

# Electrogenerated chemiluminescence sensor for itopride with $\text{Ru}(\text{bpy})_3^{2+}$ -doped silica nanoparticles/chitosan composite films modified electrode

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## Abstract

A electrogenerated chemiluminescence (ECL) sensor for itopride was developed based on tris(2,2-bipyridyl)ruthenium(II) ( $\text{Ru}(\text{bpy})_3^{2+}$ )-doped silica (RuDS) nanoparticles/biopolymer chitosan composites membrane modified glassy carbon electrode (GCE). The RuDS nanoparticles ( $52 \pm 5$  nm) were prepared by a modified Stöber synthesis method and were characterized by electrochemical, fluorometric and transmission electron microscopy technology. The  $\text{Ru}(\text{bpy})_3^{2+}$  encapsulation interior of the silica nanoparticle maintains its electrochemical activities and also reduces  $\text{Ru}(\text{bpy})_3^{2+}$  leaching from the silica matrix when immersed in water due to the electrostatic interaction. The ECL analytical performances of this ECL sensor for itopride based on its enhancement ECL emission of  $\text{Ru}(\text{bpy})_3^{2+}$  were investigated in details. Under the optimum condition, the enhanced ECL intensity was linear with the itopride concentration in the range of  $1 \times 10^{-8}$  to  $2 \times 10^{-5}$  g/mL ( $R=0.9978$ ). The detection limit was  $3 \times 10^{-9}$  g/mL, and the relative standard deviation was 2.3% for  $8 \times 10^{-8}$  g/mL itopride ( $n=11$ ). The method was successfully applied to the determination of itopride in pharmaceutical and human serum samples with satisfactory results. The as-prepared ECL sensor for the determination of itopride displayed good sensitivity and stability.

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## 1. Introduction

Itopride hydrochloride (*N*-[4-[2-(dimethylamino)-ethoxy]benzyl]-3,4-dimethoxybenzamide hydrochloride) (itopride, represented in Fig. 1) is a novel gastropkinetic agent which stimulates gastrointestinal motor activity through synergistic effects of dopamine D2 receptor blockade and acetylcholine esterase inhibition [1–3]. It is prescribed for the treatment of gastrointestinal symptoms caused by reduced gastrointestinal motility, feeling of gastric fullness, upper abdominal pain, anorexia, heartburn, nausea and vomiting, non-ulcer dyspepsia or chronic gastritis.

Very few analytical methods for detection of itopride using fluorescence [2,4,5], UV [6–8] and mass spectrometry (MS) [9] detector have been reported in the literature. These methods are

limited because of the need of arduous sample preparation and long chromatographic run-times or the price of the equipment or its low sensitivity. So, we need to look for new methods of determining itopride. Extensive literature survey revealed that no method is available for determination of itopride by electrogenerated chemiluminescence (ECL).

ECL was widely used in biological, pharmaceutical, chemical, and environmental applications due to its continuance, sensitivity, reproducibility and relative easiness to be automatically controlled [10]. Among numerous ECL reagents,  $\text{Ru}(\text{bpy})_3^{2+}$  and its derivatives are the most widely used complexes for analytical investigation and application owing to that it can give strong luminescence, electrochemically be recycled and dissolve in variety of solvents [11]. The ECL method using  $\text{Ru}(\text{bpy})_3^{2+}$  has been used extensively for detection of numerous substances, such as organic acid [12], amines [13], amino acids [14], ascorbic acid [15] and some drugs containing tertiary amine functional group [16–20]. However, its widespread application is limited by the requirement to continuously deliver the

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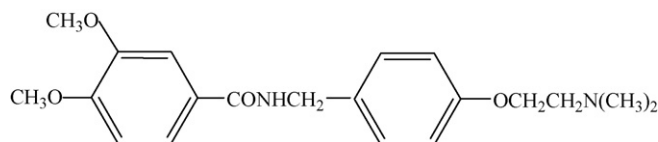


Fig. 1. Structure of itopride.

