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Preparation and evaluation of paclitaxel-imprinted polymers with a rosin-based crosslinker as the stationary phase in high-performance liquid chromatography

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ABSTRACT

In this study, molecularly imprinted polymer (MIP) microspheres for paclitaxel (PTX) were prepared by microsuspension polymerization and used as a stationary phase in high-performance liquid chromatography (HPLC) for the separation of PTX and its structural analog docetaxel (DOC). For MIP synthesis, ethylene glycol maleic rosinate acrylate and 2-vinylpyridine were used as the cross-linker and functional monomer, respectively. MIP microspheres were characterized by scanning electron microscopy, laser granulometry, nitrogen sorption porosimetry, and thermogravimetric analysis. Results indicated the formation of regular MIPs with an even pore size distribution; furthermore, these MIPs exhibited excellent thermal stability. These MIP microspheres were packed into a stainless steel column for the selective separation of PTX and DOC. Under optimum chromatographic conditions, a separation factor of 2.54 and an imprinting factor of 2.37 were obtained. In addition, thermodynamic data obtained from van't Hoff plots revealed enthalpy-driven separation and higher contribution from functional group interactions as compared with that from steric complementary interactions. Microcalorimetry was employed to investigate the binding mechanisms of the analytes on the MIP surface.

1. Introduction

Molecularly imprinted polymers (MIPs) are typically prepared using a template molecule and functional monomers with complementary structures and are subsequently crosslinked with each other. The functional monomers, which are self-assembled around the template molecule by interactions between functional groups on both template and monomers, are polymerized to form an imprinted solid matrix. So it is important to select a suitable functional monomer, along with the development of molecular imprinting technology, various kinds of methods for rapid screening of functional monomer, such as spectral method [1], chromatography [2], combinatorial methods [3] and molecular modeling [4,5]. Thus far, MIPs have been extensively used in various fields, such as solid-phase extraction [6–8], electrochemical sensing [9], controlled drug release technology [10], and biological cell separation [11,12]. Numerous studies have reported the use of MIPs as a stationary phase for HPLC, where molecularly imprinted stationary phases are mainly prepared by bulk polymerization, precipitation polymerization, seed swelling, and silica-gel surface modification [13–16]. Particularly, bulk polymerization is the most basic method; however, the polymer particles thus obtained are characterized by irregular shapes and forms [17]. As a result, when these MIPs are used as stationary phases, the particles fail to exhibit excellent column performance. On the other hand, precipitation polymerization requires a large amount of toxic organic solvents, which can easily cause environmental pollution. Seed swelling polymerization involves complicated steps and requires a long reaction time. Silica-gel surface modification can only be used to form two-dimensional recognition sites on the silica gel surface, resulting in insufficient dimensionality. Microsuspension polymerization (MSP), also known as mini-emulsion polymerization, is simple and cost-effective for synthesizing MIPs. Typically, MSP utilizes free radicals and a special emulsification system formed by cetanol (CA), sodium dodecyl sulfate (SDS), and polyvinyl alcohol (PVA), with water as the dispersed phase. The particle sizes of the

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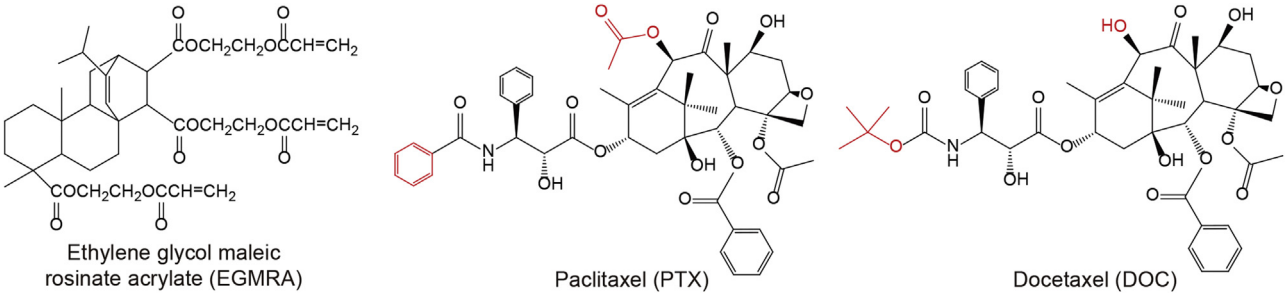

Fig. 1. Structures of ethylene glycol maleic rosinate acrylate, paclitaxel, and docetaxel.

macromolecular microspheres can be controlled to range between 0.1 and 20 μm, making these microspheres ideal for use as stationary phases in chromatography.

