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# Determination of L-tryptophan based on graphene oxide–magnetite-molecularly imprinted polymers and chemiluminescence

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ABSTRACT

A new method for determination of L-tryptophan (L-try) using the flow injection chemiluminescence (FI-CL) system of $KMnO_4$–$SnCl_2$–CHOH based on a graphene oxide–magnetite-molecularly imprinted polymer (GM-MIP) is described. The L-try GM-MIP was synthesized using graphene oxide (G) which improved the adsorption capacity as carrier, and magnetite nanoparticles which made the polymers easier to use in the sensor. The adsorption performance and properties were characterized. The GM-MIP was used in CL analysis to increase the selectivity and the possible mechanism was also discussed. The CL sensor responded linearly to the concentration of L-try over the range from $2.10\times10^{-7}$ to $7.09\times10^{-4}$ M with a detection limit of $2.11\times10^{-8}$ M ($3\sigma$). The relative standard deviation (*RSD*) for the determination of $3.0\times10^{-5}$ M L-try was 2.40% ($n=11$). On the basis of speediness and sensitivity, the sensor is reusable and shows a great improvement in selectivity and adsorption capacity over other sensors. The sensor has been used for the determination of L-try in drug samples.



## 1. Introduction

Graphene has attracted considerable attention in recent years for its two-dimensional structure and extraordinary properties [1]. This unique nanostructure holds a great promise for potential applications in nanomaterials and nanotechnology [2]. So far, chemical modification and functionalization of graphene have focused on incorporating graphene sheets in a composite material [3–6]. Recently, molecular imprinting technology has already become a highly accepted tool for the preparation of tailor-made recognition material, with cavities that are able to selectively recognize a target molecule [7,8]. However, molecularly imprinted polymers (MIP) prepared by the conventional technique have some disadvantages such as low-adsorption capacity. In recent years, graphene as a new supporter for MIP has been developed to overcome these drawbacks, which has a large surface area and high porosity 3D platform [9]. The following characteristics of graphene make it possible to increase the sensitivity and improve the binding kinetic properties such as strictly two-dimensional material, large surface area, and high surface-to-volume ratio [2].

Magnetite ($Fe_3O_4$) nanoparticles (NPs) have drawn considerable attention because of the fundamental scientific interest and the promising applications in magnetic fluids, catalysis, sensors, biomedicine, spintronics, magnetic recording devices, and environmental remediation [10]. Additionally, $Fe_3O_4$ NPs also show advantages such as low toxicity, low cost, and eco-friendliness which make them suitable as a stabilizer. Recently, the expected unique properties [10] and wide applications of the graphene–$Fe_3O_4$ composite have been explored.

L-tryptophan, an essential amino acid to life, has been used widely in the production of pharmaceuticals [11]. Recently, conventional methods of detection of L-try include electrochemical method [12,13] and high performance liquid chromatography [14,15]. Most of these methods can offer accurate determination results. However, some of them need expensive equipments and complex procedures for sample pretreatment, while the others suffer from low selectivity. Flow injection chemiluminescence (FI-CL) is known to be a powerful analytical technique that promises convenient operation, rapid determination and accuracy in recent research, but the method was limited in development and application for poor selectivity. To overcome the difficulty, molecularly imprinted technology was employed based on its selective recognition and capture capabilities were introduced into CL for improving selectivity which has high specificity recognition.

In this paper, molecularly imprinted technology (MIT) was introduced into CL for improving selectivity. Based on this MIT, a novel FI-CL sensor for L-try determination is developed. In the preparation of MIP, G was used for improving the adsorption capacity and $Fe_3O_4$ NPs were used for separation and immobilization. Due to the special binding sites on the GM-MIP, the L-try could be adsorbed selectively, improving the selectivity of CL

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analysis. And the adsorption capacity and reusability of the L-try GM-MIP were studied. The sensor was successfully used in determination of L-try in drug samples. Under optimal conditions, the CL sensor showed very high sensitivity and selectivity to L-try.

