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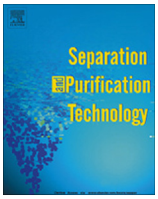

Adsorption characteristics of stevioside and rebaudioside A from aqueous solutions on 3-aminophenylboronic acid-modified poly(divinylbenzene-*co*-acrylic acid)

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ABSTRACT

3-Aminophenylboronic acid was grafted onto poly(divinylbenzene-*co*-acrylic acid), namely DA-1, to prepare the modified adsorbent DA-1M. Adsorption characteristics of stevioside (S) and rebaudioside A (RA) from aqueous solution on DA-1 and DA-1M was investigated by static and dynamic adsorption tests. DA-1M preferentially adsorbed S and exhibited better adsorption selectivity than DA-1. Kinetic data for S and RA adsorption onto DA-1 and DA-M obeyed pseudo-second-order kinetic model, and intraparticle diffusion was not the only rate-controlling step. For the single solute system, adsorption isotherm data on DA-1 was fitted to Langmuir and Freundlich isotherm model, while adsorption on DA-1M was revealed as a heterogeneous adsorbent process by Dubinin–Radushkevich isotherm model. For the binary solute system, the modified extended Langmuir model with incorporation of competitive efficiencies (θ) gave good correlation results against adsorption data with reasonable fitted parameter values. Thermodynamic data indicated that the adsorption process was spontaneous and exothermic. The separation factor of RA and S on DA-1M column eluted with water was 0.631, and the purity of RA and S achieved 96.3% and 96.0% with a recovery yield of 86.1% and 89.9%, respectively.

1. Introduction

Stevioside (S) and rebaudioside A (RA) are two major steviol glycosides in the extracts produced from the leaves of *Stevia rebaudiana* Bertoni [1]. The stevia extracts, recently approved by the US FDA as GRAS (generally recognized as safe), have been widely used as a natural sweetener or a dietary supplement in various food and beverage products. S tastes 250–300 times sweeter than sucrose but exhibits significant bitter aftertaste [2]. In contrast, RA is the sweetest component (350–450 times of sucrose) and has superior taste quality [3]. Products of steviol glycosides with large proportion of RA or high-purity RA, therefore, give a better mouthfeel and are welcomed by consumers.

Production of high-purity RA from the stevia extract was low-efficient and high-cost processing due to the structural similarity between RA and other steviol glycosides. Modern separation technologies such as re-crystallization [4] and high-speed counter-current chromatography [5] have been applied to obtain high-purity RA. These methods are not appropriate for industrial process due to several drawbacks including complicated and time-consuming operations, consumption of organic solvents and inevitably environmental contamination. Recently, Bergs et al. [6] developed a simplified and efficient chromatographic process for separation of RA from aqueous extracts of *Stevia rebaudiana* leaves. However, the process required a cascade of expensive preparative chromatographic columns.

Polymeric adsorbents such as molecular imprinted polymers (MIPs) and macroporous adsorption resins (MARs) have attracted rising attention for selective enrichment of desired components. MIPs usually possess high adsorption selectivity and have been widely used for solid phase extraction [7]; however, they are scarcely used in industrial preparation due to complicated synthesis procedure and quite low adsorption capacity. MARs have been the most commonly used type of adsorbents for large adsorption capacity and low cost. The preparative purification of RA from steviol glycosides by mixed bed of commercial MARs was recently reported [8–10]. However, ordinary MARs have poor adsorption selectivity toward individual steviol glycosides. Chemical modifications of ordinary adsorbents by introducing special functional groups onto adsorbents matrix have been widely adopted for improving adsorption selectivity [11–13]. Chen et al. [14] reported that polymeric adsorbent containing pyridine group possessed rel-

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atively good separation effects for RA. As the choice of a suitable adsorbent is the key factor of column chromatography for high efficiency, the development of desirable adsorbents with perfect adsorption selectivity never ceased.

Since boronic acids form a covalent and reversible interaction with *cis*-diols in water [15], it is a little surprising that boronic acids have been widely used as affinity ligands for the separation of carbohydrates [16], glycoproteins [17,18], and nucleotides [19]. Boronic acid-containing adsorbents with good adsorption selectivity toward *cis*-diols are usually prepared by a copolymerization procedure or a chemical modification procedure. In the copolymerization procedure, 4-vinylphenylboronic acid is a commonly used functional monomer [20]. In the chemical modification procedure, 3-aminophenylboronic acid is the most commonly used ligand [21]. Various supports that have been used to immobilize boronic acids include agarose [17], iron oxide nanoparticle [16], polymethacrylate [22], porous glass [19], and chitosan [21]. It is known that S and RA are steviol glycosides with *cis*-diol units. Therefore, the considerable interest in both extending the application of boronic acid-containing adsorbents and developing new efficient separation methods for S and RA led to our study.

The objective of the present study was to investigate adsorption characteristics S and RA from aqueous solution on a boronic acid-containing adsorbent. DA-1M, functionalized with phenylboronic acid groups, was firstly prepared from a carboxylated polymeric adsorbent DA-1 by amidation reaction. The kinetics and equilibria of S and RA adsorption onto DA-1M were thereafter tested in batch experiments with DA-1 as the reference. Thermodynamic data were analyzed and separation of S and RA on DA-1M column was also discussed.

