °C before use. The prepared Ag NPs were characterized by a transmission electron microscopic (TEM) image using a transmission electron microscope (Hitachi-7100, Japan) with an accelerating voltage of 100 kV. The TEM image of the Ag NPs (Fig. 2) shows the morphology of the particles, from which their average diameter was calculated as 15±2 nm.

### Procedure

The whole experiment for measuring all the CL, FL and UV–vis spectra was performed at room temperature. As shown in Fig. 1, the carrier water was pumped into the flow cell by pump $P_2$ at a flow rate of 3.0 mL $min^{-1}$. The $Eu^{3+}$ and $Na_2S_2O_4$ solutions were injected using a six-way valve also at a flow rate of 3.0 mL $min^{-1}$. The Ce(IV) solution and the mixture of NAP and Ag NP solution were delivered by pump $P_2$ at a flow rate of 1.5 mL $min^{-1}$, and mixed with the $Eu^{3+}$ and $Na_2S_2O_4$ solutions at the T-piece before entering the flow cell. The mixed solution stream was transferred into the flow cell in the spectrofluorimeter, accompanying the increase of the CL intensity. The CL signal produced in the flow cell was recorded by adjusting the PMT and introduced into the computer for data acquisition.

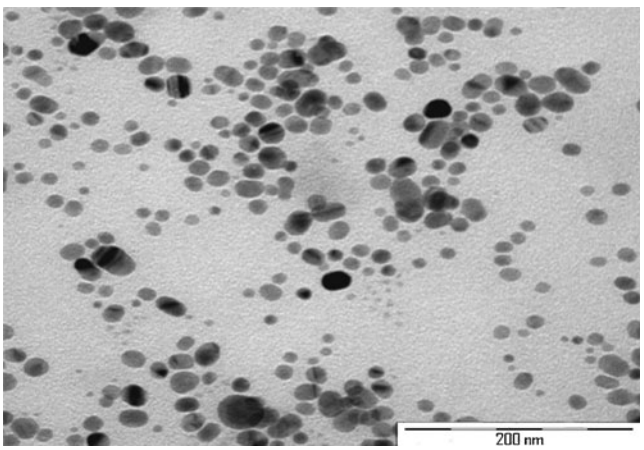



**Fig. 2** TEM image of the prepared Ag NPs

## Results and Discussion

### Enhancement of CL Intensity of $Eu^{3+}$-NAP-Ce(IV)-$Na_2S_2O_4$ by Ag NPs

The redox reaction of Ce(IV)-$Na_2S_2O_4$ produced a weak CL signal in the acidic medium (shown in Fig. 3, curve a). The CL intensity was increased when $Eu^{3+}$ was added into the Ce(IV)-$Na_2S_2O_4$ system (Fig. 3, curve b). When NAP was introduced into the $Eu^{3+}$-Ce(IV)-$Na_2S_2O_4$ system, the CL intensity was enhanced significantly (Fig. 3, curve c). Figure 3 (curve d) indicated that the CL intensity of the $Eu^{3+}$-NAP-Ce(IV)-$Na_2S_2O_4$ system was markedly enhanced in the presence of Ag NPs, and hence that the CL intensity of the presented system was markedly improved with the addition of Ag NPs.

### Optimization of Experimental Conditions

#### *Choice of Inorganic Acids*

The kind and concentration of the acid used in the reaction significantly affect the enhancement of the CL intensity.

