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Research Article

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Preparation and adsorption properties of mixed-templates molecularly imprinted polymers of epothilone B

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ABSTRACT

Epothilone B is a highly promising prospective anticancer agent. At present, the cost of producing epothilone B is very high mainly due to two reasons. The first the toxicity and feedback inhibition of epothilones lead to difficult to improve fermentation yield. The second is the fermentation broth contains a large number of epothilone homologues, leading to separation and purification costs of epothilone B are very high. Therefore, the development of epothilone B adsorption used in its fermentation and extraction will significantly reduce production costs. In the present study, Molecularly imprinted polymer(MIPs) of epothilone B was prepared by precipitation polymerization using three members of epothilone (epothilone A, epothilone B and epothilone D)as templates，methacrylic acid as functional monomer, and ethylene glycol dimethacrylate as cross-linker. MIPs can specifically recognise epothione B. By measuring the adsorbance of different mixed-templates and different ratio of templates/MAA, the best condition was determined. The results showed that the imprinting effect was best when the molar ratio of templates/MAA is 1:6 and mixed-templates are epo B and epo D (1:1), respectively. MIPs exhibited a high recognizable capacity to Epothilone B. Therefore, that was expected to be used as extraction and purification of Epothilone B in the process of fermentation.

Keywords: Epothilone B; molecularly imprinted polymer; mixed-templates; adsorption

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INTRODUCTION

The epothilone are naturally occurring 16-membered ring macrolides with a variety of biological activity, which are the second-metabolics of Sorangium cellulosum and are a vital anticancer agent [1-2]. Its components could be divided into Epothilone, A, B, C, D, E, F etc [3-4]. Their chemical structures are given in Fig. 1[5]. Also, epothilone B has the best effect. Therefore, the development of novel molecularly imprinted polymers (MIPs) as sorbents for selective separation and purification of epothilone B is of great importance. It has got a great of attention in the world recently.

Molecular imprinted technique is such a kind of technique that the polymer can match well with templates in the spatial structure and binding sites [6]. Molecularly imprinted polymers are synthetic polymers with a predetermined selectivity for a given analyte, or a group of structurally related compounds [7]. The process of polymerization happened in a certain solvent which target detection, monomer and cross-linker were added in. Target detection objects are used as templates, which combine with the monomer by covalent or non-covalent way. Finally the template molecules cleared off. The resulting polymers have well-defined cavities, which are complementary in size, geometry and functional groups orientation to the templates molecule [8-9]. These cavities show an expected

selectivity for templates and a high degree of identification of templates. Therefore, MIPs have extensive applications as separation media for chromatography.

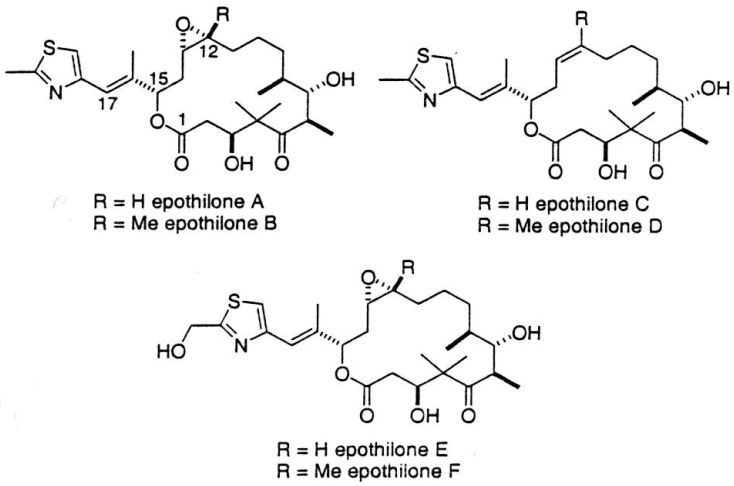

Figure 1. The structure of Epothilone

However, only a single template such as Epothilone B , Epothilone D, Epothilone A is used in most of the preparation of molecular imprinted polymer (MIPs), which synthetizes the polymers with a low affinity. In actual test, there is often not a single analyte, so MIPs synthetized with a single template have a low adsorption and selectivity for the compounds from the same family. MIPs prepared by mixed-templates can shorten the testing time, reduce the testing cost, so its application is expected to be better [10].

