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# Chemiluminescence determination of indapamide using indapamide-imprinted polymer as recognition material

Nie Fei, Lu Jiuru *, Niu Weifen

*School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, PR China*

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**Abstract**

A chemiluminescence reaction between soluble Mn(IV) and indapamide was found. An indapamide MIP was synthesized and its adsorption selectivity to indapamide in aqueous solution was evaluated. Using soluble Mn(IV)–formaldehyde–indapamide chemiluminesence system as detection system and the indapamide MIP as recognition material, a selective molecule imprinting-chemiluminesence method of determination of indapamide was established. The linearly response range of this method was from $2.0 \times 10^{-8}$ to $5.0 \times 10^{-6}$ g/mL with a linear correlation coefficient of 0.995. The detection limit was $8 \times 10^{-9}$ g/mL. The relative standard deviation for $5.0 \times 10^{-7}$ g/mL of indapamide solution was 3.5% ($n = 9$).


*Keywords:* Indapamide; Soluble Mn(IV); Molecule imprinting-chemiluminescence method

## 1. Introduction

Indapamide, 3-(aminosulfonyl)-4-chloro-*N*-(2,3-dihydro-2-methyl-1*H*-indol-1-yl)-benzamide, is an oral antihypertensive diuretic agent indicated for the treatment of hypertensive and edema.

Some methods have been developed for the determination of indapamide. HPLC and CE methods are often proposed for the determination of indapamide for biological fluids analysis [1–9]. For the detection of indapamide in pharmaceutical preparations, a liquid chromatographic method and a spectrophotometry have been proposed as a standard method in United States Pharmacopoeia (USP XXIV) and Chinese pharmacopoeia [10,11].

Chemiluminescence (CL) analysis has many attractive features, for example, the sensitivity is high, the linear range is wide, and the instruments are simple. A CL analysis method of a substance is founded on a relevant CL reaction. CL reaction for indapamide has not been seen up to now. There is an inhibitory CL method for determination of indapamide [12], but this method has some inherent shortcoming and limitation of the inhibitory analysis method. We found that the reaction between soluble Mn(IV) and indapamide can produce CL and the CL intensity could greatly be enhanced by formaldehyde. The possible mechanism has been proposed that: the dissolved oxygen in the reactant solution was transformed into singlet oxygen $^1O_2(^1\Delta_g)$ after it absorbed the energy of the redox reaction of soluble Mn(IV)–indapamide–formaldehyde; then double molecule compound of singlet oxygen $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$ emitted chemiluminescence ($\lambda_{max} = 635$ nm) when it transformed into triplet oxygen $2^3O_2(^3\Sigma_g)$. Based on this CL reaction, a CL method for the determination of indapamide could be established.

However, the selectivity of the CL method should be poor and this CL method cannot be used to determine indapamide in the complicated samples directly since there are many other substances that can react with soluble Mn(IV) to produce CL under the same condition. Therefore, to establish a selective CL method to determine indapamide in the complicated samples is very necessary.

* Corresponding author. Tel.: +86 29 85303911; fax: +86 29 85307774.
*E-mail address:* ljr@snnu.edu.cn (L. Jiuru).

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Molecular imprinting polymer (MIP) has a predetermined selectivity for the target molecule and its excellent recognition ability to the target molecule is comparable to antibody–antigen and enzyme–substrate natural molecular recognition system. MIP has been used as chromatographic stationary phase [13–15], solid phase extraction matrices [16,17], recognition elements in biosensors [18,19], and artificial receptors in drug assay [20], etc.

If a MIP is packed into a glass column which is connected into the CL flow system and used as the recognition material for analyte, when a sample solution flows through the MIP column, the analyte would be separated from coexist substances and pre-adsorbed on MIP, then the selectivity of CL method would be improved greatly. Based on this idea, a molecular imprinting-chemiluminescence (MI-CL) method for determination of indapamide was established in this paper.

In this work, the indapamide MIP was prepared using methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as cross-linker in the presence of template molecule of indapamide. The polymer sized in 74–105 μm was packed into a glass tube to make into an indapamide MIP column. The indapamide MIP column was connecting into the soluble Mn(IV)–formaldehyde–indapamide CL flow system and the CL signal was recorded. The CL intensity is linearly related to the concentration of indapamide over $2.0 \times 10^{-8}$ to $5.0 \times 10^{-6}$ g/mL range with a linear correlation coefficient of 0.995. The detection limit is $8 \times 10^{-9}$ g/mL indapamide. The relative standard deviation for $5.0 \times 10^{-7}$ g/mL indapamide solution is 3.5% ($n = 9$). This method has been used to determine indapamide in body fluids.

