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# Electrogenerated chemiluminescence sensor for metoclopramide determination based on Ru(bpy)$_3^{2+}$-doped silica nanoparticles dispersed in Nafion on glassy carbon electrode

Xu Hun[a,b], Zhujun Zhang[a,*]

[a] Department of Chemistry, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, China
[b] School of Materials & Chemical Engineering, Xi'an Technological University, Xi'an 710032, China

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ABSTRACT

A novel method for the determination of metoclopramide (MCP) using electrogenerated chemiluminescence (ECL) is presented. A tris(2,2′-bipyridyl)dichlororuthenium(II) (Ru(bpy)$_3^{2+}$)-doped silica (RuDS) nanoparticle/perfluoinated ion-exchange resin (Nafion) with nanocomposite membrane modified glassy carbon electrode (GCE) is used. The Ru(bpy)$_3^{2+}$ encapsulation interior of the silica nanoparticle maintains its electrochemical activities and also reduces Ru(bpy)$_3^{2+}$ leaching from the silica matrix when immersed in water due to the electrostatic interaction. The analytical performance of this ECL sensor for MCP is shown in detail. Under optimal experimental conditions, it has good linearity in the concentration range from $2 \times 10^{-8}$ mol/L to $1 \times 10^{-5}$ mol/L ($R = 0.9989$) with a detection limit of $7 \times 10^{-9}$ mol/L. The relative standard deviation ($n = 11$) is 3.2% for detecting $1.2 \times 10^{-6}$ mol/L MCP. The recoveries are in the range of 97.0–104.4% for sample measurements by standard-addition method. This method has been applied successfully to determine MCP in pharmaceutical preparations and in human urine. Statistical analysis (Student's *t*-test and variance ratio *F*-test) of the obtained results show no significant difference between this proposed method and the reference method.



## 1. Introduction

Metoclopramide (MCP), 4-amino-5-chloro-2-methoxy-*N*-(2-diethylamino-ethyl) benzamide, is a dopamine-receptor antagonist active on gastrointestinal motility. It is used as an anti-emetic in the treatment of some forms of nausea and vomiting and to increase gastrointestinal motility. It is also used at much higher doses for the prevention of cancer chemotherapy-induced emesis [1]. In this perspective, the wide applications of MCP in both clinical and experimental medicine have prompted extensive interest in its determination.

Current analytical methods employed for the determination of MCP can involve fluorimetry [2], spectrophotometry [3–10], chromatography [11–15], capillary electrophoresis [16,17], differential scanning calorimetry (DSC) and X-ray diffraction [18], gas chromatography–mass spectrometry (GC–MS) [19], potentiometry [20], voltammetry [21], fast stripping continuous cyclic voltammetry [22], square wave anodic stripping voltammetric [23] and $^1$H NMR spectroscopy [24].

Each method has its limitations and drawbacks. The spectrophotometric method is chiefly based on the diazotisation reaction, so that it is time-consuming and hazardous to work with. The chromatographic method is costly and also time-consuming, limiting its application. Other methods often are typically less sensitive or have their own intrinsic disadvantages such as technical complexity or require expensive instrumentation.

Recently, Al-Arfaj [25] developed a flow-injection (FI) methodology for the rapid and sensitive determination of MCP hydrochloride by using Ru(dipy)$_3^{2+}$ chemiluminescence (CL). A review of the literature reveals that up to the present time, nothing has been published concerning the electrogenerated chemiluminescence (ECL) determination of MCP.

In this paper, a novel ECL sensor for the determination of MCP was developed based on Ru(bpy)$_3^{2+}$-doped silica (RuDS) nanoparticles dispersed in a perfluorosulfonated ionomer (Nafion) on a glassy carbon electrode (GCE). In the past few years, the emergence of nanotechnology has enabled the development of specialized nanoparticles of various shapes, sizes and compositions for sensing and labeling applications in analytical chemistry [26]. One of the most widely studied sensor types has been dye-doped nanoparticle sensors [27–29]. There are several kinds of dye-doped nanoparticles, such as dye-encapsulating liposome, dye-doped

* Corresponding author. Tel.: +86 29 85308748; fax: +86 29 85308748.
*E-mail address:* zzj18@hotmail.com (Z. Zhang).