EXPERIMENTAL SECTION

Instruments and equipment

UV-Vis spectrophotometer, Constant temperature bath oscillator, Ultrasonic oscillator, Vacuum oven, Scanning electron microscope (SEM).

Preparation of mixed-templates MIPs

All of the polymers were prepared by precipitation polymerization. 0.1mmol templates, 0.6mmol MAA, 10mmol EGDMA were dissolved in a 40ml chloroform solution, put in room temperature for 1 h, then added 20mg AIBN. Ultrasonic vibrate 5-10minutes. Then deoxygenate with oxygen-free nitrogen for 5-10minutes, seal quickly under the protection of nitrogen. Polymerization took place at 50℃ for 24h in a bath oscillator with constant temperature [11]. After polymerization, the templates were removed by Soxhlet apparatus with 100ml acetic acid/methanol (1/9, v/v) for several times until the templates could not be detected. Polymers were washed with 100ml methanol to remove acetic acid. Then they were dried at 50℃ in the vacuum drying oven to reach a constant weight. Non-imprinted polymers (NIPs) were prepared under the same conditions except that there was no template during polymerization.

Determination of the amount of template molecules and monomers

Determination of the hybrid program for template molecule

Use Epo B, Epo D and Epo A as templates to synthesize MIPs When the ratio of templates/MAA was 1:6. Different Mixed-template was made. By measuring the adsorbance, the best condition was determined.

UV Spectroscopy Analysis

Choose MAA as monomer, Epo D and EpoB (1:1) as templates. The molar ratio of templates/MAA were 1:2,1:4,1:6,1:8,1:10 and 1:12. Reaction took place at 30℃ for 15h in a bath oscillator. With the corresponding concentration of monomer solution as the reference solution, detect the absorption of different molar of

MAA/templates in the 200nm ~ 450nm.

Determination of the amount of functional monomers
With the different molar ratios of templates/MAA, MIPs and NIPs were synthesized. By measuring the adsorptive quantity of the MIPs and NIPs for templates, the amount of MAA was determined.

Study on the adsorptive ability of imprinted polymers
Prepare a series of different concentrations of Epo solution, and measured the UV absorbance. Then draw the standard curve (the absorbance as ordinate, the concentration as the abscissa). Weigh polymer 30mg and put in the 10ml centrifuge tube, add different concentrations of Epo solution 8ml,then place in constant temperature bath oscillator at 30℃ for 15 h. Filter the supernatant fluid with 0.45μm filterable membrane. Determine the absorbance of each solution by UV-Vis spectrophotometer. Calculate the equilibrium concentration of each solution after adsorption according to the standard curve, and calculate the equilibrium adsorption of mixed-template polymer according to the change of the Epo concentration, with NIPs for comparison [12].

Morphology characterization of polymer
Observe the appearance and the particle size of the polymer by scanning electron microscope [13].

RESULTS AND DISCUSSION

Determination of the hybrid program for template molecule
Use Epo B, Epo D and Epo A as templates to synthesize MIPs When the ratio of templates/MAA was 1:6. By measuring the adsorbance (results in Table 1), the best condition was determined. Imprinting factor IF was defined, IF=QMIP/QNIP. The higher the value of IF, the better of the molecular imprinting is. QMIP is the adsorbance of the imprinted polymer, QNIP is the adsorbance of the non-imprinted polymer.

It showed that IF was the maximum when using Epo D/Epo B (1:1) as the templates, and the imprinting effect was best.