ECL reagent into the reaction zone because the reagent is consumed, which implies high cost and pollution. Since  $\text{Ru}(\text{bpy})_3^{2+}$  can be electrochemically recycled, these problems can be overcome by immobilizing  $\text{Ru}(\text{bpy})_3^{2+}$  on an electrode surface, which can not only minimize the consumption of  $\text{Ru}(\text{bpy})_3^{2+}$ , but also allows simpler instrumentation [13]. Up to now, a number of different approaches have been tried to immobilize  $\text{Ru}(\text{bpy})_3^{2+}$  or its derivatives on a variety of different electrode surfaces [21–30], including the immobilization of  $\text{Ru}(\text{bpy})_3^{2+}$  in Nafion films [10], the direct attachment to an electrode by a Langmuir–Blodgett techniques [22] or by self-assembly [23], the immobilization by sol–gel techniques [11,24–31]. Those methods have gained acceptance as analytical techniques. However, the Langmuir–Blodgett films are easily washed away from the electrode surface especially when organic solvent is applied, the self-assembled films are unstable during the potential scan, the sol–gel technique usually need to be calcined at high temperature and the films are easily cracked. Nafion film has no problems mentioned above but suffer from slow mass transfer through the film and the partitioning of  $\text{Ru}(\text{bpy})_3^{2+}$  into the more hydrophobic regions of Nafion. In past few years, there are several kinds of doped nanoparticles, especially the  $\text{Ru}(\text{bpy})_3^{2+}$ -doped silica nanoparticles (RuDS) is the widely studied. At first this kind of RuDS was extensively used as photostable biomarker in spectrofluorometric measurements [32,33]. Recently, Zhang et al. [34–36] found that as a typical ECL reagent,  $\text{Ru}(\text{bpy})_3^{2+}$  could still retain its ECL property even after doping inside the silica nanoparticles, and the exterior nano-silica prevented the electroactive reagent from leaching out into the aqueous solution due to the electrostatic interaction. And it has been used in the field of ECL. The inverse microemulsion polymerization was the most widely used method for the preparation of RuDS because of its simplicity of preparation process. However, this technique requires large amounts of surfactants and organic solvents. Therefore, scaling-up these procedures proved to be difficult because it is pollutional to the environment, high costs and time-consuming. In this article, we provide one improved cost-effective and environmentally friendly approach for the preparation of RuDS nanoparticles and also develop a novel method for the determination of itopride with the ECL by using RuDS nanoparticles/chitosan nanocomposites membrane modified GCE.

## 2. Experimental

### 2.1. Materials

Tris(2,2'-bipyridyl)dichlororuthenium(II) ( $\text{Ru}(\text{bpy})_3^{2+}$ ) was purchased from Sigma (St. Louis, MO, USA). Chitosan

was from Shanghai Yuanju Biochemistry Reagents Co. Ltd. (Shanghai, China). Tetraethyl orthosilicate (TEOS) and ethanol were obtained from Shanghai Chemical Plant (Shanghai, China). Ammonium hydroxide (28–30 wt.%) was purchased from Xi'an Chemical Reagent Company (Xi'an, China). Itopride hydrochloride 99.96% purity was obtained from Beijing Comens Chemical Co. Ltd. (Beijing, China). It was used without further purification. Distilled, deionized water was used throughout the analysis. Unless otherwise stated all chemicals and reagents used in this study were of analytical grade quality.

### 2.2. Apparatus

Cyclic voltammetric experiments were performed with a CHI660B Electrochemistry Working Station (CH Instruments Inc., Austin, TX, USA). All experiments were carried out with a conventional three-electrode system. The working electrode was glassy carbon electrode (GCE) coated with RuDS nanoparticles/chitosan nanocomposites membrane. A Pt wire was used as the counter electrode, and Ag wire was used as reference electrode, which is invariable during the experiment period. All the potentials were measured and reported according to this reference electrode. The ECL intensity produced in the electrolytic cell was detected and recorded by a flow injection chemiluminescence analyzer (IFFD, Xi'an Remax Electronic Science Tech. Co. Ltd., Xi'an, China), which was operated by a personal computer.

The synthesized RuDS nanoparticles were characterized by a transmission electron microscope (TEM; Hitachi H700, Hitachi, Tokyo, Japan) for the size and morphology. Scanning electron microscopy (SEM) images were determined with a Philips FEI-30 Quanta 200 SEM (FEI Company, Eindhoven, The Netherlands). For SEM imaging, the RuDS nanoparticles/chitosan composites membrane was dropped on the GCE.

### 2.3. Stock and working standard solution

Stock solution (1.0 mg/mL) of itopride hydrochloride was prepared by dissolving 50.0 mg of itopride hydrochloride working standard in 50.0 mL of water and kept in a brown volumetric flask. Five milliliters of this solution was further diluted to 50.0 mL with water to obtain a stock solution containing 0.1 mg/mL of itopride. Working standard solution of itopride was prepared daily by serial dilution of the stock standard solution.