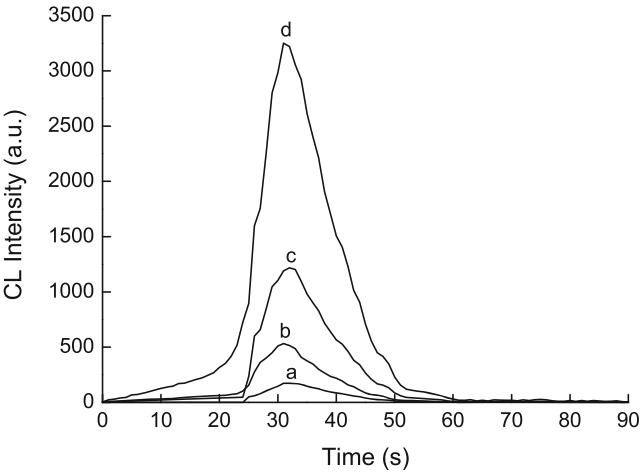



**Fig. 3** CL profiles of the Ce(IV)-$Na_2S_2O_4$-$Eu^{3+}$-NAP-Ag NPs system: **a** Ce(IV)-$Na_2S_2O_4$; **b** Ce(IV)-$Na_2S_2O_4$-$Eu^{3+}$; **c** Ce(IV)-$Na_2S_2O_4$-$Eu^{3+}$-NAP; and **d** Ce(IV)-$Na_2S_2O_4$-$Eu^{3+}$-NAP-Ag NPs. Conditions: NAP, 220 ng $mL^{-1}$; $H_2SO_4$, 40 mmol $L^{-1}$; Ce(IV), 0.13 mmol $L^{-1}$; $Na_2S_2O_4$, 0.22 mmol $L^{-1}$; $Eu^{3+}$, 0.25 mmol $L^{-1}$; Ag NPs, 0.7 mmol $L^{-1}$

Thus, several inorganic acids such as HCl, $HNO_3$, $H_3PO_4$ and $H_2SO_4$ were added to the Ce(IV) solution to investigate the effect of each acid on the CL signal. $H_2SO_4$-treated Ce (IV) exhibited the strongest CL intensity and the most stable signal. Hence, $H_2SO_4$ acid was selected for this experiment. In investigating the effect of the $H_2SO_4$ concentration, 40 mmol $L^{-1}$ $H_2SO_4$ in Ce(IV) solution showed the maximum CL signal. Hence, 40 mmol $L^{-1}$ $H_2SO_4$ acid was chosen for further study.

*Effect of Ce(IV) Concentration*

The effect of the Ce(IV) concentration on the CL intensity was investigated in the range of 0.02 to 0.25 mmol $L^{-1}$. The CL intensity was increased with increasing Ce(IV) concentration up to 0.13 mmol $L^{-1}$ (Fig. S1, supplementary material). Thus, a Ce(IV) concentration of 0.13 mmol $L^{-1}$ was chosen for the subsequent experiments.

*Effect of $Na_2S_2O_4$ Concentration*

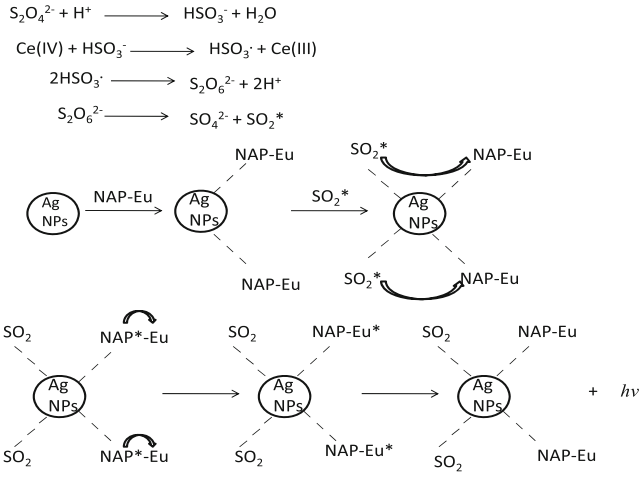
$Na_2S_2O_4$ was used as reductant in this CL system and thereby affected the system sensitivity. The effect of $Na_2S_2O_4$ concentration on the CL intensity was studied in the range of 0.13–0.3 mmol $L^{-1}$. The maximum CL intensity was obtained with a $Na_2S_2O_4$ solution concentration of 0.22 mmol $L^{-1}$ (Fig. S2, supplementary material).

