Indian Journal of Chemistry
Vol. 56A, May 2017, pp. 501-507

A chemiluminescence sensor based on molecularly imprinted polymer for determination of peimine

Hongping Han[a, d], Hong Zheng[b, c, *], Baolin Li[a, *], Hui Xiang[b] & Yilan Zhangluo[b]

[a]School of Chemistry & Chemical Engineering, Shaanxi Normal University, Shanxi, Xi'an, 710062, China

Email: baolinli@snnu.edu.cn (B-LL)

[b]Department of Chemistry, Chongqing Normal University, Chongqing, 401331, China

Email: zhenghong670@163.com (Z-H)

[c]Qinghai Institute of Salt Lakes, Chinese of Academy of Sciences, Xining, 810000, China

[d]Department of Chemistry, Qinghai Normal University, Qinghai, Xining, 810008, China

Received 18 February 2017; revised and accepted 26 April 2017

The peimine-imprinted polymer has been synthesized and the binding characteristic of the imprinted polymer to peimine evaluated by equilibrium binding experiments. Using the imprinted polymer as recognition material, a simple, rapid and sensitive chemiluminescence sensor for the determination of peimine has been developed based on the chemiluminescence reaction of peimine with potassium permanganate in acidic medium. The chemiluminescence intensity responds linearly to the concentration of peimine within the range of 5.0×10^{-7} to 5.0×10^{-5} mol L^{-1} with a detection limit of 2.0×10^{-7} mol L^{-1}. The relative standard deviation for 3.0×10^{-6} mol L^{-1} peimine solution is 3.6% (n = 11). The sensor has been applied to the determination of peimine in *Fritillaria cirrhosa* D. Don, *Fritillaria thunbergii* Miq, and *Fritillaria unibracteata* Hsiao et k. c. Hsia samples with satisfactory results.

Keywords: Molecular imprinted polymers, Sensors, Chemiluminescence sensor, Peimine

Peimine is a bioactive alkaloid and the primary active ingredient in the bulbs of species of the genus *Fritillaria*, which are used in traditional Chinese medicine under the name "Beimu". Fritillaria bulbs have antitussive, expectorant, antiasthmatic, and antibacterial effects. It has been used for the treatment of asthma, bronchitis, lymph node tuberculosis, dry cough with little sputum and so on.[1,2] Currently, the methods to determine peimine include acid dye colorimetry,[3] gravimetric method,[4] near infrared spectroscopy,[5] thin-layered chromatography,[6] gas chromatography,[7] and high performance liquid chromatography.[8] These methods often require expensive instrumentation and suffer from time-consuming and complex procedures. Our previous studies showed that in acidic conditions, potassium permanganate can oxidize peimine to produce a weak chemiluminescence (CL), and formaldehyde has a strong sensitizing effect on this chemical reaction. However, it is difficult to apply CL methods in the determination of peimine content in *Fritillaria* sample due to the various degrees of interference produced by some coexisting substances in the complex sample. Moreover, the limited selectivity of CL methods further complicates the process. Therefore, establishing rapid, accurate, and simple methods for the separation and determination of peimine has important scientific significance in *Fritillaria* samples.

Sensor technology has attracted considerable attention in recent years. Particularly, a synthetic molecular recognition material that has similarities to bio-recognition system has been studied intensively for the development of chemical sensors with long-term stability and highly selectively. Molecular imprinting is a technique used for the preparation of polymers with a predetermined selectivity for the target molecule. The recognition ability of molecular imprinted polymer (MIP) to the target molecule is comparable to antibody-antigen and enzyme-substrate natural molecular recognition systems.[9-12] Currently, MIP has been successfully applied in solid phase extraction,[13,14] chromatographic stationary phase,[15] recognition elements in biosensors,[16,17] and artificial receptors in drug assays, etc.[18,19]

Chemiluminescence methods have the advantages of high sensitivity, use of the simple instrument,[20,21] fast analysis speed, easy operation and extensive dynamic range. Moreover, CL methods have been widely used in the field of environmental chemistry, clinical testing, pharmaceutical and industrial analysis. However, the poor selectivity limits the application of this method in complex samples. Also, this method not only requires relatively complicated and expensive instruments, but in many applications also suffers from incompatibility of separation and CL detection conditions. In order to improve the selectivity of CL method, the molecular imprinting-