### 2.4. Procedure

#### 2.4.1. Synthesis of $\text{Ru}(\text{bpy})_3^{2+}$ -doped silica nanoparticles

Preparation of RuDS nanoparticles was carried out according to the method described by previous paper with some changes [37,38]. The procedure is briefly described in the following. At first, 12.5 mL ethanol, 750  $\mu\text{L}$  ammonium hydroxide and 250  $\mu\text{L}$   $\text{Ru}(\text{bpy})_3^{2+}$  solution were mixed by magnetic force stirring at room temperature (RT) for 5 min. Then, the resulting mixture was added drop by drop to a TEOS solution in ethanol (2.5 mL) under continuing stirring. When the solution

was added completely, the reaction mixture was allowed to stir for 2 h. And finally, the particles were recovered by centrifugation at 8000 rpm for 10 min. Then they were washed using centrifugation and ultrasonication with water several times to remove physically adsorbed  $\text{Ru}(\text{bpy})_3^{2+}$  dye from the particle's surface. The particles were air dried. The synthesized RuDS nanoparticles were characterized by spectrofluorometry for fluorescence intensity, transmission electron microscope (TEM; Hitachi H700) for size and morphology and SEM for images of morphology of RuDS nanoparticles/chitosan composites membrane modified GCE.

#### 2.4.2. Preparation of the modified electrode

Before modification, the GCE ( $d=3.5$  mm) was polished with 1, 0.3 and 0.05  $\mu\text{m}$  aluminum slurry on a polishing cloth, respectively, and sonicated in acetone and doubly distilled water thoroughly. Then it was allowed to dry at room temperature. A 1.0 wt.% chitosan solution was prepared by dissolving 1.0 g of chitosan flakes into 100 mL of 1.0% acetic acid and stirred for 2 h at room temperature until complete dissolution. The chitosan solution was stored in refrigerator when not in use. Amount of chitosan solution and RuDS nanoparticles solution were mixed. Then the mixture was sonicated for 3 min until a homogenous solution was obtained. A 5  $\mu\text{L}$  aliquot of the composite was hand-cast on the surface of a GCE. The film was uniform and consistently salmon pink. The film was allowed to dry at room temperature. When not in use, the modified electrode was kept in the dry state at room temperature.

#### 2.4.3. The analytical procedure

Five-milliliter blank solution which contained 0.1 mol/L phosphate buffer solution (PBS) was added to the ECL cell and a stable blank ECL signal was recorded when the electrolytic potential was applied to the working electrode. The sample or standard itopride solution which contained an appropriate concentration of itopride in 0.1 mol/L PBS was added to the ECL cell, and the ECL signal was recorded. The concentration of itopride was quantified via the peak height of the ECL emission intensity that was obtained by subtracting the blank ECL emission intensity from that of the sample or standard itopride solution.

#### 2.5. Sample preparation

For determination of itopride in biological fluids (real serum samples), the serum samples of healthy people collected from volunteers who received a single oral dose of itopride tablet and then liquid–liquid extraction (LLE) was performed with the addition of extraction solvent (*tert*-butyl methyl ether and dichloro methane, 70:30, v/v). The sample was vortexed for 2.0 min and was allowed to settle for 15.0 min. About 5.0 mL of supernatant was transferred to another test tube and was evaporated to dryness in a thermostatically controlled water-bath maintained at 40 °C under the stream of nitrogen for about 25.0 min. After drying, the residue was reconstituted in 1 mL water, then analyzed according to the proposed procedure.

### 3. Results and discussion

#### 3.1. $\text{Ru}(\text{bpy})_3^{2+}$ -doped silica nanoparticles formation

Silica nanoparticles can be formed by either an acid-catalyzed reaction or a base-catalyzed (Stober) reaction. The base-catalyzed reaction, using ammonia, ethanol, water, and TEOS, can be controlled to yield spherical silica particles with low size polydispersity [39]. In this paper, the RuDS nanoparticles were prepared with a modified Stöber synthesis method. The Stöber synthesis method was also applied to prepare the dye-doped silica nanoparticles, but most of them need of conjunction of dye and organoalkoxysilanes coupling agent [40,41]. We found that the electroactive  $\text{Ru}(\text{bpy})_3^{2+}$  can be embedded into  $\text{SiO}_2$  nanoparticles, which maybe was attributed to strong electrostatic attractions between the positively charged ruthenium complex and the negatively charged silica. Unlike inverse microemulsion-based method often used to prepare luminescent silica particles this technique completely avoids the use of potentially toxic organic solvents and surfactants and also is time-saving. Further conjugation of the particles to biomolecules is easier because there is no need to wash the particles off surfactant molecules, which often requires multiple washing steps when inverse microemulsion techniques are used to prepare nanoparticles. So this method would be used easily in the research field of biology and medicine.