*Effect of $Eu^{3+}$ Concentration*

The effect of $Eu^{3+}$ concentration on the CL signal was investigated over the range of 0.02–0.47 mmol $L^{-1}$. The CL intensity was increased with increasing $Eu^{3+}$ concentration (Fig. S3, supplementary material). By considering the sensitivity and reagent consumption, 0.25 mmol $L^{-1}$ $Eu^{3+}$ was selected for further study.

*Effect of Ag NP Concentration*

The concentration of colloidal Ag NPs can greatly influence the CL intensity of the system. The effect of the Ag NP solution was studied over the concentration range of the colloidal solution from 0.2 to 1.2 mmol $L^{-1}$. The CL intensity of the system peaked with 0.7 mmol $L^{-1}$ colloidal solutions of Ag NPs (Fig. S4, supplementary material). When the colloidal solution of Ag NPs was added to the $Eu^{3+}$-NAP complex, the local refractive index around the complex may have been changed, leading to the modification of the electric dipole transition rate. The colloidal solution concentration of more than 0.7 mmol $L^{-1}$ may have distorted the local field arising from the self interaction of plasmon electrons of Ag NPs [31]. This effect may have resulted in the quenching of luminescence intensity. Hence, the Ag NP colloidal solution of 0.7 mmol $L^{-1}$ was selected in this study in order to maximize the CL signal.




**Scheme 1** Possible reaction mechanism of the Ag NP-enhanced $Eu^{3+}$-NAP-Ce(IV)-$Na_2S_2O_4$ chemiluminescence (CL) system

## Possible Interaction Mechanism

The reaction of Ce(IV) with $S_2O_4^{2-}$ in an acidic medium has been studied in detail. In an acidic medium, $Na_2S_2O_4$ can produce $HSO_3^-$, which can be oxidized by Ce(IV) to produce the hydrogen sulfite radical $HSO_3$*. The $HSO_3$* radical then form $S_2O_6^-$ and excited intermediate product $SO_2$*. When $SO_2$* relaxes to its ground state, a photon is emitted [32] in the spectral region of 300 to 450 nm [33]. However, the CL intensity is weak because of the low luminescence

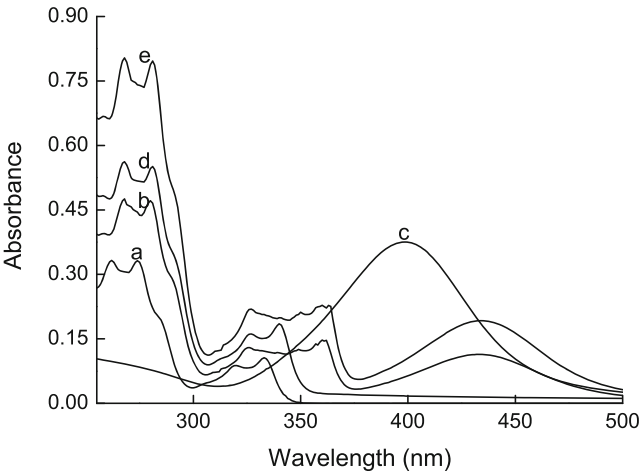



**Fig. 4** UV-visible absorption spectra of the system: **a** NAP; **b** $Eu^{3+}$-NAP; **c** Ag NPs; **d** NAP-Ag NPs; and **e** $Eu^{3+}$-NAP-Ag NPs. Conditions; NAP, 220 ng $mL^{-1}$; $Eu^{3+}$, 0.25 mmol $L^{-1}$; Ag NPs, 0.7 mmol $L^{-1}$