## 2. Experimental

### 2.1. Materials and reagents

L-try was purchased from Shanghai Chemical Reagent Company (Shanghai, China); ethylene glycol dimethacrylate (EGDMA, AR) was supplied from Aladdin Reagent Co., Ltd.; and 2,2-azobisisobutyronitrile (AIBN, AR) was supplied from Sigma-Aldrich. Acrylamide (AM, AR), graphite powders, $NaNO_3$, $KMnO_4$, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, and $NH_4Fe(SO_4)_2 \cdot 12H_2O$ were purchased from Tianjin Chemical Reagent Co., Ltd.; methanol, acetic acid and all other chemicals used and obtained from Tianjin Chemical Co., Ltd., were of analytical reagent grade.

The EGDMA was distilled to remove inhibitors. AIBN was recrystallized prior to its use.

The L-try stock solution ($4.0 \times 10^{-2}$ M) and $KMnO_4$ stock solution ($1.0 \times 10^{-3}$ M) were prepared and stored at 4 °C. Double distilled water was used throughout the work.

### 2.2. Apparatus

The schematic of the system used in this study is shown in Fig. 1. The IFFM-E flow injection CL analyzer (Xi'an Remex Electronic Instrument High-Tech Ltd., China) was equipped with an automatic injection system and a detection system. Polytetrafluoroethylene tube (0.8 mm i.d.) was used to connect all the components in the flow system. Two tubes filling with GM-MIP and graphene oxide–magnetite-molecularly non-imprinted polymer (GM-NIP) are positioned in front of the FI-CL analyzer. The GM-MIP and GM-NIP were fastened using magnetic-iron as shown in Fig. 1. A switch was used to change the flow direction. The CL signal was analyzed with a computer.

### 2.3. Preparation of GM-MIP of L-try

Graphene oxide nanosheets were prepared according to the literature [16]. Graphite oxide was prepared from nature graphite powders by a modified Hummers method [17]. The graphene oxide magnetic composites (GM) were synthesized according to the literature [18–23]. The precipitate was isolated in the magnetic field, and the supernatant was separated from the precipitate by decantation. The impurities in the GM samples were removed by washing with copious amounts of double-distilled water and the precipitate was isolated by a permanent magnet. The obtained GM composites were then washed with absolute alcohol for three times. Subsequently, the composite was dried under vacuum.

In a typical experiment [2], 1.0 g of GM was dispersed in 100.0 mL of $SOCl_2$ and 20.0 mL of benzene. The mixture was stirred for 24 h under reflux at 70 °C. The obtained solid (GM–Cl) was washed with ultra-dried tetrahydrofuran (THF) three times and dried under vacuum at 25 °C. In the next step, 0.5 g GM–Cl, 2.0 mL 2-hydroxylethyl-2'-bromoisobutyrate ($HOCH_2$–$CH_2O$-$COC(CH_3)_2Br$, HEBrIB) and 20.0 mL anhydrous toluene were mixed under nitrogen protection followed by addition of 0.5 mL anhydrous triethylamine. The mixture was refluxed for 48 h. The solid was separated by filtration, then washed with ethanol and ether, and vacuum-dried for 12 h to yield GM–Br. Subsequently, 14.5 mL phenyl magnesium bromide was dispersed in 130.0 mL THF, and then 3.5 mL $CS_2$ was added drop wise. After stirring for 2 h, 1.0 g GM–Br was added under nitrogen protection at 60 °C for 64 h. The reaction was stopped by adding ice hydrochloric acid (1 M, 50.0 mL). The obtained product was washed with distilled water three times, and then washed with ether three times. Subsequently, 0.1 mmol L-try and 0.4 mmol AM were dispersed into a 20 mL acetone solution, and the mixture was stirred for 1 h. After sealing, shaking, and purging, the obtained product was added in the mixture, and then 2.0 mmol EGDMA, and 15 mg AIBN were added under nitrogen protection at 65 °C for 18 h. The polymerization was stopped by freezing. The obtained product was separately washed three times with methanol–acetic acid (9:1, v/v) solution to remove the catalyst and unreacted reagents. Finally, the resultant product was dried under vacuum at room temperature for 24 h. The GM-NIP was prepared and processed in the same way, but in the absence of L-try as template.