CL detection system for peimine was developed. Typically, in this procedure, an MIP with special recognition ability for the target molecule is packed into a glass column which is connected into the CL flow system and used as the recognition material for analytes. When the analyte flows through the MIP column, it is separated from the coexisting substances in the sample and adsorbed on MIP due to the MIP's ability to recognize and capture the analyte molecules. When the CL reagent is run through the MIP column, it reacts with the analyte adsorbed on the MIP to produce chemilumenescence, thus improving the selectivity of the CL method greatly.[22,23]

The aim of the present study is to develop a simple, rapid and sensitive molecular imprinting-chemiluminescence (MI-CL) sensor for the determination of peimine. The peimine-imprinted polymer was prepared by using epoxy resin as functional monomer, polyethylene glycol as the porogen and diethylenetriamine as a cross-linker in the presence of a template molecule of peimine. The MIP column prepared with the resultant polymer particles size of 70–100 μm was connected to the flow CL system for selective and temporary adsorption of peimine. Then, the combined stream of acidic potassium permanganate solution and formaldehyde solution flowed through the MIP column and reacted with peimine adsorbed on the polymer to generate CL signal. During the CL reaction, the peimine molecules were destroyed, leaving cavities for new determination. Using MIP for CL analysis can greatly improve the selectivity of the CL sensor. The sensor was applied in the determination of peimine in *F. cirrhosa* D. Don, *F. thunbergii* Miq and *F. unibracteata* Hsiao et k. c. Hsia samples with satisfactory results.

Experimental

The schematic diagram of the MIP-CL flow system used in this study is shown in Fig. 1. The sensor

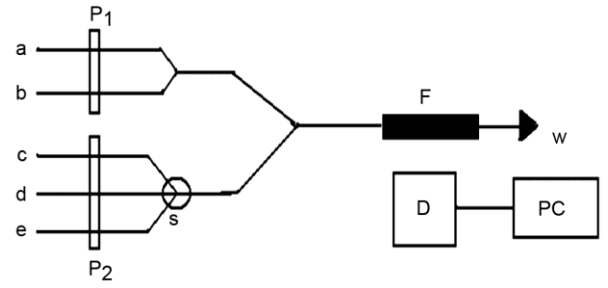

Fig. 1—Schematic diagram of the MIP-CL flow system. [(a) Potassium permanganate; (b) hydrochloric acid; (c) formaldehyde solution; (d) standard solution or sample solution; (e) water; (P1, P2) peristaltic pumps; (s) switch valve; (D) detector; (F) MIP column or NIP column; (PC) computer; (W) waste solution].

design in detail is given in Fig. S1 (Supplementary data). Peristaltic pumps were used to deliver all solutions and PTFE tubing (0.8 mm id) was used as the connection material in the flow system. CL measurements were performed using an IFFM-D CL analyzer (Xi'an Remax Electronic High-Tech Ltd, China). The data acquisition and treatment were performed using with IFFM-D flow injection CL data processing software (Xi'an Remax Electronic High-Tech Ltd, China). Absorbance was measured on a Mini 1240 UV-vis spectrophotometer (Shimadzu Corporation, Japan).

Peimine, peiminine and peimisine were purchased from Shanghai Wanjiang Biotechnology Co. Ltd (Shanghai, China). *F. cirrhosa* D. Don, *F. thunbergii* Miq, and *F. unibracteata* were purchased from Qinghai Xining Chenkang New and Special Drug Pharmacy (Xining, China). Epoxy resin, diethylenetriamine and polyethylene glycol-1500 were purchased from Tianjin Chemical Reagent Wholesale Company (Tianjin, China). Hydrochloric acid and formaldehyde were purchased from Shanghai reagent plant (Shanghai, China). All reagents used were of analytical reagent grade except for epoxy resin.

