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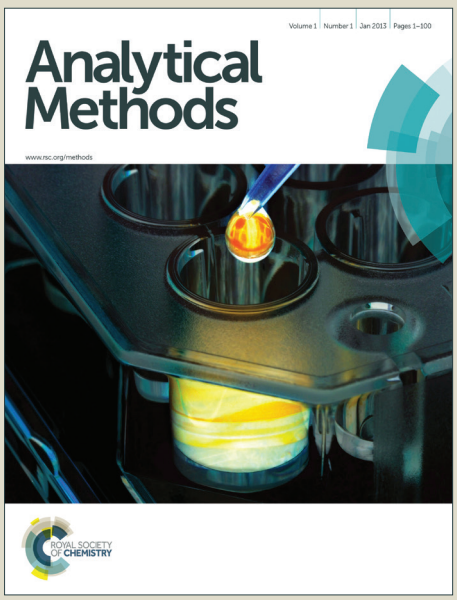

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Molecularly imprinted polymer coated on carbon nanotubes for matrix solid phase dispersion extraction of camptothecin from *Camptotheca acuminate*

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A molecularly imprinted polymer (MIP) – matrix solid phase dispersion (MSPD) method for extraction of camptothecin from *Camptotheca acuminate* was developed. The MIPs were synthesized by surface imprinting technique using carbon nanotubes as support, camptothecin as template, methacrylic acid as functional monomer, ethylene glycol dimethacrylate as crosslinker. The MIPs were characterized by scanning electron microscopy, transmission electron microscopy and Fourier transform-infrared spectroscopy. The isothermal adsorption, dynamic adsorption and selectivity adsorption experiments were also carried out in this study. The MIP-MSPD method coupled with high performance liquid chromatography was applied for determination of camptothecin in *Camptotheca acuminate* fruit, bark and leaf. Factors affecting the extraction yield such as sorbent/sample ratio, dispersion time, and washing and elution solvents were investigated. Under the optimized conditions, a good linearity of camptothecin was obtained in a range of 1–200 μg mL^{-1}, and the detection limit of the method was 0.13 μg mL^{-1}. The extraction yields of camptothecin obtained were 0.1055%, 0.0628% and 0.0930% for *Camptotheca acuminate* fruit, bark and leaf, respectively. The recovery of camptothecin was in the range of 96.1–103.3%. The results showed that this method is fast, selective, cost-effective and environment-friendly compared with other extraction methods such as stirring extraction, ultrasonic extraction and Soxhlet extraction used for extraction of camptothecin.

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1. Introduction

Camptotheca acuminate is a species of indigenous tree in southern China. It produces antitumor alkaloids, most notably camptothecin.[1] Camptothecin is attracting considerable attention worldwide because of its promising antitumor characteristic, which was discovered in the 1960s during the screening of plant extracts for antitumor activity. Its structure (Fig. 1) was determined by Wall *et al*.[2] Camptothecin and its analogs have demonstrated effectiveness in killing cells of various cancer types such as small and non-small lung cancers, ovarian cancer, pancreatic cancer, myelomonocytic leukemia and related disorders.[3]

Many conventional methods have been used for the extraction of camptothecin, such as homogenate extraction,[1] stirring extraction,[4] Soxhlet extraction,[5,6] heat reflux extraction,[7] ultrasound-assisted extraction,[3,8] microwave-assisted extraction,[9] column chromatographic extraction[10] and cloud point extraction[11]. Various concentrations of ethanol or methanol, [5, 7] alkaline solution,[12] acidic solution,[13] ionic liquid aqueous solution [3] and non-ionic surfactant X-114 solution[11] were reported to be effective solvents for camptothecin extraction. However, these extraction methods are based on maceration extraction, which need long extraction time, and are relatively low in efficiency as well as being costly.

Matrix solid-phase dispersion (MSPD) is an extraction procedure which combines aspects of several analytical techniques allowing sample homogenisation, disruption, extraction, fractionation and clean-up within a single process.[14] In MSPD, the solid sample is blended in a mortar with an appropriate sorbent to obtain complete disruption and dispersion of the sample on the solid support. The blend is packed into a column from which the analytes are eluted with a relatively low solvent volume.[15] In recent years, MSPD has been widely used in the pre-treatment of food, plant, biological samples, environmental samples and cosmetics.[16] However, due to the lack of special selectivity, MSPD using traditional sorbents was confronted with the difficulty of selectively extracting target analytes from complex samples.[17] Therefore, further improving the selectivity of MSPD is still very crucial and significant.

Molecularly imprinted polymers (MIPs) are artificially synthesized macromolecular materials, which are highly cross-linked polymers and are able to recognize the target molecules by imprinting the molecules during polymer synthesis through covalent or noncovalent interactions.[18] So far, MIP has been researched and utilized extensively in such fields as biochemical separation,[19] chiral resolution,[20, 21] enzyme catalysis,[22, 23] chromatographic analysis,[24] biosensors,[25] drug delivery,[26] and so on. In recent years, MIPs have been extensively used for the selective enrichment and pretreatment of target compounds which exist in a complex matrix sample. It is a new trend that MIPs are used as selective MSPD sorbents to achieve simultaneous extraction and purification of analytes, which can significantly reduce the labor and cost of the analysis.[27]

The main challenge for the traditional imprinted materials is the generated cavities that are not at the surface or in the proximity of the materials' surface. The high resistance to mass transfer will still hinder target species from accessing the deep imprinted cavities, thus reducing the kinetics of binding target analyte. Fortunately, several research groups have made efforts to prepare core–shell structural MIPs, which combine the advantageous properties of both molecular imprinting technology and support material.[28, 29]

Carbon nanotubes (CNTs) have enjoyed widespread attention for their high electrical and thermal conductivity properties.[30] CNTs have unique mechanical properties and extremely large surface areas. This material was used as support which endows MIPs with large surface areas. If the MIPs were prepared onto the surface of CNTs, the binding sites in the outer layer of the composite would improve the accessibility of template molecule and reduce the binding time.[31]

In this work, we present a facile process for the preparation of imprinted polymers coated on CNTs. Given the CNTs possessing high surface area and chemical inertness, it can be grafted vinyl groups in appropriate synthesis conditions. Vinyl groups could directly copolymerize with methacrylic acid and cross-linkers in the presence of template camptothecin. Thus the MIPs were grafted on the surface of CNTs. Then the MIP was used as MSPD sorbent for selective extraction of camptothecin in *Camptotheca acuminate* samples. The factors affecting the MSPD and the

applicability of the method were discussed.