## 2. Experimental

### 2.1. Apparatus

The schematic diagram of MI-CL flow system used in this work is shown in Fig. 1. The reagent solutions and sample solution were delivered using two peristaltic pumps. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. CL measurements were performed using an IFFM-D flow injection CL analyzer (Xi'an Remax Electronic High-Tech Ltd.). The data acquisition and treatment were performed with the IFFM-D flow injection CL data processing software (Xi'an Remax Electronic High-Tech Ltd.).

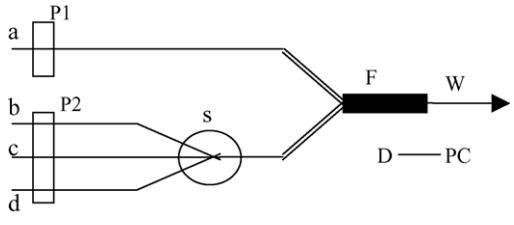


Fig. 1. Schematic diagram for MI-CL method; P: peristaltic pump; D: detector; F: MIP column; PC: computer; W: waste; s: switch valve; a: Mn(IV) solution; b: water; c: formaldehyde solution; d: sample solution.

### 2.2. Reagents

Indapamide was purchased from Leader Chemical Co. Ltd. (Jiangsu, China). EGDMA were purchased from Sigma (St. Louis, MO). MAA and 2,2′-azobis(2-methylpropinitrile) (AIBN) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Other reagents were purchased from Xi'an Chemical Reagent Factory (Xi'an, China). All reagents used were of analytical reagent grade except for AIBN, which was chemical purity grade. EGDMA and MAA were redistilled and AIBN was recrystallized prior to use.

Stock standard solution of indapamide ($1.00 \times 10^{-4}$ g/mL) was prepared by dissolving 5.0 mg of indapamide with 0.1 mol/L sodium hydroxide and then diluting to 50 mL with water. Working standard solutions of indapamide were prepared of diluting this stock solution with water.

Mn(IV) solution ($4.0 \times 10^{-3}$ mol/L) was prepared by the following: 2 g of newly made $MnO_2$, which was produced from the reaction between sodium formate and potassium permanganate, was added into 500 mL phosphoric acid. This mixture of $MnO_2$ and phosphoric acid were oscillated with ultrasonic for 30 min. Then it was placed at room temperature for 24 h [21]. The concentration of this solution was determined by iodimetry and it shows no significant change in a month.

Doubly distilled water was used throughout the experiments.

### 2.3. Synthesis of the polymer

Into a 50 mL round-bottomed flask, 1 mmol of indapamide, 5 mL ethylnitrile, and 4 mmol of MAA was added. The mixture was oscillated with ultrasonic for 4 h to let MAA sufficiently mixed with indapamide. Then 50 mg of AIBN and 20 mmol of EGDMA were added. The mixture was purged with nitrogen for 15 min and sealed under vacuum. The polymerization reaction was carried out at 60 °C in a water bath for 24 h. The obtained polymers were crushed, ground and sieved to collect the particles of the size between 74 and 105 μm.

### 2.4. Binding experiments

Before binding experiments, the indapamide molecules in the MIP were removed by washing with the mixture of methanol–acetic acid (9:1 v/v) until the absorbance of indapamide had been no longer detected in the elution solution. The polymer was dried to a constant weight at 60 °C under vacuum. Then 20.0 mg MIP was mixed with 5.0 mL of

various concentrations of indapamide solution in a 10 mL conical flask and oscillated for 12 h at room temperature. After centrifuging at 3000 rpm for 10 min, the concentration of free indapamide in the supernatant was detected by UV spectrophotometry. The amount of indapamide bound to the polymer was calculated by subtracting the concentration of free indapamide from the initial indapamide concentration. The data obtained were used for the Scatchard assay.