#### 3.2. $\text{Ru}(\text{bpy})_3^{2+}$ -doped silica nanoparticles and composite film characterization

The modified Stöber synthesis method yielded relatively uniform RuDS nanoparticles. These nanoparticles were characterized using microscopic method. The results showed that the particle sizes of nanoparticles were about  $52 \pm 5$  nm (Fig. 2 left). In addition, SEM was also used to characterize the composite film on the GCE. As seen from Fig. 2 (right), the composite film is homogenous and the nanoparticles disperse evenly in the film. In addition, spectrofluorometric measurements were used to characterize the RuDS nanoparticles because  $\text{Ru}(\text{bpy})_3^{2+}$  is also fluorophore. Free Rubpy dye emitted fluorescence light at 595 nm when excited at 470 nm in aqueous solution. However the emission maximum of the RuDS nanoparticles shifted with 4 nm to the longer wavelength, indicating that the spectral characteristics of the Rubpy dye were changed only insignificantly after encapsulation in the nanoparticles.

Furthermore, the fluorescence intensity of RuDS nanoparticles solution was investigated. It was found that the fluorescence intensity of the RuDS nanoparticles solution varied with the variation of the concentration of the  $\text{Ru}(\text{bpy})_3^{2+}$  solution. The  $\text{Ru}(\text{bpy})_3^{2+}$  concentration in the preparation solution was optimized to yield RuDS nanoparticles with the fluorescence intensity. We observed that the fluorescence intensity was enhanced significantly when the  $\text{Ru}(\text{bpy})_3^{2+}$  concentration was increased from 4, 8, 12, 16 to 20 mmol/L. However, at concentrations >20 mmol/L the fluorescence intensity became more flattened. In the future experiment we also found that the intensity of ECL emission increased with the increasing of con-

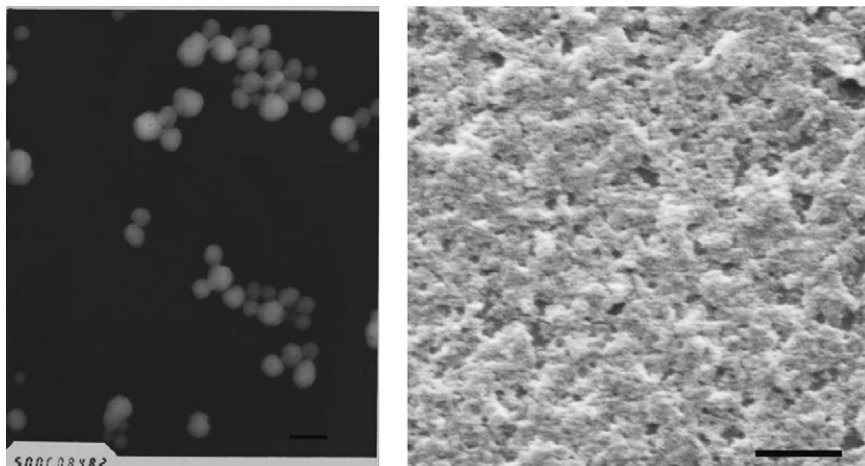


Fig. 2. TEM image of RuDS nanoparticles (left) (bar scale is 100 nm) and SEM of the RuDS nanoparticles/chitosan composite film on GCE (right) (bar scale is 500 nm).

centration of  $\text{Ru}(\text{bpy})_3^{2+}$  from 4, 8, 12, 16 to 20 mmol/L in the absence or in the presence of itopride (Fig. 3), but the maximal signal/noise was obtain when 16 mmol/L  $\text{Ru}(\text{bpy})_3^{2+}$  was used in the preparation solution. So the RuDS nanoparticles prepared with 16 mmol/L  $\text{Ru}(\text{bpy})_3^{2+}$  was used to investigation of ECL.

### 3.3. Electrochemistry and ECL behavior

Cyclic voltammetry and ECL study were performed to characterize the modified electrode. Fig. 4 shows cyclic voltammograms (CVs) of RuDS nanoparticles/chitosan composite film in the absence (a) and presence (b) of itopride at the scan rate of 100 mV/s in PBS (pH 7.2). The presence of itopride made the oxidation current of  $\text{Ru}(\text{bpy})_3^{2+}$  increase clearly while the reduction current decreased, which is consistent with the electrocatalytic reaction mechanism as tripropylamine (TPA)– $\text{Ru}(\text{bpy})_3^{2+}$ . Meanwhile, the ECL signal increased considerably in the presence of itopride. This is due to the ECL reaction of  $\text{Ru}(\text{bpy})_3^{2+}$  and itopride. This result showed that the  $\text{Ru}(\text{bpy})_3^{2+}$  could retain its ECL efficiency when doped in the silica nanoparticles. Moreover, with such a unique immobilization

method, thousands of  $\text{Ru}(\text{bpy})_3^{2+}$  could dope inside the silica nanoparticles, which led to the strong ECL signal. Such ECL signal enhancement could facilitate the ultrasensitive analyte determination. As can be expected from the  $\text{Ru}(\text{bpy})_3^{2+}$ –tertiary amine (such as TPA) ECL mechanism, the onset of luminescence occurred near 0.90 V, and then the ECL intensity rose until it reached a maximum about 1.14 V, which was consistent with the oxidation potential of  $\text{Ru}(\text{bpy})_3^{2+}$ .