2. Materials and methods

2.1. Chemicals and reagents

The camptothecin standard was purchased from the F.S. Biological Development (Baoji, China). Multi-walled carbon nanotubes with diameter of 30-50 nm and length of 5-15 μm (purity > 97 wt%) were purchased from Nanoport (Shenzhen, China). Ethylene glycol dimethacrylate (EGDMA) was purchased from Aladdin (Shanghai, China). Ammonia and acetic acid were purchased from Guangfu (Tianjin, China). Methanol, ethanol, nitric acid, hydrochloric acid, N,N-dimethyl acrylainide (DMF), acrylamide, methacrylic acid (MAA) and azo-isobutyronitrile (AIBN) were obtained from Kermel (Tianjin, China). Acetonitrile of chromatographic grade was purchased from Fisher (Pittsburgh, PA, USA). High purity water was obtained from a Milli-Q WaterSystem (Millipore, Billerica, MA, USA).

The *Camptotheca acuminate* samples were purchased from Huqiao (Haozhou, China).The dry samples of *Camptotheca acuminate* were powdered using a cyclone mill and then sieved (60 mesh). The stock standard solution of camptothecin (0.5 mg mL^{-1}) was prepared by dissolving an appropriate amount of the compound in chloroform–methanol (1:1, v/v) solution. The solution was stored in a refrigerator at 4 ^{o}C. The working standard solution was prepared daily by diluting the stock standard solution.

2.2 Apparatus

The MIPs were characterized by scanning electron microscopy (SEM; JEM-6700 F, JEOL, Tokyo, Japan), transmission electron microscopy (TEM, H7650, Hitachi, Japan) and Fourier-transform infrared spectrometry (FT-IR 360, Nicolet, Madison, WI, USA). Sample analysis was performed using a Shimadzu liquid chromatography (Kyoto, Japan). The DZKW-D-1 water bath (Shuli, Shanghai, China) was used in the synthetic process of the polymer. A mechanical shaker

(Shengtang, Jintan, China) was used in adsorption process.

2.3 Preparation of MIPs

The impurities such as amorphous carbon and metallic catalyst in the CNTs (1.0 g) were removed by using HNO_3 (100 mL) solution at 80 ^oC for 6 h. Then the CNTs were washed with water. The activated CNTs (CNTs–COOH) were mixed with sulfoxide chloride (50 mL) in a round bottom flask at 70 ^oC for 12 h. The sulfoxide chloride in the mixture was removed at 90 ^oC by distillation. After being washed with DMF, the acylating CNTs (CNTs–COCl) were dried at 60 ^oC under vacuum overnight. Then the dried CNTs–COCl and 6.0 g acrylamide were added into 100 mL DMF. After ultrasonic for 10 min, the reaction was performed at 45 ^oC for 24 h. The vinyl groups functionalized CNTs (CNTs–CONHCOCH=CH_2) were washed with 0.12 mol L^{-1} hydrochloric acid and water, respectively.

Camptothecin (0.4 mmol) was dissolved in 50 mL chloroform–methanol (1:1, v/v) solution, and then 0.15g CNTs–CONHCOCH=CH_2, 2 mmol MAA, 10 mmol EGDMA and 0.1 g AIBN were added. The solution was degassed with sonication for 5 min. The reaction was allowed to proceed at 60 ^{o} C for 24 h with vigorous stirring (300 rpm). In order to extract the template, the polymers were subjected to Soxhlet extraction with methanol: acetic acid (8:2, v/v) until no camptothecin could be detected by HPLC. Finally, the polymers were dried in vacuum overnight, and stored at room temperature. Fig. 2 shows the protocol for synthesis of the MIPs. Non-imprinted polymers (NIPs) were prepared and processed similarly as above, except that the template camptothecin was not added.

2.4 Binding experiment

The adsorption experiments were performed by adding 20.0 mg MIPs or NIPs in a glass tube containing 2.0 mL of camptothecin standard solution. The solution was incubated at room temperature to obtain the maximum binding of camptothecin to polymers, and then the supernatant

was isolated and analyzed by HPLC. The amount of camptothecin bound to the polymers was calculated by subtracting the free concentration from initial concentration of camptothecin added to the mixture.

To investigate the adsorption kinetics of the sorbent, 20.0 mg of MIPs or NIPs was added into 2.0 mL of camptothecin standard solution and was shaken for 5-240 min.

The selectivity of the MIPs was investigated with 7-ethyl-10-hydroxycamptothecin (Fig. 1b) as the structural analogue of camptothecin template and bergenin (Fig. 1c) as reference compound. Compared with camptothecin, bergenin also has hexatomic ring, hydroxyl and carbonyl groups. The experiment was conducted by adding 20.0 mg MIPs or NIPs into 2.0 mL camptothecin, 7-ethyl-10-hydroxycamptothecin and bergenin standard solution, respectively.

2.5 MIP-MSPD procedure

The powdered *Camptotheca acuminate* (0.1 g) with 0.1 g of MIP was placed into a glass mortar and ground for 5 min using a pestle to obtain homogeneous mixture. The homogenized sample was loaded into a cartridge (6 mL pre-fritted, 20 μm porosity, polypropylene tubes). The cartridge was rinsed with 5.0 mL 10% aqueous methanol, and then the analyte was eluted with 4.0 mL acetic acid-methanol (5:95, v: v) at the flow rate of 1.0 mL min^{-1}.

2.6 Other extraction methods for comparison

Different methods which refer to the previously reported methods [5] were used for the extraction of camptothecin from *Camptotheca acuminate* fruit, bark and leaf.

For stirring extraction, 5.0 g of *Camptotheca acuminate* sample was placed into a 250 mL flask with 100 mL 90% methanol aqueous solution and stirred for 60 min at 70 ^{o}C.