### 2.5. Preparation of the indapamide MIP column

In MI-CL analysis, Mn(IV) solution and formaldehyde solution had to be combined first before they flowed through the MIP column and reacted with indapamide adsorbed in the MIP producing CL. The CL signal would have been very low if Mn(IV) solution and formaldehyde solution had been mixed together for a relative long time. Perhaps it is because of the waste of the each other. In order to obtain a good sensitivity of the determination, the mix time before Mn(IV) solution and formaldehyde solution flowed into the MIP column must be shortened. A Y-like tube has been developed aiming at this. Indapamide MIP column is a 4 mm i.d. × 15 mm length colorless glass tube whose shape is just like a capital letter Y. In the straight part of the Y-tube, 20.0 mg of above-collected polymer was packed and plugged with a small amount of glass wool at both ends. The column was connected into CL system during determination. For a new MIP column, indapamide template molecules in the MIP have not been removed. Before the MIP column was used, let the combining stream of Mn(IV) solution and formaldehyde flowed through the column, reacted with indapamide to produce CL. The CL signal declined with the waste of indapamide in the MIP. It cannot be considered that indapamide in the MIP had all been reacted until the signal declined to the baseline. Then, the column was washed by water to clean the reaction products.

### 2.6. Procedures for MI-CL method

The schematic diagram for MI-CL method was shown in Fig. 1 and the procedure could be summarized as four steps.

- Step 1: Adsorption of indapamide.
  In this step, pump 1 was stopped; switch valve was in connection of sample solution. Pump 2 pumped indapamide solution through the MIP column for 80 s and indapamide was selectively adsorbed in the cavities of the polymer.
- Step 2: Washing to remove other substances except indapamide.
  In this step, pump 1 was stopped; switch valve was in connection of formaldehyde solution. Pump 2 pumped formaldehyde solution through the MIP column continuously for 70 s to remove other substances except indapamide.
- Step 3: Chemiluminescence detection.
  In this step, switch valve was in connection of formaldehyde solution. Pumps 1 and 2 were both started to pump the combined stream of soluble Mn(IV) solution and formaldehyde solution flowed through the MIP column for 90 s and reacted with indapamide adsorbed in the MIP to produce CL until the signal declined to the baseline.
- Step 4: Cleaning the MIP column.
  In this step, pump 1 was stopped; switch valve was in connection of formaldehyde solution. Pump 2 pumped formaldehyde solution through the MIP column continuously for 60 s to clean the polymer for the next determination.

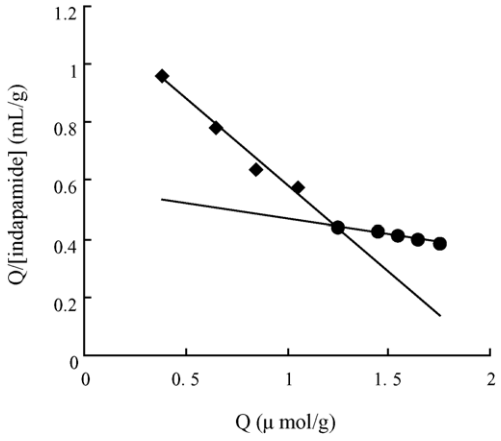


Fig. 2. Scatchard plot to estimate the binding nature of indapamide-imprinted polymer.

## 3. Results and discussions

### 3.1. Binding characteristic of the MIP

The binding characteristic of the indapamide MIP was investigated by equilibrium binding experiments.

In molecule imprinting technique, the Scatchard equation is usually used to estimate the binding characteristic of the MIP. The Scatchard equation can be expressed as $Q/[\mathrm{MET}] = (Q_{\max} - Q)/K_{\mathrm{d}}$, in which $Q$ is combining amount, $K_{\mathrm{d}}$ the dissociation constant of binding sites, $Q_{\max}$ the apparent maximum combining amount and [MET] is the equilibrium concentration of indapamide. The equilibrium binding experiments were carried out by varying indapamide concentration from $1 \times 10^{-5}$ to $10 \times 10^{-5}$ mol/L. The obtained data were plotted according to the Scatchard equation and the plot was shown in Fig. 2.

