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Determination of 2-methoxyestradiol in serum samples and pharmaceutical preparations by silver nanoparticles-enhanced chemiluminescence

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ABSTRACT

Silver nanoparticles (AgNPs) exhibited better chemiluminescence (CL) catalysis activity and smaller nanoparticles have stronger catalysis ability in luminol–$K_3Fe(CN)_6$ system among the synthesized AgNPs of different size. 10 ± 2 nm nanoparticles was used as catalysts to enhance the reaction sensitivity. It was found that the CL intensity of AgNPs–luminol–$K_3Fe(CN)_6$ was strongly inhibited in the presence of 2-methoxyestradiol (2-ME) and the relative CL intensity was in linear correlation with the concentration of 2-ME. Thus, the silver nanoparticles-enhanced CL method for the determination of 2-ME was developed. The proposed method has a detection limit ($3\ S_b/K$) of 5.0×10^{-10} mol L^{-1} with a relative standard deviation of 0.75% for 5.0×10^{-8} mol L^{-1} 2-ME. The method was successfully applied for determination of 2-ME in human serum and pharmaceutical preparations. The possible CL reaction mechanism was also discussed briefly. Oxygen radicals played an important role in the catalytic process.

1. Introduction

2-methoxyestradiol (2-ME) is a physiological metabolite of the endogenous estrogen estradiol-17β (Fig. 1). 2-ME can inhibit the proliferation, migration and invasion of endothelial cells in vitro and has antiangiogenic effects in several in vivo models. Nowadays, 2-ME is being tested clinically in patients with metastatic breast cancer, prostate cancer and various other solid tumors in a number of Phase I and Phase II trials [1,2].

Many means have been reported for 2-ME determination, including radioimmunoassay [3], LC/UV [4], LC/FID [5], LC/MS [6], and GC/MS [7]. However, these methods often suffer from drawbacks of low sensitivity, poor reproducibility and complicated pretreatment or expensive instrumentation. Therefore, it was very necessary to develop a simple, fast and sensitive method for the determination of 2-ME in pharmaceutical preparations and biological fluids. On account of its excellent sensitivity, wide linear dynamic range, short analysis time and inexpensive apparatus, the flow injection-CL method has received much attention in various fields [8–10].

In recent years, more and more attention has been paid to nanomaterials participating in CL systems to improve sensitivity and stability [11]. Nanoparticles used as catalysts have been rapidly growing for use in a variety of organic and inorganic reactions [12–14]. In the present work, silver nanoparticles could participate in the CL reactions of luminol with a variety of oxidants, including H_2O_2 [15,16], $K_3Fe(CN)_6$ [17], $KMnO_4$ [18] and $AgNO_3$ [19].

In this work, we studied the behavior of AgNPs in the CL reaction of luminol with $K_3Fe(CN)_6$. It was found that AgNPs could catalyze luminol–$K_3Fe(CN)_6$ CL and the catalysis ability of AgNPs varied from their sizes. The results demonstrated that smaller particle size have stronger catalysis ability. When 2-ME is injected into the AgNPs–luminol–$K_3Fe(CN)_6$ system, the CL signal could be greatly inhibited. Based on this phenomenon, a simple, rapid and sensitive flow injection-CL method for 2-ME determination was proposed. The proposed method has a detection limit of 5.0×10^{-10} mol L^{-1} for 2-ME and has been applied to the analysis of 2-ME in human serum and pharmaceutical samples. The CL spectra were analyzed and CL mechanism has been proposed. Oxygen radicals played an important role in the catalytic process.

2. Experimental

2.1. Reagents

Silver nitrate ($AgNO_3$, $\geq 99.8\%$) was obtained from Beijing Fine Chemicals Co., Ltd. (Beijing, China). Sodium borohydride ($NaBH_4$, $\geq 99.0\%$) and trisodium citrate dehydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, $\geq 99.0\%$) were purchased from Tianjin Chemical Reagent Corporation (Tianjin, China).

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All chemicals were of analytical reagent grade and were used without further purification. All solutions were diluted using deionized water. 2-ME (Zhengzhou University, China) stock solution (5.0×10^{-4} mol L^{-1}) was prepared by dissolving 15.12 mg 2-ME in 100 mL of methanol and working standards were prepared by diluting with deionized water as required. The stock solution of 2-ME was stored at 4 °C in refrigerator to avoid exposure to light and air. Luminol (Solarbio, China) solution (1.0×10^{-2} mol L^{-1}) was prepared with 0.1 mol L^{-1} NaOH in a 100 mL brown volumetric flask and stored at 4°C, working solutions of luminol were prepared by diluting the stock solution with 0.1 mol L^{-1} NaOH solution. $K_3Fe(CN)_6$ (Kermel Chemical Reagent Co., Ltd, Tianjin, China) stock solution (5.0×10^{-3} mol L^{-1}) was prepared by dissolving the required amount of compound in deionized water and stored in the dark. Fresh solutions were prepared daily.