### 3.4. Selection of the ECL reaction medium

The medium of the proposed ECL reaction system not only affected the enhancing ECL effect of itopride but also was the key factor that affected the reproducibility of proposed ECL method. In order to obtain better analytical performances, some medium, such as 0.1 mol/L  $\text{Na}_2\text{CO}_3$ ,  $\text{NaHCO}_3$ , NaAc, borate and PBSs were investigated. The experimental result suggested that the PBS offered best ECL sensing performances for itopride. Therefore, PBS was selected as optimum ECL reaction medium for detecting itopride in the subsequent research works. Since

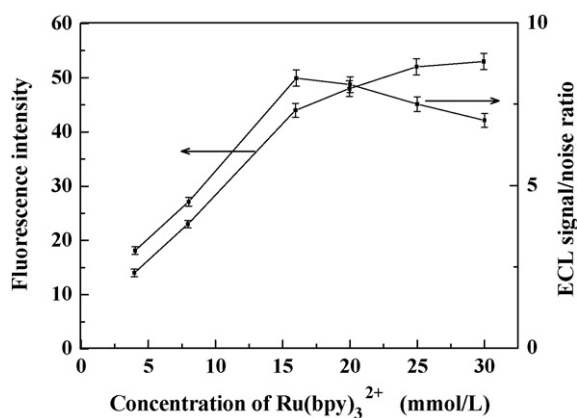


Fig. 3. Effect of concentration of  $\text{Ru}(\text{bpy})_3^{2+}$  in the preparation solution on the fluorescence intensity (left-hand scale) and the signal-to-noise ratio of ECL (right-hand scale).

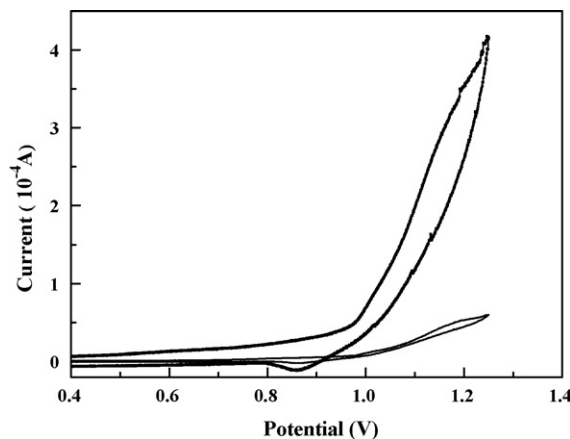


Fig. 4. Cyclic voltammograms for the RuDS nanoparticles/chitosan composite film modified GCE in the absence (dash) and presence (solid) of  $5 \times 10^{-6}$  g/mL itopride in PBS (pH 7.2) at the scan rate of 50 mV/s.



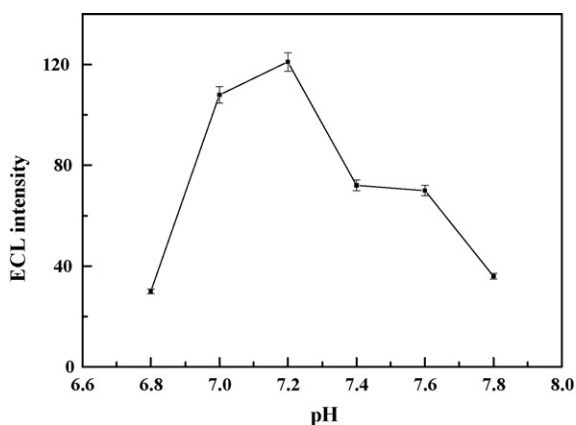


Fig. 5. Effect of pH on the ECL intensity in PBS containing  $3.5 \times 10^{-7}$  g/mL itopride with the scan rate of 50 mV/s.