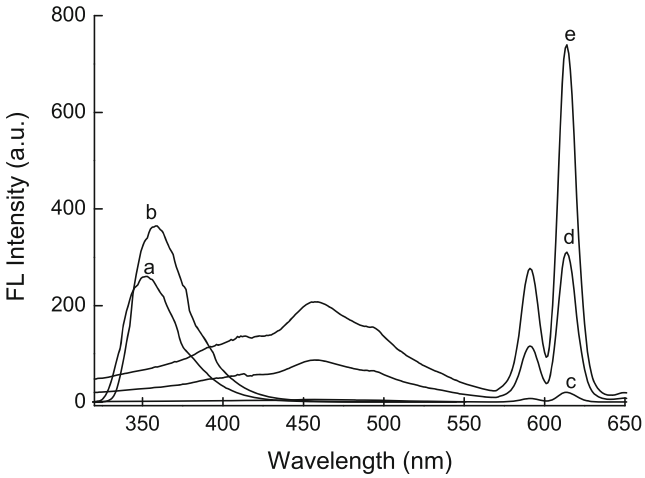



**Fig. 5** Fluorescence emission spectra of the system: **a** NAP; **b** NAP-Ag NPs; **c** $Eu^{3+}$; **d** $Eu^{3+}$-NAP; and **e** $Eu^{3+}$-NAP-Ag NPs. Conditions; NAP, 220 ng $mL^{-1}$; $Eu^{3+}$, 0.25 mmol $L^{-1}$; Ag NPs, 0.7 mmol $L^{-1}$

efficiency of $SO_2$*. The excited $SO_2$* transfers its energy to a fluorophore with an absorption band at 300~450 nm [34], which is used to enhance the CL intensity. NAP can form a chelate with $Eu^{3+}$ ($Eu^{3+}$-NAP). When $Eu^{3+}$ and NAP are added to the Ce(IV)-$Na_2S_2O_4$ system, the energy of the excited $SO_2$* is transferred to $Eu^{3+}$ through NAP (shown in Scheme 1). It was assumed that the excited $SO_2$* and $Eu^{3+}$-NAP are both adsorbed by Ag NPs and the excited $SO_2$* transfers its energy to the $Eu^{3+}$-NAP more easily because of the high electronic sensitivity of the Ag NPs (Scheme 1). Hence, $Eu^{3+}$ was well excited with the aid of Ag NPs which enhanced the CL intensity significantly.

To investigate the mechanism, the UV-visible absorption and fluorescence spectra of NAP, $Eu^{3+}$, Ag NPs and their mixture were studied and the results are shown in Figs. 4 and 5. NAP exhibited two absorption peaks at 261 and 273 nm (Fig. 4a). The absorption spectra of NAP were increased with a red shift from 261 and 273 nm to 268 and 282 nm, respectively, (Fig. 4b) when $Eu^{3+}$ was added to NAP solution, indicating the formation of the $Eu^{3+}$-NAP complex. Ag NPs showed a notable absorption peak at 399 nm (Fig. 4c), which was red shifted to 435 nm (Fig. 3d) when NAP was mixed with Ag NPs solution. In the presence of Ag NPs, the absorption intensity of the $Eu^{3+}$-NAP complex was increased (Fig. 4e), indicating that more energy had been transferred to $Eu^{3+}$.

The fluorescence spectra of NAP, Ag NPs-NAP, $Eu^{3+}$, $Eu^{3+}$-NAP and $Eu^{3+}$-NAP-Ag NPs are presented in Fig. 5. In the fluorescence spectra, NAP exhibited a characteristic fluorescence signal at about 352 nm (Fig. 5a). When Ag NPs were added to the NAP solution, the fluorescence signal was increased (Fig. 5b), indicating that Ag NPs were favorable for the excitation of NAP. $Eu^{3+}$ showed characteristic fluorescence peaks at 591 and 614 nm (Fig. 5c), corresponding to the $^5D_0$-$^7F_1$ and $^5D_0$-$^7F_2$ transitions of the $Eu^{3+}$ ion, respectively. When NAP was mixed with $Eu^{3+}$ solution, the fluorescence intensity was increased significantly (Fig. 5d), which indicated the formation of the $Eu^{3+}$-NAP complex. The characteristic fluorescence peak was enhanced markedly by the introduction of Ag NPs into the $Eu^{3+}$-NAP system. This phenomenon indicated that the formation of the $Eu^{3+}$-NAP complex enabled more energy to be transferred to $Eu^{3+}$ with the aid of Ag NPs. Moreover, according to Förster theory, there should have some overlaps between the FL spectrum of the donor and the absorption spectrum of the acceptor to promote energy transfer efficiently. It can be illuminated that energy transfer occurred easily between NAP and $Eu^{3+}$ because of the strong spectral overlap between the FL spectra of donor (NAP) and the absorption spectra of accepter ($Eu^{3+}$) (Fig. S5, supplementary material). The above explanation further supports the CL reaction mechanism.