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polymerization nanoparticles, rare earth-doped nanoparticles and dye-doped silica nanoparticles. Among the various dye-doped nanoparticle choices, $Ru(bpy)_3^{2+}$-doped nanoparticles have been the most thoroughly studied. They were extensively used as a photostable biomarker in spectrofluorometric measurements [29]. Recently, Zhang and co-workers [30–32] found that as a typical ECL reagent, $Ru(bpy)_3^{2+}$ could still retain its ECL property even after doping inside the silica nanoparticles. The exterior nano-silica boundary prevented the electroactive reagent from leaching out into the aqueous solution due to the electrostatic interaction. Its ECL intensity was increased greatly because lot of $Ru(bpy)_3^{2+}$ was encapsulated into silica nanoparticles. Other authors also found that electroactive reagent doped in the silica nanoparticles showed high electron-transfer efficiency in electrochemical detection [33,34]. To the authors' best knowledge, although the preparation and the conjugation of biomolecules for the fluorescence labeling with RuDS nanoparticles have been developed, detailed information about the application of ECL by using this kind of RuDS nanoparticles in the issued papers is lacking [30–32]. It was the aim of the study presented here to develop and validate a novel method for the determination of MCP with the ECL by using RuDS nanoparticles/Nafion nanocomposites membrane modified GCE.

The present paper describes the development of a novel ECL method for the determination of MCP based on RuDS nanoparticles dispersed in a Nafion on a GCE. The stability, electrochemical activity and ECL of RuDS nanoparticles/Nafion modified electrode have been examined. These results show that this method has the advantages of sensitivity, a lower detection limit and good stability for the determination of MCP. The data obtained with the proposed method are compared with the standard control method with good agreement.

## 2. Experimental

### 2.1. Materials

Tris(2,2′-bipyridyl)dichlororuthenium(II) ($Ru(bpy)_3Cl_2$) was purchased from Sigma (St. Louis, MO, USA). Nafion (perfluoinated ion-exchange resin, 5% (w/v) solution in a solution of 90% aliphatic alcohol 10% water mixture), tetraethyl orthosilicate (TEOS), *n*-hexanol, Triton X-100 (TX-100) and cyclohexane were obtained from Shanghai Chemical Plant (Shanghai, China). Ammonium hydroxide (28–30 wt%) was purchased from Xi'an Chemical Reagent Company (Xi'an, China). MCP hydrochloride was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). And MCP tablets (5 mg/tablet) were purchased from local market and made by Shanxi Linfen Jianmin Pharmaceutical Ltd. Distilled, deionized water was used for the preparation of all aqueous solutions. Unless otherwise stated, all the other chemicals and reagents used in this study were of analytical grade quality.

A $1.0 \times 10^{-3}$ mol/L standard aqueous solution of MCP was prepared by dissolving MCP hydrochloride in 100 mL of water and kept in a brown volumetric flask. The solution was stable for at least 60 days in a refrigerator (4 °C). MCP working standard solution was prepared daily by serial dilution of the stock standard solution.

### 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 GCE coated with RuDS nanoparticles/Nafion composite film. A platinum wire was used as the counter electrode, and Ag wire was used as reference electrode. 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 photomultiplier tube (PMT) used in this analyzer was operated in current mode. And potential supply of the photomultiplier tube was 800 V. The ECL cell was placed directly in front of the PMT window and was enclosed in a light-tight box. The experimental set-up was shown in Fig. 1.