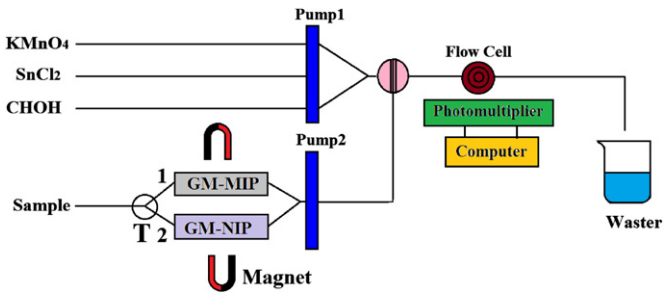


**Fig. 1.** Schematic of CL system.

### 2.4. Preparation of different polymers and adsorption study

In order to fully prove the effect of G and GM, seven different polymers were prepared and the adsorption of seven prepared different polymers for L-try was investigated. The seven prepared polymers were as follows: graphene oxide (G), graphene magnetic (GM), molecularly imprinted polymer (MIP), magnetic-molecularly imprinted polymer (M-MIP), graphene oxide-molecularly imprinted polymer (G-MIP), GM-MIP and GM-NIP.

G, GM, GM-MIP and GM-NIP were prepared in section 2.3.

The preparation process of MIP for L-try was as follows: 1 mmol of L-try, 4 mmol of AM and 20 mmol of EGDMA were added into a glass vial and dissolved in 20 mL acetone. Then 15 mg AIBN was added, and the solution was purged with nitrogen for 5 min and polymerized at 65 °C for 24 h. The resultant bulk polymers were crushed, ground and sieved to collect the particles of size between 100 and 200 μm as MIP.

M-MIP was prepared in the absence of G.

G-MIP was prepared in the absence of $Fe_3O_4$.

The seven different polymers were added into the L-try solutions for adsorption under the same conditions. After full adsorption, the precipitate was isolated by centrifugation and the supernatant was tested by flow injection chemiluminescence. Adsorption capacity was obtained according to the regression equation.

### 2.5. Procedure for determination of L-try

The schematic for the CL sensor is shown in Fig. 1 and the determination could be summarized in six steps, as follows:

*First step*: recognition and adsorption of L-try. Switch valve (T) was connected with 1, sample solution was delivered to flow through GM-MIP, and L-try in the sample solution was selectively adsorbed.

*Second step*: chemiluminescence detection of sample solution without L-try. Switch valve (T) was connected with 1. The merged stream of $KMnO_4$, HCOH and $SnCl_2$ flowed to react with sample solution without L-try in 'Flow Cell' to produce $CL_1$.

*Third step*: transit of L-try. Switch valve (T) was in connection with 2, sample solution was delivered to flow through the GM-NIP and L-try cannot been adsorbed.

*Fourth step*: chemiluminescence detection of sample solution with L-try. Switch valve (T) was in connection with 2. The merged stream of $KMnO_4$, HCOH and $SnCl_2$ flowed to react with L-try in sample solution to produce $CL_2$.

*Fifth step*: cleaning the GM-MIP and GM-NIP for reusability. Switch valve (T) was in connection with 1 and 2, eluent and ultrapure water flowed through the GM-MIP and GM-NIP to remove L-try for next determination.

*Sixth step*: calculation of the concentration of L-try. According to regression equation, the concentration of L-try was obtained by

$$\Delta I = CL_2 - CL_1 \quad (1)$$

## 3. Results and discussion

### 3.1. Characterization of G and GM

In this work, the preparation of G and GM was an important part of GM-MIP and GM-NIP which as recognition materials in the sensor, the characterization of G and GM was done in this work. As a result, the Fourier transform infrared (FTIR) spectra, X-ray diffraction (XRD) measurements, scanning electron microscopy (SEM) and UV–vis absorption spectra of G and GM are shown in Fig. 2.