Table 1 the effect of ratio of template molecule and adsorption properties of polymers

Polymers	Epo / MAA	Q_{MIP} [mg/g]	Q_{NIP} [mg/g]	IF
MIPs(Epo B) to Epo B [1]	1:6	15.274	10.515	1.453
MIPs(Epo A)	1:6	16.625	11.821	1.406
MIPs(Epo D)	1:6	17.023	12.025	1.415
MIPs(Epo B/Epo A)	1:6	18.743	12.420	1.509
MIPs(Epo B/Epo D)	1:6	23.107	14.120	1.636
MIPs(Epo A/Epo D)	1:6	19.225	12.587	1.527
MIPs(Epo B/Epo A/Epo D)	1:6	17.426	11.603	1.502
MIPs(Epo B) to Epo D [2]	1:6	14.876	10.537	1.412
MIPs(Epo A)	1:6	16.002	11.521	1.389
MIPs(Epo D)	1:6	16.924	11.910	1.421
MIPs(Epo B/Epo A)	1:6	18.345	12.613	1.455
MIPs(Epo B/Epo D)	1:6	22.534	14.524	1.552
MIPs(Epo A/Epo D)	1:6	18.883	12.745	1.482
MIPs(Epo B/Epo A/Epo D)	1:6	17.014	11.756	1.447
MIPs(Epo B) to Epo A [3]	1:6	15.034	10.542	1.426
MIPs(Epo A)	1:6	16.725	12.126	1.379
MIPs(Epo D)	1:6	16.027	10.923	1.467
MIPs(Epo B/Epo A)	1:6	18.794	12.445	1.510
MIPs(Epo B/Epo D)	1:6	22.406	14.528	1.542
MIPs(Epo A/Epo D)	1:6	18.129	12.407	1.461
MIPs(Epo B/Epo A/Epo D)	1:6	17.126	11.875	1.442

[1] is the adsorption of MIPs to Epo B, the followings as the same
[2] is the adsorption of MIPs to Epo D, the followings as the same
[3] is the adsorption of MIPs to Epo A, the followings as the same

Preparation of MIPs

UV scanning spectra of Epothilone B

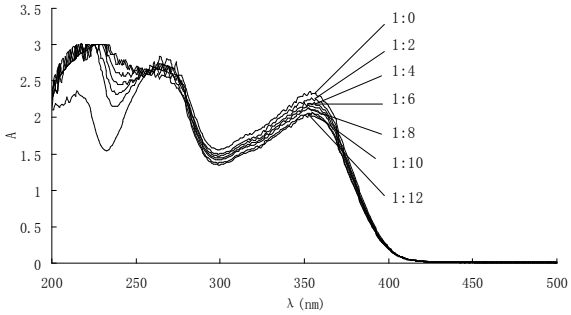

Figure 2. UV scanning spectrum of Epo B

It was shown in the Figure 2, that spectral occurred red-shift with the increasing of MAA concentration, indicating that the template and monomer MAA took effect.

The effect of the amount of functional monomer on the adsorption property of polymers

When the amount of cross-linker and templates was constant, the ratio of templates/MAA was changed as follows, examine the adsorption of polymers to Epo by combining tests. The result is in Table 2.

It showed that IF was the maximum when the molar ratio of templates/MAA is 1:6, and the imprinting effect was best.

Table 2 the effect of adsorption properties of monomer on polymers

Polymers	Epo / MAA	Q_{MIP} [mg/g]	Q_{NIP} [mg/g]	IF
MIPs(Epo A/Epo D) to Epo B [1]	1:2	19.212	13.186	1.457
MIPs(Epo A/Epo D)	1:4	21.425	14.236	1.505
MIPs(Epo A/Epo D)	1:6	23.826	14.897	1.600
MIPs(Epo A/Epo D)	1:8	16.107	11.802	1.365
MIPs(EpoA/EpoD)	1:10	14.526	10.872	1.336
MIPs(Epo A/Epo D)	1:12	13.175	9.960	1.323
MIPs(Epo A/Epo D) to Epo D [2]	1:2	16.780	11.423	1.468
MIPs(EpoA/EpoD)	1:4	19.314	12.758	1.514
MIPs(EpoA/EpoD)	1: 6	21.551	14.108	1.528
MIPs(Epo A/Epo D)	1:8	14.978	11.114	1.348
MIPs(EpoA/Epo D)	1:10	13.564	11.036	1.229
MIPs(Epo A/Epo D)	1:12	12.988	10.796	1.203
MIPs(Epo A /Epo D) to Epo A [3]	1:2	19.107	13.861	1.379
MIPs(Epo A/Epo D)	1:4	20.124	14.006	1.437
MIPs(Epo A/Epo D)	1:6	22.760	14.986	1.519
MIPs(Epo A/Epo D)	1:8	16.415	11.527	1.424
MIPs(Epo A/Epo D)	1:10	14.156	10.872	1.302
MIPs(Epo A/Epo D)	1:12	13.854	11.317	1.224