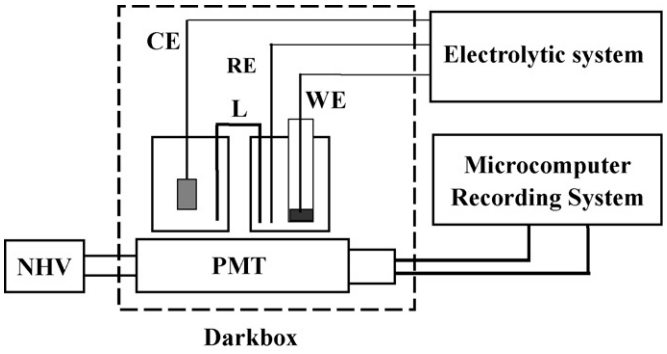


**Fig. 1.** Schematic diagram of ECL experimental set-up. WE: working electrode; RE: reference electrode; CE: counter electrode; L: $KNO_3$ salt bridge; PMT: photomultiplier; NHV: negative high-voltage supply.

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

### 2.3. Procedure

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

Synthesis of RuDS nanoparticles was carried out according to methods described by a previous paper with little change [28]. First, the water-in-oil microemulsion (W/O microemulsion) was prepared at room temperature first by mixing 1.77 mL surfactant TX-100, 7.5 mL oil phase cyclohexane and 1.8 mL cosurfactant *n*-hexanol. 0.2 mL $Ru(bpy)_3^{2+}$ solution was then added. Then the resulting mixtures were homogenized with magnetic stirring to form a W/O microemulsion. In the presence of 100 μL of TEOS, a hydrolyzation reaction was initiated by adding 60 μL of $NH_3 \cdot H_2O$ under stirring. The reaction was allowed to stir for about 24 h. After the reaction was completed, acetone was added to break the microemulsion and recover the particles. The contents were then centrifuged and washed with ethanol and water several times to remove surfactant molecules and physically adsorbed $Ru(bpy)_3^{2+}$ from the particles' surface. The particles were air dried at room temperature.

#### 2.3.2. Preparation of the modified electrode

To prepare the ECL sensor, the GCE ($d$ = 3.5 mm) was polished with 1, 0.3 and 0.05 μm aluminum slurries on a polishing cloth, respectively, and sonicated in acetone and doubly distilled in water thoroughly. The polished GCE was then allowed to dry at room

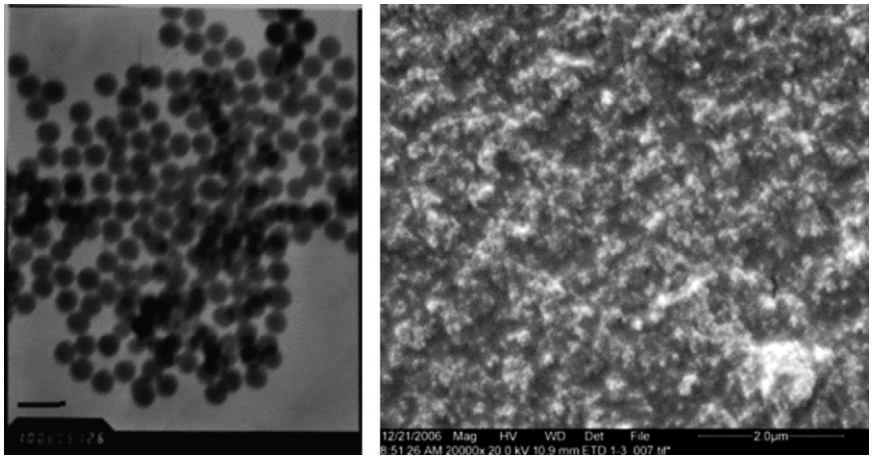

**Fig. 2.** TEM image of RuDS nanoparticles (left) (bar scale is 100 nm) and SEM of the RuDS nanoparticles/Nafion composite film on GCE (right).

temperature. The Nafion solution and RuDS nanoparticles solution were then mixed. This mixture was then sonicated for 10 min until a homogenous solution was obtained. A 5 μL aliquot of the composite was hand-cast on the surface of the GCE. This 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. Sample preparation

#### 2.4.1. Pharmaceutical preparation

Not less than 20 tablets were ground to fine powder. A sample containing approximate 20 mg of MCP was weighed accurately, transferred into a 100 mL brown calibrated flask into which water was added to give 100 mL of solution.

