Using chiral magnetic surface molecularly imprinted polymers for chiral separation of gossypol

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Abstract

The aim of this study was to prepare a method for quick resolution of gossypol. The chiral magnetic surface molecular imprinting polymers (chiral MMIP) were utilized. A chiral reagent (N-octyl-D-glucosamine) acted as a monomer, and magnetic nanoparticles were used as carriers to obtain chiral MMIPs. The chiral MMIPs could combine with (+)-gossypol molecules, and enable the separation of (−)-gossypol from racemic gossypol.The characterization of chiral MMIPs were detected by Infrared spectroscopic analysis and transmission electron microscopic (TEM). The results showed that the presence of monomeric methacrylate in the chiral MMIPs and the particle size of chiral MMIP was about 150 nm. The binding capacity of gossypol chiral MMIP indicated that the chiral MMIPs prepared in the laboratory were capable of binding well to gossypol. The polymer was then used as the stationary phase for column chromatography, and (-)-gossypol solution was thus isolated

through chromatography. The product was tested by ultraviolet spectrum and optical rotation tests and was confirmed to be (-)-gossypol. Thus, we established a simple, rapid, and cost-effective method for separating chiral compounds.

Keywords: gossypol; magnetic surface molecularly imprinted polymer; chiral reagent; ultraviolet spectrum

Introduction

Gossypol is a polyphenol compound that exists as yellow crystals in various organs of cotton plants.It has been shown to be toxic for the heart, kidneys, liver, nervous system, and blood （AA Conceição，2018）. However, recent studies have revealed that, besides being toxic, gossypol also has a high medicinal value, and has contraceptive（Wen N，2018）, anti-tumor （L Zeng，2017；H Chen，2017；Zeng Y，2017）, antiviral（B Zhang，2017）, and anti-bacterial effects （Chen CW，2018）. The anti-tumor effects of gossypol are particularly valued. Studies have found that (–)-gossypol has a stronger inhibitory effect on cancer cells than gossypol （S Shen，2018）, and could effectively induce apoptosis in PC-3 and DU-145 cells. In the USA, (–)-gossypol has shown significantly encouraging results in phase-3 clinical trials. Figure 1 shows the structures of (–)- and (+)-gossypol.
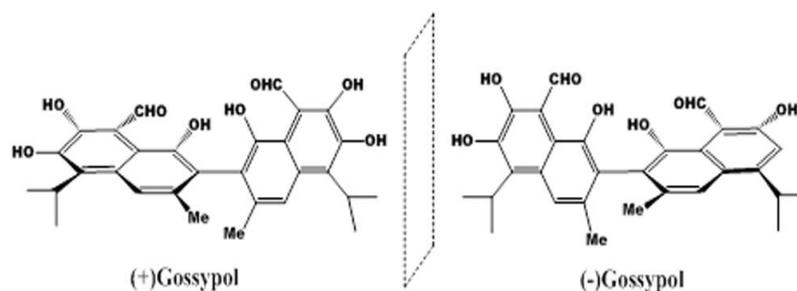

Fig. 1 Structures of (+)-gossypol and (-)-gossypol

Chiral resolution of compounds is a major challenge worldwide. Although various methods for chiral resolution, such as gas chromatography (GC), high performance liquid chromatography (HPLC), column electrophoresis (CE), and capillary electrophoresis, exist, these methods do not show high specificity to target molecules, causing errors in the separation of these pharmaceutical molecules, thus affecting the purity of drugs. GC is a

relatively new technique for separation and analysis of compounds, and is widely used in industry, agriculture, defense, construction, and scientific research. HPLC, although more precise, is disadvantaged in that samples require a complex and cumbersome pre-processing. In addition, HPLC is a time-consuming and expensive technique unsuitable for rapid detection. Therefore, effective, simple, and rapid resolution methods need to be developed. Magnetic molecularly imprinted polymers (MMIPs) could represent a solution to this problem. MMIPs are generated by combining magnetic materials with molecularly imprinted polymers (MIPs) （Zhao C，2017；Chen Z，2013）. Application of an external magnetic field results in the rapid separation of MMIPs. MMIPs are known to be capable of actively distinguishing and rapidly separating template molecules （Chen Z，2016；Chen Z，2016）. Iron (II, III) oxide, Fe_3O_4, is the most widely used magnetic material due to its low toxicity, low cost, and high stability.