A stock standard solution of peimine (0.10 g L^{-1}) was prepared by dissolving 10 mg peimine in 100 mL doubly distilled water. Working standard solutions of peimine were prepared by diluting the stock solution with water.

The stock solution of potassium permanganate (1.0×10^{-2} mol L^{-1}) was prepared by dissolving 1.58 g of potassium permanganate in 1000 mL of boiling water, filtered through a glass fiber filter, and stored for one week at room temperature before use. Formaldehyde solution was prepared with distilled-deionized water. Hydrochloric acid (2.0 mol L^{-1}) was used. Doubly distilled water was used throughout the experiments.

The peimine-imprinted polymer was prepared As follows: Epoxy resin (4 g) and polyethylene glycol (8 g) were evenly mixed, followed by the addition of 1.0 mmoL of peimine and 1.0 g of diethylenetriamine. Under vigorous stirring, the mixture was transferred into an empty glass tube. When the early vigorous exothermic reaction was complete, the system viscosity was very high. The glass tube was placed in an incubator at 60 °C to allow the reaction to continue for 24 h without stirring. The white solid produced in the tube was collected, followed by cooling down. The product was repeatedly washed with water to remove the polyethylene glycol, and the column was

then washed with deionized water until neutral pH. After being dried at 60 °C, the sample was ground and passed through a sieve. The polymer powder with a particle size of 70–200 μm was collected for future use. A non-imprinted polymer (NIP) in the absence of the template molecular was prepared and treated in the same manner.

A portion of the above synthesized polymer particles (10.0 mg) was packed into a colorless glass tube (3 mm i.d. × 15 mm length) and plugged with a small amount of glass wool at both ends. This glass tube was connected to the CL flow system and placed in front of the window of photomultiplier tube. For a new MIP column, a merged stream of acidic potassium permanganate solution and formaldehyde solution was allowed to flow through the MIP column to clean the peimine absorbed on the MIP column until a stable baseline was recorded. Then, doubly distilled water was flushed through the MIP column to clean the polymer.

The binding properties of peimine MIP were measured by shaking-adsorption method. The polymer was dried to a constant weight at 60 °C under vacuum, then 50 mg of MIP was mixed with 5.0 mL peimine solution of various concentration in 25 mL colorimetric tubes and oscillated for 12 h at room temperature. After centrifuging at 3000 rpm for 10 min, the concentration of free peimine in the supernatant was detected by UV spectrophotometry at 270 nm. The amount of peimine bound to the polymer was calculated by subtracting the concentration of free peimine from the initial peimine concentration. The data was used for the Scatchard analysis.

The procedure for determining peimine is summarized in four steps:

Step 1: Adsorption of peimine: Pump 1 was stopped and the switch valve was in contact with sample solution. Pump 2 delivered peimine solution through the MIP column for 50 s, and peimine molecule in the sample solution was selectively adsorbed on the polymer.

Step 2: Removing other substances except peimine: Pump 1 was stopped and the switch valve was connected with the formaldehyde solution. Pump 2 continuously pumped formaldehyde solution through the MIP column for 80 s to remove other substances except peimine in the MIP column.

Step 3: Chemiluminescence detection. Pump 1 and pump 2 were both started and the switch valve was connected to formaldehyde. The merged stream of acidic potassium permanganate and formaldehyde flowed through the MIP column for 30 s to react with peimine adsorbed in the MIP to produce CL.

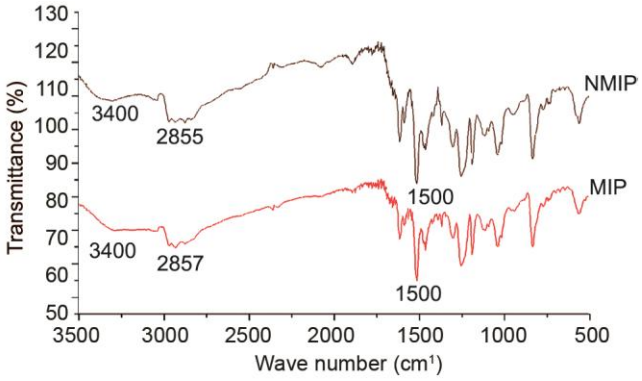

Fig. 2–FT-IR spectra of the peimine MIP and NIP adsorbents.