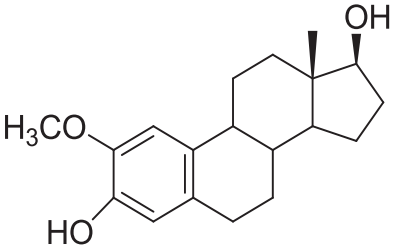


Fig. 1. Chemical structure of 2-ME.

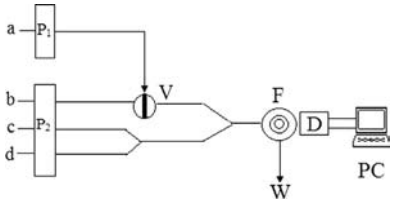


Fig. 2. Schematic diagram of the flow system for determination of 2-ME: (a) $K_3Fe(CN)_6$ (3.0×10^{-5} mol L^{-1}); (b) AgNPs (2.5×10^{-9} mol L^{-1}); (c) luminol (1.0×10^{-5} mol L^{-1}) prepared in 0.1 mol L^{-1} NaOH; and (d) 2-ME; P – peristaltic pump; V – injection valve; F – flow cell; W – waste; D – detector; and PC – personal computer.

2.2. Apparatus

A schematic diagram of the flow system employed in this work is shown in Fig. 2. Two pumps of Luminescence Analyzer (IFFM-E, Remax Analytical Instrument Limited Co., Xi'an, China) were used to deliver flow streams. Polytrafluoroethylere (PTFE) tubing (0.8 mm i.d.) was used to connect all components in the flow system. The flow cell was a 10 cm length of spiral glass tubing (0.5 mm i.d.) and the distance between injection valve and flow cell was about 10 cm. The CL signal was detected by the photomultiplier tube (PMT) voltage of 500 V placed near the flow cell and was recorded with a computer. UV absorption spectra were measured on UV-2550 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were recorded with a RF-5301 fluorospectrophotometer (Shimadzu, Japan) to study the luminescence characteristics.

2.3. Synthesis of AgNPs

AgNPs were synthesized by the reduction of $NaBH_4$ and stabilized using $Na_3C_6H_5O_7$ according to the published method [20]. First, 100 mL of a solution containing $AgNO_3$ and $Na_3C_6H_5O_7$, both in a concentration of 0.25 mmol L^{-1}, was prepared and stirred for 30 s. Then, 3 mL 1.0 mmol L^{-1} solution of $NaBH_4$ (freshly prepared) was added quickly to the mixture. The solution immediately turned to yellow and was stirred for 60 s and then stored in refrigerator at 4 °C. The size and monodispersity of the obtained nanoparticles was determined by Transmission electron microscopy (TEM) (Tecnai G2 20, Czech) and the diameter of particles was 10 ± 2 nm as shown in Fig. 3(a). The prepared AgNPs were characterized with UV–visible absorption spectra, and the surface plasmon resonance peak of AgNPs was at about 390 nm (Fig. 3(b)).

2.4. Procedures

Using the flow system schematically shown in Fig. 2, flow lines were inserted into the $K_3Fe(CN)_6$ solution, carrier stream (AgNPs), luminol and 2-ME solution, respectively. $K_3Fe(CN)_6$ (3.0×10^{-5} mol L^{-1}) solution was injected into the AgNPs solution by a six-way injection valve, merged at a T-piece with the mixture of luminol (1.0×10^{-5} mol L^{-1}) prepared in 0.1 mol L^{-1} NaOH and 2-ME solution, then reached the flow cell, producing the CL