**Table 1** Merits of comparable methods for determining NAP

| Methods/reagents | Analytical ranges | LODs | Ref. |
|---|---|---|---|
| Spectrophotometry | 500–3,500 ng $mL^{-1}$ | 160 ng $mL^{-1}$ | [2] |
| Spectrofluorimetry | 50–200 ng $mL^{-1}$ | 30 ng $mL^{-1}$ | [6] |
| Phosphorimetry | 10–400 ng $mL^{-1}$ | 2.7 ng $mL^{-1}$ | [8] |
| HPLC | 40–2,000 ng $mL^{-1}$ | 16 ng $mL^{-1}$ | [14] |
| Chemiluminescence (Ce(IV) in acidic medium) | 100–1,000 ng $mL^{-1}$ | 15 ng $mL^{-1}$ | [17] |
| Chemiluminescence (sulfite-SDBS system in HCl) | 1.0–700 ng $mL^{-1}$ | 0.9 ng $mL^{-1}$ | [18] |
| Chemiluminescence ($KMnO_4$-$Na_2SO_3$) | 4.0–1,000 ng $mL^{-1}$ | 100 ng $mL^{-1}$ | [19] |
| Chemiluminescence ($Eu^{3+}$-$KIO_4$-$H_2O_2$) | 50–5,000 ng $mL^{-1}$ | 500 ng $mL^{-1}$ | [21] |
| Chemiluminescence (Ce(IV)-$Na_2S_2O_4$-$Eu^{3+}$-NAP-Ag NPs) | 1.0–420 ng $mL^{-1}$ | 0.11 ng $mL^{-1}$ | Proposed method |

*LOD* Limit of detection

**Table 2** Analytical results of NAP in pharmaceutical samples

| Sample | Amount (mg) | | Standard addition method | | |
|---|---|---|---|---|---|
| | Active ingredient label | Found by the proposed method ± RSD[a] | Added ($\times10^{-7}$ g mL$^{-1}$) | Observed ($\times10^{-7}$ g mL$^{-1}$) ± RSD[a] | Recovery (%) |
| 1 | 50 | 51.05±1.11 | 1.0 | 1.03±0.82 | 103 |
| | | | 3.0 | 2.97±1.05 | 99 |
| | | | 5.0 | 4.91±1.47 | 98.2 |
| 2 | 50 | 48.86±1.65 | 1.0 | 1.02±1.35 | 102 |
| | | | 3.0 | 3.11±1.45 | 103.66 |
| | | | 5.0 | 5.07±1.12 | 101.4 |

[a] Relative standard deviation for five replicate measurements

## Analytical Performance

Under the optimum conditions described above, a calibration graph of the CL intensity versus the NAP concentration was obtained. The CL intensity was increased with increasing NAP concentration in the linear range of 1 to 420 ng mL$^{-1}$, according to the regression equation of $Y$=6.02$C_{NAP}$ +956 ($r$=0.9992), where $C_{NAP}$ is the NAP concentration and Y the CL intensity in arbitrary unit (a.u.). The limit of detection (LOD) as defined by IUPAC, $C_{LOD}$ = 3 * Sb/m (where Sb is the standard deviation of the blank signals and m is the slope of the calibration graph), was 0.11 ng mL$^{-1}$ and the relative standard deviation (RSD) was 1.15% for 5 replicate determinations of 20 ng mL$^{-1}$ NAP. The sensitivity of the presented method is compared with that of other reported methods in Table 1: the presented method offered higher sensitivity for determining trace amounts of NAP.