ECL of  $\text{Ru}(\text{bpy})_3^{2+}$ -itopride is a pH-dependent reaction, we also studied the effect of pH on the ECL response. At first, the ECL intensity increases gradually with increasing the pH. But when pH becomes higher than 7.2, ECL begins to decrease (Fig. 5). The ECL signal increases from 6.5 to 7.2, implying that deprotonation of itopride is required during ECL process. At high pH value,  $\text{OH}^-$  ions are assumed to be at considerable concentration level [42], which caused the competitive reaction between  $\text{Ru}(\text{bpy})_3^{2+}$  and  $\text{OH}^-$  ions. Therefore, pH 7.2 was selected for subsequent experiments.

### 3.5. Selection of the concentration of the $\text{Ru}(\text{bpy})_3^{2+}$ -doped silica nanoparticles and chitosan

$\text{Ru}(\text{bpy})_3^{2+}$  plays an important role in the process of ECL. We investigated the effect of RuDS nanoparticles and chitosan concentration on the ECL intensity in the presence of  $2 \times 10^{-7}$  g/mL itopride in PBS (pH 7.2) at the scan rate of 50 mV/s. As seen from Fig. 6, the ECL intensity increases accordingly with the increase of RuDS nanoparticles concentration at first, which may be attributed to the fact that more  $\text{Ru}(\text{bpy})_3^{2+}$  could be

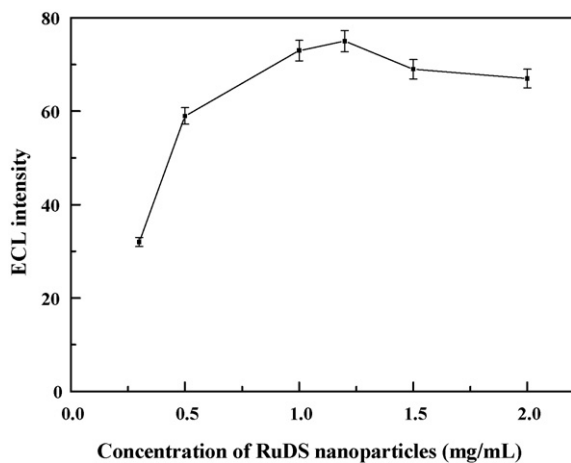


Fig. 6. Effect of RuDS nanoparticles concentration on the ECL intensity in the presence of  $2.0 \times 10^{-7}$  g/mL itopride in PBS (pH 7.2) with the scan rate of 50 mV/s.

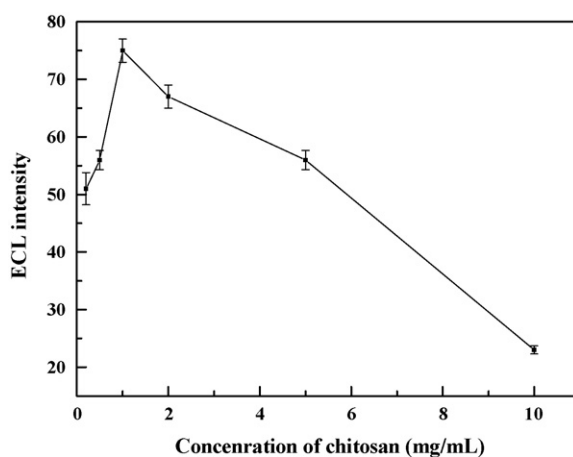


Fig. 7. Effect of chitosan concentration on the ECL intensity in the presence of  $2.0 \times 10^{-7}$  g/mL itopride in PBS (pH 7.2) with the scan rate of 50 mV/s.

immobilized on the electrode with more RuDS nanoparticles. But when the RuDS nanoparticles concentration is higher than 1.0 mg/mL, ECL begins to fall. This could be explained as the increased RuDS nanoparticles amount might absorb and scatter the ECL emission within the films. Therefore, we choose 1.0 mg/mL RuDS nanoparticles in our experiment.

As one of the useful materials, chitosan has been widely used in fabricating the ECL sensors since it offered not only better light-transferring, but special chemical properties such as complex metal ion ability, strong hydrogen bond function with some hydroxyl-based containing species [43]. Based on these considerations, chitosan was selected as the immobilization material for nanoparticles because of hydroxyl-based group on the surface of the RuDS nanoparticles.

It was found that the concentration of chitosan in composites membrane obviously affected the analytical performances of the ECL sensor. So the influence of chitosan concentration on the ECL intensity also was investigated. The relating investigating results showed (as shown in Fig. 7) that the ECL intensity increased with concentration of chitosan. When 1 mg/mL chitosan was added, the response reached the largest. And at this concentration the coating of the composite films did not come off during the test period, indicating that the RuDS nanoparticles/chitosan composite film was well adhered to the GCE. As the concentration kept increasing, the signal decreased slightly maybe because the chitosan polymer may act as a mass transfer blocking layer at the high concentration. So 1 mg/mL chitosan was used in all other experiments.

