FOOD AND BIOPRODUCTS PROCESSING XXX (2013) XXX–XXX

Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Separation of glycyrrhizic acid from licorice root extract using macroporous resin

*Trupti W. Charpe, Virendra K. Rathod**

Chemical Engineering Department, Institute of Chemical Technology, Matunga, Mumbai 400019, India

ABSTRACT

Glycyrrhizic acid (GA) is the major active ingredient of licorice which has many pharmacological activities. In the present study, separation of GA from licorice root extract has been carried out by adsorption on five different macroporous resins. Static and dynamic adsorption of GA from crude licorice root extract is studied on ion exchange resins followed by desorption. Indion 810 shows the maximum adsorption as well as desorption capacity. The adsorption experiments indicate that equilibrium can be achieved in 360 min. The adsorption equilibrium data is well fitted in the Langmuir isotherm. The separation process is optimized by investigating the effect of pH on adsorption capacity and effect of concentration of ethanol on desorption capacity. The dynamic adsorption is carried out in a column packed with Indion 810 resin and effect of feed flow rate and initial concentration of GA in extract has been studied. The results showed that increase in feed flow rate as well as initial feed concentration of GA lowers the dynamic binding capacity and mass transfer coefficient while increases the HETP. The purity of GA is increased from 14.3% to 71.5% by the dynamic desorption with 60% ethanol. Indion 810 resin can efficiently separate GA from licorice root extract with the HPLC recovery of 63.6%. This study forms the basis for large scale preparation of GA by resin adsorption.

Keywords: Adsorption; Glycyrrhizic acid; Licorice; Macroporous resin

1. Introduction

Licorice, the root and rhizome of the *Glycyrrhiza glabra* plant species has been used as herbal medicine all over the world especially in Asia and southern Europe since ancient age. It is known to have anti-inflammatory, anti-viral, anti-allergic, anti-oxidant, gastro-protective, anti-ulcer anti-hepatotoxic and anti-cancerous properties. It is a powerful natural sweetener and hence widely used in food, confectionery and pharmaceutical products, such as cough syrups, herbal supplements, chewing gums, drinks and candy (Mukhopadhyay and Panja, 2008). The major active and most studied ingredient of licorice is glycyrrhizic acid (GA). In many countries, GA is used as a major therapeutic agent to treat chronic viral hepatitis and allergic dermatitis (Tian et al., 2008). It is also one of the leading natural compounds for clinical trials of chronic active viral hepatitis and HIV infections (Baltina, 2003).

GA is a triterpenoid glycoside compound and a weak acid which has three carboxyls and five hydroxyl groups. The chemical structure of GA is shown in Fig. 1. GA is commercially purified from licorice by several steps such as crystallization and preparative chromatography. However, it suffers from various disadvantages which include poor recovery (only 20 and 30%) and higher process cost. Existing HPLC methods used to analyze the GA are not suitable for its large-scale isolation (Jiang et al., 2004). Hence, there is a need to develop an alternative protocol which could overcome the drawbacks of existing processes.

The preliminary enrichment and separation of phytochemicals from plant extracts is an important step in the commercialization of manufacturing process of pure phytochemical. There are several methods, such as liquid–liquid extraction, membrane filtration, available in the literature for the enrichment and separation of active constituents. Advanced methods like supercritical CO_2 extraction, ultrasound assisted extraction, microwave assisted extraction, pressurized hot water extraction, etc. are also employed for extraction of these natural ingredients. In our previous work,

* *Corresponding author.* Tel.: +91 22 33612020; fax: +91 22 33612010.
E-mail addresses: vk.rathod@ictmumbai.edu.in, virendrakrathod@gmail.com (V.K. Rathod).
Received 25 June 2013; Received in revised form 29 October 2013; Accepted 5 November 2013
0960-3085/$ – see front matter © 2013 The Institution of Chemical Engineers. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fbp.2013.11.002

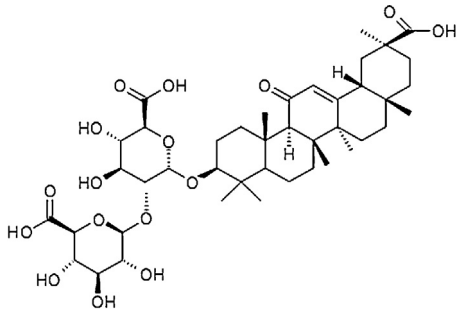

Fig. 1 – Molecular structure of GA.

the ultrasound assisted extraction of GA has been successfully carried out with good yield (Charpe and Rathod, 2012). Since, the licorice extract obtained contains many other constituents; it is must to purify and obtain GA in a pure form for the specific application like drug formulation. Adsorption seems to be the most suitable method for this due to its high efficiency, easy scalability and simple procedure.