For ultrasonic extraction, 5.0 g of *Camptotheca acuminate* sample was added to a 100 mL volumetric flask with 100 mL 90% methanol aqueous solution and sonicated for 30 min.

For Soxhlet extraction, 5.0 g of *Camptotheca acuminate* sample was put into 200 ml Soxhlet

thimble. The apparatus was fitted with 250 ml round bottom flask containing 100 ml of 90% methanol aqueous solution. The extraction was performed at 70 ^{o}C for 120 min.

CNTs without coating MIPs were also used as MSPD sorbent for the extraction of camptothecin. The extraction process was performed according to MIP-MSPD procedure.

2.7 HPLC analysis

Camptotheca acuminate sample analysis was performed using liquid chromatography with a Hypersil ODS2 column (150 mm × 4.6 mm, 5 μm) (Elite, Dalian, China). The chromatographic mobile phase was a mixture of acetonitrile–water (40 : 60, v/v). The flow rate was 1.0 mL min^{-1} and the detection wavelength was set to 254 nm. Extraction solutions were filtered through 0.45 μm filter papers before HPLC analysis.

3. Results and discussion

3.1. Characterizations of the MIPs

SEM and TEM were used to characterize the morphologies of the MIPs. As shown in Fig. 3, the diameter of MIPs increases obviously compared with crude CNTs, which revealed that the imprinted polymer layer was attached on the CNTs surface successfully. Moreover, the MIPs still keep the hollow and tubular structure of CNTs.

Fig. 4 presents the FT-IR spectrum of MIPs. The major peaks for MIPs can be assigned as follows: the adsorption band around 3432 cm^{-1} unveiling the stretching vibration of O-H group, and the adsorption band around 2974 cm^{-1} unveiling the telescopic vibration of C-H group. The band around 1730 cm^{-1} is attributed to the C=O stretching vibration. The peaks at 1456 and 1386 cm^{-1} are attributed to the existence of C-H bending vibration. The peak at 1635 cm^{-1} is attributed to the existence of C=C stretching vibration. The peak at 1258 cm^{-1} is attributed to the existence of C-O stretching vibration.

3.2. Isothermal adsorption experiment

The binding isotherm was studied by changing camptothecin concentration. The results were shown in Fig. 5. It could be seen that the amount of camptothecin bound to both MIPs and NIPs at equilibrium concentration increased along with the increase of the initial concentration of camptothecin, but the binding amount of camptothecin on MIPs was greater than that on NIPs. This result indicated that the MIPs had a specific binding capacity for the template molecule.

The static adsorption experiments were employed and the data were processed with Scatchard analysis according to the equation:[32]

$$\frac{Q}{C} = \frac{(Q_{\max} - Q)}{K_d}$$

where Q is the amount of camptothecin bound to the polymers at equilibrium (mg g^{-1}); C is the free camptothecin concentration at equilibrium (μg mL^{-1}); K_d is the dissociation constant (mg L^{-1}) and Q_{max} is the apparent maximum binding amount (mg g^{-1}). The values of K_d and Q_{max} can be calculated from the slope and intercept of the linear line plotted in Q/C versus Q.

It is observed that two straight lines were obtained in the plot region (Fig. 5b), which indicated that there are two kinds of binding sites for MIPs. The linear regression equations for the left and right slope of the biphasic curve is Q/C= -0.5229Q+0.8026 and Q/C= -0.0183Q +0.1478. From the slope and the intercept of the biphasic curve, the K_d were 1.91 and 54.6 mg L^{-1}, the Q_{max} were 1.53 and 8.08 mg g^{-1}, respectively. As can be seen from Fig. 5c, NIPs showed one kind of binding site. The K_d was 40.1 mg L^{-1} and the Q_{max} was 4.32 mg g^{-1}.

3.3. Adsorption kinetics

Adsorption kinetics studies were carried out to investigate the adsorption process. As shown in Fig. 6, the adsorption rate of the MIPs and NIPs toward camptothecin increased rapidly in the early 120 min, and then the rate of adsorption increased slowly with the time extension.

Two of the most widely used kinetic models, i.e. pseudo-first-order equation and

pseudo-second-order equation were used to research the adsorption kinetic behavior of camptothecin onto polymers. The pseudo-first-order kinetic model is expressed by the following equation:[33]

$$\ln(Q_{eq}-Q_t) = \ln Q_e - K_1 t$$

The plot of $\ln(Q_{eq}-Q_t)$ against t provides a linear relationship from which K_1, constant of pseudo-first-order adsorption (min^{-1}) and Q_e (mg g^{-1}), adsorption capacity at equilibrium are calculated from the slope and intercept of the plot, respectively, given that Q_t (mg g^{-1}) is the adsorption capacity at time t. Another kinetic model is pseudo-second-order model, which is expressed by:[34]

$$t/Q_t = 1/K_2 Q_e^2 + t/Q_e$$

The plot of t/Q_t against t provides a linear relationship from which K_2, rate constant of pseudo-second-order adsorption (g mg^{-1} min^{-1}) and Q_e (mg g^{-1}), adsorption capacity at equilibrium are calculated from the slope and intercept of the plot, respectively. Plots of pseudo-first-order and pseudo-second-order kinetic models of camptothecin adsorption onto MIPs and NIPs are shown in Fig. 6b and c, respectively. Different kinetic parameters of camptothecin adsorption onto MIPs and NIPs are shown in Table 1. All the experimental data of camptothecin adsorption onto MIP showed better compliance with pseudo-second-order kinetic model in terms of higher correlation coefficient values (R^2=0.999), and closer values between Q_e and Q_{eq}. The experimental data showed that the adsorption behavior of camptothecin onto NIP was better conform to pseudo-first-order kinetic model in terms of closer values between Q_e and Q_{eq}, and higher correlation coefficient values (R^2=0.999).

3.4. The selectivity of MIP

The results about the study on selectivity of MIP were shown in Fig. 7. The amount of camptothecin and 7-ethyl-10-hydroxycamptothecin bound to the MIP was higher than that of NIP. Because these two compounds have similar structure, the MIP also has selectivity to

7-ethyl-10-hydroxycamptothecin. However, the MIP has higher specificity to camptothecin which used as template. The selectivity is mainly due to the molecular size recognition of MIP to template molecule and the hydrogen bonding interactions between the carboxylic group in the MIP, and carbanyl group and hydroxyl group in camptothecin at identical positions. There was no obvious difference between the MIP and NIP to adsorb the reference compound bergenin indicating that the adsorption of bergenin is non-specific even it also has carbanyl group and hydroxyl group.