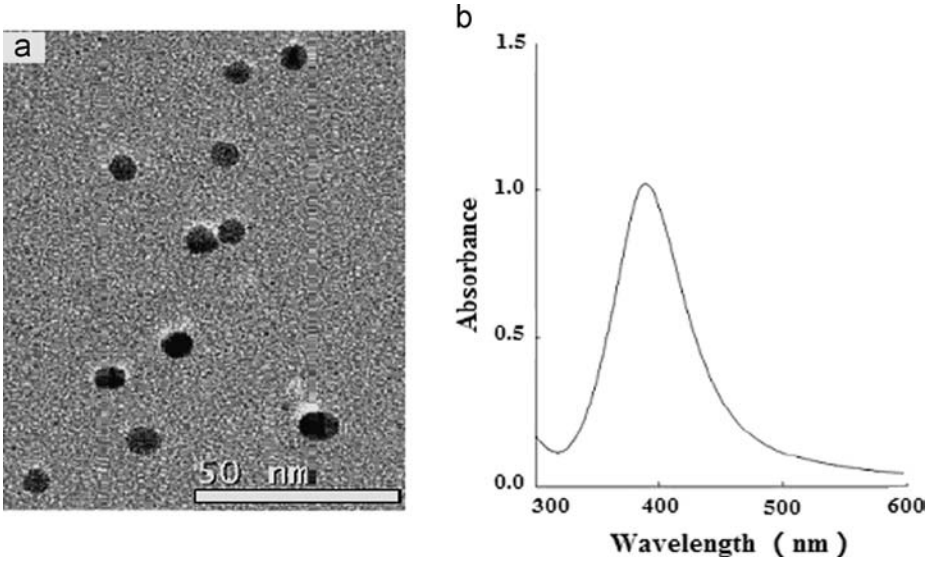


Fig. 3. TEM image (a) and UV–vis absorbent spectrum (b) of the AgNPs.

emission. The concentration of the 2-ME was quantified by measuring the relative CL intensity $\Delta I = I_0 - I_s$, where I_s and I_0 are the CL intensities of sample solution and blank solution. The flow rates of P_1, P_2 were set at 1.8 and 2.4 mL min^{-1}, respectively.

2.5. Sample preparation

2-ME injection samples were home-made by the School of Pharmaceutical Sciences, Zhengzhou University. Injections were diluted appropriately with water prior to measurement so that the concentration of 2-ME was in its linear response range.

The human serum samples were obtained from the Red Cross Blood Center in Henan Province (China). Three different known amounts of standard solution of 2-ME (5.0×10^{-4} mol L^{-1}) were added to 200 μL of serum samples, respectively, and then were treated with 600 μL methanol and thoroughly vortex-mixed for 3 min. After centrifugation at 12,000 rpm for 15 min, the supernatants were collected and diluted with water appropriately so that the final concentration was in the linear range of determination. A blank solution was prepared by treating the drug-free serum in the same way.

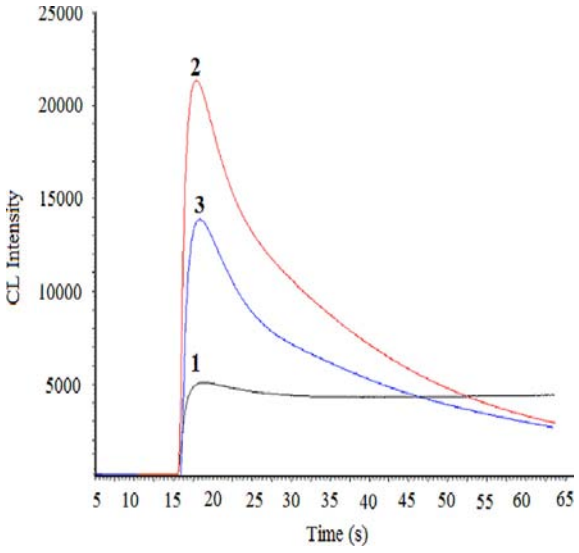


Fig. 4. The kinetic curve of CL reaction. Conditions: luminol, 1.0×10^{-5} mol L^{-1} (0.1 mol L^{-1} NaOH); $K_3Fe(CN)_6$, 3.0×10^{-5} mol L^{-1}; AgNPs, 2.5×10^{-9} mol L^{-1}; 2-ME, 5.0×10^{-8} mol L^{-1}. (1), luminol+$K_3Fe(CN)_6$; (2), (1)+AgNPs; (3), (2)+2-ME.

3. Results and discussion

3.1. Kinetic characteristics of the CL reaction and catalyzed by AgNPs

Before carrying out the flow injection method, the batch method for the CL dynamic profiles was applied. From Fig. 4, it can be seen that a CL signal of luminol–$K_3Fe(CN)_6$ was observed (curve 1). But the CL intensity was greatly enhanced when the AgNPs were added in the solution (curve 2). After adding 2-ME into the above mixing solution, the CL of the AgNPs–luminol–$K_3Fe(CN)_6$ system was strongly inhibited (curve 3). The time interval between the start of CL and its maximum is about 3.0 s. After 3.0 s, the CL intensity gradually declined to the baseline. It can be seen that the CL reaction was a flash-type luminescent one.