#### 2.4.2. Urine samples

Urine samples of healthy people were collected from volunteers who received a single oral dose of 10 mg of MCP tablet. The treatment procedure of urine samples used here was carried out according to method described by a previous paper with little change [25]. 1 mL urine sample was pipetted into clean 10 mL centrifugation vial. 0.1 mL of 0.1 mol/L NaOH solution was added, shaken for few seconds, followed by the addition of 5 mL dichloromethane. The mixture was vortex mixed at high speed for 2 min, and then centrifuged at 3000 rpm for 10 min. The resulting supernatant was transferred to a small conical flask. The extract was evaporated to dryness at 60 °C and the residue was dissolved in 0.5 mL water and then analyzed according to the proposed procedure.

### 2.5. The analytical procedure

A 5 mL 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 MCP solution which contained an appropriate concentration of MCP in 0.1 mol/L PBS was added to the ECL cell, and the ECL signal was recorded. The concentration of MCP 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 MCP solution.

## 3. Results and discussion

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

Among many nanoparticles preparation techniques, W/O microemulsion polymerization is one of the most widely used methods for the preparing nanomaterials of small size [35–39]. In this study, we used this method for the formation of silica nanoparticles by a base-catalyzed reaction. With this method, reversed micelles are formed, i.e. water nanodroplets surrounded by a monolayer of surfactant molecule are formed in an organic oil medium in the presence of cosurfactant and used as nanoreactors for the formation of nanoparticles [40]. As may be expected, the particle size will be strongly influenced by the ratio of cosurfactant to surfactant, the ratio of surfactant to oil, and the ratio of water to oil [41–43]. The experimental results suggest that when the ratio of cosurfactant to surfactant and the ratio of surfactant to oil are kept fixed, the ratio of water to oil can be carefully adjusted to control the particle size [44,45].

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

The W/O microemulsion method yielded uniform RuDS nanoparticles. These nanoparticles were characterized using microscopic methods. The results showed that the particle sizes of nanoparticles were about $34 \pm 4$ 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.

### 3.3. Electrochemistry and ECL behavior

Cyclic voltammetry and ECL study were performed to characterize the modified electrode. Fig. 3 shows cyclic voltammograms (CVs) of RuDS nanoparticles/Nafion composite film in the absence (a) and presence (b) of MCP at the scan rate of 50 mV/s in PBS (pH 7.8). The presence of MCP made the oxidation current of $Ru(bpy)_3^{2+}$ increase clearly while the reduction current decreased, which is consistent with the electrocatalytic reaction mechanism as TPA-$Ru(bpy)_3^{2+}$. Meanwhile, the ECL signal increased considerably in the presence of MCP. This is due to the ECL reaction of $Ru(bpy)_3^{2+}$ and MCP. This result showed that the $Ru(bpy)_3^{2+}$ could retain its ECL efficiency when doped in the silica nanoparticles. Moreover, with such a unique immobilization method, thousands of $Ru(bpy)_3^{2+}$

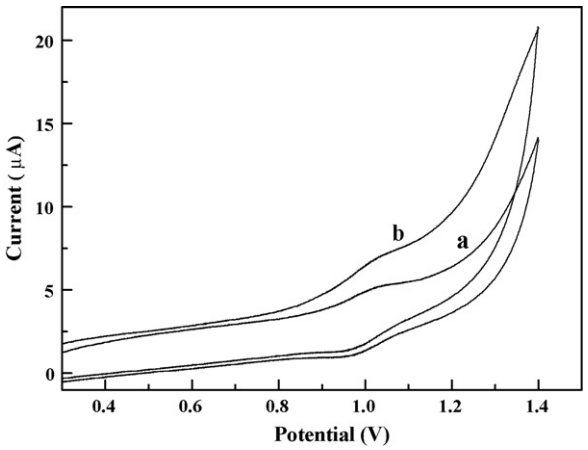


**Fig. 3.** Cyclic voltammograms for the RuDS nanoparticles/Nafion composite film modified GCE in the absence (a) and presence (b) of $5 \times 10^{-6}$ mol/L MCP in PBS (pH 7.8) at the scan rate of 50 mV/s.