3.5. Optimization of the MSPD procedure

The first step for the development of a MSPD method was selecting a suitable mass ratio of sorbent/sample, in order to allow complete adsorption of the sample component and to facilitate the sample transfer onto the cartridge. The sorbent/sample ratios typically ranged from 4:1 to 1:1 using traditional SPE sorbents in the previously reported works.[35] In our study, different sorbent/sample ratios were evaluated (1:2, 2:3, 1:1 and 3:2) using 100 mg of the sample (Fig. 8a). The results showed that a sorbent/sample ratio of 1:1 was sufficient for complete retention of camptothecin, with extraction yield of 0.1057%. Further increasing the proportion of sorbents gave no improvement for the extraction yield. However, less MIP sorbent resulted in heterogeneous sample mixture and low extraction yield. Therefore, the sorbent/sample ratio of 1:1 was finally selected for further investigations.

In the dispersion procedure, the sample needs to be completely dispersed in the sorbent.[36] However, the homogenization process of the sample with the sorbent is laborious. The dispersion time of 2, 5, 10, and 15 min were evaluated (Fig. 8b). Five minutes were used in this study, because the satisfactory extraction yield was obtained with saving time.

For the washing step, different concentrations of methanol aqueous solution (2%, 5%, 10% and 20%) as washing solutions were compared and the results showed that the best extraction yield of camptothecin obtained using 10% methanol. For the purpose of efficiently rinsing of interferences with the minimum volume of washing solution, different volumes of 10% methanol ranged from

1.0 to 10.0 mL were investigated and 5.0 mL was found to be the optimum washing volume.

The kind of elution solvent was important since the target analyte should be efficiently desorbed. In this case, a variety of solvents including the mixtures of acetonitrile, methanol with acetic acid as elution solvents were evaluated and the results were shown in Fig. 8c. The lower extraction yield of camptothecin was obtained using organic solvents without acetic acid. The most satisfactory result for the same compound was achieved when acetic acid–methanol (5:95, v/v) was used. The optimum volume of 5% acetic acid–methanol was evaluated using different volumes (1.0-6.0 mL) and the result revealed that the yield of camptothecin increased with the increase of elution volume from 1.0 to 4.0 mL and then retained constant even with the further increase to 6.0 mL (Fig. 8d). Considering the elution efficiency and solvent consumption, 4.0 mL was selected as the optimum volume of elution solvent.

3.6 Analytical performance

A calibration curve for camptothecin was obtained by plotting the peak area versus the theoretical concentration of camptothecin. Linearity was obtained in the concentration range of 1–200 μg mL^{-1}, and the regression equation and correlation coefficient were as follows:

$$A = 6.21 \times 10^4 C + 3.48 \times 10^2,\ R^2 = 0.9995$$

The result showed that a good correlation exists between the HPLC peak area (A) and the concentration (C) of camptothecin.

The sensitivity of the method was described by the limit of detection (LOD). The LOD defined as three times the ratio of signal to noise was 0.13 μg mL^{-1}.

The precision of the proposed method was studied from six replicated experiments for *Camptotheca acuminate* fruit samples. The average extraction yield of camptothecin was 0.1055%, with a relative standard deviation (RSD) of 5.4% (n=6). The low RSD also indicated that the MIP has good reproducibility. Different batches of synthetic MIPs were used in MSPD procedure for analyzing the same sample.

3.7 Application of the method and comparison to different methods

In order to demonstrate the applicability, the proposed method was used for the determination of camptothecin in *Camptotheca acuminate* fruit, bark and leaf. Furthermore, other methods including stirring extraction, ultrasonic extraction, Soxhlet extraction and CNT-MSPD were also used to extract camptothecin. The content of camptothecin is different in different *Camptotheca acuminate* parts (Table 2). The extraction yields of camptothecin from Camptotheca acuminate fruit (0.1055%), bark (0.0628%) and leaf (0.0930%) achieved by the proposed method were all higher than those by other methods. However, the difference among the MIP-MSPD, CNT-MSPD and Soxhlet extraction is not obvious. Comparing to conventional techniques, the proposed method reduces the extraction time and simplifies the extraction process. Because the MIP has better selectivity, the use of MIP resulted in less matrix interferences (Fig. 9). Moreover, the recovery of camptothecin from *Camptotheca acuminate* fruit, bark and leaf was studied by analyzing spiked samples with the proposed method. The recovery obtained was in the range of 96.1–103.3%.

4. Conclusions

In this work, a novel camptothecin MIP was prepared using CNTs as the support matrix via surface imprinting technique. The pseudo-second-order kinetic model was more accurate to describe the adsorption behavior of camptothecin onto MIPs. In Scatchard analysis there are two kinds of binding sites for this material. The MIP was used as the sorbent of MSPD for extraction of camptothecin from *Camptotheca acuminate*. The developed method combines the high affinity and selectivity of MIP technology with the simple, rapid and efficient sample pretreatment of MSPD to achieve significant time reduction of the total analytical process. In addition to the analytical advantages, the method has other practical improvements over conventional methods of sample treatment, such as lower cost and simple instrumentation involved.

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Figure captions:

Fig. 1. The chemical structures of camptothecine, 7-ethyl-10-hydroxycamptothecin and bergenin.

Fig. 2. The protocol for synthesis of the MIPs.

Fig. 3. The SEM image of MIP (a), and TEM image of CNT (b) and MIP (c).

Fig. 4 The FTIR spectrum of MIPs.

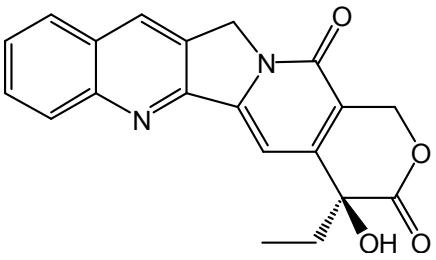
Fig.5 Binding isotherms (a) and Scatchard plot analysis of the binding of camptothecine onto the MIP (b) and NIP (c).