3.2. Optimization of the reaction conditions

The reaction conditions were optimized for 2-ME–AgNPs–luminol–$K_3Fe(CN)_6$ CL system as shown in Fig. 5. The CL reaction was performed in alkaline condition and the concentration of NaOH was examined from 0.01 to 0.20 mol L^{-1}, and the maximal emission was obtained at 0.1 mol L^{-1} NaOH (Fig. 5(a)). Thus, 0.1 mol L^{-1} NaOH was the optimal concentration for the experiments. The effect of the luminol concentration on the CL intensity was examined in the range of 1.0×10^{-6}–1.0×10^{-5} mol L^{-1}. It was found that the CL intensity increased linearly with the luminol concentration increasing (Fig. 5(b)). Therefore, 1.0×10^{-5} mol L^{-1} luminol was chosen for the following studies. The effect of $K_3Fe(CN)_6$ concentration on the CL was studied in the range of 1.0×10^{-6}–5.0×10^{-5} mol L^{-1}, the maximun ratio of signal-to-noise and stability was obtained with 3.0×10^{-5} mol L^{-1} (Fig. 5(c)). Thus, 3.0×10^{-5} mol L^{-1} $K_3Fe(CN)_6$ was used in all subsequent experiments. The effect of the concentration of Ag nanoparticles was also investigated. According to the calculation method of literature [21], the prepared AgNPs concentration was 8.2 nmol L^{-1}. As shown in Fig. 5(d), the CL intensity increased with the concentration of AgNPs in the range of 8.0×10^{-11}–4.0×10^{-9} mol L^{-1}. Considering the linear and the consumption of the reagents, 2.5×10^{-9} mol L^{-1} AgNPs was selected for further studies.

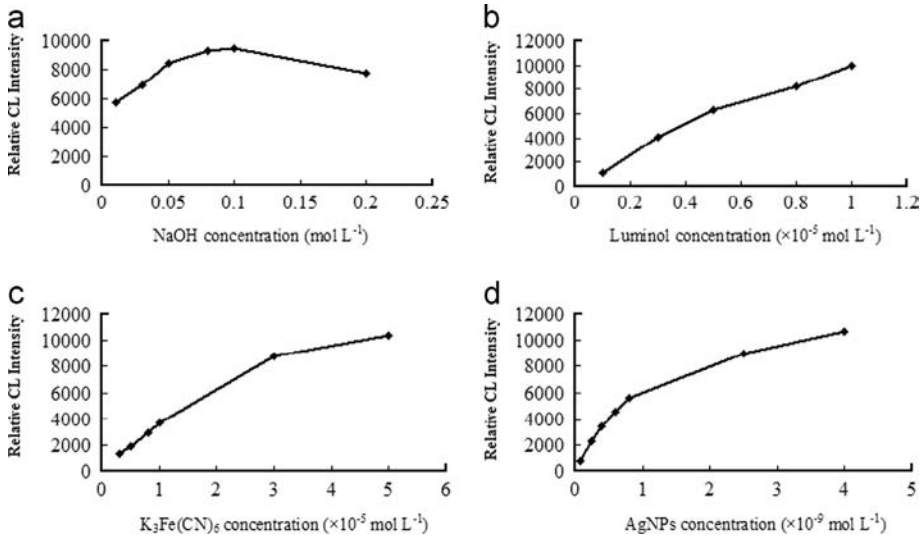


Fig. 5. Effect of NaOH, luminol, $K_3Fe(CN)_6$ and AgNPs concentration on the CL intensity for 5.0×10^{-8} mol L^{-1} 2-ME.

3.3. Analytical characteristics of the FI–CL system

Under the optimum conditions mentioned above, the calibration graphs were obtained for 2-ME determination by plotting the CL intensity (ΔI, relative unit) versus 2-ME concentration (C,) which gave a linear range from 5.0×10^{-9} to 1.0×10^{-7} mol L^{-1}, with a detection limit ($3S_b/K$) of 5.0×10^{-10} mol L^{-1} (S_b: the standard deviation of the blank value; K: the slope of the standard curve). The regression equation was $\Delta I=589.9+1486.6C$ (C, $\times10^{-8}$ mol L^{-1}) with a correlation coefficient of 0.9988 ($n=7$). The relative standard deviation (RSD) was 0.75% for 5.0×10^{-8} mol L^{-1} 2-ME in 8 replicate measurements.