[1] is the adsorption of MIPs to Epo B, the followings as the same
[2] is the adsorption of MIPs to Epo D, the followings as the same
[3] is the adsorption of MIPs to Epo A, the followings as the same

Study of adsorption properties of polymers on template molecules

The isothermal adsorption curve of MIPs (Epo B/Epo D) on different concentrations of Epo B was determined by static adsorption. The results showed in Figure 3. It could be seen from the figure that the adsorption capacity of imprinted polymer on Epo B was increasing with the initial concentration of Epo B increasing. When the initial concentration of Epo B was identical, the adsorption capacity of MIPs was significantly higher than NIPs, which showed that MIPs (Epo B/Epo D) had specific recognition ability with Epo B, and they could adsorb each other by hydrogen bonds or spatial matching. But for NIPs, its interior might not have the selective binding sites. It mainly depended on the non-specific adsorption of the polymer surface, and could not recognize the templates effectively.

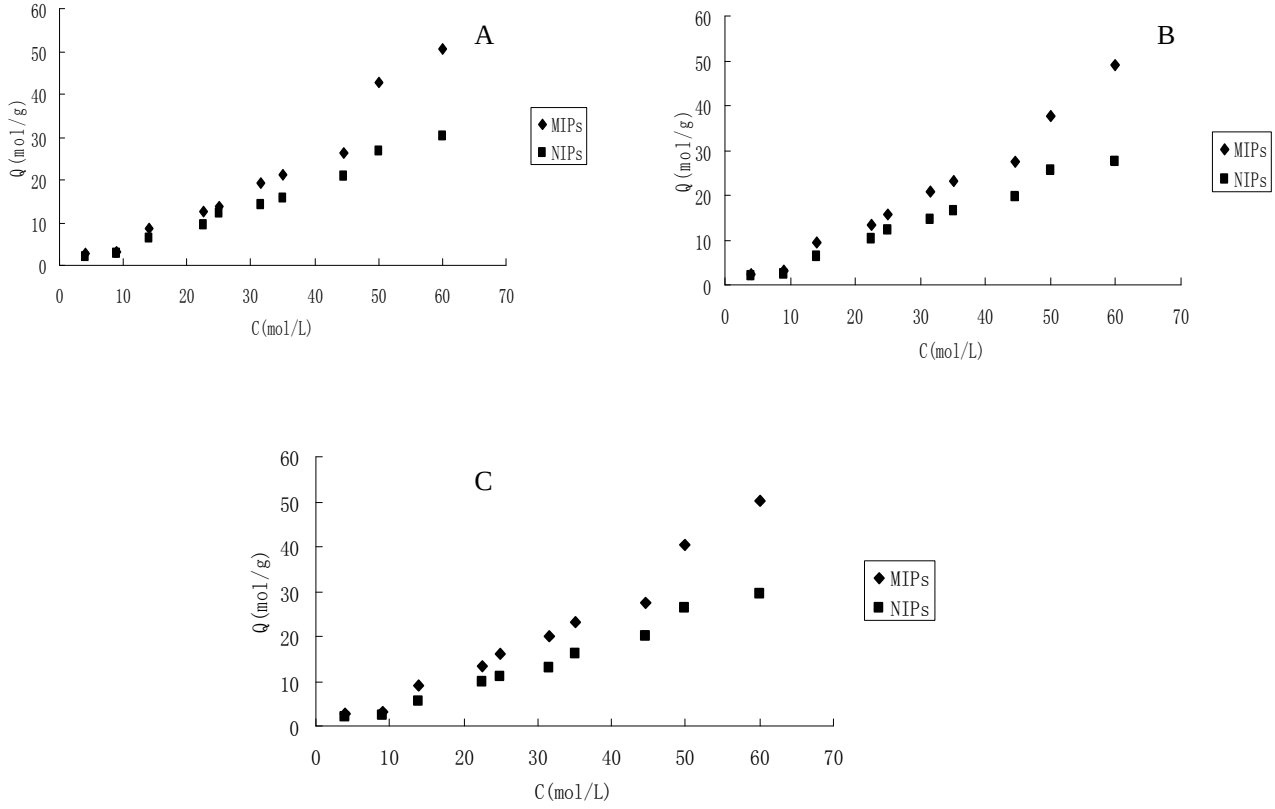

Figure 3. The adsorption isotherms of MIPs and NIPs （A: The adsorption isotherms of MIPs and NIPs to Epo B, B: The adsorption isotherms of MIPs and NIPs to Epo D, C: The adsorption isotherms of MIPs and NIPs to Epo A）

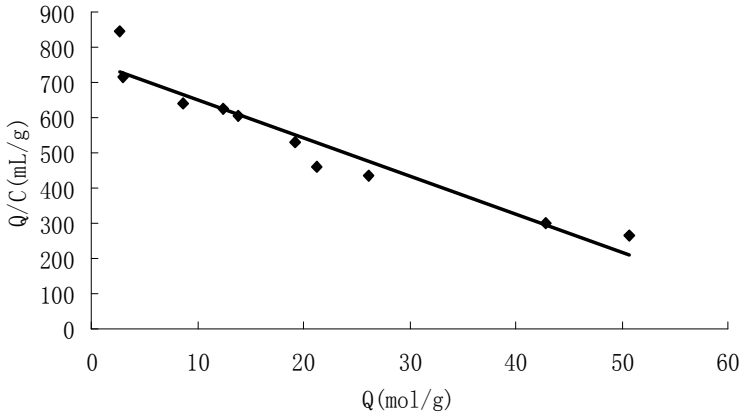

Figure 4. The Scatchard equation analysis of MIPs

The Scatchard model enables to assess the binding site heterogeneity of a solid material, such as MIPs. The sites can be classified in several families, depending on their binding energy beside the template. Each family is characterized by its equilibrium dissociation constant (K_d), as well as by its maximum site number (Q_{max}).

The Scatchard equation reports two variables, Q and C, measured at the equilibrium. C is the concentration of free Epo B in the solution and Q, the number of occupied sites. The quantity of Epo B bound to the material is equal to

the number of occupied sites and was calculated by the subtraction of the concentration of free Epo B (C) to the initial concentration of Epo B.

Formula was Q /C=(Q_{max}-Q) / K_d. Mapping with Q / C as ordinate, Q as the abscissa. As illustrated in Figure 4, the combination of imprinted polymer with the template molecule mainly belonged to type 1 binding sites. Regression equation was Q/C = 726.82-9.997Q, K_d (equilibrium dissociation constant) was 0.096mmol/L, Q_{max} (maximum apparent adsorption) was 51.347mol/g.

Characterization of MIPs

The morphology of the obtained MIPs was observed on SEM as shown in Figure 5. Depending on different volume of chloroform solution, the imprinted polymer particles had drastically different size and shape. When the chloroform solution was 40 ml, the shape of MIPs was similar to megalith.