The use of MMIPs for chiral resolution satisfies the need for rapid detection, is highly sensitive, has low cost, and has the potential for a wide range of applications. For preparing MIPs, a chiral reagent is used as a monomer, while racemic drugs are used as template molecules, thus solving the problem of obtaining pure enantiomers for use as templates during chiral separation. The MIP technology could overcome challenges that occur during the binding and elution of template molecules and MIPs, as well as the problem of low number of sites for MIP binding.

The aim of this study was to achieve the quick resolution of gossypol using MMIP technology.

Methods

Materials

$FeCl_2 \cdot 4H_2O_2$, $FeCl_3 \cdot 6H_2O$, ammonia (20%), ethylene glycol diglycidyl ether (EGDE), isopropanol, and tetraethyl orthosilicate (TEOS) were purchased from TCI Development Co., Ltd, Shanghai, China. Methanesulfonic acid (Chinese Medical Chemicals, Shanghai, China), triethoxyvinylsilane (TTS, Sigma-Aldrich, USA), N-n-Octyl-D-glucamine (Sigma-Aldrich, USA), and ammonium persulfate (Chinese Medical Chemicals, Shanghai, China) were of analytical grade.

Methods

Preparation of gossypol chiral MMIP

Preparation of magnetic nanoparticles (Fe₃O₄)

FeCl$_2$·4H$_2$O (1.72 g), FeCl$_3$·6H$_2$O (4.72 g), and deionized water (80 ml) were added to a flask with a continuous stream of nitrogen gas, and then heated and stirred using a magnetic stirrer. When the temperature had reached 80 ℃, ammonia (10 mL) was slowly added and allowed to react for 30 min; black precipitates were obtained. The product was washed 4-6 times with deionized water to remove unreacted substances and impurities; they were then dried to obtain Fe$_3$O$_4$ nanoparticles.

Synthesis of SiO₂-encapsulated magnetic nanoparticles (SiO₂-Fe₃O₄)

Three hundred milligrams of the magnetic nanoparticles, deionized water (4 mL), and isopropanol (50 mL) were mixed and subjected to ultrasonic treatment for 15 min. Ammonia (5 mL) and tetraethyl orthosilicate (TEOS) (2 mL) were sequentially added and continuously stirred at 25℃ for 12 h for reaction. After reaction, the product was washed 4-6 times with distilled water. The product was enriched under a magnetic field and then isolated and dried to obtain SiO$_2$-encapsulated magnetic nanoparticles (SiO$_2$-Fe$_3$O$_4$)

Preparation of chiral MMIPs

Ten grams of the SiO$_2$-Fe$_3$O$_4$ was soaked in methanesulfonic acid solution (wt %: 50%) for 24 h, and then rinsed with acetone and distilled water, before drying to obtain activated particles. Triethoxyvinylsilane (TTS) (30 mL), 20 g activated SiO$_2$-Fe$_3$O$_4$ nanoparticles, and ethanol (ethanol: water = 1:1) (400 mL) were added to a round-bottomed flask and allowed to react at 50 ℃ for 31 h. The product was rinsed with ethanol and distilled water, and then dried to obtain TTS- SiO$_2$-Fe$_3$O$_4$. TTS-SiO$_2$-Fe$_3$O$_4$ (3 g), N-n-Octyl-D-glucamine (10 g), distilled water (200 mL), and ammonium persulfate (0.018 g) were added to a round-bottomed flask and allowed to react at 70 ℃ for 7 h. The product thus obtained was washed with ethanol and distilled water, and then enriched under a magnetic field, isolated, and dried. This product was then dissolved with 4 mmol/L gossypol in chloroform solution (100 mL), and nitrogen gas was added for 5 min. After agitation for 6 h, the ethylene glycol diglycidyl ether (EGDE) was added and the solution was mixed for 8 h at 50 ℃, and then eluted (elution solution, ethanol: methanol: water = 2:7:1, v/v/v). The product was enriched under a magnetic field. And the MMIPs were dried at 80℃.