3.4. Interference

The influence of foreign species was studied by analyzing a standard solution of 5.0×10^{-8} mol L^{-1} 2-ME to which increasing amounts of foreign species were added. A substance was considered no interference if the variation of the CL intensity was within $\pm5\%$. The results are listed in Table 1, most of metal and nonmetal ions do not interfere, however, halide ions were easily adsorbed on the surface of Ag nanoparticles with getting well with the report [22]. It is deduced that halide ions may change the catalytic activity of the Ag nanoparticles due to the adsorption. Therefore, halide ions should not be introduced into the AgNPs system in order to avoid producing influences.

3.5. Analytical application

Following the procedure described previously, the proposed CL method was applied to the determination of 2-ME in injection samples. The determination results are listed in Table 2, which agrees well with those obtained by the HPLC method. The recovery of 2-ME in human serum was determined by the proposed method with the standard addition method. The determination results of recovery in human serum are listed in Table 3, which suggested that the proposed method can be satisfactorily used for the determination of 2-ME in serum samples.

Table 1
Effect of foreign substances on the determination of 5.0×10^{-8} mol L^{-1} 2-ME under optimum conditions.

Substance	Tolerance concentration ratio
Na^+, $NO3^-$, CO_3^{2-}, Lactose	1000
Mg^{2+}, Ca^{2+}, Glucose, Sodium citrate	500
K^+, NH_4^+, SO_4^{2-}, Cu^{2+}, cysteine	200
Al^{3+}, SO_3^{2-}, EDTA, estradiol, ascorbic acid	100
Cl^-, HP-β-CD	50
I^-	10

3.6. Possible mechanism

In order to obtain the possible mechanism, the CL spectra were performed using the modified RF-5301 spectrofluorimeter with the light source taken off. From Fig. 6, it can see that the maximum wavelength of luminol–$K_3Fe(CN)_6$, luminol–$K_3Fe(CN)_6$–AgNPs and luminol–$K_3Fe(CN)_6$–AgNPs–2-ME was at 425 nm, indicating that the luminophor in the AgNPs–luminol–$K_3Fe(CN)_6$ reaction with and without 2-ME is the same species, which is the oxidation product 3-aminophthalate (3-AP*) of luminol [23].

Du et al. [24] had reported the possible reaction mechanism of gold-catalyzed CL of luminol. It was deduced that CL reaction of luminol catalyzed by AgNPs might have the similar reaction mechanism. AgNPs as the catalyst in the reaction, hydroxyl radical ($^{\bullet}OH$) and superoxide radical ($O_2^{\bullet-}$) were frequently reported [16,25]. The adsorption of oxygen proceeded directly on nanoparticles [26] and then formed peroxides and superoxides [27]. As shown in Scheme 1, under the catalytic action of AgNPs, the formed $O_2^{\bullet-}$ participated in the reactions to generate H_2O_2. The generated H_2O_2 adsorbed on the surface of AgNPs. Partial electrons were transferred to H_2O_2 by the catalytic effect of AgNPs, resulting in the broken of O–O band of H_2O_2 into double $^{\bullet}OH$ radical [28]. $^{\bullet}OH$ radicals reacted with HO_2^- and luminol anion, generating $O_2^{\bullet-}$ and luminol radicals. It was followed by the reaction between $O_2^{\bullet-}$ and luminol radicals and generated CL in alkaline condition. To examine whether these oxygen radicals participate in the CL reaction, the effect of scavengers on the reaction was studied. The CL signal was inhibited 90% by common oxygen radical scavengers of ascorbic acid (0.03 mmol L^{-1}). The addition of methanol (10%) inhibited 68% of the CL signal. 5.0 mmol L^{-1} mannitol and sodium azide inhibited 20%, respectively. Adding to oxygen radical scavengers, the CL signal was inhibited to some extent. Therefore, it was concluded that these oxygen radicals participated in the CL reaction.

Based on the above discussion and related literature [16,29], the possible CL mechanism can be summarized as follows: luminol was oxidated by $K_3Fe(CN)_6$ to form luminol radical in alkaline conditions. Luminol radical was catalyzed by oxygen radicals on the surface of AgNPs and generate the key intermediate more easily [29]. The phenolic hydroxyl group in the molecular structure

Table 3
Recovery for 2-ME in human serum samples.