As shown in Fig. 2, the Scatchard plot was not linear within the whole indapamide concentration range, which indicated that the binding sites in the MIP are non-uniform. It is observed that two distinct sections in the plot can be regarded as straight lines. This revealed that there are two classes of binding sites in the MIP. $K_{\mathrm{d1}}$ and $Q_{\max 1}$ for the higher affinity binding sites were calculated to be 1.68 mmol/L and

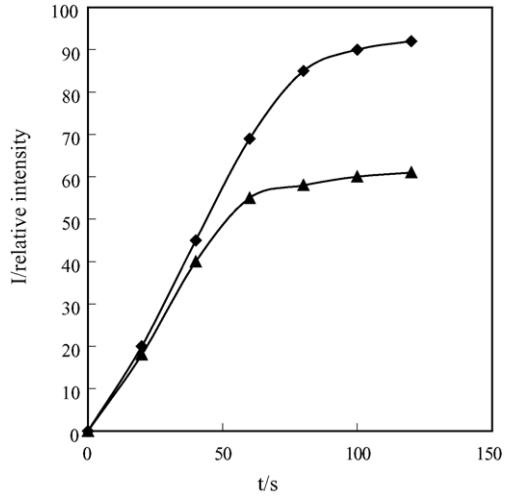


Fig. 3. The adsorption time curve of the MIP and NIP to indapamide. Concentration of indapamide: $5.0 \times 10^{-7}$ g/mL; flow rate: 1.6 mL/min; (■) MIP; (▲) NIP.

l.98 μmol/g for dry polymer. $K_{d2}$ and $Q_{max2}$ for the lower affinity binding sites were calculated to be 9.49 mmol/L and 5.43 μmol/g.

### 3.2. Evaluation of adsorption selectivity of the MIP to indapamide in aqueous solution

It is generally considered that the MIP shows a selective recognition towards the template molecules only in organic media and the MIP would show non-specific binding to all organic compounds in the aqueous solution owing to the hydrophobic interaction. In order to examine the adsorption performance of the MIP to indapamide in the aqueous solution, the following experiments were performed.

#### 3.2.1. Adsorption time curve of the MIP and NIP to indapamide

The MIP column and the NIP column were connected into the flow system (see Fig. 1) to examine the adsorption performance of the MIP and the NIP to indapamide, respectively. The adsorption time curve of the MIP and the NIP to indapamide was drawn (see Fig. 3). The results show that both the MIP and the NIP can adsorb the indapamide in the aqueous solution, but the adsorption capacity of the MIP is higher than that of the NIP. So, it may be considered that the adsorption properties of the MIP and the NIP to indapamide are different.

#### 3.2.2. Washing curve

Formaldehyde was the enhancer in the CL reaction. In the experiments, washing effect of 4% formaldehyde and water was examined, respectively. The washing time curve was drawn (see Fig. 4). As shown in Fig. 4, water cannot wash out indapamide in the MIP and NIP columns, and the indapamide adsorbed in the NIP can be completely washed out by formaldehyde with a washing time of 70 s, but only a little

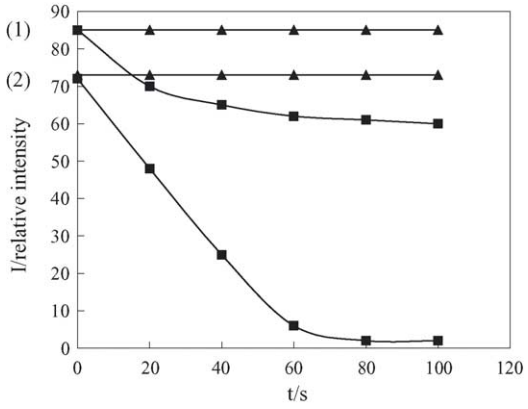


Fig. 4. Washing curve. Concentration of indapamide: $5.0 \times 10^{-7}$ g/mL; adsorption time: 80 s; flow rate: 1.6 mL/min; (1) MIP; (2) NIP; (▲) washing with water; (■) washing with formaldehyde.

indapamide adsorbed in the MIP is washed out by formaldehyde with the same washing time, and increasing the washing time, the amount of indapamide retained on the MIP column remains almost constant. These experimental results suggest that the adsorption behaviors of the MIP and the NIP to indapamide are essentially different. The adsorption of the NIP to indapamide is possibly only weakly non-specific binding based on the hydrophobic interaction and electrostatic interaction, whenas there are both the strong specific binding based on molecule recognition and the weakly non-specific binding in the adsorption of the MIP to indapamide, and the specific binding is dominating.

#### 3.2.3. Separating effect of the MIP to coexisted substances

In order to further validate the selectivity of the MIP to indapamide, indomethacin which was similar to indapamide in the structure and can react with soluble Mn(IV) and formaldehyde producing CL and sulphamethoxazole which can also react with soluble Mn(IV)–formaldehyde producing CL under this condition, were selected as indicators (the structures of the three substances are shown in Fig. 5). The separation effect of the MIP to indapamide and the coexisted substances had been examined. The results were shown in Table 1.