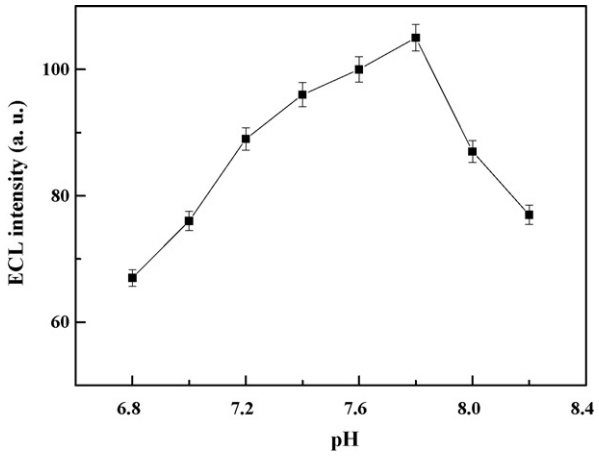


**Fig. 5.** Effect of pH on the ECL intensity in PBS containing $3.5 \times 10^{-7}$ mol/L MCP with the scan rate of 50 mV/s.

could dope inside the silica nanoparticles, which led to the strong ECL signal as demonstrated in Fig. 4. Such ECL signal enhancement could facilitate ultrasensitive analyte determination. As can be expected from the $Ru(bpy)_3^{2+}$-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 $Ru(bpy)_3^{2+}$. The difference between the ECL–potential curves in the presence of and absence of MCP is the attenuation speed of ECL which is faster in the presence of MCP. The reason for this phenomenon is not yet well understood.

### 3.4. Selection of the ECL reaction medium

The medium of the proposed ECL reaction system not only affected the enhancing ECL effect of MCP but also was the key factor that affected the reproducibility of this proposed ECL method. In order to obtain better analytical performance, various media, such as 0.1 mol/L $Na_2CO_3$, $NaHCO_3$, NaAc, borate and phosphate buffer solutions were investigated. The experimental result suggested that the phosphate buffer solution offers best ECL sensing performance for MCP. Therefore, PBS was selected as optimum ECL reaction medium for detecting MCP in the subsequent research works. Since ECL of $Ru(bpy)_3^{2+}$-MCP 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 the pH becomes higher than 7.8, ECL begins to decrease (Fig. 5). While as the pH increases continuously, the corresponding ECL intensity decreases. This phenomena is consistent with the electrocatalytic reaction mechanism as TPA-$Ru(bpy)_3^{2+}$. The MCP was tertiary amine. The ECL signal increases from 6.8 to 7.8, implying that deprotonation of MCP is required during ECL process. At high pH values, $OH^-$ ions undergo a competing reaction with $Ru(bpy)_3^{2+}$ [46,47]. Therefore, a pH of 7.8 was selected for subsequent experiments.

### 3.5. Selection of the concentration of RuDS nanoparticles

Because $Ru(bpy)_3^{2+}$ plays an important role in the process of ECL, the influence of RuDS nanoparticles concentration on the ECL intensity in the presence of $2.5 \times 10^{-7}$ mol/L MCP in PBS (pH 7.8) at the scan rate of 50 mV/s was also investigated. As seen from Fig. 6, with the concentration of RuDS nanoparticles increasing, the ECL intensity increased at first, which may be attributed to the fact that more $Ru(bpy)_3^{2+}$ could be 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 from two aspects: (1) the increased RuDS nanoparticles amount might absorb and scatter the ECL emission within the films [32]; (2)

