Determination of Morphine by Molecular Imprinting–Chemiluminescence Method

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Abstract

A molecular imprinted polymer of morphine was synthesized. Using the imprinted polymer as the recognition material, sodium sulfite as the protective agent, and a potassium permanganate-morphine chemiluminescence system as the detection system, a molecular imprinting–chemiluminescence method for the determination of morphine was established. The linear response range of this method was $5.0 \times 10^{-9}$–$1.0 \times 10^{-6}$ g/mL ($r = 0.9981$) and the detection limit was $2 \times 10^{-9}$ g/mL. The coefficient of variation for $1.0 \times 10^{-7}$ g/mL morphine solution was 2.8% ($n = 9$). This method was applied to the determination of morphine in the urine of the heroin abusers with satisfactory results.

Introduction

Morphine, a narcotic and analgesic used in the area of medicine, is one of the toxicants that were abused generally. Morphine can be eliminated from the human body without metabolism, and it is also the main metabolite of codeine and heroin. So, determination of morphine has an important significance on toxicant analysis, drugger cognizance, and effective estimation of refraining from drug.

At present, many analytic techniques have been developed for the determination of morphine including gas chromatography–mass spectrometry (1–3), immunoanalysis (4,5), high-performance liquid chromatography (6–8), and so on. Townshend has reported a method for the determination of morphine with the potassium permanganate-morphine chemiluminescence (CL) system in 1986 (9). This method, with many advantages (such as high sensitivity, extensive dynamic range, inexpensive instrumentation, and high analysis efficiency) attracts intensive attention, and some related papers have been published in succession (10–13). However, because potassium permanganate can react with many organic compounds to produce CL under the same condition, reliable results would hardly be obtained when this method was applied directly to determine morphine in complicated samples such as urine and serum. The molecular imprinting technique has emerged as a useful tool for the preparation of molecular imprinted polymer (MIP) with synthetic recognition sites that have a predetermined selectivity for analytes. If a minicolumn made of MIP was connected into the CL flow system, utilizing its fine recognition and capture ability to target a molecule and separate the target molecule from other coexistent substances, the selectivity of the CL method would be improved greatly. A molecular imprinting (MI)–CL method for the determination of epinephrine in serum has been successfully developed in our laboratory (14).

The aim of this work is to develop a MI–CL analytical method for morphine using a morphine-imprinted polymer as recognition material and morphine-potassium permanganate CL system as the detection system. During the CL reaction, potassium permanganate can also react with unsaturated bonds in the MIP and destroy the recognition sites in MIP. In order to overcome this problem, sodium sulfite was used as the protective agent in the experiments to prevent the recognition sites in MIP from being destroyed. The method has good selectivity and has been successfully applied to the determination of morphine in the urine of the heroin abusers.

Experimental

Apparatus

The schematic diagram of the MI–CL flow system is shown in Figure 1. Peristaltic pumps were used to deliver all solutions, and 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 CL analyzer (Xi'an Remax Electronic High-Tech, Ltd.). The data acquisition and treatment was performed with the IFFM-D CL data processing software (Xi'an Remax Electronic High-Tech, Ltd.).

Reagents

Morphine was purchased from the National Institute for the
Control Pharmaceutical and Biological Products. Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma (St. Louis, MO). Methacrylic acid (MAA) and 2,2'-azobis(2-methylpropionitrile) (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, MAA, and acetonitrile were redistilled, and AIBN was recrystallized prior to use.

Stock standard solution of morphine (1.00 × 10^{-3} g/mL) was prepared by dissolving 0.1000 g of morphine in 10 mL of 0.2 mol/L hydrochloric acid and then diluting to 100 mL with water. Working standard solutions of morphine were prepared by diluting this stock solution with water. All morphine solutions were stored in the refrigerator and protected from light.

Potassium permanganate solution (5.0 × 10^{-2} mol/L) was prepared by dissolving 0.3950 g of potassium permanganate in 50 mL water and protected from light. Sodium sulfite solution (2.0 × 10^{-2} mol/L) was prepared by dissolving 0.1220 g of sodium sulfite in 50 mL water and protected from light. The concentration of polyphosphoric acid used was 0.1 mol/L. Doubly distilled water was used throughout the experiments.