The data in Table 1 indicate the MIP displays good separation effect in separating indapamide from its coexisted substances and has fine adsorption selectivity to indapamide.

Table 1
Separating effect of the MIP to coexisted substances

| Washing reagent | A | B | C | B + C | A + B + C |
|---|---|---|---|---|---|
| $H_2O$ | 90 | 70 | 78 | 95 | 121 |
| HCHO | 65 | 0 | 2 | 2 | 70 |

The data in the table are the relative CL intensity that was detected when the CL reagents were flowing through the MIP column after washing. Adsorption time: 80 s; washing time: 70 s. A: indapamide ($5.0 \times 10^{-7}$ g/mL); B: indomethacin ($2.5 \times 10^{-6}$ g/mL); C: sulphamethoxazole ($5 \times 10^{-4}$ g/mL).

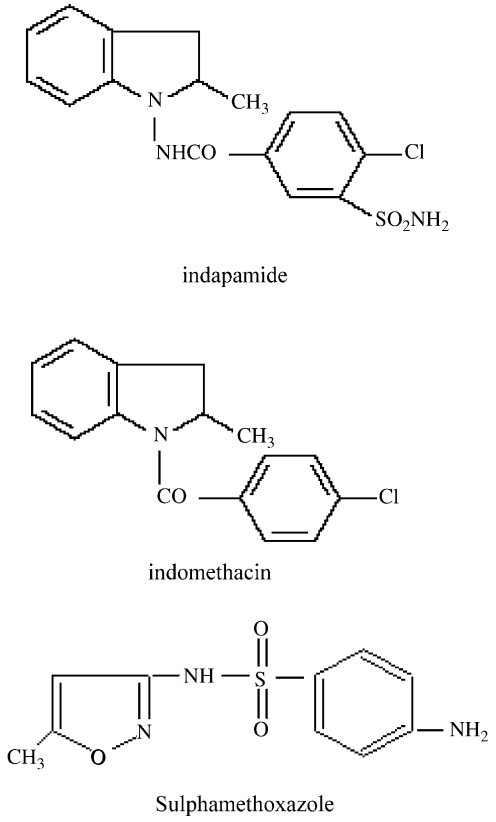


Fig. 5. The structures of indapamide, indomethacin, and sulphamethoxazole.

The above experiments adequately clarified that MIP has a specific adsorption character to target molecule in aqueous solution.

### 3.3. Conditions of CL reaction

#### 3.3.1. Concentration of Mn(IV) solution

Mn(IV) solution was used as the oxidant in this CL reaction. Its concentration can effect on the intensity of CL signal. The effect of Mn(IV) solution was examined in the range of $8.0 \times 10^{-5}$ to $8.0 \times 10^{-4}$ mol/L. The CL signal was the maximum when the concentration of soluble Mn(IV) solution was $4.0 \times 10^{-4}$ mol/L.

#### 3.3.2. Enhancer

The enhanced effects were compared of some substances such as sodium dodecanesulfonic, beta-cyclodextrin, sodium sulfite, and formaldehyde. It was observed that formaldehyde was the best suitable enhancer. In view of the blank signal and enhanced effect, 4% formaldehyde was selected.

### 3.4. Procedures of determination

#### 3.4.1. Adsorption time

The adsorption time is the time standard solution or sample solution flowing through the MIP column. It determines the amount of indapamide adsorbed in the MIP column, and then determines the sensitivity of the detection and the linear range of the method. The adsorption time is relevant to the concentration of indapamide, the binding capacity of the polymer and the flow rate. When the amount of polymer was 20 mg, the flow rate was fixed at 1.6 mL/min and the concentration of indapamide was $5.0 \times 10^{-7}$ g/mL, the relation between the CL intensity and the adsorption time within the range 20–140 s was examined (Fig. 6). The CL intensity was increased with the increase of adsorption time. Above 90 s, the CL intensity almost remained constant. Considering analytical efficiency and the linear range of this method, 80 s was finally selected as adsorption time. When a sample with low indapamide content should be determined, the adsorption time may be prolonged appropriately to improve the sensitivity of the determination.