Magnetic non-imprinted polymers (MNIPs) were prepared in the same manner, with the exception that the target compound, gossypol, was not added.

Detection of the binding capacity of chiral MMIPs

A gossypol standard curve was first prepared. Gossypol MMIPs (0.05 g) were then added to different concentrations of gossypol solution and agitated for 2 h to ensure binding between MMIPs and gossypol. The supernatant was removed from the mixture and UV spectrophotometry was used to detect the absorbance of the solution at 349 nm. The concentration after reaction was calculated based on the standard curve, and the Q value was calculated based on formula (1):

$$Q = \frac{V(c_1 - c_2)}{m} \tag{1}$$

where c_1 is the initial gossypol concentration (mg/L); c_2 is the equilibrium gossypol concentration (mg/L); V is the volume of gossypol (L), m is the mass of MMIPs (g), and Q is the unit mass of MMIP that was bound to gossypol at equilibrium.

The binding rate for NIPs was detected in a similar manner, with the exception that MNIPs were added to the gossypol solution.

Chromatographic separation and specific rotatory power of gossypol

Chromatographic separation of gossypol

Half a gram of the sample and 8 times the sample weight of MMIPs were weighed (4 g). Ethyl acetate (2 mL) was added to the sample to form gossypol solution. Two volumes of solvent (ethyl acetate) was added to MMIPs and mixed with a glass stirrer; the mixture was left to stand for 24 h, and a homogenous suspension was obtained after stirring. The column was then prepared for loading and loaded with this mixture. The prepared MMIP paste was injected uniformly into the column through a funnel while stirring. Twice the volume of the column of the remaining MMIPs was then added at a low flow rate. When the precipitation was complete, the device was clamped to iron pillars and prepared for the baseline run. After connecting the device, the baseline run was initiated and the chromatogram was obtained in 1 h. In this experiment, the sample was wet-loaded. After the sample entered the column containing MMIPs, the elution solution was used for column elution at a flow rate of 3 mL/min. The collection device was used to collect the eluent for UV spectroscopy.

Specific rotatory power of gossypol

The polarimeter was pre-warmed for 30 min and zeroed using the blank solvent. The test sample was accurately weighed and adjusted to a fixed concentration using a volumetric flask.

It was then sealed before insertion into the polarimeter for determining its specific rotatory power.

Results

Infrared spectroscopic analysis of gossypol chiral MMIPs

The standard wave number of Fe_3O_4 is 580 cm^{-1}. Figure 2(a) shows a wide and strong absorbance peak at 561 cm^{-1} in the infrared spectrum of Fe_3O_4. This was likely because of a "purple shift" in the Fe_3O_4 infrared spectrum, caused because the dimensions of the Fe_3O_4 crystals produced were in the nanoscale. Figure 2(b) shows a strong absorbance peak at 1092 cm^{-1} in the infrared spectrum, likely due to asymmetrical stretching vibrations of Si—O—Si, while the absorbance peaks at 798 cm^{-1} and 468 cm^{-1} likely indicate symmetrical stretching vibrations and bending vibrations of Si—O—Si, respectively. These observations indicated the formation of a complex containing SiO_2 and showed that the Fe_3O_4 on the surface was bound to SiO_2, forming SiO_2-Fe_3O_4. Lastly in Figure 3(c), the absorbance peaks at 1634 cm^{-1} and 3447 cm^{-1} likely represent the stretching vibration peaks of the carbonyl (C=O) bond and the carboxyl (—COOH) bond, respectively, indicating the presence of monomeric methacrylate in the MMIPs.
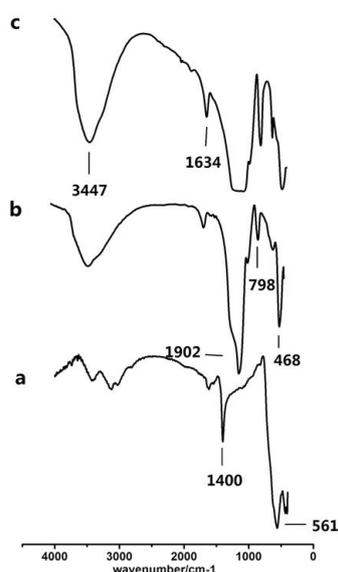