In the FTIR spectrum of G, the peak at 3419 $cm^{-1}$ has strong intensity and broad shape; it is the O–H stretch. 1670 $cm^{-1}$ is the characteristic of C=O bending band. Their bands at 2935, 2495, and 1385 $cm^{-1}$ are the characteristics of benzene ring, and the peak at 1091 $cm^{-1}$ is characteristic of C–OH, which confirms the presence of graphene oxide. In the FTIR spectrum of GM, 544 $cm^{-1}$ is the characteristic of $Fe_3O_4$ which gives evidence of the successful preparation of the GM.

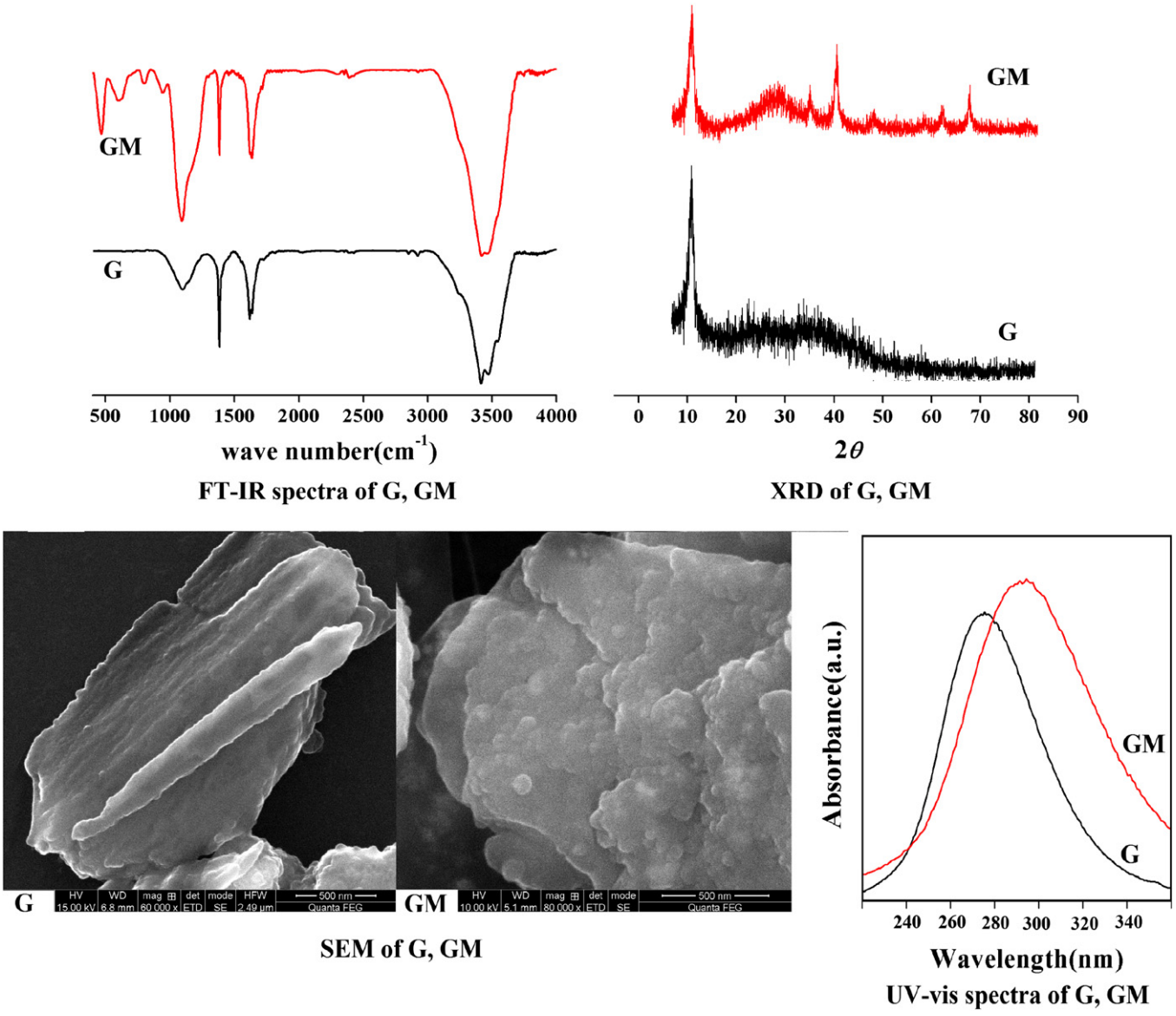


**Fig. 2.** Characterization of G and GM.

X-ray diffraction (XRD) measurements were employed to investigate the phase the and structure. As shown, the XRD pattern of the graphene oxide shows a sharp peak at $2\theta=10.9^{\circ}$. The typical XRD patterns of the GM composite show peaks at $2\theta$ values of 30.0°, 35.3°, 42.9°, 53.4°, 56.9°, and 62.5° which are consistent with the standard XRD data of $Fe_3O_4$.