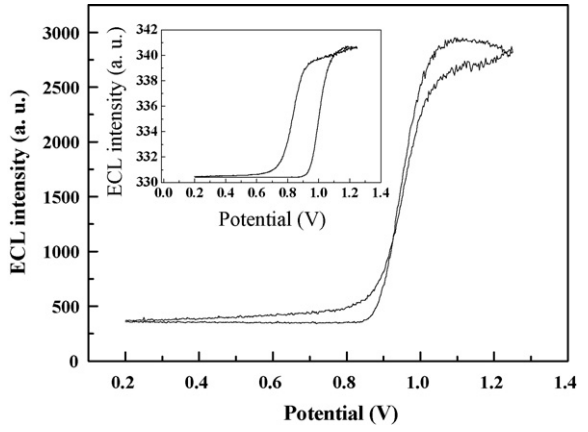


**Fig. 4.** ECL–potential curves for the RuDS nanoparticles/Nafion composite film modified electrode in the presence of $8 \times 10^{-6}$ mol/L MCP in PBS (pH 7.8) and absence of MCP (inset). Scan rate: 50 mV/s.

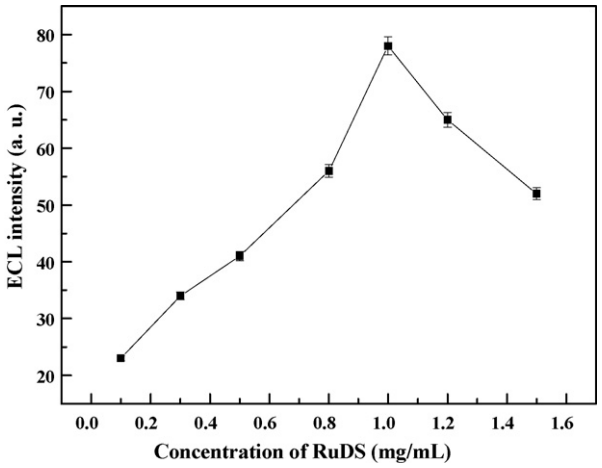


**Fig. 6.** Effect of RuDS nanoparticles concentration on the ECL intensity in the presence of $2.5 \times 10^{-7}$ mol/L MCP in PBS (pH 7.8) with the scan rate of 50 mV/s.

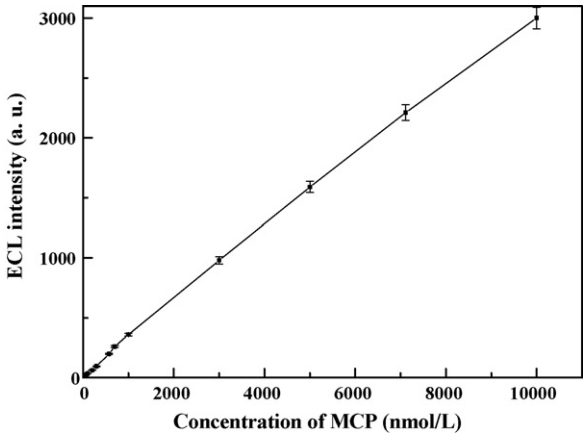


**Fig. 7.** Standard curve of the ECL intensities for MCP.

the increased RuDS nanoparticles concentration led to an increase in thickness of the films which would prevent the MCP from transferring from aqua solution to the interior of the films. This result comes with the experimental phenomenon reported by Zhang and Dong [31,32]. Therefore, we choose 1.0 mg/mL RuDS nanoparticles in future experiments.

### 3.6. Selection of the concentration of Nafion

The Nafion acted as a role of forming the film in this kind of modified electrode. This was different from the earlier reported study issued by our and other groups [48–51]. In that study, $Ru(bpy)_3^{2+}$ was immobilized into the $TiO_2$/Nafion nanocomposites membrane with the action of ion-exchange. And the ECL intensity increased when the Nafion was added and the concentration of the Nafion increased. The dependence of ECL intensity on the concentration of Nafion mixed with the nanoparticles added to the GCE surface was examined over the range of 0.1–0.5%. If the Nafion was insufficient to encapsulate the nanoparticles into the film, the nanoparticles would easily diffuse into the solution when the modified electrode was dipped in the solution, resulting in lower ECL signal. The experimental results showed that the ECL intensity increased with increasing Nafion concentration. ECL intensity peaked (data not shown) when 0.3% Nafion was used. As the concentration kept increasing, the signal decreased slightly. Because the film became thicker, this would prevent the physical diffusion of MCP when Nafion concentration increased. Therefore, 0.3% Nafion was used in all other experiments.