Step 4: Cleaning the MIP column. Pump 1 was stopped and the switch valve was connected to water. Water flowed through the MIP column to clean the MIP for 70 s for next determination.

Results and discussion

The FT-IR spectra of peimine MIP and NIP adsorbent show bands around 3400 cm^{-1} in NIP and MIP, indicates presence of –OH (Fig. 2). The band around 2600–3000 cm^{-1} is due to C-H vibrations and the peak of 1500 cm^{-1} is indicative of epoxides. In MIP, the presence of bands around 1350 cm^{-1} indicates the pentabasic cyclic structure. All these bands demonstrate the successful peimine molecular imprinting.

The morphological structure characterization of the peimine-MIP and NIP were studied by SEM. as shown in Fig. 3(a&b). The morphological structure of NIP shows presence of particles about 10 μm in size with smooth, uniform particle size distribution and good dispersion, while the morphological structure of peimine-MIP has small cavities, which can further explain that peimine-MIP has higher adsorption quantity than NIP.

The binding characteristeristics of the MIP and NIP were investigated by equilibrium binding experiments with varying concentrations of peimine (0.0–10.0 mmol L^{-1}) in the presence of 50.0 mg of MIP or NIP (Fig. 4). As it can be seen from the figure, not only the MIP but also the NIP can adsorb peimine in organic solvent. However, the adsorption capacity of the MIP is obviously larger than that of NIP.

The data obtained for amount of peimine bound to the MIP was used for the Scatchard analysis to estimate the binding parameters of the MIP. As shown

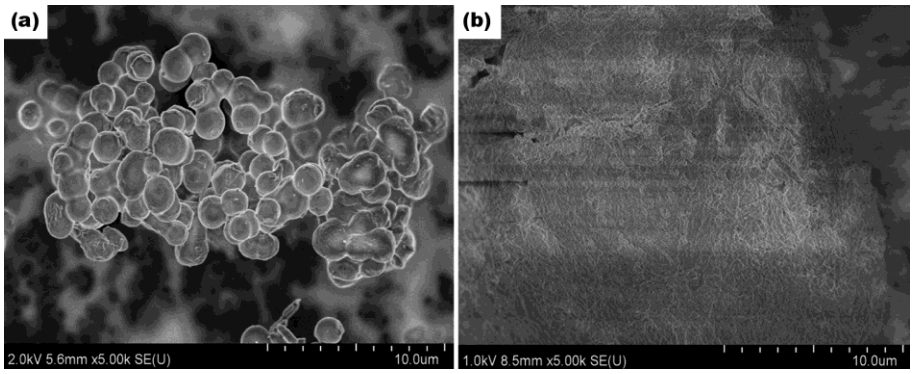

Fig. 3–SEM images of (a) MIP and (b) NIP.

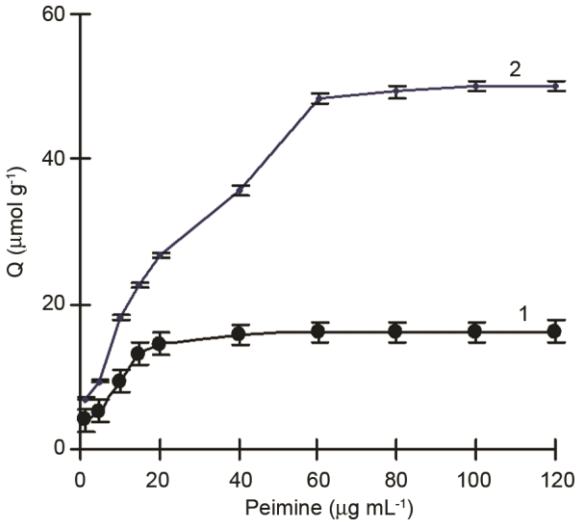

Fig. 4–Adsorption of varying concentrations of peimine on NIP (1) and MIP (2).