Representative SEM images of the obtained G and GM are shown. It can be seen that, as the roughness of two synthesized nanocomposites were different, $Fe_3O_4$ NPs have been coated on the G surface. We considered that the $Fe_3O_4$ NPs were stably attached to the G surface through chemical bonding.

The UV–vis absorption spectra of G and GM for comparison were shown. G presented a characteristic peak at 269 nm corresponding to $\pi\rightarrow\pi^*$ transitions of aromatic C–C bonds. While for GM, the absorption peak was blueshifted to 285 nm, suggesting that the covalent attachment of $Fe_3O_4$ onto graphene oxide surface by the amidation reaction changed the structure of G.

### 3.2. Adsorption study of different polymers

The adsorption results are shown in Fig. 3. According to the results, the adsorption capacity of G-MIP ($11.4\times10^{-5}$ mol/g) was much higher than common MIP ($1.2\times10^{-5}$ mol/g). The results confirmed that the addition of G improved adsorption capacity. The addition of $Fe_3O_4$ has no role for adsorption capacity but made the separation from solution easier. And the adsorption capacity of GM-MIP ($11.9\times10^{-5}$ mol/g) was much higher than GM-NIP ($2.5\times10^{-5}$ mol/g); the large difference made the GM-MIP more appropriate to use in the CL sensor.

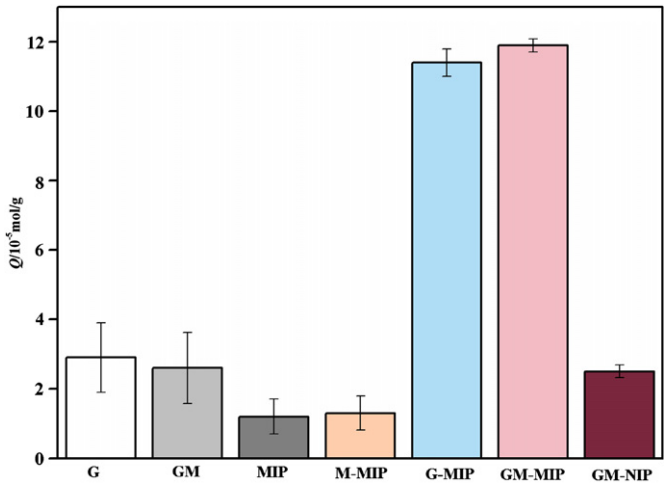


**Fig. 3.** Adsorption study of different polymers.

### 3.3. Optimization of GM-MIP-CL sensor

The schematic of the flow system shown in Fig. 1 was used to optimize the reagents concentrations for the CL determination of L-try, and the results are shown in Fig. 4.

With the $KMnO_4$ concentration in the range from $8.0\times10^{-5}$ to $4.0\times10^{-4}$ M, the CL intensity increased by raising the concentration of $KMnO_4$ up to $2.0\times10^{-4}$ M. Above $2.0\times10^{-4}$ M, the CL intensity decreased. Thus, the $2.0\times10^{-4}$ M $KMnO_4$ was used for further work.

The effect of HCOH concentration was examined from 5% to 30%, the CL intensity reached maximum when HCOH was 15%.

The effect of $SnCl_2$ was examined over $6.0\times10^{\times4}$ to $2.0\times10^{-3}$ M range, and the CL intensity reached a maximum value when $1.0\times10^{-3}$ M $SnCl_2$ was used. But higher concentration of $SnCl_2$ lowered the CL intensity of this system.