### 3.7. Interference study

The effect of foreign substances was tested by analyzing a standard solution of MCP ($1.0 \times 10^{-7}$ mol/L) to which increasing amounts of interfering substances was added. The tolerable concentration ratios for interference at the 5% level were over 1000 for $Na^+$, $K^+$, $Cl^-$, glucose, dextrin, starch and granisetron, 100 for $Mg^{2+}$, $Fe^{3+}$, $Ca^{2+}$, $NH_4^+$, $Pb^{2+}$, $Zn^{2+}$, $SO_4^{2-}$, $CO_3^{2-}$, dexamethasone and ondansetron, 10 for $NO_3^-$, Vc, oxalic acid and uric acid, and 1 for $Cu^{2+}$, $S^{2-}$ and glycin, respectively.

### 3.8. ECL analytical performances of the proposed ECL sensor for metoclopramide

Under the selected conditions, the proposed ECL sensor could linearly sense MCP in the concentration range of $2.0 \times 10^{-8}$ mol/L to $1.0 \times 10^{-5}$ mol/L and with a $7.0 \times 10^{-9}$ mol/L detection limit for MCP. The regression equation was $I = 5.817\ (\pm 0.267) + 0.3026\ (\pm 0.0042)$ [MCP] (nmol/L) (Fig. 7). The correlation coefficient was 0.9989. The relative standard deviation was less than 5% for detecting $1.2 \times 10^{-6}$ mol/L MCP ($n = 11$). We also evaluated the intra-assay precision of the method by analyzing the same concentration samples five times with multiple replicates and the inter-assay precision by analyzing the same concentration samples on 5 consecutive days. Intra- and inter-assay precision tests indicated good repeatability of our method for ECL intensity (Table 1).

**Table 1**
Intra- and inter-assay precision data

| Concentration (mol/L) | Relative S.D. (%) | |
|---|---|---|
| | Intra-assay | Inter-assay |
| $5.0 \times 10^{-8}$ | 5.9 | 5.2 |
| $5.0 \times 10^{-7}$ | 4.1 | 3.4 |
| $5.0 \times 10^{-6}$ | 2.2 | 2.3 |

### 3.9. Analytical application

#### 3.9.1. Application to dosage forms

The MCP in its commercial pharmaceutical preparation tablets (with a nominal MCP content of 5 mg/tablet) was determined in the optimized conditions by the proposed method. The results of the determination of MCP in pharmaceutical preparations are given in Table 2. The accuracy of MCP in pharmaceutical preparations was evaluated by determining the recovery of MCP by a standard-addition method, into which a known quantity of MCP was added. The results show that the concentrations obtained by the proposed method are in good agreement with those given by spectrophotometry ($\lambda_{max} = 308$ nm) (pharmacopoeia method) [10].

#### 3.9.2. Application to urine samples

The proposed ECL sensor was also applied to the determination of MCP concentrations in human urine. The urine samples of healthy people collected from volunteers who received a single oral dose of 10 mg of MCP tablet. The results of the determination of MCP in urine are given in Table 3. The accuracy of MCP in human urine was also evaluated by determining the recovery of MCP by a standard-addition method, into which a known quantity of MCP was added. The results show that the concentrations obtained by the proposed method are in good agreement with those given by spectrophotometry ($\lambda_{max} = 308$ nm) (pharmacopoeia method) [10]. The results obtained by the proposed method were given in Table 3 with recovery varying from 97% to 104.4% and R.S.D. of less than 4%.