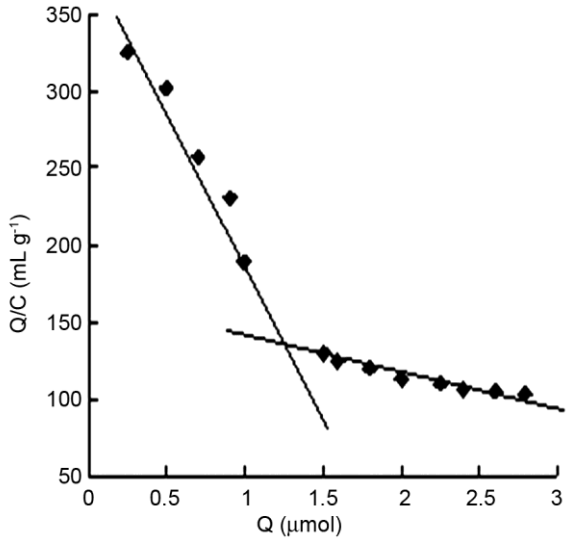

Fig. 5–Scatchard plot to estimate the binding characteristic of peimine imprinted polymer.

in Fig. 5, the Scatchard plot was not linear within the entire peimine concentration range studied, indicating that the binding sites in the MIP are non-uniform. However, it is observed that two distinct sections within the plot can be regarded as straight lines. This revealed that there were two classes of binding sites in the MIP. The equilibrium dissociation constant (k_{d1}) and the apparent maximum amount (Q_{max1}) for the higher affinity binding sites were calculated to be respectively 7.59×10^{-4} mol L^{-1} and 70.12 μmol g^{-1} for the dry polymer. By the same treatment, K_{d2} and Q_{max2} for the lower affinity binding sites were calculated to be respectively 4.31×10^{-3} mol L^{-1} and 232.19 μmol g^{-1}.

Peimine, peiminine and peimisine have similar structures and were used as substrates to study the binding selectivity of peimine MIPs on the substrates. An equilibrium binding method was employed to determine the binding capacities of peimine MIPs and NIP (see Table 1).

Table 1—Amounts of substrates bound on peimine-MIP and NIP polymer. [Amt of polymer: 50.0 mg; binding time: 12 h; initial conc. of substrates: 2.0 mmol L-1; vol.: 5.0 mL. temp: 25 °C]

Substrate	Peimine-IMP (μmol g^{-1})	NIP (μmol g^{-1})
Peimine	87.5	39.6
Peiminine	32.1	33.9
Peimisine	30.3	31.7

The binding capacity of MIPs with peimine was significantly higher than that of NIPs on peimine, showing a good selectivity. The binding capacities of MIPs with peiminine and peimisine were lower than that of NIPs with peiminine and peimisine due to the three-dimensional configuration formed by MIPs, which is suitable for the spatial structure of peimine. The fact that the binding capacity of MIPs with peiminine and peimisine were lower than that of NIPs

demonstrates the good selectivity of MIPs. Thus, the interaction between MIPs and imprinted molecules is a synergistic interaction of spatial structure with shape and functional groups.

The size of the MIP particles packed in the MIP column is an important parameter. If the polymer particles were too small, it would need higher pressure for transfusing a solution through the MIP column. If the polymer particles were too big, the adsorption capacity of the MIP column would decrease because the effective surface area of the MIP column is smaller. Two kinds of particles (sized 70–100 μm and 100–200 μm) were examined and the experimental results revealed that the most appropriate particle size was in the range of 70–100 μm.

To investigate the stability of the MIP, 10 mg of peimine was filled in the selected vertical glass column (dia. = 3 mm, length = 15 mm), connected to the CL flow system. Formaldehyde mixed quickly with potassium permanganate and reacted with the peimine adsorbed on the column, producing strong CL signals. The prepared MIP columns were relatively stable, and no significant changes were observed after being used 120 times. During the experiment, the CL signals of the standard solutions with the same concentrations were the same, and there were no significant differences in the detection limit of the method indicating a good stability and reusability of peimine MIP monolithic column under the determined conditions.