The adsorption time which depends on peristaltic pump's speed was an important parameter for the amount of L-try adsorbed on the GM-MIP. So the relation of CL intensity with the pump's speed was observed under the condition with 5–40 r/min. It was observed that the adsorption reached maximum at 15 r/min. Simultaneously, according to the experiment results, the other substances in the samples would be adsorbed by GM-MIP when the pump's speed was too small. L-try molecules cannot be adsorbed by GM-MIP completely under too large pump's speed. So the 15 r/min pumps speed was chosen for the determination of L-try with good results.

### 3.4. The analytical performance of the sensor

Under optimal conditions, the CL intensity responded linearly to the concentration of L-try. In the range from $2.10\times10^{-7}$ to $7.09\times10^{-4}$ M a detection limit of $2.11\times10^{-8}$ M ($3\sigma$) was observed, which is lower than conventional methods. The regression equation is $I_{CL}=4.24\times10^{2}+13.2c$ ($c$ being the L-try concentration) with a correlation coefficient of 0.9982.

### 3.5. Interferences study

Under the chosen conditions ($KMnO_4$: $2.0\times10^{-4}$ M, HCOH: 15% and $SnCl_2$: $1.0\times10^{-3}$ M, pump's speed: 15 r/min), coexisting substances were added into L-try solution ($4.0\times10^{-5}$ M) to investigate the effect on CL intensity. The tolerable fold of interfering substances in sample with and without GM-MIP column was compared when relative error was less than ±5% and the tolerance times are shown in Fig. 5.

These results showed that GM-MIP can be used as recognition material in the CL analysis and improved the selectivity of the CL method. Because the contents of coexisting substances were all lower than their tolerable concentrations, the proposed sensor