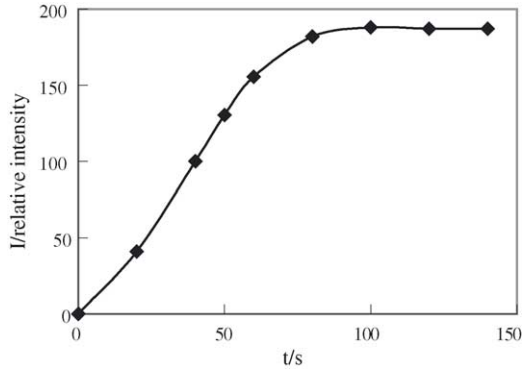


Fig. 6. Effect of adsorption time on CL reaction. Concentration of indapamide: $5.0 \times 10^{-7}$ g/mL, flow rate: 1.6 mL/min.

#### 3.4.2. Washing time

Following the adsorption step, it is necessary to wash the MIP column to remove the other substances absorbed by non-specific binding. A suitable washing time should be able to remove other substances completely and did not cause the loss of indapamide adsorbed by specific binding. To select the washing time, indomethacin was selected as interference indicator and added into the indapamide standard solution (indapamide $5.0 \times 10^{-7}$ g/mL, indomethacin $2.5 \times 10^{-6}$ g/mL). Formaldehyde was used as washing reagent. The effect of washing time was examined in the range 20–100 s with the flow rate at 1.6 mL/min. The experimental results showed that when the washing time was beyond 70 s, the interference indicator could be effectively removed and the CL intensity showed no obvious difference with that of $5.0 \times 10^{-7}$ g/mL indapamide standard solution. So, 70 s is selected as the washing time.

#### 3.4.3. Reaction time

When the stream of CL reagent flowed through the column, the indapamide adsorbed on the polymer should react with soluble Mn(IV) and formaldehyde producing CL. The indapamide was completely exhausted when the CL

signal declined to the stable baseline. The experimental results showed that the whole process needs 90 s.

### 3.4.4. Cleaning time

After indapamide adsorbed in the polymer reacted with soluble Mn(IV), its molecule structures had been destroyed and it was desorbed from the MIP. Therefore, the reaction products can be easily removed from MIP column with water flowed through the MIP column and the cavities can be emptied out for the next determination. The effect of the cleaning time in the range 20–100 s was examined by alternately measuring the blank signal and the CL signal from $5.0 \times 10^{-7}$ g/mL indapamide solution. It was observed that when the cleaning time was over 60 s, both the blank signals and the CL signal from $5.0 \times 10^{-7}$ g/mL indapamide solution had good repeatability. So 60 s is selected as the cleaning time.

## 3.5. Analytical parameter

At the optimized conditions, using the flow system depicted in Fig. 1, the relation between the CL intensity and the concentration of indapamide was examined. The CL intensity is linearly related to the concentration of indapamide over $2.0 \times 10^{-8}$ to $5.0 \times 10^{-6}$ g/mL with a linearly regression equation of $I = 1.55C + 21.74$ ($r = 0.995$), where $I$ is the CL intensity (relative unit) and $C$ is the concentration of indapamide ($\times 10^{-7}$ g/mL). The relative standard deviation for $5.0 \times 10^{-7}$ g/mL of indapamide solution is 3.5% ($n = 9$). According to suggestion of IUPAC, the detection limit is determined as $8 \times 10^{-8}$ g/mL.

## 3.6. Selective experiment

In this study, the interference for the determination was investigated with $5.0 \times 10^{-7}$ g/mL indapamide standard solution using the MI-CL method and the FI-CL method, respectively. The interfering species were selected from those foreign species normally existed in urine and the substances having CL behaviors in soluble Mn(IV)–formaldehyde CL system. The results were shown in Table 2. From Table 2, it is obvious that this method exhibited high selectivity compared with the FI-CL method.

## 3.7. Sample determination

In order to test whether other substances in urine would interfere with the determination of indapamide in this MI-CL method, a blank urine sample determination had been performed. From each of two healthy volunteers, 5 mL blank urine sample was collected, respectively, and centrifuged at 3000 rpm for 30 min. The supernatant was transferred into a 50 mL volumetric flask and diluted to the mark with doubly distilled water. The blank urine was determined by MI-CL and FI-CL methods, respectively. The results were shown in Table 3. As seen from Table 3, the CL signal of urine sample is higher than the blank signal in the FI-CL determination while there is no significant difference of the two kinds of signals in the MI-CL determination. It means the MI-CL method can eliminate the interferences from the species coexisted in the urine, it has good selectivity to the determination of indapamide and the recovery test can be used to estimate the accuracy of this method.

