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Enhancement of the isolation selectivity of isoflavonoid puerarin using oligo-β-cyclodextrin coupled polystyrene-based media

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Abstract

The isolation selectivity of the isoflavonoid puerarin, a well-known traditional Chinese medicine, was studied when native and oligo-β-cyclodextrin (CDP) coupled polystyrene-based macroporous resins were used as adsorbents by static tests. The research results indicated that the CDP coupled resin HPD-100-CDP offered the best adsorption and desorption capacity for puerarin than others and its equilibrium adsorption data at 25 °C fit best to the Freundlich isotherm. The performance of separation of puerarin on HPD-100-CDP column in one step was evaluated. Based on the above experimental data, a novel medium PS-CDP was synthesized and its chromatographic retention behaviors were also explored. ESI-MS/MS, ^{1}H NMR spectroscopy and UV absorption spectrum were used for the detection and characterization of puerarin in isolated fraction. Under the optimum mobile phase, methanol/acetic acid/water = 5.0/6.6/88.4 (v/v/v), the purity and recovery of puerarin were 95.3% and 86.7%, respectively, by HPLC analysis. In conclusion, the PS-CDP medium can enhance the isolated selectivity of puerarin and it can be applied in preparative scale operations.

Keywords: Oligo-β-cyclodextrin; Puerarin; Polystyrene-based media; Coupled; Chromatography; Isolation

1. Introduction

Radix puerariae, one of the most popular traditional Chinese herbal medicines, is the root of *Pueraria lobata* (Willd.) *Ohwi*. It was officially recorded in *Chinese Pharmacopoeia* under the monograph 'Gegen' (*Radix Puerariae*, RP) [1]. Extracts of RP are rich in isoflavonoids and have been employed to relieve fever and dysentery, promote the production of body fluid, facilitate eruption, lessen stiffness and pain of the nape, control alcoholism, and for the treatment of cardiovascular diseases, e.g. hypertension, myocardial infarction and arrhythmia [2–5]. Depending on its growing conditions, the total isoflavonoids in *Radix puerariae* root varies from 1.77 to 12.0%. RP contains significant amount of the isoflavonoid puerarin (daidzein 8-*C*-glucoside), which has been demonstrated to be the major efficient components of RP in pharmacology and clinical use [6]. Traditionally, the approaches to prepare the main component of RP, puerarin, include hydrolyzing, solvent extraction, precipitation and adsorption based on resin adsorbents [7–9]. A literature introduced a method for the separation and purification of puerarin from a crude extract of *Pueraria lobata* using HSCCC (high-speed counter-current chromatography) [10]. Nevertheless, these techniques, which are limited by excessive solvent usage or the lack of excellent selectivity towards puerarin results in low recovery and purity, consequently make the process impractical for scale-up. In recent years, the increasing requirement of puerarin on clinical use and pharmacological study necessitates the development of an efficient preparative separation method for this individual active herb component.

Due to the ability to form inclusion complexes (host–guest complexes), moreover the formation of the complexes may be influenced by the shape and size of the guest molecule, hydrogen bonding, and hydrophobic interaction, β-cyclodextrin (CD) and its derivatives are extensively used as ligand as well as stationary phases or mobile phase additives in liquid chromatography and capillary electrophoresis [11–14]. Compared to monomeric CD, immobilization of polymeric CD may be obtained the considerably higher local concentration of the CD group because the large soluble molecular aggregates were formed via the polymerization of the CD monomer before

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coupling. In our previous work [15,16], water-soluble CD oligomers (oligo-β-cyclodextrin or CDP) have been successfully coupled to allyl-substituted Sepharose HP. The experimental results indicated that high concentration of the CD group attributed to pendants of CDP is favourable to improving the separation efficiency of native matrix. The CDP-substituted Sepharose HP medium exhibited high selectivity of separation of puerarin from *Radix puerariae* flavones. But the agarose support medium is limited by its lack of mechanical rigidity, even in heavily cross-linked varieties, thus restricting its application in preparative scale operations.