## Interference Study

The effects of the potentially interfering species were examined in the determination of NAP for application of the presented method in pharmaceutical and biological samples. The tolerance level was defined as the amount of foreign species that produce an error not exceeding 5% in determining the analytes. The effect of potential interferents was therefore investigated by preparing a set of solutions, each one with 100 ng mL$^{-1}$ of the NAP plus a different concentration of the chemical species to be tested. The results implied that the foreign species did not interfere in the determination of NAP at the level of 1,000 fold for $K^+$, $Mg^{2+}$,$Ca^{2+}$, $Ni^{2+}$, $NO^{3-}$, and $CO_3{}^{2-}$, 800 fold for dextrin, urea, uric acid, $Zn^{2+}$, $Ba^{2+}$, $Cu^{2+}$, and $Cd^{2+}$, 500 fold for starch, glucose, and lactose, 100 fold for $Al^{3+}$, $Mn^{2+}$, $Fe^{2+}$, $Fe^{3+}$, and $Co^{2+}$, 50 fold for mannitol, sorbitol, and cellulose, and 10 fold for ascorbic acid, and salicylic acid. These results demonstrated the good selectivity of the presented method and its potential for successful application to determine NAP in pharmaceutical and biological samples.

## Analytical Application

### Determination of NAP in Pharmaceutical Samples

The presented method was applied to assay NAP in commercially available NAP tablets. Ten NAP tablets were weighed and ground into fine powder by pestle in a mortar. An accurately weighed portion of powder equivalent to 50 mg of NAP was accurately weighed and dissolved with DI water. The dissolved solution was filtered through a Millipore membrane filter paper, diluted appropriately with DI water and determined by the proposed method. The obtained results are

**Table 3** Analytical results of NAP in urine samples

| Sample | Added (μg mL$^{-1}$) | Found (μg mL$^{-1}$) ± RSD[a] | Standard addition method | | |
|---|---|---|---|---|---|
| | | | Added ($\times10^{-7}$ g mL$^{-1}$) | Found ($\times10^{-7}$ g mL$^{-1}$) ± RSD[a] | Recovery (%) |
| Urine | 1.0 | 1.04±1.19 | 2.0 | 1.98±1.03 | 99 |
| | | | 4.0 | 4.06±1.71 | 101.5 |
| | | | 6.0 | 6.02±0.82 | 100.33 |
| | | | 8.0 | 7.89±1.11 | 98.62 |
| | | | 10.0 | 10.13±1.23 | 101.3 |

[a]Relative standard deviation for five replicate measurements

summarized in Table 2 and indicated that the determination of NAP in the pharmaceutical samples was in good agreement with the labeled contents. Recoveries were found in the range of 98.2–103.66% for NAP.

### Determination of NAP in Spiked Human Urine

The proposed method was applied to determine NAP in spiked human urine. Samples of 1.0 mL of urine were collected from a healthy person. A known amount of NAP standard solution was added to the prepared spiked urine sample and diluted within the working range of determination. The results are listed in Table 3. The recoveries of NAP contents in the urine sample were 98.62–101.5%, which demonstrated the accuracy of the proposed method in determining NAP in urine samples.

## Conclusion

In the present study, Ag NPs exhibited strong catalytic activity and markedly enhanced the CL intensity of the $Eu^{3+}$-NAP-Ce(IV)-$Na_2S_2O_4$ system in determining NAP. This was attributed to the assumed behavior of Ag NPs in enhancing the CL intensity of the system by accelerating the energy transfer process from $SO_2$* to NAP and from NAP to $Eu^{3+}$ in aqueous solution. This enhancement of CL intensity was proportional to the NAP concentration, which showed a good linear relationship over the range of 1–420 ng $mL^{-1}$ with a low LOD (0.11 ng $mL^{-1}$). The presented method displayed good results in an assay of NAP in tablets and a urine sample. Based on these experimental results, the introduction of Ag NPs and $Eu^{3+}$ to the Ce(IV)-$Na_2S_2O_4$-NAP system enhanced the CL intensity, thereby demonstrating the potential role of Ag NPs as an enhancer in the CL system capable of expanding the analytical applications of nanomaterials and of meliorating the CL method.

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