Fig. 2 Infrared spectra of (a) Fe_3O_4, (b) SiO_2-Fe_3O_4, and (c) MMIP.

Transmission electron microscopic (TEM) analysis of gossypol chiral MMIPs

TEM (JEM-2100) was used for examining the Fe_3O_4, SiO_2-Fe_3O_4, and MMIP at a magnification of 10,000-× and an accelerating voltage of 75 kV. As shown in Figure 4, the particle size distribution of MMIPs was basically uniform, with particle sizes around 50 nm

(Figure 3(a)). As shown in Figure 3(b), the particle size of SiO_2-Fe_3O_4 was around 90 nm, indicating an increase in the particle size after encapsulation with SiO_2. As shown in Figure 3(c), the particle size of MMIP was around 150 nm, indicating that SiO_2-Fe_3O_4 acted as a carrier and that MIPs were synthesized by the binding of crosslinking agents and functional monomers on its surface, resulting in the increased particle size.
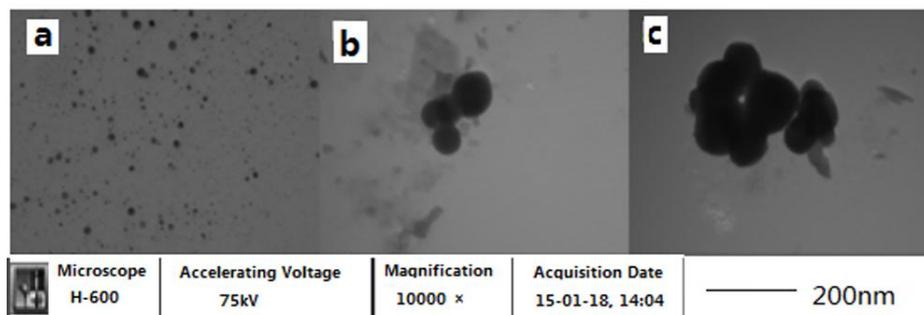

| Microscope H-600 | Accelerating Voltage 75kV | Magnification 10000 × | Acquisition Date 15-01-18, 14:04 | ——— 200nm |

Fig. 3 TEM images of (a) Fe_3O_4, (b) SiO_2-Fe_3O_4, and (c) MMIP.

Determination of binding capacity of chiral MMIP

The UV standard curve of gossypol was used to calculate the concentration of MMIP and MNIP before and after addition into the solvent. The binding rates of MMIP and MNIP to different concentrations of gossypol were calculated using formula (1). Figure 4 shows an isothermal graph of the binding rate of gossypol MMIPs. The binding capacity of MNIP was significantly lesser than that of MIPs; as the concentration of gossypol solution increased, the binding capacity of MMIPs also increased. When the concentration was 0.03 mg/L, the binding capacity was gradually saturated, as evidenced by the binding curve. These observations indicated that the gossypol MMIPs prepared in the laboratory were capable of binding well to gossypol.
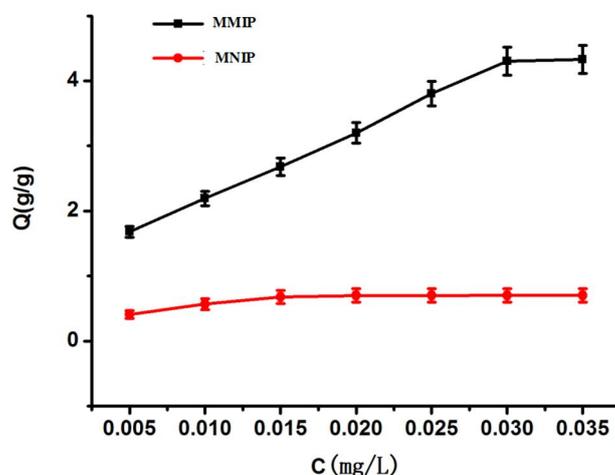

Fig. 4 Adsorption isotherm of gossypol for MMIP and MNIP.