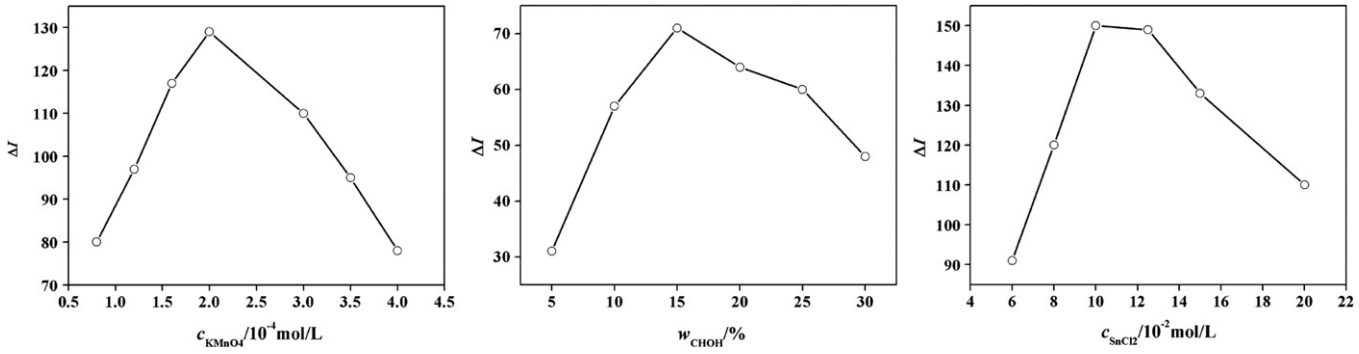


**Fig. 4.** Optimization results.

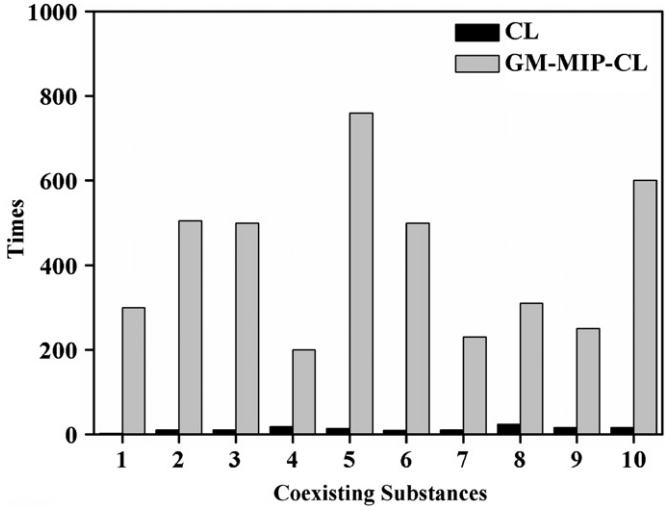


**Fig. 5.** Tolerable ratio of interfering species to L-try with and without GM-MIP. 1: L-lysine; 2: $Ag^{+}$; 3: $Fe^{3+}$; 4: L-phenylalanine; 5: $Ce^{2+}$; 6: $Ba^{2+}$; 7: L-glycine; 8: L-leucine; 9: L-serine; 10: $Cd^{2+}$.

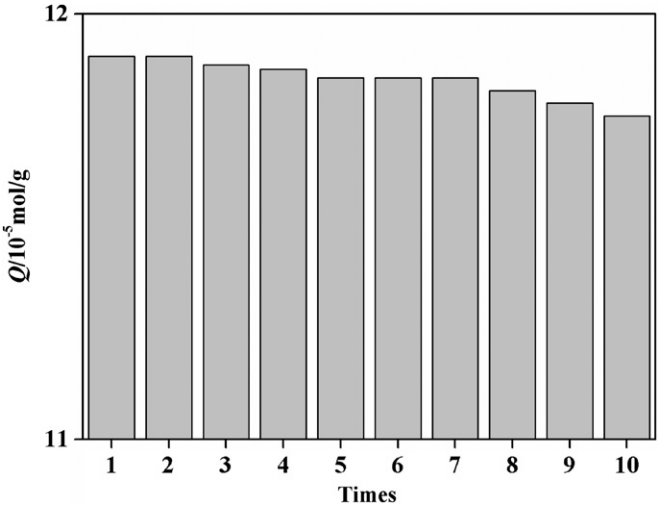


**Fig. 6.** Reusability of GM-MIP-cell.

**Table 1**
Results of recovery tests ($n=6$).

| Samples | Adding ($10^{-5}$ mol/L) | L-try content (%) | Found ($10^{-5}$ mol/L) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| 1# | 1 | 87.8 | 1.02 | 102.0 | 3.08 |
| 2# | 5 | 86.9 | 4.84 | 96.8 | 2.49 |
| 3# | 10 | 87.6 | 9.97 | 99.7 | 3.03 |

could be used directly to determinate the L-try in drug samples with high sensitivity.

### 3.6. Application of GM-MIP-CL sensor

Drug samples were obtained from the drugstore, and were diluted by double distilled water for analysis. The results of the recovery test are shown in Table 1. As can be seen from Table 1, the recoveries of added L-try can be quantitative and recoveries between 96.8% and 102.0% are at confidence level of 95%.

### 3.7. Reusability

The reusablility of the sensor was evaluated by comparing the adsorption of GM-MIP. According to the experiments, the GM-MIP was extracted with methanol/acetic acid (9/1, v/v) overnight after use, and then by adsorption to get the adsorption capacity. The results are shown in Fig. 6; the difference between ten times is tolerable. The sensor could be used more than hundreds of times before the adsorption began to decrease. This was possibly due to the loss of binding sites; however it was easy to replace the GM-MIP cell and the GM-NIP cell in the channel.

## 4. Conclusions

In this work, the L-try-GM-MIP was used as a molecule recognition material in the CL analysis. The characterization of the selective binding function of L-try GM-MIP to L-try molecules enables the method to have the advantage of high selectivity, making it possible to be applied for the analysis of L-try in drug samples directly. In the preparation of GM-MIP, G and $Fe_3O_4$ were added for optimizing the determination process. G was used for improving adsorption capacity and $Fe_3O_4$ NPs were used for fastening more easily in the GM-MIP cell. The application of the method was validated by testing the recovery of known amounts of L-try in the drug samples. The sensor was successfully applied to the determination of L-try in drug samples with satisfactory results. And the obtained GM-MIP-CL sensor has shown to provide a sensitive and fast method for on-site determination of L-try, or other drug and antibody.

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