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Adsorption characteristics of rebaudioside A and stevioside on cross-linked poly(styrene-*co*-divinylbenzene) macroporous resins functionalized with chloromethyl, amino and phenylboronic acid groups

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ABSTRACT

The adsorptive separation of each steviol glycoside from aqueous solutions by polymeric adsorbents has attracted a lot of interest in recent years. The adsorption properties of chloromethylated cross-linked poly(styrene-*co*-divinylbenzene) macroporous resins, functionalised with chloromethyl, amino and phenylboronic acid groups, towards rebaudioside A and stevioside were studied. The results revealed that the resins with amino and phenylboronic acid groups preferred to adsorb stevioside rather than rebaudioside A, and their adsorption kinetics fitted a pseudo-second-order model. Isothermal equilibrium curves of rebaudioside A and stevioside showed a good fitness with the Langmuir and Freundlich models. The adsorption of rebaudioside A and stevioside onto resins was a spontaneous and exothermic process as indicated by the negative values in free energy and enthalpy. Results from the resin-packed column demonstrated that an effluent rich in rebaudioside A (purity 98%) was obtained prior to the breakthrough point of stevioside.

1. Introduction

Steviol glycosides that are extracted from *Stevia rebaudiana* Bertoni are used in the food industry as non-nutritive high-intensity sweeteners. The use of stevia extract as a sweetener, with a total amount of steviol glycosides of higher than 95%, was approved by the 73rd meeting of the Joint FAO/WHO Expert Committee on Food Additives. Steviol glycoside products with purity above 97% were approved as GRAS in the US in 2008. More than ten steviol glycosides have been identified in *Stevia rebaudiana* Bertoni, of which rebaudioside A and stevioside are the most important. Rebaudioside A produces a clean, sweet taste with no significant undesirable taste characteristics (Prakash, DuBois, Clos, Wilkens, & Fosdick, 2008). Stevioside presents a slightly bitter taste and aftertaste (Abelyan, Balayan, Ghochikyan, & Markosyan, 2004; Brandle, Starratt, & Gijzen, 1998). Moreover, stevioside exhibits several physiological activities such as lowering blood pressure (Chan et al., 2000), lowering blood sugar (Gregersen, Jeppesen, Holst, & Hermansen, 2004), anti-inflammatory (Boonkaewwan, Toskulkao, & Vongsakul, 2006) and anti-tumor (Yasukawa, Kitanaka, & Seo, 2002). Usually, rebaudioside A and stevioside co-exist in commercially available stevia extracts, which has led to the limited utilization of these products in food systems due to their aftertaste and bitter taste caused by the stevioside. Therefore, the separation of rebaudioside A from stevioside should promote the application of stevia extracts. Recrystallization was previously applied in separating rebaudioside A from stevioside in stevia extracts. However, the recrystallization process requires considerable energy consumption, organic solvent and is time-consuming. Some alternative methods for separation of rebaudioside A from stevia extracts were developed in recent years, such as high-speed counter-current chromatography (Huang, Fu, & Di, 2010) and preparative HPLC (Bergs, Merz, Delp, Joehnck, & Martin, 2012). Although high-purity rebaudioside A can be obtained, these methods are not appropriate for industrial process because of high-cost and time-consuming processing, utilization of organic solvents and inevitably environmental pollution.

Adsorption resins have received considerable attentions due to their excellent performance in isolation and purification of fine

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chemicals, pharmaceuticals and food additives (Bretag, Kammerer, Jensen, & Carle, 2009; Ma et al., 2009; Dotto & Pinto, 2011; Wang, Deng, Jin, & Huang, 2012). The adsorption resins have been successfully applied in enriching the steviol glycosides from crude stevia extracts in China, Japan, and other Asia countries. However, the main problem in the attempt to separate specific steviol glycoside is the poor adsorption selectivity of adsorption resins and the structural similarity of steviol glycosides. Some researchers studied the application of adsorption resins mixed bed in rebaudioside A purification and they claimed that the adsorption selectivity was enhanced by mixing several resins in optimum ratios (Liu et al., 2011; Chen, Wei, Li, & Di, 2012; Li, Chen, & Di, 2012). In order to obtain better adsorption selectivity for a specific compound, chemical modification of ordinary adsorbents is often adopted by introducing some special functional groups onto the adsorbent matrix (Wang et al., 2012; Liu et al., 2011). For instance, by introducing a group, the resin exhibited higher adsorption selectivity toward stevioside (Chen et al., 1999). In our previous study (Ye et al., 2013), a 3-aminophenylboronic acid modified polymeric adsorbent was synthesized. It exhibited improved adsorption selectivity toward stevioside, which was ascribed to the reversible binding of steviol glycosides to phenylboronic acids of the adsorbent (Yan, Springsteen, Deeter, & Wang, 2004).

In this study, a chloromethylated styrene-*co*-divinylbenzene copolymer (SD-0) was modified by hexamethylenetetramine via amination reaction and a novel adsorbent with amino groups (SD-1) was obtained. SD-1 was consecutively modified by *p*-formylphenylboronic acid to obtain SD-2 with phenylboronic acid groups. The adsorption of rebaudioside A and stevioside on the resins with chloromethyl, amino and phenylboronic acid groups was studied.