Statistical analysis of the results obtained by the proposed method, and those given by the comparison method was performed using the Student's *t*-test and the variance ratio *F*-test [52]. As illustrated in Tables 2 and 3, the calculated values did not exceed the theoretical ones, indicating no significant difference in the performance of the compared methods in accuracy or precision.

### 3.10. Stability of ECL sensor

The sensor stability in air and immerged in solution at room temperature was also 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 MCP in 0.1 M PBS (pH 7.8) with intermittent usage (every 2 days) performed as Zhang and Dong described [31,32]. The coating of the

**Table 2**
Results of determination of MCP in pharmaceutical preparations

| Sample no. | Claimed (mg/tablet) | Found (mg/tablet)[a] | Reference (mg/tablet) [10] | *t*-Value[b] | *F*-value[b] |
|---|---|---|---|---|---|
| 1 | 5 | 4.91 ± 0.09 | 4.87 | 2.64 | 4.90 |
| 2 | 5 | 4.88 ± 0.11 | 4.91 | 2.12 | 4.74 |
| 3 | 5 | 4.85 ± 0.14 | 4.90 | 2.47 | 4.87 |
| 4 | 5 | 5.00 ± 0.09 | 4.99 | 2.36 | 3.9 |
| 5 | 5 | 5.02 ± 0.12 | 5.04 | 2.20 | 3.69 |

[a] The average of five determinations (±S.D.).
[b] The theoretical values for *t*- and *F*-values are equal to 2.78 and 5.05, respectively ($P = 0.05$).

**Table 3**
Results of determination of MCP in human urine

| Sample no. | Original (μmol/L) | Added (μmol/L) | Found (μmol/L)[a] | Recovery (%) | Reference (μg/mL)[b] [10] | *t*-Value[c] | *F*-value[c] |
|---|---|---|---|---|---|---|---|
| 1 | 2.11 | 1.0 | 3.13 ± 0.10 | 102.0 | 2.13 | 2.01 | 3.10 |
| 2 | 1.84 | 1.0 | 2.81 ± 0.08 | 97.0 | 1.77 | 1.97 | 2.82 |
| 3 | 1.93 | 2.0 | 3.86 ± 0.15 | 96.5 | 1.93 | 2.56 | 3.65 |
| 4 | 2.35 | 2.0 | 4.37 ± 0.14 | 101.0 | 2.29 | 2.10 | 3.22 |
| 5 | 0.56 | 1.0 | 1.60 ± 0.06 | 104.4 | 0.57 | 2.07 | 4.10 |

[a] The average of five determinations (±S.D.).
[b] The reference method was used only for determination of MCP originally present in human urine.
[c] The theoretical values for *t*- and *F*-values are equal to 2.78 and 5.05, respectively ($P = 0.05$).

composite films did not come off during the test period, indicating that the RuDS nanoparticles/Nafion 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 10% compared with the initial steady state value after 1 month of storage. 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 MCP in 0.1 M PBS (pH 7.8) with intermittent usage (every 2 h). The ECL intensity only decreased 6% 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. The electrochemical stability of the RuDS nanoparticles/Nafion composite film modified electrode is likely due to the shell of nano-silica prevents the incorporated $Ru(bpy)_3^{2+}$ from partitioning into hydrophobic regions of the Nafion film.

## 4. Conclusion

A novel ECL sensor based on RuDS nanoparticles/Nafion modified GCE has been shown to be suitable for the determination of MCP. The sensitivity of this ECL sensor is significantly enhanced by encapsulation of thousands of $Ru(bpy)_3^{2+}$ inside the silica nanoparticles on the modified electrode surface. The results also suggest that the proposed ECL sensor has a good stability due to the shell of nano-silica preventing the incorporated $Ru(bpy)_3^{2+}$ from partitioning into hydrophobic regions of the Nafion film. This kind of ECL sensor shows a great potential not only in the application of bioanalysis because of the biocompatibility of the silica nanoparticles and the easy preparations of the electroactive component-doped nanoparticles and the modified GCE but also in vivo and on-line analyses for biological samples and drug metabolism due to the easy micromation of the electrode. Further work is in progress.

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