Potassium permanganate is a strong oxidant, and it caused the color of MIP in the column to change from white to brown. This phenomenon indicated that potassium permanganate can react with the unsaturated bond in the MIP, which would destroy the recognition sites in the MIP and influence the recognition ability of the MIP to peimine. The experiments showed that this problem could well be solved by introducing some reducing agents, described as the protective agents, into the reaction system. The protective ability of different reducing agents including sodium sulfite, sodium thiosulfate, and formaldehyde were compared. The results showed that in an acidic medium, the CL signal generated by potassium permanganate-formaldehyde was the strongest. Formaldehyde was selected as the protective and sensitizing agent for this CL system. The protective effect of formaldehyde on MIPs within the mass fraction range of 10–60 g kg^{-1}, and the sensitizing effect on potassium permanganate-peimine reaction was studied. When the formaldehyde mass fraction was 30 g kg^{-1}, the recognition capacity of MIP columns on peimine was strong and substantially stable, and the highest chemiluminescent intensity of this reaction was observed.

A series of experiments was conducted to optimize the experimental conditions for the determination of peimine. The luminescent reaction for potassium permanganate-formaldehyde system takes place in acidic media, and therefore, different acids have different effects on the luminescence intensity of the system. The effects of H_2SO_4, HNO_3, HCl, and H_3PO_4 (of equal concentration) on the CL reaction of this system were investigated. The results showed that in an HCl medium, the CL signal of the system was the strongest and stable, hence HCl was selected as the reaction medium. Additionally, the impact of HCl in the concentration range of 0.5–8 mol L^{-1} on the CL intensity of the system was studied. The maximum CL signal was obtained at a concentration of 1.5 mol L^{-1}, above which the CL intensity begins to decline. The best signal-to-noise ratio in the CL reaction was obtained at a concentration of 1.5 mol L^{-1} and hence was chosen as the best condition for further studies.

Potassium permanganate is the oxidant for the system, and the magnitude of its concentration not only directly affects the CL intensity and signal-to-noise ratio, but also the linear range of the measurement. The study of the effect of potassium permanganate solution at a concentration range of 5.0×10^{-7}–1.0×10^{-2} mol L^{-1} on the luminescence intensity of the system showed that the highest signal-to-noise ratio of luminescence intensity for the system was obtained when the potassium permanganate solution concentration was 5.0×10^{-4} mol L^{-1}. The effect of the concentration of potassium permanganate solution within the range of 0.1×10^{-4} to 9.0×10^{-4} mol L^{-1} on the luminescence intensity was further investigated.

The CL intensity increased when increasing potassium permanganate concentration. The CL intensity was the highest and remained stable when the concentration of potassium permanganate reached 2.5×10^{-4} to 7.0×10^{-4} mol L^{-1}. Therefore, the concentration of the potassium permanganate used in further experiments was 3.0×10^{-4} mol L^{-1}.

Adsorption time is the time standard solution or the sample solution flowing through the MIP column. It also determines the amount of peimine adsorbed on

MIP column and affects the sensitivity and linear range of the measurement. The adsorption time is relevant to the concentration of peimine, the binding capacity of the polymer and the flow rate. With the amount of polymer as 40 mg and the flow rate as 1.5 mL min^{-1}, the relation between the CL intensity and the adsorption time within the range 10–120 s was examined using 5.0×10^{-4} mol L^{-1} peimine solution. It was observed that the CL intensity increased with increasing absorption time. After 40 s, CL intensity tended towards constant value and reached equilibrium. In view of analytical efficiency and the linear range of this method, 50 s was finally selected as the adsorption time. It should be mentioned that for the analysis of a sample with lower peimine content, the sensitivity of the detection can be improved by increasing the adsorption time.