Microporous resins are very popular and widely investigated in research for the separation of natural ingredients as they have high mechanical strength, good acid and alkali resistance, many functional groups, porous availability, high surface area and long lifetime (Liu et al., 2010b). In case of adsorption on macroporous resins, separation is based on differences in molecular weight, functional group, polarity and shape of different molecules in the solution because of which they show difference in affinity for the particular adsorbent. Resins are durable, non-polar and polar polymers, having a high adsorption capacity with possible recovery of the adsorbed molecules, relatively low cost and easy regeneration. They can be used for selective adsorption and recovery of constituents from aqueous solutions as well as from nonaqueous systems through electrostatic force, hydrogen bonding interaction, complexation and size sieving action, etc. (Liu et al., 2010a). Many phytochemicals from plant source such as genistein and apigenin from extracts of pigeon pea roots, lycopene from tomato skins extracts, rosavin from Rhodiola rosea are investigated for purification by adsorption (Gao et al., 2013; Liu et al., 2010b; Ma et al., 2009). Fu et al. (2005) had studied adsorption of GA and licorice flavonoids on XDA-1 resin, but the main focus of their work was to remove GA in order to obtain a deglycyrrhizinated licorice product.

In the present work, static and dynamic adsorption of GA from crude licorice root extract was carried out on five different ion exchange resins. Various process parameters which affect the separation process such as pH and concentration of ethanol for desorption are optimized. Desorption of GA has also been carried out to identify the best suitable resin. The main objective of the study was to develop the simple and efficient process for preliminary enrichment of GA from licorice root extract.

2. Materials and methods

2.1. *Reagents and material*

Licorice powder was purchased from local market. Acetonitrile and acetic acid used as solvent for High Pressure Liquid Chromatography were analytical grade purchased from Hi Media Ltd., Mumbai, India. Ethanol purchased from Hi Media Ltd., Mumbai, India was analytical grade. Water used as a solvent was freshly prepared de-ionized water from Millipore Milli-Q 50. Standard i.e. glycyrrhizic acid (mono-ammonium salt hydrate $\geq$ 70%) was purchased from Aldrich Chemical Company, USA.

2.2. *Adsorbent*

Macroporous resins including Indion 810, Indion 850 and Indion 860 were supplied by Ion exchange (India) Ltd. Duolite A161 and Duolite A368 resin were supplied by Auchtel Products Ltd., Mumbai, India. Their physical properties are listed in Table 1. All the adsorbent beads were regenerated prior to use by 3% NaOH and then thoroughly washed with deionized water to remove the remaining NaOH. Finally, it was washed with methanol and dried at 60 °C.

2.3. *Analytical method*

The glycyrrhizic acid was analyzed using HPLC. The HPLC instrument was of Agilent 1260 series equipped with a diode-array UV–vis detector (DAD, model G1315D). Analysis was performed on a XBD-C18 reversed-phase column (Eclipse, 250 mm × 4.6 mm, 5 μm) at the wavelength of 254 nm. The mobile phase consisted of a mixture of 70% methanol and 30% acidified water (1% acetic acid) and the flow rate was maintained at 1 ml/min.

2.4. *Static adsorption and desorption study*

Batch adsorption experiments were performed by contacting weighed quantities of resins in 100 ml flasks containing 15 ml of licorice roots extract. The sample pH was adjusted to the desired value with the buffer solution. The flasks were agitated at 150 rpm for 6 h at a constant temperature. The solution was then filtered and the concentrations of glycyrrhizic acid were analyzed by HPLC.

Adsorption kinetics tests were conducted by mixing a 250 mg of resin with 15 ml of licorice extract in 100 ml flask. The flasks were then kept in an incubator shaker at 130 rpm. The samples were withdrawn after every 30 min in the first hour and every 1 h until equilibrium was reached. All the samples were further analyzed using HPLC. Adsorption isotherms were studied at temperature of 25, 30 and 35 °C. Effect of pH was studied by contacting 15 ml of sample solution with known amount of resin and adding buffer of pH range 4–9.

Once the adsorption equilibrium was reached, the same resins were further used for the desorption studies. The resins were first filtrated from the solutions, and then adequately washed by deionized water. 30 ml of ethanol–water (60:40, v/v) solution containing 1% NaOH was added to 100 ml flasks containing the adsorbate-laden resins. The flasks were shaken at 150 rpm for 6 h at a constant temperature of 50 °C. The desorbed glycyrrhizic acid from different resins was analyzed by HPLC.

2.5. *Dynamic adsorption and desorption study*

Dynamic adsorption and desorption experiments were performed in a lab-scale glass column of 1 cm internal diameter packed with 2 g (dry weight) of the Indion 810 resin and 5.5 cm of packed bed length. Sample solution was passed through the bottom of the glass column at the fixed flow rate using peristaltic pump. Samples were collected at definite time interval and analyzed by HPLC. To investigate the effect of flow rate on adsorption, experiments were carried out at 0.5 ml/min,

Please cite this article in press as: Charpe, T.W., Rathod, V.K., Separation of glycyrrhizic acid from licorice root extract using macroporous resin. Food Bioprod Process (2013), http://dx.doi.org/10.1016/j.fbp.2013.11.002