Growing attention has been taken to separation and purification of pharmaceutical and natural products using polymeric resins because of their convenience, low cost, high chemically stability, easy regeneration and adjusted selectivity by modification of their surface chemistry and controlling their pore structure [17,18]. Although the wide variations in functionality, surface area, and porosity available for polymeric resins present the possibility of customizing resins for the separation of specific natural product species, the adsorption selectivity of these resins is generally low. This results from the sorption of sorbates on these resins driven by a single type of weak interaction. If CDP is anchored on polymeric supports capable of adsorbing for isoflavonoid puerarin, the selectivity of the media will be enhanced. We have successfully synthesized polyacrylate-based media D152-CDP for purification of puerarin in our laboratory [19], but the more efficient media should be manufactured in order to be employed on scale-up with high yield and purity. More recent studies in Chinese journals [20–24] depicted that polystyrene-based macroporous resins such as AB-8, HPD-100, HPD-300 and NKA-9 can be utilized to separate puerarin. Based on our previous works [15,19,25], the selectivity of these media for puerarin could be enhanced via CDP bonded on the bare supports. Moreover, the water wettability of these polystyrene-type adsorbents, which possess hydrophobic chemical moiety in molecular structure, can be improved by modifying the surface with the water-soluble CDP. Facile contact with aqueous solution will extend their broad application in the separation of natural product [26]. As far as we know, no observations of isolation of isoflavonoid puerarin have been reported for CDP coupled polystyrene-based media. The objectives of the present study, therefore, were to investigate the adsorption and desorption properties of puerarin on different macroporous resins before and after coupling CDP, and a one-step column chromatographic method for isolation of puerarin was performed with the optimal coupled resin. In accordance with the data of the above matrix, a polystyrene-type uniform porous microsphere medium was selected as support. Using this stationary phase, a simplified and efficient purification approach for puerarin from crude extract was established.

2. Experimental

2.1. Materials and reagents

CD was purchased from the Nankai University Fine Chemicals Factory, Tianjin, China. It was recrystallized, vacuum dried at 110 °C for 24 h before use. Epichlorohydrin was obtained from Beijing Yili Fine Chemicals Factory, Beijing, China. Polystyrene-based macroporous resins including AB-8, S-8, NKA-9, NKA-II, HPD-100, HPD-450, HPD-600, YWD01B, YWD03F, YWD04, YWD06, D201 (chloromethylated polystyrene, content of chlorine: 16.5%) were purchased from Chemical plant of Nankai University, Cangzhou Baoen Chemical Ltd. and Cangzhou Yuanwei Chemical Ltd. Polystyrene-type uniform porous microsphere medium PS was the gift from Institute of Process Engineering Chinese Academy of Science. The physical properties of adsorbents are summarized in Table 1. The adsorbents were pre-treated by 1.0 M HCl and NaOH solutions successively to remove the monomers and porogenic agents trapped inside the pores during the synthesis process, then dried at 60 °C under vacuum. Prior to the immobilization experiments, an amount of adsorbents were soaked in ethanol and subsequently washed by deionized water thoroughly, dried under vacuum. A crude extract powder of *Radix puerariae* called "*Radix puerariae* flavone", and reference puerarin with a purity of >98% were bought from Luye Biology, Huainan, China. Sodium hydride (NaH) was purchased from Tianjin Huanwei Fine Chemicals Factory, Tianjin, China. Phase transfer catalyst, i.e. tetrabutyl ammonium bromide (TBAB) and Potassium iodidum (KI) were purchased from Shanghai Reagents Factory, Shanghai, China. Chloromethyl methylether was obtained from Langfang Chemical Co. Ltd. Langfang, China. Ethanol, methanol, acetic acid, acetone, acetonitrile, sodium hydroxide, zinc chloride, *N*,*N*-dimethylformamide (DMF), and others were of analytical grade and obtained from Beijing Chemicals Factory, Beijing, China.

Table 1
Physical properties of the adsorbents used for immobilization reaction

Name	Polarity	Particle diameter (mm)	Surface area ($m^2\ g^{-1}$)	Average pore diameter (nm)
AB-8	Weak-polar	0.3–1.25	480–520	13.0–14.0
S-8	Polar	0.3–1.25	100–120	28.0–30.0
NKA-9	Polar	0.3–1.25	250–290	15.5–16.5
NKA-II	Polar	0.3–1.25	160–200	14.5–15.5
HPD-100	Non-polar	0.3–1.25	600–630	10.0–12.0
HPD-450	Medium-polar	0.3–1.25	700–730	7.0–8.0
HPD-600	Polar	0.3–1.25	520–550	8.0–9.0
YWD01B	Non-polar	0.2–0.6	600–630	5.0–5.5
YWD03F	Weak-polar	0.2–0.6	500–530	12.0–13.0
YWD04	Medium-polar	0.2–0.6	500–530	9.0–10.0
YWD06	Polar	0.2–0.6	250–280	15.0–16.0
D201	Non-polar	0.3–1.25	220–250	14.5–15.5
PS	Non-polar	0.030	740.2	11.7

2.2. Preparation of the coupled media

Beads of adsorbents mentioned above in 2.1 were chloromethylated according to the previously reported procedure [18]. CD was swollen in DMF solution then reacted with NaH for 12 h. About 5.0 g chloromethylated resin beads and 9.0 g treated CD were, respectively, added into a three-necked round-bottomed flask equipped with a mechanical stirrer, a thermometer and a reflux condenser. In addition, about 3.0 g TBAB