Following the adsorption step, the MIP column should be washed to clean the remaining test solution and other nonspecifically adsorbed materials on the column. Hence, a suitable washing time should remove other substances completely and not cause the loss of peimine adsorbed on the MIP column. In order to select the appropriate washing time, peiminine was selected as the interference indicator because its structure is similar to peimine, and its CL reactions take place under the same conditions. Peiminine was added to peimine standard solution (peimine: 5.0×10^{-4} mol L^{-1}, peiminine: 5.0×10^{-4} mol L^{-1}). The effect of washing time on the CL intensity was examined in the range 10–180 s. The results showed that the luminescence intensity decreased between 10 s and 80 s, indicating that peiminine was gradually eluted from the column. The luminescence intensity remained unchanged between 90 s and 180 s, and showed no significant difference with that measured with the peimine standard solution without the interference indicators. Therefore, only peimine reacted with potassium permanganate to produce CL. Therefore, 90 s was selected as the washing time.

When the combined stream of the CL reagents flowed through the MIP column, they reacted with the peimine adsorbed on the column to produce an enhance CL signal. The CL signal declined to the baseline which indicated the peimine adsorbed on the MIP column has been consumed completely. For a complete reaction, time of 30 s was found sufficient, therefore, 30 s was selected as the CL reaction time.

The CL reaction between potassium permanganate and peimine is an oxidation-reduction reaction. In this reaction, the molecule structure of peimine adsorbed on the polymer was destroyed and peimine was desorbed from the MIP. The reaction products could be cleaned from the MIP when water flowed through the MIP column. The effect of the cleaning time in the range 10–120 s was examined by alternately measuring the blank and CL signals from 5.0×10^{-4} mol L^{-1} peimine solution. The results showed that when the cleaning time is up to 70 s, both the blank signals and the CL signal from 5.0×10^{-4} mol L^{-1} peimine solution have a good repeatability. Therefore, 70 s was selected as the cleaning time.

In order to examine the selectivity of the sensor, the interference of foreign species on the determination of 5.0×10^{-4} mol L^{-1} peimine was investigated in the presence and absence of MIP. The interference studied herein were that due to coexisting substances in the *Fritillaria* samples and the substances showing CL behavior in potassium permanganate CL system. The tolerable limit of such foreign species were taken as that producing relative error less than ±5%. The results in Table 2 show that most interferents studied herein were tolerated nearly 100-folds and could be completely removed by use of the MIP, because the material was specific peimine.

Using the selected experimental conditions, the relation between the CL intensity and peimine

Table 2—The tolerable ratio of interfering species to peimine in presence and absence of MIP

Interfering substances	MIP	Without MIP
K^+	300	100
Na^+	1000	500
NH_4^+	100	10
Cu^{2+}	100	10
Fe^{3+}	100	50
Al^{3+}	150	15
Ca^{2+}	200	50
SO_4^{2-}	100	5
Cl^-	1000	400
NO_3^-	100	5
Starch	1000	200
Vitamin B_1	500	200
Vitamin B_2	100	50
Vitamin B_6	100	20
Vitamin B_{12}	50	5
Vitamin C	100	30
Urea	1000	100
Glucose	100	5
Ascorbic acid	200	5
Maltose	1000	100
Salicylic acid	500	100
Fructose	1000	500

concentration was examined. The CL intensity was linearly related to the concentration of peimine in a range of 5.0×10^{-7} to 5.0×10^{-5} mol L^{-1}, and the linear regression equation was $I = 7.8931c + 27.191$ ($n = 5$, $r^2 = 0.9969$), where I is the CL intensity (relative unit), and c is the concentration of peimine. For the peimine solution of 3.0×10^{-6} mol L^{-1}, the relative standard deviation was 3.6% for eleven parallel experiments. According to the International Union of Pure and Applied Chemistry (IUPAC) rules, the detection limit of the method was determined as 2.0×10^{-7} mol L^{-1}.

To study the practical application of the studied method, samples of *F. cirrhosa* D. Don, *F. thunbergii* Miq, and *F. unibracteata* samples (1.000 g each) were weighed, ground, and transferred to 100 mL volumetric flasks. The samples were soaked for five hours and distilled water was used to dilute the solution up to the mark. Subsequently, the samples were treated in an ultrasonic cleaner for 50 min, followed by centrifugation. The supernatant (10 mL) was collected, and 10 mL of 8 % polyethylene glycol was added, and the mixture was mixed evenly and allowed to stand for five hours. CL measurements were conducted in triplicate according to the procedure described above. The results show that the recoveries of added peimine were quantitative, and the t-test showed that there was no significant difference between the recoveries and were 100% at a confidence level of 95% (Supplementary data, Table S1). These results show that the MI-CL sensor has good accuracy for the determination of peimine in *Fritillaria* samples.