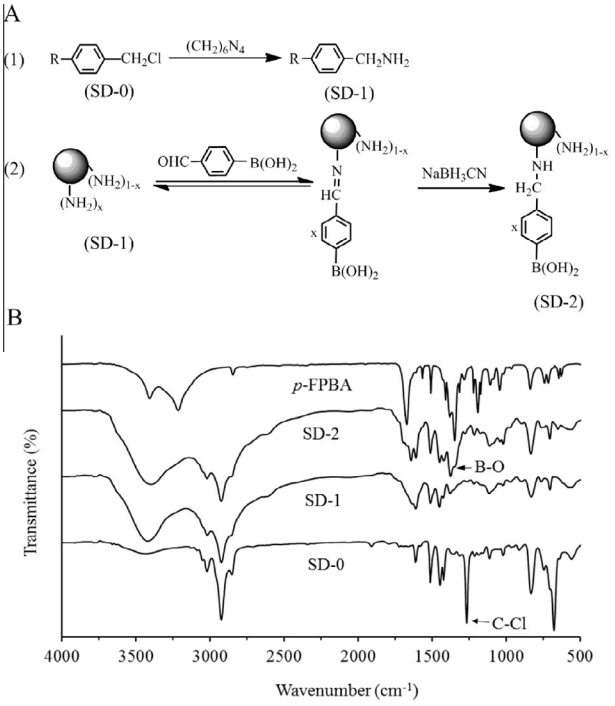

Fig. 1. (A) The scheme of the procedure for preparation of hexamethylenetetramine modified resin SD-1 and *p*-formylphenylboronic acid modified resin SD-2; (B) FT-IR spectra of chrolomethylated resin SD-0, SD-1, SD-2 and *p*-formylphenylboronic acid.

2. Materials and methods

2.1. Materials

Chloromethylated cross-linked poly(styrene-*co*-divinylbenzene) resins (SD-0, the chloride content 17.4%) were kindly provided by Tianjin Nankai Hecheng S & T Co., Ltd. (Tianjin, China); *p*-formylphenylboronic acid (*p*-FPBA, purity 95%), hexamethylenetetramine and sodium cyanoborohydride were obtained from Sigma–Aldrich (Shanghai, China). Authentic stevioside (99%) and rebaudioside A (97%) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan); The extract, namely, Stevia *reb-A* (total steviol glycoside 90%, stevioside 39% and rebaudioside A 44%) was purchased from Jining Aoxing Stevia Products Co., Ltd. (Shandong, China).

2.2. Synthesis of cross-linked poly(styrene-co-divinylbenzene) resins functionalized with amino and phenylboronic acid groups

The reaction scheme for the preparation of SD-1 and SD-2 on the basis of SD-0 is shown in Fig. 1A. SD-1 with amino groups was synthesized through an amination reaction (Ricard, Villemin, & Ricard, 1981). SD-0 was swelled thoroughly with anhydrous *N*,*N*-dimethylformide for 24 h, and mixed with excess hexamethylenetetramine in a 500 mL three-necked round bottom flask equipped with a mechanic stirrer and a reflux condenser. The mixture was held at 413 K for 9 h. The resin was filtered out and poured into ethanol: hydrochloric acid (1:3) solution at 303 K for 6 h, followed by filtration and rinsing with 1 mol/L sodium hydroxide and successively with a large amount of distilled water. The resultant SD-1 resin with amino groups (4.8 mmol/g dry resin) was obtained. SD-2 with amino and phenylboronic acid groups was synthesized via *p*-FPBA modification. Forty grams of SD-1 were swollen by 120 mL methanol in a 500 mL three-necked round bottom flask equipped with a mechanic stirrer and a reflux condenser for 8 h at room temperature. At a moderate stirring speed (150 r/min), 9.0 g *p*-FPBA were then added into the flask at 303 K. About half an hour later, the reaction mixture was held at 333 K for 10 h. The phenylboronic acid groups were covalently bound to the resin matrix via the reaction between formyl groups of *p*-FPBA and amino groups on the resin, by which a Schiff's base was formed (Donia, Atia, & Elwakeel, 2008). The flask was then put into an ice water bath and the reaction mixture was cool down to the temperature around 277 K. The sodium cyanoborohydride powder was added portionwise to the flask to reduce the Schiff's base. The resin was filtered out and packed in an extractor, and washed with methanol and then distilled water. SD-2, a pale yellow resin, was obtained.

2.3. Characterization of the adsorbents

Infrared spectra of *p*-FPBA, SD-0, SD-1 and SD-2 were collected by KBr disks on a Nicolet IS10 FT-IR spectrometer (Nicolet, USA). The chorine content of the resin was measured by Volhard method. The amino content of the resin was measured by the method of National Standard of China (GB 5760-86). The density of phenylboronic acid groups on the resin (mmol/g dry resin) was determined by calculating *p*-FPBA (λ_{max} 256 nm) content on a UV-3600 spectrometer (Shimazu, Japan) before and after the reaction. The regression line for *p*-FPBA was $A = 0.1696C - 0.0047$ ($R^2 = 0.9999$; $n = 9$), where A was the absorbance at 256 nm and C was the concentration of *p*-FPBA in methanol solution. The calibration curve showed excellent linearity over the range of 0.667–20.0 μmol/L. The BET surface area of SD-0, SD-1 and SD-2 was determined from the nitrogen adsorption curves at 77 K by an automated system (ASAP 2020, Micromeritics Instrument Co., USA). Before the BET