Sample no.	Added (10^{-8} mol L^{-1})	Detected [a] (10^{-8} mol L^{-1})	Recovery (%)	RSD (%, $n=3$)
1	1.00	0.962 ± 0.02	96.2	2.1
2	5.00	5.15 ± 0.05	103.0	2.4
3	8.00	7.55 ± 0.03	94.4	0.9

[a] Mean ± SD of three measurements.

Table 2
Recovery results for the determination of 2-ME in pharmaceutical preparations.

2-ME	HPLC method (10^{-8} mol L^{-1})	FI–CL method (10^{-8} mol L^{-1})	Added (10^{-8} mol L^{-1})	Found [a] (10^{-8} mol L^{-1})	Recovery (%)	RSD(%, $n=3$)
1	2.89	2.92	1.00	3.90 ± 0.02	98.0	1.7
			2.00	4.84 ± 0.04	96.0	2.3
			5.00	7.93 ± 0.03	100.2	0.8
2	2.43	2.40	1.00	3.38 ± 0.02	98.0	1.3
			2.00	4.42 ± 0.05	101.0	0.7
			5.00	7.39 ± 0.02	99.8	0.8
3	2.70	2.72	1.00	3.69 ± 0.06	97.0	1.2
			2.00	4.69 ± 0.03	98.5	1.6
			5.00	7.63 ± 0.05	98.2	2.5

[a] Mean ± SD of three measurements.

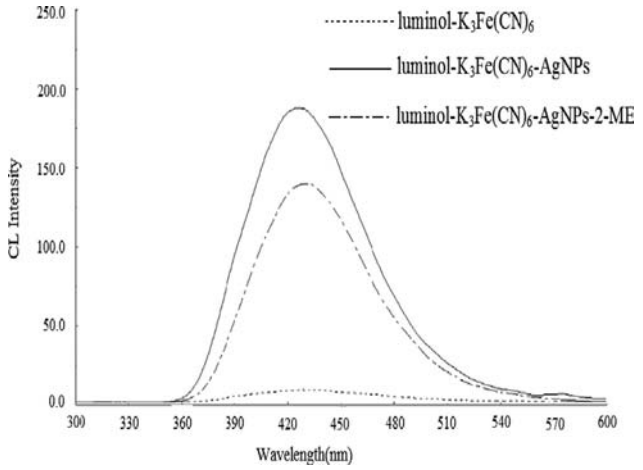
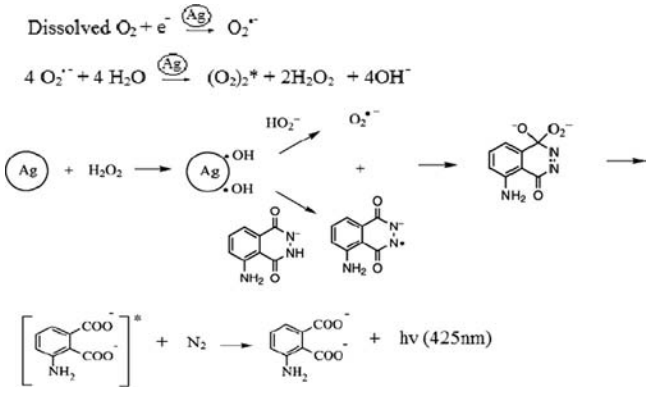


Fig. 6. CL spectra of the reactions. (1) luminol–$K_3Fe(CN)_6$; (2) (1)+AgNPs; (3) (2)+2-ME. Condition: luminol, 5.0×10^{-5} mol L^{-1} (0.2 mol L^{-1} NaOH); $K_3Fe(CN)_6$, 5.0×10^{-5} mol L^{-1}; AgNPs, 2.5×10^{-9} mol L^{-1}; and 2-ME, 1.0×10^{-5} mol L^{-1}.



Scheme 1. Possible mechanism of CL reaction catalyzed by AgNPs.

of 2-ME reacts with luminol intermediates to reduce the luminophor [16], leading to weakening of the signal.

4. Conclusions

It was found that AgNPs could act as a nanocatalyst on the luminol–$K_3Fe(CN)_6$ system to generate CL. In the presence of 2-ME, the CL intense of AgNPs–luminol–$K_3Fe(CN)_6$ was inhibited. The CL emitter is 3-aminophthalate, which is the oxidation product of luminol. The proposed method has the advantages of simplicity and sensitivity, which is also suitable for the detection of 2-ME in human serum and pharmaceutical preparations with satisfactory results.

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