2. Materials and methods

2.1. Materials

Poly(divinylbenzene-*co*-acrylic acid) (DA-1), a polymeric adsorbent containing carboxyl groups (9.5 mmol g^{-1}), was supplied by Tianjin Nankai Hecheng S&T Co., Ltd (Tianjin, China); 3-aminophenylboronic acid monohydrate (APBA, purity 98%) was obtained from Sigma–Aldrich (Shanghai, China); *N*-hydroxysuccinimide (NHS, purity 98%) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, purity 98%) was obtained from Shanghai Demo Medical Tech Co., Ltd. (Shanghai, China); Water used in all of the experiments was purified by a Milli-Q system (Millipore, Milford, MA, USA); Authentic S (99%) and RA (97%) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan); Stevia *reb-A* (RA 98% purity) and Stevia ST (S 90% purity) were purchased from Jining Aoxing Stevia Products Co., Ltd. (Shandong, China).

2.2. Preparation of DA-1M

The chemical modification of DA-1 was performed via EDC/NHS coupling chemistry [23], as shown in Fig. 1. The transformation of the carboxyl groups into *N*-hydroxysuccinimide esters, a process known as activation, was conducted in an ethanol solution, containing a mixture of EDC (0.1 mol L^{-1}) and NHS (0.2 mol L^{-1}), for 60 min at room temperature. After activation, the adsorbent was exposed to APBA solution (0.1 mol L^{-1}), and allowed to react over night at 313 K. The modified absorbent named DA-1M was washed several times with ethanol followed by water.

2.3. Characterization of the adsorbents

Infrared spectra of DA-1 and DA-1M were recorded on a Nicolet IS10 FT-IR spectrometer (Nicolet, USA) with discs of powdered potassium bromide and adsorbent. X-ray photoelectron spectroscopy (XPS) was used to evaluate the boron and nitrogen of DA-1M. The XPS spectra were recorded on a PHI 5000 VersaProbe spectrometer (ULVAC-PHI Inc., Japan) using an Mg Kα excitation source. The BET surface area of DA-1 and DA-1M were determined from the nitrogen adsorption curves at 77 K by an automated system (ASAP 2020, Micromeritics Instrument Co., USA). Before the BET surface area measurement, the adsorbents were dried at 323 K and degassed at 333 K for 16 h on the degas port of the analyzer. The surface morphology of DA-1 and DA-1M was investigated by scanning electron microscopy (SEM; SU1510, Hitachi Co., Japan).

2.4. Analysis of S and RA

Analysis was performed on a Hitachi LaChrom Elite L-2000 series HPLC System (Hitachi Co., Japan) with a Shodex Asahipak NH2P-50 4E column (4.6 mm × 250 mm; Showa Denko K. K, Japan) using acetonitrile–water (75:25, v/v) as mobile phase at flow rate of 1.0 mL min^{-1} at 308.15 K. The wavelength of the diode array detector was set at 210 nm. The injection volume was 5 μL. Quantification of S and RA concentration was performed by an external calibration curve.

2.5. Adsorption of S and RA onto DA-1 and DA-1M at different initial solution pH

About 39.0 g of pretreated adsorbent (equal to 10.0 g of dry mass) was immerged into 200 mL of S and RA binary solution (S/RA = 1:1) in a 500 mL of conical flask with stopper. The initial concentration of S and RA was approximately 4.0 mg mL^{-1}. The initial pH values (5.0, 6.0, 7.0, 8.0, 9.0, and 10.0) of S and RA solutions were adjusted by 1.0 mol L^{-1} HCl and NaOH solution. The flasks were shaken in SHA-2A thermostatic oscillator (120 rpm) at 298.15 K for 12 h to achieve adsorption equilibrium. The initial and final pH values of the tested solutions were detected by a FEP20-FiveEasy Plus pH meter (Mettler-Toledo Co., Germany). The initial and final S and RA concentrations were determined by HPLC. All samples were analyzed in triplicate.

The equilibrium adsorption capacity in batch experiments, q_e (mg g^{-1} dry adsorbent), was calculated as:

$$q_e = (C_0 - C_e) \times \frac{V}{m} \quad (1)$$

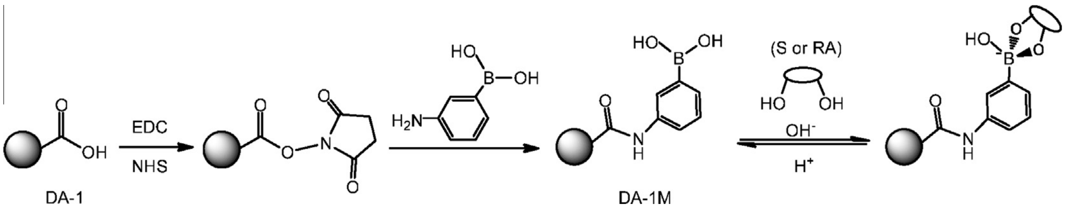

Fig. 1. The scheme of the procedure for preparation of 3-aminophenylboronic acid modified adsorbent DA-1M and the representation of reversible formation of covalent bonds from diol units of steviol glycosides and phenylboronic acid groups.