Table 2
Tolerable ratios of interfering species to the determination of indapamide

| Species | MI-CL method | FI-CL method |
|---|---|---|
| Sulfanilamide | 50 | 0.05 |
| Epinephrine | 20 | 0.01 |
| $Fe^{2+}$ | 10 | 0.1 |
| Uric acid | 10 | 0.1 |
| Ascorbic acid | 10 | 0.1 |
| Starch | – | 1000 |
| Glucose | 1000 | 500 |
| Trimethoprim | 50 | 0.5 |
| Indomethacin | 5 | 1 |
| Carbamide | – | 1000 |
| Tryptophan | 1 | 0.01 |
| $Na^+$ $K^+$ $Mg^{2+}$ $Cu^{2+}$ $Fe^{3+}$ | – | 1000 |
| $Ca^{2+}$ | 1000 | 100 |

The data in the table are the tolerable limit of an interfering species to indapamide ($5.0 \times 10^{-7}$ g/mL), when the deviation of the CL signals of indapamide solution with the interfering species and without the interfering species is less than 5%.

The MI-CL method was applied to the determination of indapamide in the urine of person who took indapamide tablets 8 h before determination. The urine was treated and determined in the same way with the blank urine and the determination results were showed in Table 4. The recoveries of added indapamide are quantitative and *t*-test assumes that there is no significant difference between the recovery efficiency and 100% at confidence level of 95%. These results showed that the MI-CL method has good accuracy to the determination of indapamide in urine sample.

## 3.8. Characteristics of the CL reaction

The CL kinetic characteristics of the reactions were examined using the batch system of the IFFM-D multifunction CL analysis. Fig. 7 shows the CL intensity–time curve. When Mn(IV) was injected into the mixed solution of indapamide and formaldehyde, a strong CL reaction was initiated and the maximum CL intensity was obtained in 2 s. About 13 s later, the CL reaction terminated and the CL signal declined to baseline. The results of the study on the CL

Table 3
Comparison of the CL signal of different methods

| Method | Sample | | |
|---|---|---|---|
| | Double-distilled water | Sample 1 | Sample 2 |
| Intensity (FI-CL) | 105 | 332 | 359 |
| Intensity (MI-CL) | 250 | 258 | 261 |

The data in the table denote the intensity of CL signal (relative intensity).

Table 4
Results of the determination of urine samples

| Sample | Found[a] ($\times10^{-7}$ g/mL) | Added ($\times10^{-7}$ g/mL) | Found[a] ($\times10^{-7}$ g/mL) | Recovery (%) |
|---|---|---|---|---|
| No. l | 4.85 | 0.50 | 5.57 | 104.1 |
| | | 1.00 | 5.93 | 101.4 |
| | | 2.00 | 6.79 | 99.1 |
| | | 1.00 | 6.37 | 101.9 |
| No. 2 | 5.25 | 2.00 | 7.42 | 102.3 |
| | | 4.00 | 9.14 | 98.8 |
| | | 1.00 | 6.17 | 96.4 |
| No. 3 | 5.40 | 2.00 | 7.26 | 98.1 |
| | | 4.00 | 9.71 | 103.3 |

[a] Average of three measurements.

kinetic characteristics indicate that the CL reaction of soluble Mn(IV)–formaldehyde–indapamide is a fast CL reaction.

The crucial problem of study on the mechanism of a CL reaction is to ascertain the emitter. The emitter for a CL reaction may be the reactant, the resultant or some other substances related to the reaction. If some substance is the emitter of a CL reaction, the fluorescence spectrum of this substance should accord with the CL spectrum of the CL reaction. In order to find out the emitter of soluble Mn(IV)–formaldehyde–indapamide CL reaction, the CL spectrum of the reaction (Fig. 8) was drawn and the fluorescence characteristics of all substances involved in the reaction were examined.

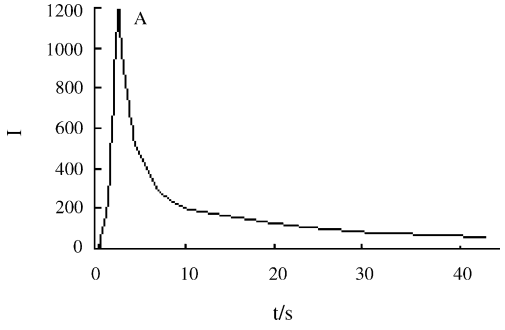


Fig. 7. Kinetic curve of chemiluminescence. A: $4.0\times10^{-4}$ mol/L Mn(IV) + 4.0% HCHO + $5.0\times10^{-6}$ g/mL indapamide.