In summary, a simple, rapid and sensitive MIP-CL sensor was developed for the determination of peimine by using peimine-imprinted polymer as recognition material and acidic potassium permanganate-peimine CL reaction as the detection system. The sensor exhibited high selectivity to peimine due to the specific binding of the MIP to peimine. This sensor showed high sensitivity owing to the preconcentration of the peimine on the MIP and the sensitive CL detection method. The sensor was successfully applied for the determination of peimine in the *Fritillaria* samples.

Supplementary data

Supplementary data associated with this article, viz., Fig. S1 and Table S1, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_56A(05)501-507_SupplData.pdf.

Acknowledgement

This study was supported by the QingHai Science and Technology Department (Grant No 2013-Z-752), the National Natural Science Foundation of China (No. 21267018), the Program of "One Hundred Talented People" of the Chinese Academy of Science, and Chongqing Education Committee Science and Technology Research Project (No. KJ130650), PR China).

References

1 Xie Z M & Liu R X, *J Chinese Med Maters*, 24 (2001) 600.
2 Yu X L, Ji H, Wang C L & Li P, *J Chinese Trad Herbal Drugs*, 31 (2000) 313.
3 Peng S M & Gao M Y, *Chinese Pharmaceutical Affairs*, 20 (2006) 687.
4 Sha S Y, *Methods of Analysis the Effective Components in Chinese Trad Medicine*, Vol. 1, (Beijing: People's Medical Publishing House Press, PR Chine), 1982, p.59.
5 Zhao Y D, Du W F, Wang S B, Cong X D, Cai B C, & Ge W H, *Chinese Archives Trad Chinese Med*, 31 (2013) 756.
6 Lu B, Shi R B, Zhou Y & Zhou J F, *Chinese J Expt Trad Medical Form*, 7 (2001) 8.
7 Li S L, Li P, Lin G, Chan S W & Hu Y P, *J Chromatogr A*, 873 (2000) 221.
8 Li S L, Lin G, Chan S W & Li P, *J of Chromatogr A*, 909 (2001) 207.
9 Wang Z & Cao X j, *Process Biochem*, 7 (2015) 1136.
10 Revaz O Solomonia, Brian J & McCabe, *Neurosci Biobehav Rev*, 50 (2015) 56.
11 Wulff G, Sarhan A & Zabrocki K, *Tetrahedron Lett*, 44 (1973) 4329.
12 Cao B Q, Pan Y & Zhao J J, *J Modern Scient Instr*, 6 (2006) 29.
13 Yi L X, Rou F & Chen G H, *J Chromatogr Sci*, 45 (2014) 608.
14 Gao R X, Cui X H, Hao Y, Zhang L L, Liu D C & Tang Y H, *J Food Chem*, 194 (2016) 1040.
15 Yan H Y & Kyung Ho Row, *Biotechnol Bioprocess Engng*, 11 (2006) 357.
16 Wang Z H, Li J S, Liu X L, Yang J M & Lu X Q, *Anal Bioanal Chem*, 405 (2013) 2525.
17 Nie F, Lu J R, He Y H & Du J X, *Talanta*, 66 (2005) 728.
18 Andersson G L I, Muller R & Mosbach K, *Nature*, 361 (1993) 645.
19 Guo H Y, Zheng H, Zhang J, Song L, Xia X S & Gao X Z, *J Anal Sci*, 29 (2013) 196.
20 Qi Y Y & Xiu F R, *J Luminescence*, 171 (2016) 2385.
21 Zhao P N, Yu J H, Liu S Q, Y M, Zang D J & Gao L, *Sensors Actuators B* , 162 (2012) 166.
22 He Y H, Lu J R, Zhang H G & Du J X, *Microchim Acta*, 149 (2005) 239.
23 Du J X, Shen L H & Lu J R, *Anal Chim Acta*, 489 (2003) 183.