Synthesis of the polymer

The morphine-imprinted polymer was synthesized similar to the method reported previously (15). A 1.5 mmol of morphine and 6 mmol of MAA were dissolved in 6 mL of acetonitrile in a 50 mL round-bottom flask, then 24 mmol of EGDMA and 56 mg of AIBN were added, and the solution 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 size between 74 and 105 μm.

Preparation of the MIP column

Morphine MIP column was a 4-mm i.d. × 15-mm length colorless glass tube packed with a 10.0 mg of polymer (synthesis described previously) and plugged with a small amount of glass wool at both ends. The MIP column was connected into the CL flow system and placed in front of the window of the photomultiplier tube. For a new MIP column, the merged stream of acidic potassium permanganate and sodium sulfite flowed through the MIP column for 45 s and reacted with morphine adsorbed on the MIP column. The further experiments showed this problem could be well solved by introducing some reducing agents into the reaction system. We described such a reducing agent as the protective agent. The protective ability of different agents was compared, including sodium sulfite, sodium hypochlorite, and formaldehyde. The experiments showed that sodium sulfite was the most suitable one. Sodium sulfite can not only protect the MIP well but also enhance the potassium permanganate-morphine CL reaction. The effect of sodium sulfite was examined in the range of 1.0–5.0 × 10^{-4} mol/L. The suitable concentration of sodium sulfite was 2.5 × 10^{-4} mol/L.

Optimization of experimental conditions

Using the schematic diagram shown in Figure 1, a series of experiments were conducted to optimize the experimental conditions for the determination of morphine.

Adsorption time

The adsorption time is the time that it takes for the standard solution or sample solution to flow through the MIP column.
The adsorption time determines the amount of morphine adsorbed in the MIP column, thereby influencing the sensitivity of the detection and the linear range of the method. The adsorption time is relevant to the concentration of morphine, binding capacity of the polymer, and flow rate. When the amount of the polymer was 10.0 mg and the flow rate was fixed at 1.5 mL/min, the relation between the CL intensity and the adsorption time within the range 30–200 s was examined using 1.0 × 10^{-7} g/mL morphine solution (Figure 2). The CL intensity was increased with the increase of adsorption time up to 120 s. Above 120 s, the CL intensity remained constant. Considering the analytical efficiency and linear range of this method, 90 s was finally selected as the adsorption time. It should be mentioned that for analysis of a sample with lower morphine content, the sensitivity of the detection could be improved by increasing the adsorption time.

Washing time

Following the adsorption step, it is necessary to remove the other substances in the MIP column absorbed by nonspecific interaction. A suitable washing time should remove other substances completely and not cause the loss of morphine adsorbed in MIP column. To select the washing time, codeine, which was similar to morphine in structure and can also react with potassium permanganate to produce CL in an acidic medium, was selected as interference indicator and added in the morphine standard solution (morphine 1.0 × 10^{-7} g/mL, codeine 5.0 × 10^{-7} g/mL). At the same time, sodium sulfite was used as washing reagent in order to protect the MIP from being destroyed in next step. The effect of the washing time was examined in the 10–90 s at the flow rate of 1.5 mL/min. The experimental results showed that when the washing time was > 60 s, the interference indicator, codeine, can be effectively removed. The CL intensity showed no obvious change with that of the same concentration (1.0 × 10^{-7} g/mL) of morphine standard solution in the absence of codeine. So 60 s was selected as the washing time.

CL reaction time

When the merged stream of CL reagents flowed though the MIP column, they reacted with morphine adsorbed on the polymer to produce CL. The CL signal declined to the baseline, which indicated the morphine adsorbed on the MIP column has been consumed completely. The experimental results showed 40 s was enough for a complete reaction. Therefore, 40 s was selected as the CL reaction time.

Cleaning time

It is necessary to clean the MIP column after the reaction. The CL reaction between potassium permanganate and morphine is an oxidation-reduction reaction. During the reaction, the molecular structure of morphine adsorbed on the polymer was destroyed, and morphine was desorbed from the MIP. The reaction products can be easily removed from the MIP column when water flowed through the MIP column. The effect of the cleaning time in the range 20–70 s was examined by alternately measuring the blank signal and the CL signal from 1.0 × 10^{-7} g/mL morphine solution. It was observed that when the cleaning time was up to 40 s, both the blank signals and the CL signal from 1.0 × 10^{-7} g/mL morphine solution had good repeatability. Therefore, 40 s was selected as the cleaning time.

