Separation and Purification Technology 164 (2016) 70–80

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

A novel chitosan base molecularly imprinted membrane for selective separation of chlorogenic acid

Qinqin Liu [a], Yan Zhao [a], Jiefeng Pan [a], Bart Van der Bruggen [b], Jiangnan Shen [a,*]

[a] College of Ocean, Technology of Zhejiang University, Hangzhou 310014, Zhejiang, China
[b] Process Engineering for Sustainable Systems, Department of Chemical Engineering, KU Leuven, W. de Croylaan 46, BE-3001 Heverlee, Belgium

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 5 March 2016
Accepted 13 March 2016
Available online 16 March 2016

Keywords:
Chlorogenic acid
Chitosan
Membrane
Selectivity
Permeability

ABSTRACT

Using molecularly imprinted technology and the phase inversion method, a chlorogenic acid molecularly imprinted chitosan membrane was prepared. Chlorogenic acid was used as the template molecule and chitosan as the film-forming material. Sulfuric acid was chosen as the cross-linking agent, in order to inhibit the swelling of chitosan membranes. The selectivity of these imprinted membranes in terms of permeability and adsorption capacity was studied. Furthermore, the chemical composition and surface structure of the membranes as well as their thermo-stability were characterized. Scatchard results show that there are two kinds of adsorption sites present on the molecularly imprinted membranes, one with high affinity sites and one with low affinity sites. For the high affinity sites, the maximum adsorption capacity was 139.57 μmol g^{-1} and that for the low affinity sites was 287.86 μmol g^{-1}. In contrast, for the non-imprinted membranes, only one kind of sites was found, with a maximum adsorption capacity of 83.84 μmol g^{-1}. The adsorption isotherms indicate that the adsorption isotherm curve of chlorogenic acid binding onto the imprinted membrane agrees with the Freundlich model while that of the non-imprinted membrane conforms to the Langmuir model. The permeation experiments proved that the imprinted membrane has a selective adsorption capacity for chlorogenic acid.

1. Introduction

Chlorogenic acid is a phenolic acid, which is structurally composed of caffeic acid and quinic acid [1]. It is widely distributed in plants [2] such as eucommia bark, honeysuckle and coffee [3]. It also occurs in potato, carrot, spinach, apples and other fruits [4]. In recent years, chlorogenic acid has attracted significant attention due to its wide spectrum of pharmacological properties including anti-oxidation [5,6], anti-bacterial and anti-viral, anti-cancer and anti-tumor effect, cardiovascular protection as well as reducing blood-lipid and blood-sugar [7,8]. Therefore, chlorogenic acid of high purity requires a highly efficient method for extraction. The conventionally used chemical separation and purification methods mainly include organic solvent extraction, microwave-assisted extraction [9], ultrasonic-assisted aqueous extraction [10], enzymatic extraction, alcohol extraction-water precipitation [11], high-speed counter-current chromatography, preparative liquid chromatography, macro-porous resins and ultrahigh pressure extraction [12].

However, these conventional preparation methods are time-consuming and are generally multi-step procedures [13]. High-speed counter-current chromatography is a one-step procedure but it is a particularly troublesome technique leading to high process costs accounting for the long separation time and high solvent consumption [14]. Adsorption on macro-porous resins is low-cost but the condition of pretreatment for the resin is extremely strict to avoid the loss of active ingredients [15].

In recent years, researchers have been seeking for better methods to obtain chlorogenic acid of high purity, and the molecularly imprinted technique was found to have good recognition and selection properties for the imprinted molecules [16]. The molecularly imprinted technique refers to using an artificial method to prepare polymers which have a "memory" effect with the specific template molecule, in order to achieve a specific separation from the perspective of bionics. This approach was explained in the field of medicine although published studies are limited for the application of molecularly imprinted polymers (MIPs) to adsorb chlorogenic acid [17–19]. For instance, a molecularly imprinted polymer for chlorogenic acid by modified precipitation polymerization was prepared and successfully applied for extraction of chlorogenic acid from the leaves of *Eucommia ulmodies* [20]. A molecularly imprinted polymer for hollow fiber–solid phase

* Corresponding author.

http://dx.doi.org/10.1016/j.seppur.2016.03.020
1383-5866/

micro-extraction of chlorogenic acid in medicinal plants was also synthesized and evaluated [21]. Moreover, a hollow chlorogenic acid imprinted polymer was prepared by surface imprinted on nano-TiO_2 as sacrificial material and its recognition behavior was tested [22]. Compared to molecularly imprinted polymers, molecularly imprinted membranes (MIMs) [23–27] have advantages, e.g., MIMs have larger adsorption areas and therefore obtain a higher adsorption capacity; secondly, MIMs are more convenient for usage because they do not need crushing. Moreover, since MIPs and the mixtures comprising chlorogenic acid are both powder, the separation process will be inconvenient if using MIPs. Therefore, it is more feasible to separate chlorogenic acid from mixtures by using MIMs than MIPs. To date, there have been few reports about purification for chlorogenic acid with MIMs which have the characteristics of molecularly imprinted technology and membrane separation technology. Thus, innovatively utilizing MIMs to purify chlorogenic acid is worthy to be studied.