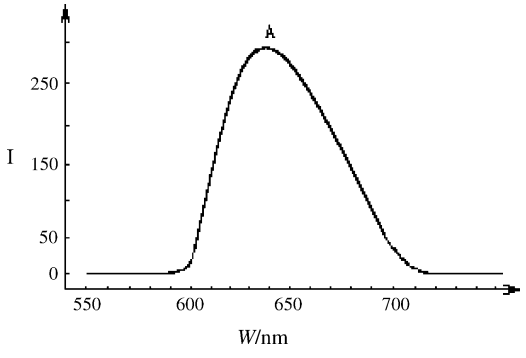


Fig. 8. Chemiluminescence spectrum of the reaction, A: 4% formaldehyde + $4.0\times10^{-4}$ mol/L Mn(IV) + $5.0\times10^{-6}$ g/mL indapamide.

In the reactants, soluble Mn(IV), formaldehyde and indapamide are all non-fluorescence substance. So all the reactants were not the emitter. The fluorescence spectrum for the mixture solution after the CL reaction was recorded in the range of 300–700 nm. No fluorescence peak had been observed in the whole wavelength range, which indicates that none of the resultants is fluorescence substance. So all the resultants were not the emitter.

The CL spectra were recorded in the range of 400–750 nm and it showed that the maximum emission wavelength ($\lambda_{max}$) of the soluble Mn(IV)–formaldehyde–indapamide CL reaction was 635 nm. It was reported that the $\lambda_{max}$ of the $ClO^-$–$H_2O_2$–$OH^-$ CL system is also 635 nm and they presumed that the emitter of the $ClO^-$–$H_2O_2$–$OH^-$ CL system is $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$, because when $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$ transformed into $^3O_2(^3\Sigma_g)$, CL is produced and the maximum emission wavelength ($\lambda_{max}$) of the CL is 643 nm in theory [22]. So we thought that the emitter of the soluble Mn(IV)–formaldehyde–indapamide CL system may be $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$ too.

Additionally, the reactant solutions are deoxidized with $N_2$ for different time, the result was shown in Table 5. From Table 5, it can be seen that the intensity of CL reduced distinctly, which suggested that dissolved oxygen in the reactant solutions should be the source of the emitter ($^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$) in the CL system.

From these experimental results mentioned above (fluorescence and CL spectra, deoxygenization experiment), we thought the possible mechanism of this CL reaction should be: the dissolved oxygen in the reactant solution was transformed into singlet oxygen $^1O_2(^1\Delta_g)$ after it absorbed the energy of the redox reaction of soluble Mn(IV)–indapamide–formaldehyde; then double molecule compound of singlet

Table 5
Results of deoxygenization experiment

| Relative CL intensity | Deoxygenization time (min) |
|---|---|
| 83 | 0 |
| 65 | 2 |
| 51 | 5 |

Concentration of indapamide: $5.0\times10^{-6}$ g/mL; concentration of HCHO: 4%; concentration of Mn(IV): $4.0\times10^{-4}$ mol/L.

oxygen $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$ emitted chemiluminescence ($\lambda_{max} = 635$ nm) when it transformed into triplet oxygen $^3O_2(^3\Sigma_g)$. This process can be expressed using the following equations:

$$\text{Mn(IV)} + \text{HCHO} + \text{indapamide} + O_2 \rightarrow {}^1O_2(^1\Delta_g) + \text{other resultants}$$

$$2\,{}^1O_2(^1\Delta_g) \rightarrow {}^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$$

$$^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g) \rightarrow 2\,{}^3O_2(^3\Sigma_g) + h\nu\,(\lambda_{max} = 635\,\text{nm})$$

## 4. Conclusion

In this work, we found a new soluble Mn(IV)–formaldehyde–indapamide CL system, proposed the possible mechanism of this CL reaction, synthesized the indapamide MIP and evaluated its adsorption characteristics in the aqueous solution. Using the indapamide MIP in the soluble Mn(IV)–formaldehyde–indapamide CL system, a MI-CL method had been established and this method had been used to determine indapamide in body fluids successfully.

This work shows that the new CL system can be applied to establish a sensitive method for the determination of indapamide and the selectivity of the method can be improved by combining with molecule imprinting technique.

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