### 3.6. Interference study

The effect of foreign substances was tested by analyzing a standard solution of itopride ( $1.0 \times 10^{-7}$  g/mL) to which increasing amounts of interfering substances was added. The tolerable concentration ratios for interference at the 5% level were over 1000 for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , glucose, dextrin and starch, 100 for magnesium stearate, carbamide, uric acid, sucrose, lactose, fructose, dopamine,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{SO}_4^{2-}$  and  $\text{CO}_3^{2-}$ , 10 for  $\text{NO}_3^-$ , Vc and oxalic acid, and 1 for

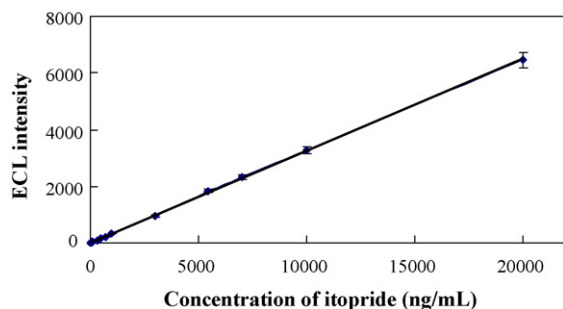


Fig. 8. Standard curve of the ECL intensities for itopride.

$\text{Cu}^{2+}$ ,  $\text{S}^{2-}$  and glycine, respectively. It was found in high amounts (50 nmol/g) of dopamine in a region of the brain known as the “caudate nucleus”. The very low concentration of dopamine was in the “extracellular fluid” of the caudate nucleus. But no dopamine was detected in healthy blood serum in the issued papers and in our experiment [44,45]. So the proposed could be used to detect itopride without the interference of dopamine D2 receptor blockade.

### 3.7. ECL analytical performances of the proposed ECL sensor for itopride

Calibration curves for itopride have been constructed using the present ECL sensor based on the RuDS nanoparticles/chitosan composite films (shown in Fig. 8). Under the selected conditions, the proposed ECL sensor could linearly sense itopride in the concentration range of  $1 \times 10^{-8}$  to  $2 \times 10^{-5}$  with a distinguished detection limit of  $3 \times 10^{-9}$  g/mL. The regression equation was  $I = 0.3274 [\text{itopride}] (\text{ng/mL}) + 2.632$ . The correlation coefficient was 0.9978. Multiple determinations ( $n=11$ ) gave relative standard deviation of 2.3% at concentrations of  $8 \times 10^{-8}$  g/mL. A typical ECL intensity for the determination of itopride is shown in Fig. 9. The results displayed a good reproducibility of the proposed sensor. The linear ranges and detection limits were compared with those obtained by different methods in the literature. Table 1 indicates that the proposed method has a wider linear range and lower detection

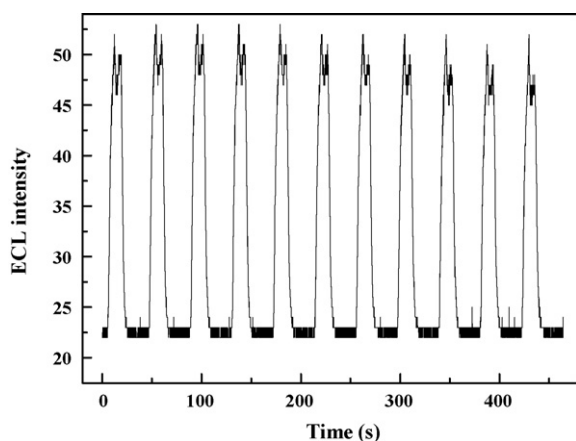


Fig. 9. Observed ECL signals for duplicate measurements of itopride at the concentration of  $8 \times 10^{-8}$  g/mL with the scan rate of 50 mV/s.

Table 1

Figures of merits of comparable detection methods for determination of itopride by ECL detection using modified GCE

Detection method	Linear range	Detection limit	Literature cited
Fluorescence	14–1000 ng/mL	14 ng/mL	[5]
UV	10–100.0 $\mu\text{g/mL}$	100 ng/mL	[7]
UV	500–5000 ng/mL	162.35 ng/mL	[8]
UV	10–70 $\mu\text{g/mL}$		[6]
Mass spectrometry	0.5–1000 ng/mL		[9]
ECL	10–20,000 ng/mL	3 ng/mL	This work

limit than UV and fluorescence detection. Notwithstanding, the proposed method shows a little bad analytical characteristics compared with mass spectrometric detection, the cost efficient instrument and simple method would make it useful in bioanalytical and clinical application. The remarkable sensitivity could be attributed to the following two points: a large amount of  $\text{Ru}(\text{bpy})_3^{2+}$  is three-dimensionally immobilized in the matrix of the nanoparticles which greatly increases ECL response. The nano-silica has larger surface, more chemical activities and electrocatalysis and be able to accelerate electron transfer efficiently [10,35,46,47].