In HPLC, the stationary phase must exhibit high mechanical strength for maintaining excellent linearity between the flow rate and back pressure. Currently, ternary cross-linkers, such as trimethylolpropane trimethacrylate (TRIM) and ethylene glycol dimethacrylate (EGDMA), are used for synthesizing molecularly imprinted stationary phases, affording highly crosslinked polymers with a low degree of swelling; however, as these polymers do not possess parent nuclei in their structures, particle collapse at high pressure occurs when these polymers are used as stationary phases in HPLC. Thus, these polymers are mainly used to prepare monolithic columns. Moreover, although divinylbenzene is an aromatic compound with excellent rigidity, when it is used for the separation and analysis of drugs, the residuals may pose potential damage to the human body. On the other hand, ethylene glycol maleic rosinate acrylate (EGMRA), a new cross-linker synthesized herein, exhibits a tricyclic phenanthrene skeleton with excellent structural rigidity and can maintain a steady imprinting recognition space when it is used as the stationary phase for HPLC [18,19]. Meanwhile, EGMRA helps to increase the degree of crosslinking during polymerization. Moreover, the raw material used for synthesizing this cross-linker, rosin, is a non-toxic, environment-friendly natural product collected from pine [20].

Paclitaxel (PTX), a diterpene compound with an extremely unique structure extracted from yew, exhibits excellent anticancer effects against ovarian and breast cancer; in addition, it exhibits specific efficacy against lung and colon cancer, as well as melanoma. Thus, PTX is in high demand. Nevertheless, yew only contains a marginal amount of PTX; in addition, yew contains structural analogs and other impurities with complex compositions, leading to high costs associated with extraction and separation. Several studies have been reported on the molecular imprinting of PTX. For example, Zhu et al. [21] have extensively investigated the interactions between PTX and some common functional monomers, such as methacrylic acid (MAA), acrylamide (AM), 2-vinylpyridine (2-VP), and 4-vinylpyridine, in different solvents by ultraviolet spectrophotometry and found that the strongest interaction between PTX and 2-VP in chloroform is observed at a ratio of 1:6. Fatemeh et al. [22] have employed MSP using EGDMA for preparing MIPs for PTX with a high degree of crosslinking and found that the highest binding capacity for PTX is 48.4%; however, the particle sizes of the MIPs are mainly distributed around 100 nm. Hence, despite its excellent imprinting effect, the MIPs cannot be further used for separation and analysis.

In this study, MIP microspheres for PTX were synthesized by MSP with PTX, 2-VP, and EGMRA as the template, functional monomer, and cross-linker, respectively; these MIPs were further characterized by field-emission scanning electron microscopy (FE-SEM), laser granulometry, N_2 adsorption, and derivative thermogravimetric analysis (DTG). After loading the as-synthesized MIPs onto a chromatographic column, PTX and its structural analog docetaxel (DOC) were separated, and their thermodynamic behaviors were investigated. Microcalorimetry was employed to determine the adsorption heat of PTX adsorbed on MIPs and non-molecularly imprinted polymers (NIPs).

2. Materials and methods

2.1. Reagents

PTX (98%), DOC (98%), 2-VP, and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). EGDMA was supplied by Alfa Aesar (Qingdao, China). PVA (1755 ± 50), cetyl alcohol (CA), chloroform, methanol (MeOH, HPLC), ethanol, and glacial acetic acid were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). EGMRA was provided by the Guangxi Key Laboratory of Forest Products Chemistry and Engineering (Nanning, China). Chromatographic-grade methanol was used, and all other chemicals were analytical-grade reagents. 2-VP and EGDMA were subjected to rotary evaporation before use to remove the polymerization inhibitor, and chloroform was distilled before use to remove ethanol. AIBN was recrystallized in methanol before use.

2.2. Preparation of MIPs, NIPs, and PEV

MIPs for PTX were prepared by MSP as follows: First, PTX (0.625 mmol), 2-VP (5 mmol), EGMRA (10 mmol), chloroform (18 mL), and AIBN (0.1550 g) were mixed together as the oil phase and subjected to ultrasonication for 10 min. Second, the resulting mixture was added into 3.0 *wt%* PVA/water (200 mL) with CA (0.38 g) in a glass vessel, followed by dispersion using a high-speed homogenizer (IKA T18, Ultra-Turrax, Germany) for 10 min at 4500 rpm. Polymerization was performed at 80 °C for 24 h with stirring under N_2, affording spherical microparticles. The reaction solution containing polymer particles was centrifuged at 5000 rpm for collecting the product, which was washed using hot distilled water for removing PVA, followed by Soxhlet extraction with glacial acetic acid/methanol (10/90) and ethanol until no PTX was detected by UV–vis spectrophotometer (UV1800, Shimadzu, Japan) in the washings [22]. NIPs were prepared following the same procedure but without the addition of PTX. Similarly, poly(EGDMA-co-2-VP) (PEV) was prepared in the same manner as the NIPs, but EGMRA was replaced by EGDMA.

2.3. Chromatographic evaluation of polymers

The MIPs were packed into a stainless-steel chromatographic column (250 mm × 4.6 mm i.d.) by the slurry method using a slurry packer (Scientific Systems, USA) for the analysis column at 35 MPa with chloroform–methanol (1:1, v/v) as the slurry solvent. The MIP-packed chromatographic column was connected to an HPLC