Fig. 6. Kinetics adsorption (a), pseudo-first-order kinetic for adsorption of MIP and NIP (b), pseudo-second-order kinetic for adsorption of MIP and NIP (c).

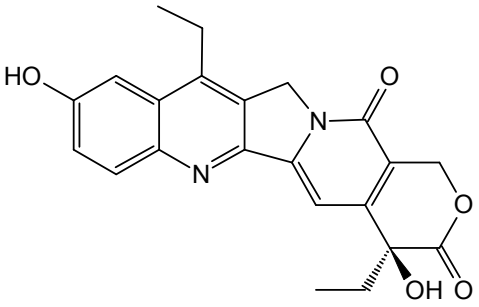
Fig.7. The specificity adsorption of the MIP and NIP.

Fig. 8. The effect of MIP/sample ratio, dispersion time, the type of elution solution and volume of elution solution on the extraction yield of camptothecine.

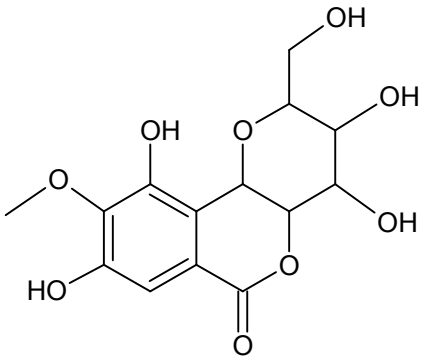
Fig.9. The chromatograms of camptothecin in *Camptotheca acuminate* fruit extracted with MIP-MSPD (a), Soxhlet extraction (b) and CNT-MSPD (c).



Camptothecin



7-ethyl-10-hydroxycamptothecin



Bergenin

Fig. 1

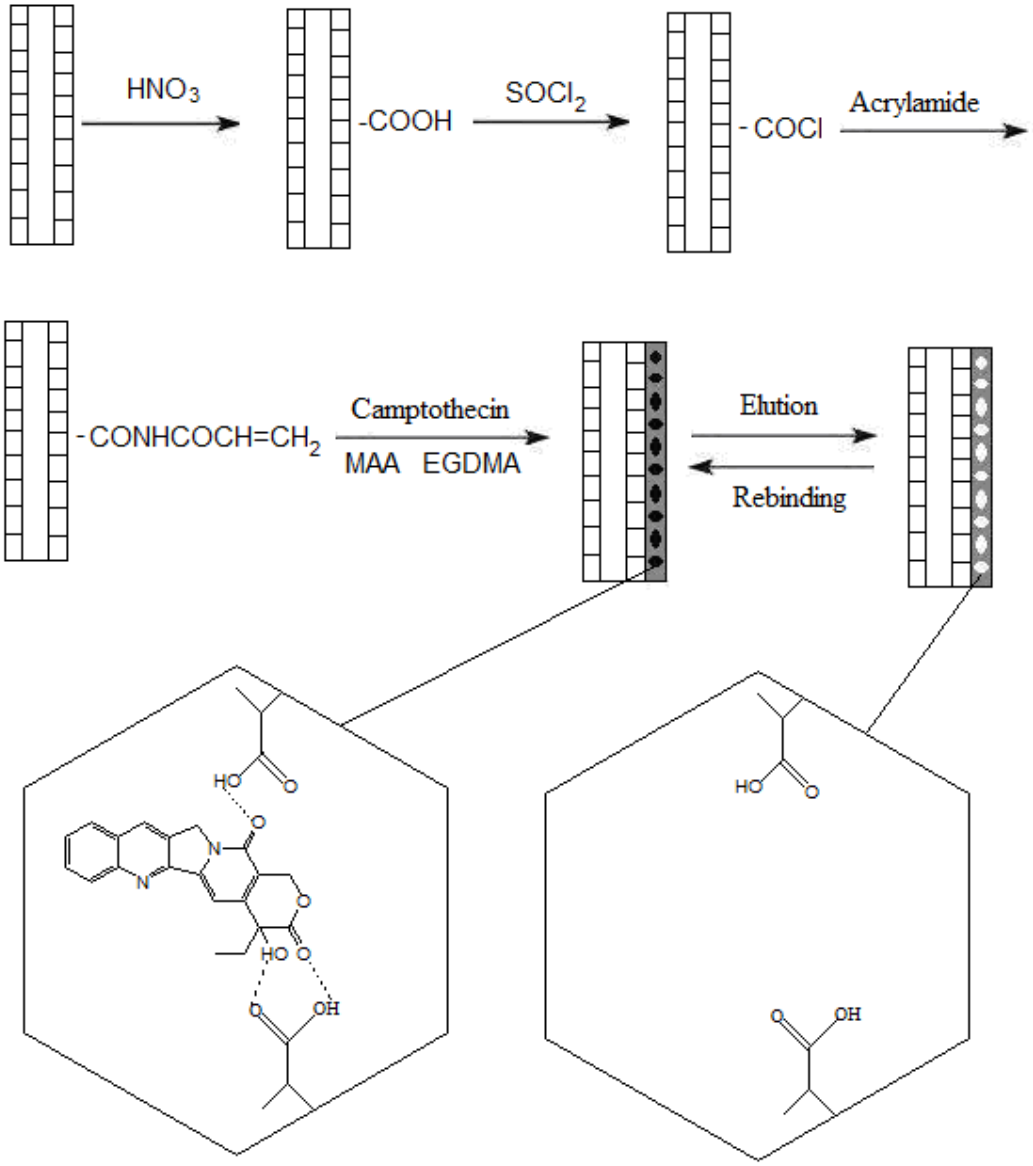
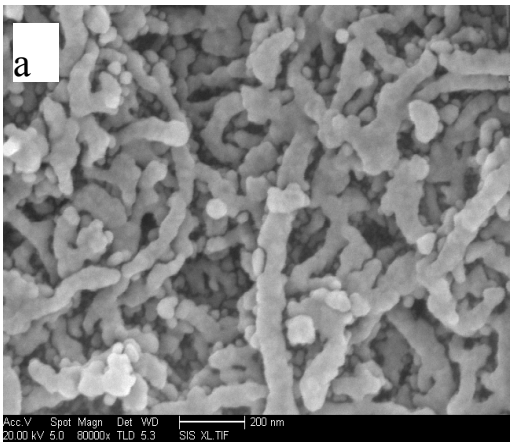
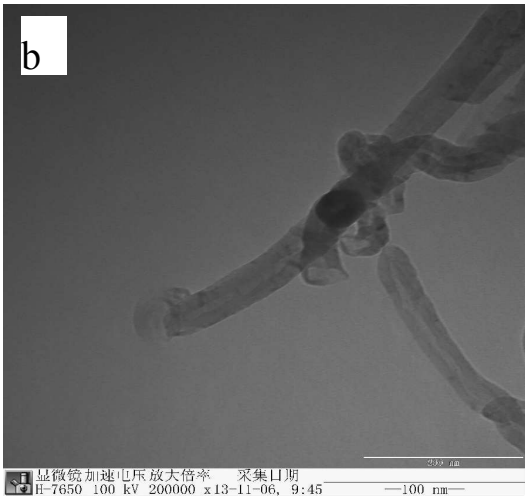