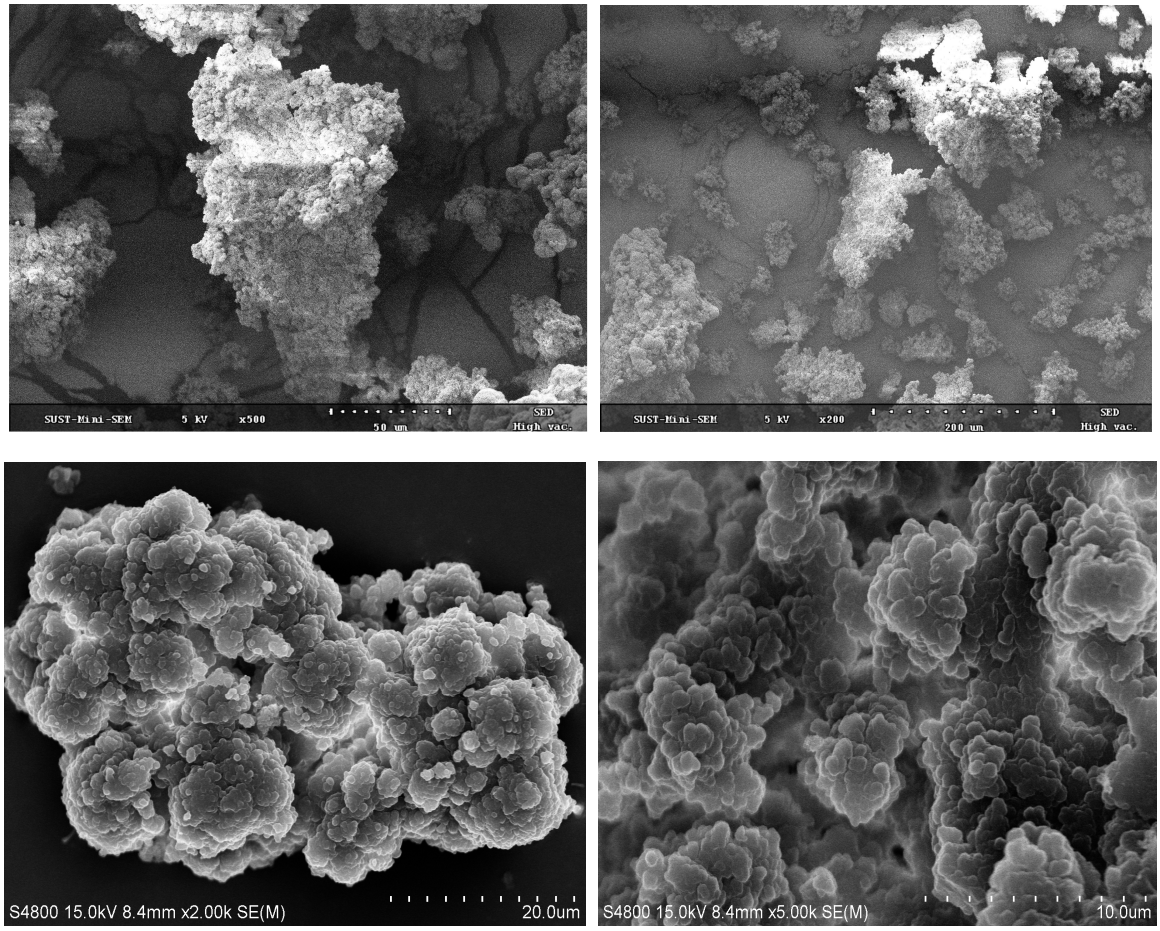

Figure 5. Mixed-template MIPs SEM

In addition, the shape of MIPs was irregular, which might result from low dissolubility of templates in porogenic solvent. It is Interesting that the shape of the mixed-templates MIPs displays dual characters by combining the sphericity and porosity. It was shown that the volume of solvent appeared to control the size and shape of polymers to small. Furthermore, it was shown in the Figure 5 that the cavity size of MIPs obtained by precipitation polymerization was 0.10μm~0.30μm. The mixed-templates MIPs had narrower cavity size distribution than other MIPs, which warrant their commercial use to purify and concentrate Epo B in practical samples.

CONCLUSION

The present study has demonstrated the possibility of preparing a novel MIPs using epothilone A, Epothilone B and Epothilone D as the mixed-templates for recognizing a group of Epo. And the imprinting effect was best when the molar ratio of templates/MAA is 1:6 and mixed-templates are epo B and epo D (1:1), respectively. The adsorption property of polymers was studied through static adsorption, and Scatchard analysis was carried out at the same time. The results indicated that mixed-templates MIPs exhibited a high recognizable capacity to Epo group, the recognition ability of mixed-templates MIPs for Epo B was explained by the chemical structures of templates and the imprinting effect. In a word, the results suggested that mixed templates MIPs showed better selectivity for Epo B than those of single-template MIPs. Therefore, mixed-templates MIPs provided favorable condition for the separation and purification of Epothilone B. And it was expected to be used as a kind of newer separation media of Epothilone B in the process of fermentation production.

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