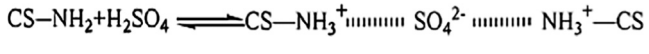
Chitosan is a natural bio-organic resource, which is second to cellulose in production volumes [28]. It has been proved to be an ideal film-forming material with attractive properties. To be specific, it is nontoxic, cheap, bio-degradable [29] and bio-compatible. Due to the existence of amino and hydroxyl groups on chitosan, it can be well combined with chlorogenic acid, which has carboxyl and hydroxyl groups by the ionic effect and hydrogen bonding. However, chitosan membranes would easily swell in water, which would have a bad impact on the properties of the membranes. Chitosan membranes are often modified by adding functional cross-linking agents, in order to decrease the degree of swelling and improve the mechanical performance. There are two kinds of cross-linking systems: one is chemical cross-linking, using agents such as glutaric dialdehyde; the other is ionic cross-linking, using sulfuric acid or tripolyphosphate. As sulfuric acid is not only nontoxic but also cheap, it is a promising material to be used as the cross-linking agent. Furthermore, there is a Coulomb interaction between the amino groups in chitosan and the sulfate ions in sulfuric acid, as shown in Scheme 1.

In this paper, chlorogenic acid molecularly imprinted chitosan membranes [30] have been prepared under suitable synthesis conditions through phase inversion. For the purpose of evaluating its performance, swelling experiments, adsorption tests and permeation measurements have been designed. Caffeic acid is used to be the competitive analogue of chlorogenic acid with two main reasons. Firstly, the mixtures in plants usually contain not only chlorogenic acid but also caffeic acid. Secondly, the chemical structure of caffeic acid is a part of the chlorogenic acid chemical structure. To further clarify this point, the structure of these two compounds are shown in Fig. 1. As chitosan is also one of the three main compounds used in this work, its structure is given as well in Fig. 1.

2. Methods and materials

2.1. Reagents and materials

Chlorogenic acid (98%) and caffeic acid (99%) were obtained from Xiya Reagent Co., Ltd. (Chengdu, China). Chitosan (D. D > 95%) was received from Jinhu Crust Product Co., Ltd. (Qingdao, China). Methanol, ethanol, acetic acid and sulfuric acid (98%) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical reagent grade. All solutions were prepared with deionized water.



Scheme 1. The cross-linking reaction between chitosan and sulfuric acid.

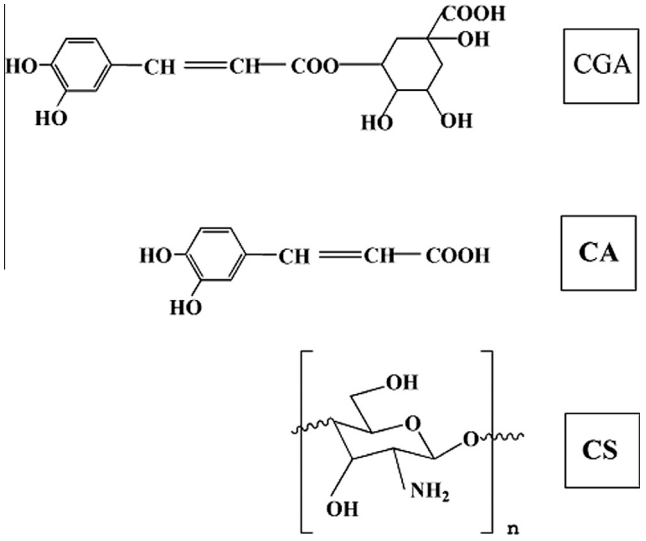


Fig. 1. Chemical structure of the three main compounds used in this work (CGA: chlorogenic acid; CA: caffeic acid; CS: chitosan).

2.2. Membranes preparation

Molecularly imprinted membranes and non-imprinted membranes (NIMs) were prepared by the phase inversion method. Briefly, chlorogenic acid (0.125 g) and chitosan (1.25 g) were dissolved in 50 mL 2% acetic acid solution at the same time, then reacted for almost 6 h under stirring at 60 °C. After that, the mixture was filtered to remove impurities. The casting solution that was obtained after vacuum de-aeration was poured onto a clean glass plate and dried at 60 °C for 12 h. The thickness of the membrane thus obtained was in the range of 40–50 μm. For the purpose of preventing swelling of the chitosan membrane, it was necessary to use sulfuric acid (0.5 M) as the cross-linking agent and it was also essential to use sufficient sulfuric acid to immerse the membranes. For extraction of template molecules to obtain the recognition cavities, the membrane was washed repeatedly with ethanol-acetic acid-distilled water (2:3:7, v/v/v) until the imprinted molecules could not be detected anymore by UV–vis at 327 nm, which is the characteristic wavelength of chlorogenic acid according to the literature [11]. The NIM was prepared in the same way but without the addition of the imprinted molecules.

2.3. Instruments and apparatus

The functional groups of the materials were characterized by the Nicolet NEXUS-470 Fourier transform infrared spectroscopy (FTIR) apparatus (U.S.A.). The surface morphology of MIM and NIM were studied by using a JSM-7500F scanning electron microscope (SEM) (Japan). Thermal gravimetric analysis spectra were measured with the NETZSCH TG 209 F3 thermogravimetric analyzer (Germany). The chlorogenic acid concentration in adsorption tests was determined by a UV–vis spectrophotometer (UV 3200PCS, Shanghai, China) and permeation tests were carried out by a LC-20AT high performance liquid chromatograph (HPLC) (Shimadzu, Japan). A thermal shaker (THZ-C, Taicang, China) was used to keep the temperature constant during batch adsorption experiments.

2.4. Characterization techniques

The surface morphology of MIM and NIM was observed by scanning electron microscopy. Samples were prepared by pasting on a