Fig.2





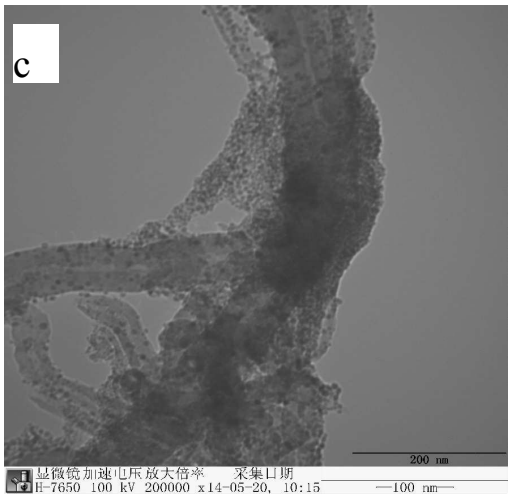


Fig. 3

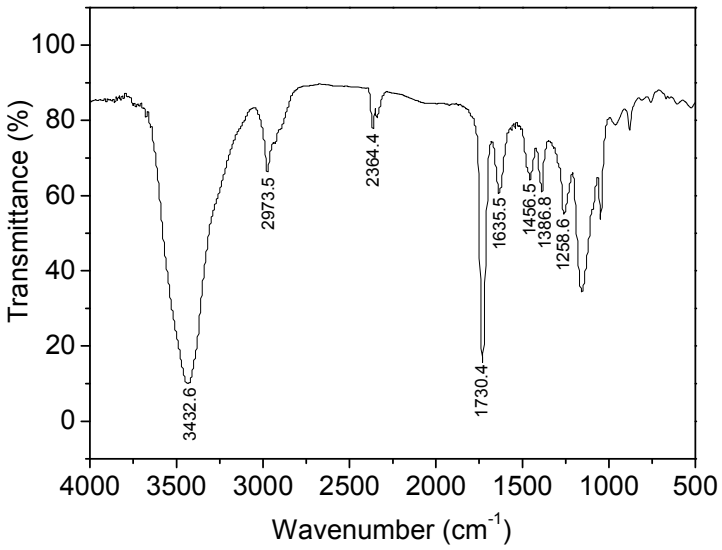


Fig. 4

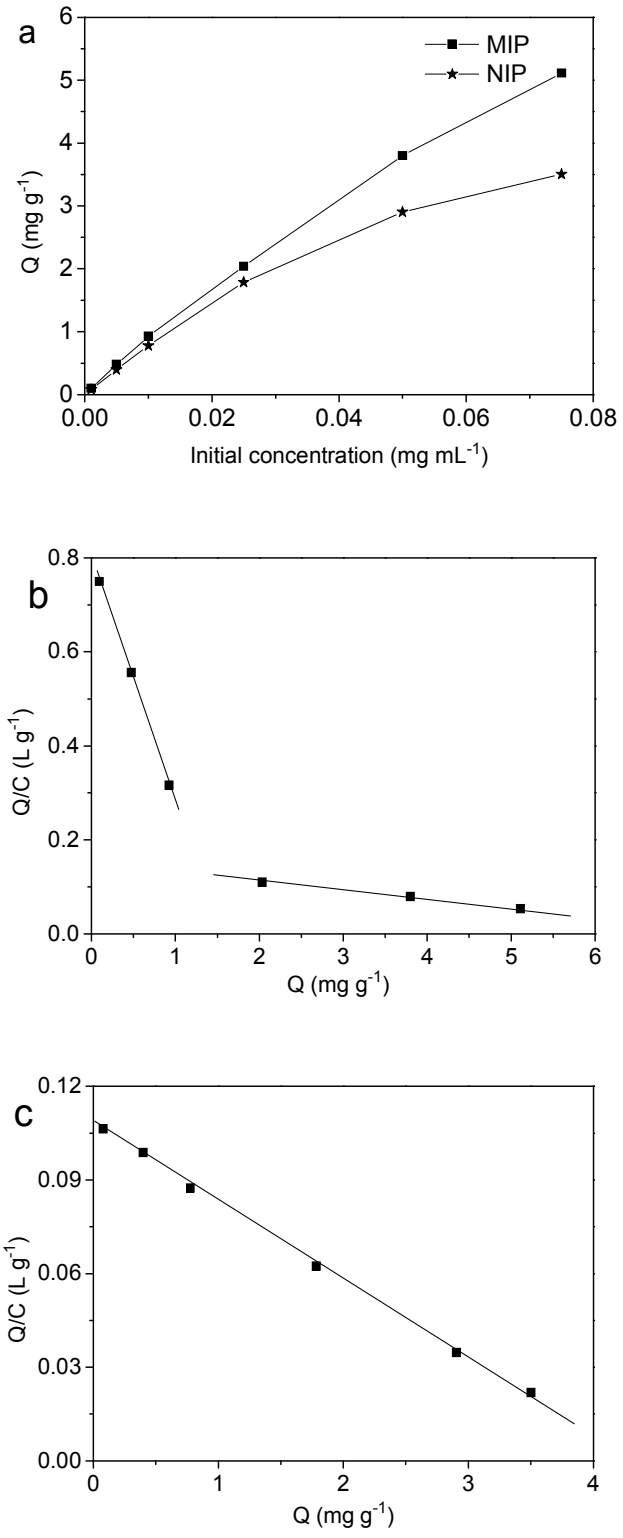


Fig. 5

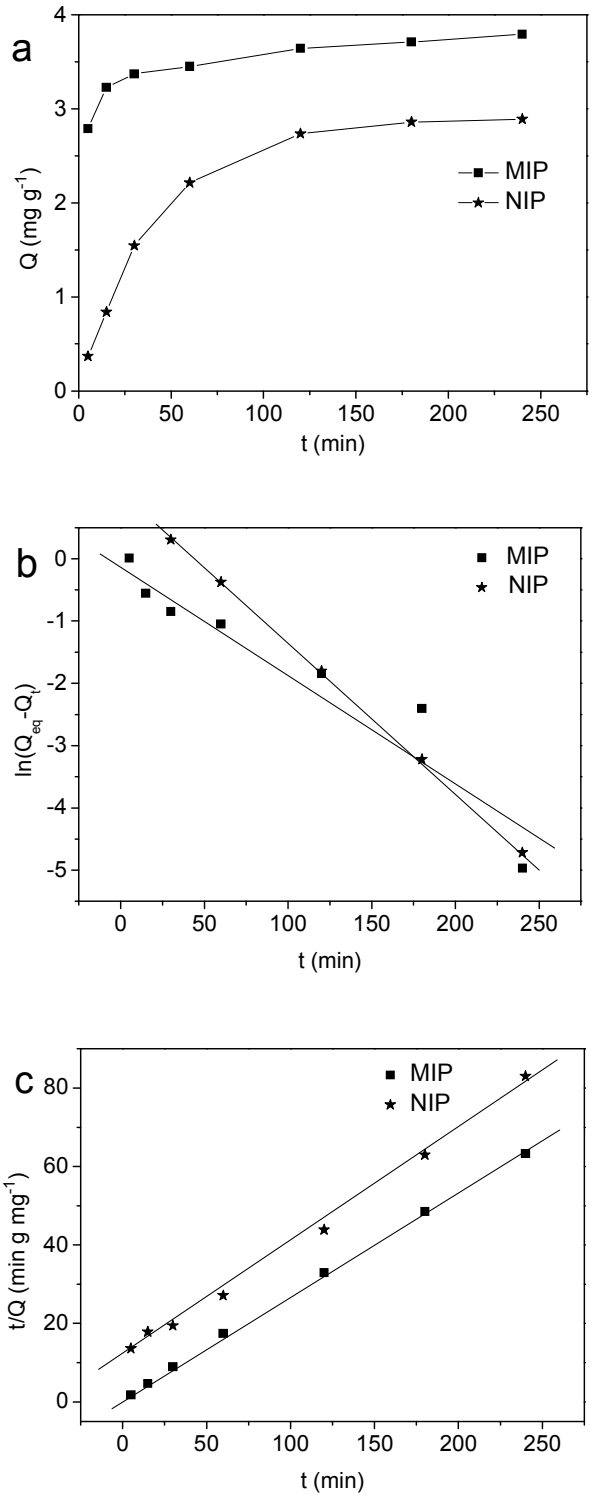


Fig. 6

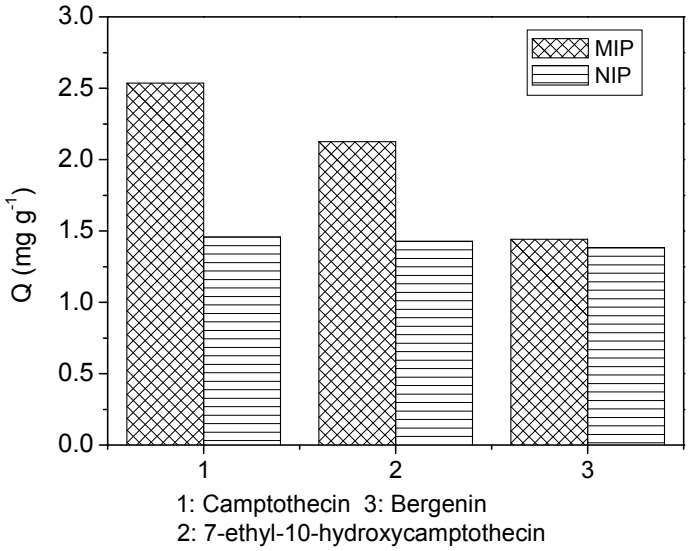


Fig. 7

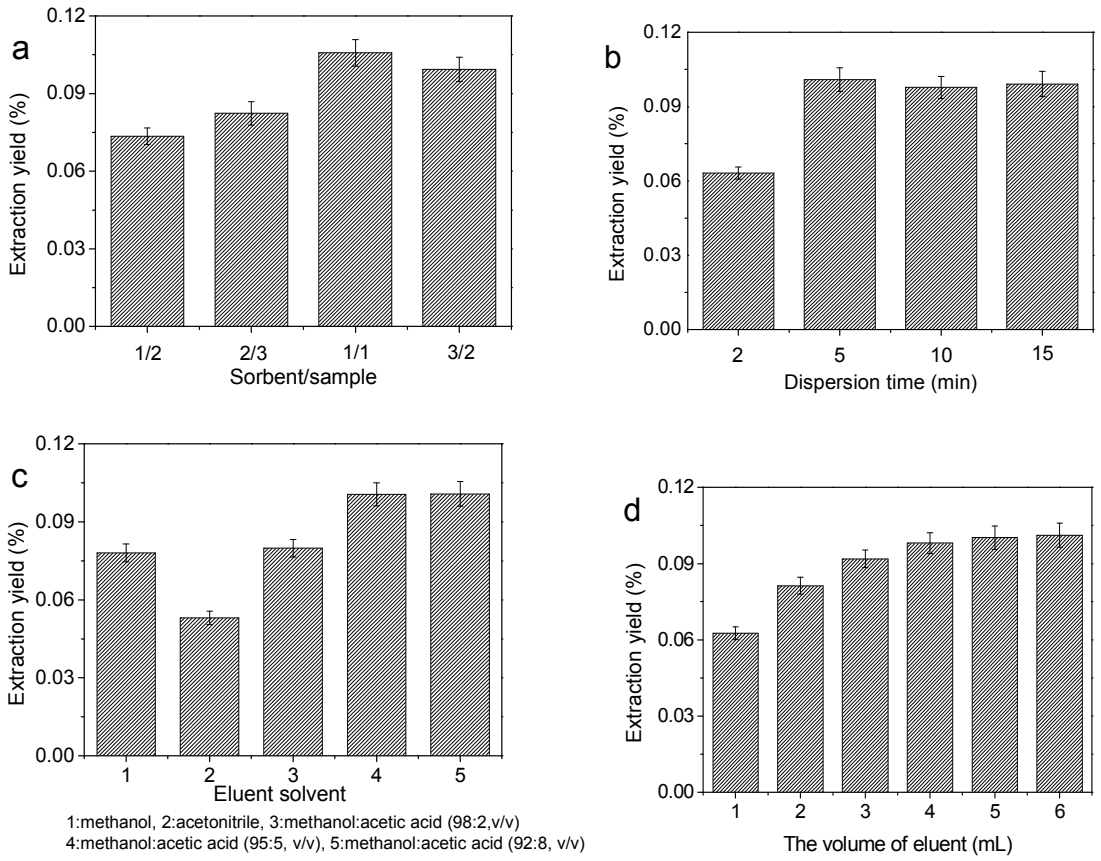


Fig. 8

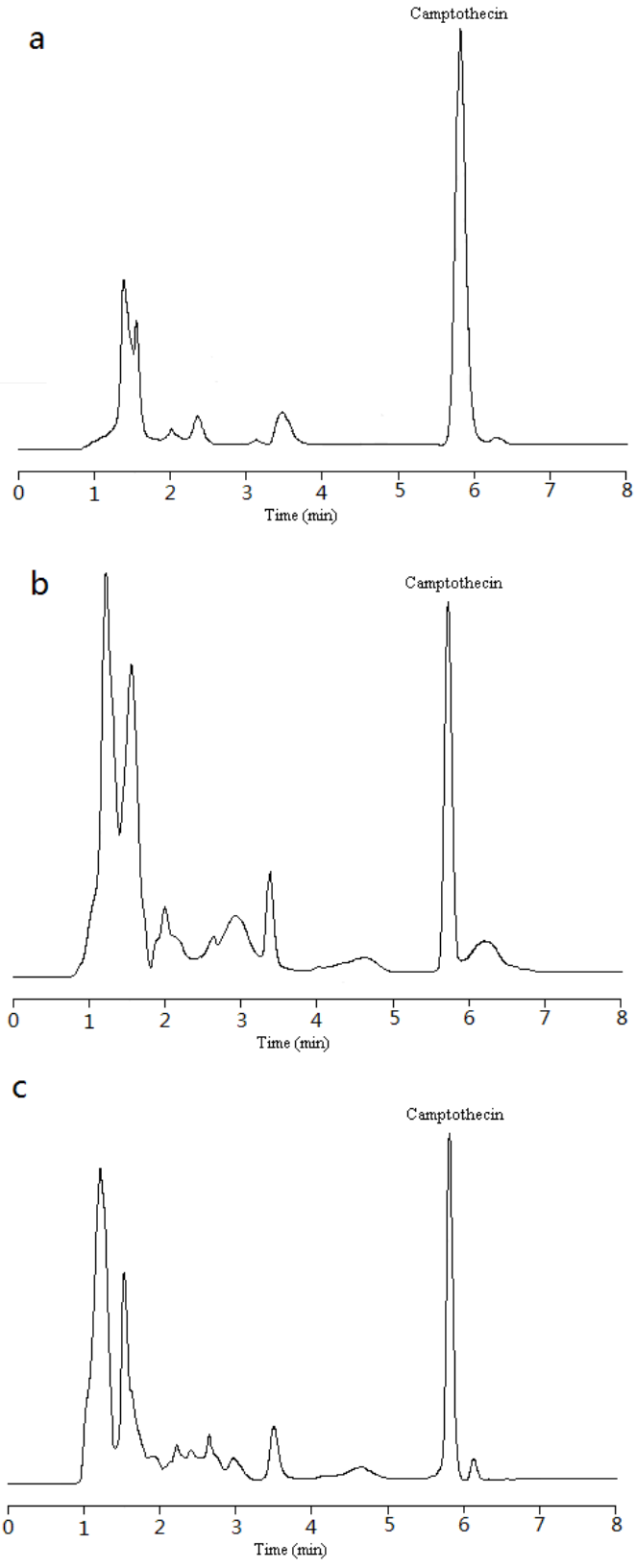


Fig. 9

Table 1. First-order and second-order kinetic constants for MIP and NIP

Adsorption material	Q_{eq} (mg g^{-1})	Pseudo-first-order			Pseudo-second-order		
		K_1 (min^{-1})	Q_e (mg g^{-1})	R^2	K_2 (g mg^{-1} min^{-1})	Q_e (mg g^{-1})	R^2
MIP	3.8	0.018	0.97	0.916	0.067	3.81	0.999
NIP	2.9	0.024	2.88	0.999	0.008	3.42	0.996

Table 2 Comparison of different methods used for the extraction of camptothecin (n=5)

Extraction method	*Camptotheca acuminate* fruit		*Camptotheca acuminate* bark		*Camptotheca acuminate* leaf	
	Extraction yield (%)	RSD (%)	Extraction yield (%)	RSD (%)	Extraction yield (%)	RSD (%)
Stirring extraction	0.0629	5.9	0.0429	6.7	0.0599	4.7
Ultrasonic extraction	0.0752	6.2	0.0431	5.0	0.0624	6.1
Soxhlet extraction	0.0912	4.1	0.0534	5.6	0.0803	3.5
MIP-MSPD	0.1055	4.7	0.0628	4.8	0.0930	5.5
CNT-MSPD	0.0869	5.5	0.0527	6.2	0.0774	4.0