### 3.8. Analytical application

In order to assess the validity of the proposed method, the proposed ECL sensor was applied to the determination of itopride concentrations in pharmaceutical preparations. Not less than 10 tablets were weighed and ground to fine powder. And then a sample containing approximate 50 mg of itopride was weighed accurately, transferred into a 50 mL brown calibrated flask and made up to volume with water. Then pharmaceutical preparation samples were determined directly after being diluted appropriately. The results of the determination of itopride in pharmaceutical preparations are given in Table 2, which agreed well with that obtained by spectrophotometry ( $\lambda_{\text{max}} = 258 \text{ nm}$ ) [7].

The determination result of itopride in serum samples was shown in Table 3. Moreover, recovery studies were also carried out in serum samples to which known amounts of itopride were added. As shown in Table 2, the recoveries were between 95.7 and 104.0% ( $n=5$ ). This proposed method could therefore be applied to analysis of itopride in serum samples.

Table 2

Results of determination of itopride in pharmaceutical preparations (mg/tablet)

Sample no.	Claimed	Found <sup>a</sup>	Reference <sup>b</sup>
1	50	48.7 ( $\pm 3.4\%$ )	48.0
2	50	46.5 ( $\pm 2.7\%$ )	46.7
3	50	49.1 ( $\pm 3.6\%$ )	49.3
4	50	47.3 ( $\pm 1.9\%$ )	47.1
5	50	48.0 ( $\pm 2.0\%$ )	48.2

<sup>a</sup> The average of five determinations.

<sup>b</sup> The reference method was used only for determination of itopride originally present in pharmaceutical preparations.

Table 3  
Results of determination of itopride in human serum

Sample no.	Original (ng/mL)	Added (ng/mL)	Found (ng/mL) <sup>a</sup>	R.S.D. (%)	Recovery (%)
1	255	200	463	3.1	104.0
2	316	300	603	2.9	95.7
3	243	400	661	1.8	104.5
4	344	500	845	3.4	100.2
5	190	600	784	3.0	99.0

The reference method was used only for determination of MCP originally present in human serum.

<sup>a</sup> The average of five determinations.

### 3.9. Stability of ECL sensor

Two kinds of stability about this sensor, namely, kept in air and immersed in solution at room temperature, are both tested. To investigate the storage stability of proposed ECL sensor kept at room temperature in air, the modified electrode was kept at room temperature for about 1 month. The following cyclic voltammetry measurements were performed by monitoring ECL intensity of the modified electrode response to itopride in 0.1 M PBS (pH 7.2) with intermittent usage (every 2 days) performed as Zhang described [26,27]. The coating of the composite films did not come off during the test period, indicating that the RuDS nanoparticles/chitosan composite film was well adhered to the GCE. The peak potential was essentially unchanged for more than 1 month, and ECL intensity decreased less than 15.1% compared with the initial steady state value after 1 month of storage. On the other hand, because the sensor was used in water solution in this experiment, the stability of the sensor kept in PBS solution was also investigated. The cyclic voltammetry measurements were also performed by monitoring ECL intensity of this sensor for itopride in 0.1 M PBS (pH 7.2) with intermittent usage (every 2 h). The ECL intensity only decreased 9.2% compared with the initial steady state value after 24 h of immersion in PBS solution. The result suggested that the modified electrode has a good stability.

## 4. Conclusion

A novel ECL sensor based on RuDS nanoparticles/chitosan nanocomposites membrane modified GCE has been shown to be suitable for the determination of itopride. The result suggested that the Ru(bpy)<sub>3</sub><sup>2+</sup> encapsulation interior of the silica nanoparticle maintains its electrochemical activities and also reduces Ru(bpy)<sub>3</sub><sup>2+</sup> leaching from the silica matrix when immersed in water due to the electrostatic interaction and the sensitivity of this ECL sensor is significantly enhanced by encapsulation of thousands of Ru(bpy)<sub>3</sub><sup>2+</sup> inside the silica nanoparticles on the modified electrode surface. This kind of ECL sensor shows great potential not only in the application of bioanalysis because of the biocompatibility of the silica nanoparticles and the easy, cost-effective and environmentally friendly approach for the preparations of the electroactive component doped nanoparticles but also in vivo and on line analysis for biological samples and drug metabolism due to the easy micromation of the electrode. The further work is under going.

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