Experimental chromatogram

A nearly parallel baseline was obtained when the baseline run was performed. As shown in Figure 5, peaks were observed at 0.5 min , indicating the detection of (–)-gossypol. As the prepared MMIP could bind specifically to (+)-gossypol, (–)-gossypol would be eluted at 0.5 min; thus, the eluent collected at 0.5 min would be (–)-gossypol.
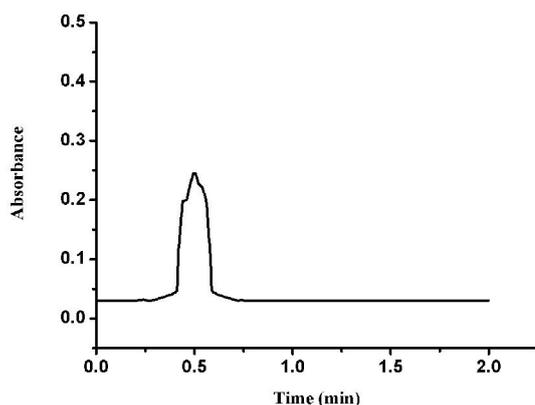

Fig.5 Column chromatogram of (–)-gossypol .

Specific rotatory power of (–)-gossypol

Similar to melting and boiling points, specific rotation is a physical constant for optically active substances. By determining the specific rotation of unknown substances, allows us to infer their identity. Determining the specific rotation of known substances allows us to calculate their optical purity. Thus, determining the specific rotation of optically active substances is one of the commonly used qualitative and quantitative methods.

The specific rotation ($[\alpha]_{\lambda}^{t}$) can be calculated from the concentration of the tested substance (C) and the optical rotation (α) measured using the polarimeter, using the formula:

$$[\alpha]_{\lambda}^{t} = 100\alpha/l \times C \qquad\qquad (2)$$

where t is the temperature at which the measurement was taken (generally 20 °C), λ is the wavelength of the light source (the commonly used sodium lamp has a wavelength of 589 nm, and is represented by the symbol D), α is the measured optical rotation (°), C is the concentration of the measured solution (g/100 mL), and l is the length of the tube (dm).

The measured (–)-gossypol concentration was C = 0.1, and the final specific rotation obtained was α = –130.2°. From these results, we concluded that the final solid obtained was (–)-gossypol.

Discussion

This study established a method for the rapid resolution of gossypol. Gossypol is an important drug with anti-tumor effects, and (–)-gossypol has been shown to have better anti-tumor activity than racemic gossypol. The separation of synthesized drugs has always been a challenge in the pharmaceutical industry. Therefore, developing effective, simple, and rapid resolution methods is a priority. The chiral reagent, N-n-Octyl-D-glucamine was used as monomers and magnetic nanoparticles were used as carriers to obtain chiral MMIPs. This method helped overcome the challenge of obtaining pure enantiomers for use as template molecules during chiral MIP preparation. Moreover, MIP technology can be used to overcome the challenge of binding and that of eluting template molecules and MIPs, as well as the low number of binding sites. Compared to other typical chiral separation methods, this method is advantageous in that it is rapid, simple, and inexpensive. These chiral MMIPs could specifically bind to (+)-gossypol molecules, enabling the separation of (–)-gossypol from racemic gossypol. The optical rotation and specific rotation of the obtained (–)-gossypol was measured in order to prove the feasibility of this method.

Conflicts of Interests Statement

The authors declare no conflicts of interest.

Funding:

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