Reagents concentrations

The effects of reagents concentrations including potassium permanganate (5.0 × 10^{-5}–5.0 × 10^{-3} mol/L), polyphosphoric acid (0.01–0.3 mol/L), and sodium sulfite (1.0–5.0 × 10^{-4} mol/L) on the CL reaction were also examined. The optimum concentrations for potassium permanganate, polyphosphoric acid, and sodium sulfite were 5.0 × 10^{-4} mol/L, 0.1 mol/L, and 2.5 × 10^{-4} mol/L, respectively.

Analytical performance

At the selected experimental conditions and using the flow system depicted in Figure 1, the relation between the CL intensity and the concentration of morphine was examined. The CL intensity was linearly related to the concentration of morphine over 5.0 × 10^{-9}–1.0 × 10^{-6} g/mL range with a regression equation of I = 127C − 158 (n = 5, r = 0.9981), where I was the CL intensity (relative unit) and C was the concentration of morphine (× 10^{-9} g/mL). The coefficient of variation for 1.0 × 10^{-7} g/mL of morphine solution was 2.8% (n = 9). According to suggestion of International Union of Pure and Applied Chemistry (IUPAC), the detection limit can be determined as 2 × 10^{-9} g/mL.

Selectivity

In this work, the interferences of foreign species on the determination of 1.0 × 10^{-7} g/mL morphine were investigated using the MI–CL method and the flow injection (FI)–CL method, respectively. The determination procedure of morphine with FI–CL method is according to reference (9). The foreign species selected were the substances that normally exist in urine and substances having CL-like behavior in an acidic potassium permanganate CL system. In this selectivity study, the CL signal of 1.0 × 10^{-7} g/mL morphine solution was determined, which was used as a standard. Then, a certain amount
of the interfering species was added into a 1.0 × 10⁻⁷ g/mL morphine solution, and a CL signal of this mixing solution was obtained through a same determination procedure. The tolerance ratio, which means the ratio of the amount of interfering species to the analyte in the sample solution when the deviation of their CL signals is less than 5%, is shown in Table I. As can be seen in Table I, the MI-CL method exhibited an excellent selectivity for the determination of morphine, and the tolerable ratios for all foreign species were improved greatly.

Application
The blank urine was collected from healthy volunteers and centrifuged at 3000 rpm for 10 min. The supernatant (2.0 mL) was transferred into a 50-mL volumetric flask, diluted with water to the mark, and used as the sample solution. The sample solution was analyzed by the MI-CL method and the FI-CL method, respectively. The results are shown in Table II. As can be seen in Table II, the CL signals of urine sample are stronger than that of water in the FI-CL method. This indicates that there are interfering species in the urine sample. However, the CL signals of different urine samples and water have no significant differences with each other in MI-CL determination. These results indicated that the other species that coexisted in the urine sample can be effectively removed from the urine samples by MIP column and do not interfere with the determination of morphine.

The MI-CL method was applied to the determination of morphine in the urine of the abusers who had used heroin within 24 h. The urine samples were treated with the procedures described for the blank urine and determined using the MI-CL method. The results of the determination of urine sample are shown in Table III. As can be seen in Table III, the recoveries of added morphine were quantitative, and the t-test assumed that there is no significant difference between recovery efficiency and 100% at a confidence level of 95%.

Conclusions
Poor selectivity is the obvious disadvantage of the general CL method. Using MIP with fine recognition ability to target the molecule, selectivity of the CL method can be improved greatly. In this work, the morphine MIP was synthesized and the MI-CL method for the determination of morphine was developed. The method had been successfully applied to the determination of morphine in urine samples.

Although MIP is not as good as the inartificial antibodies in some aspects, it also showed some attractive features such as good mechanical strength, reusability, and stability in harsh environments. With improvement of the method step by step, it can be foreseen that these advantages will translate into a more economical approach to morphine detection and measurement in the future.

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References
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