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The application of macroporous resins in the separation of licorice flavonoids and glycyrrhizic acid

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Abstract

Glycyrrhizic acid (GA) and licorice flavonoids (LF) are the two classes of bioactive components in licorice with known pharmacological effects. But long-term excessive intake of GA may cause sodium retention and hypertension. In this study, the performance and adsorption characteristics of four widely used macroporous resins for the separation of deglycyrrhizinated, flavonoids enriched licorice has been critically evaluated. The sorption and desorption properties of LF and GA on macroporous resins including XDA-1, LSA-10, D101 and LSA-20 have been compared. The adsorption capacity was found to depend strongly on the pH of the feed solution. XDA-1 offers much higher adsorption capacity for GA and LF than other resins, and its adsorption data fit the best to the Freundlich isotherm. XDA-1 also shows much higher adsorption affinity towards LF than that of GA based on calculated results from the measured adsorption isotherms. Dynamic adsorption and desorption experiments have been carried out on a XDA-1 resin packed column to obtain optimal parameters for separating GA and LF. An enriched LF extract (about 21.9% purity) free of GA, and an enriched GA extract with 66% purity can be separated from crude licorice extract in one run.

Keywords: Macroporous resins; Licorice; Flavonoids; Glycyrrhizic acid; DGL; Adsorption; Desorption

1. Introduction

Licorice (gancao in Chinese) is the roots and rhizomes of some *Glycyrrhiza* species (*G. glabra, G. uralensis, G. inflata, G. eurycarpa, G. aspera, and G. korshinskyi*) [1]. Besides being a popular food additive, licorice is also one of the most widely used medicinal herbs in China. The two groups of active compounds in licorice are glycyrrhizic acid (GA) and licorice flavonoids (LF). GA, along with its aglycone, glycyrrhetinic acid (GTA), is known to have anti-inflammation [2], anti-ulcer [3], anti-hepatotoxic [4] and antivirus [5,6] activities. Recently, GA has also been applied clinically to treat patients with AIDS [7]. However, clinical data showed that a long-term excessive intake of licorice products containing more than 1 gram of GA daily for longer than six weeks would induce a syndrome of sodium retention and potassium excretion, causing oedema and hypertension [8]. The pseudo-aldesterone-like effects are generally attributed to the GTA, the hydrolytic metabolite of glycyrrhizic acid, which binds to mineralocorticoid receptors in the same way as aldosterone [9]. Different from GA, the licorice flavonoids (LF) demonstrated significant antitumorigenic [10], antimicrobial, antiulcer [11], and antioxidant [12] activities, but without the unwanted side effects of GA.

To develop active licorice products without the mineralocorticoid-like side effects, an effective separation procedure is established to remove GA from the licorice extract. The procedure results in a deglycyrrhizinated licorice product, commonly abbreviated as DGL, composed mainly of licorice flavonoids. Several studies have demonstrated that DGL reduces inflammation and is as effective as some

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prescription drugs for gastric and duodenal ulcers [13,14]. In addition, it could enhance the effectiveness of antiulcer medications such as cimetidine [15]. In fact, some flavonoids and deglycyrrhizinated licorice extracts have been marketed as dietary supplement products for ulcer treatment. In preparing these products, GA was generally removed by precipitation with acids. Although these methods were simple and inexpensive, the products still contained certain amounts of GA and a great deal of non-active impurities such as saccharides. Recently, separation methods based on macroporous resin are gaining popularity in pharmaceutical applications and have also been used for GA separation [16]. The process, however, is good for GA production but not flavonoids. For the latter species strong alkali solvents would be needed for desorption, and would destroy the bioactivities of flavonoids. Towards this objective, experiments have been carried out in this study to investigate the adsorption and desorption properties of GA and LF on different macroporous resins. The information is of significance in the selection of adsorption resins for the preparative separation of licorice extract or other herbal materials in general.

2. Experimental

2.1. Reagents

Glycyrrhizic acid (mono-ammonium salt hydrate) was purchased from Aldrich Chemical Company, Inc., (Milwaukee, WI, USA). Acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Trifluoroacetic acid was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Anhydrous aluminum chloride, ethanol obtained from Shanghai Chemical Co. (Shanghai, P.R. China) was of Analytical-grade. Rutin was purchased from National Institute for the Control of Pharmaceutical and Biological Products. All solutions were filtered through 0.45 μm membranes (Fisher Scientific) before HPLC. Freshly deionized water (18.2 MΩ) prepared by a Millipore Milli-Q^{50} (Millipore Corp., Waltham, MA, USA) was used in all experiments.

2.2. Adsorbents

Macroporous resins including XDA-1, LSA-10, D101, LSA-20 were supplied by Grancent Exchange & Adsorbent Material Ltd.(Xi'an, China). Their physical properties are listed in Table 1. The adsorbent beads were pre-treated to remove the monomers and porogenic agents trapped inside the pores during the synthesis process. Finally, it was dried at 60 °C under reduced pressure [17]. Prior to use in the adsorption experiments, preweighed amounts of adsorbents were wet with ethanol and subsequently the ethanol was thoroughly replaced with deionized water [18].

Table 1
Physical characteristics of macroporous resins

Grade	Surface area (m^2/g)	Ave. pore diam. (Å)	Polarity
XDA-1	800–1000	26–32	Polar
LSA-20	420–500	85–90	Non-polar
D101	480–550	12–15	Non-polar
LSA-10	500–540	13–18	Moderate polarity

2.3. Preparation of crude licorice extracts

Licorice (*Glycyrrhiza uralensis* Fisch.) root was obtained from Elion Resources Group Co., 25 g of licorice sample was minced, ground into 180 μm powder by a disintegrator and extracted with 500 ml of a solution of ethanol/water (70:30, v/v) by sonication in an ultrasonic bath (SK3200LH, Shanghai KUDOS Ultrasonic Instrument Co., Ltd., China) for 30 min with intermittent stirring using a glass rod. The extraction solution was centrifuged at 6000 rpm for 10 min using a centrifuge (MIKRO 22R, Hettich Zentrifugen GmbH&Co. KG, Germany). The supernatant clear extract was concentrated to one tenth of the original volume by removing the ethanol solvent in a rotary evaporator (SBW-1, Shanghai Shenbo Instrument Co., China) at 50 °C.

2.4. Analytical methods

2.4.1. HPLC analysis of glycyrrhizic acid

Quantification of the glycyrrhizic acid concentration was carried out by HPLC on a Agilent 1100 series HPLC system equipped a diode-array UV–vis detector (DAD, model G1315B). Analysis were performed on a KR100-5C18 reversed-phase column (Kromasil®, 150 mm × 4.6 mm, I.D., 5 μm) (Eka Chemicals AB, Bohus, Sweden). The UV detector was set at the wavelength of 254 nm with a sensitivity of 0.1 a.u.f.s. The column temperature was maintained at room temperature (25–30 °C). Gradient elution was used in HPLC runs. The gradients were formed by varying the proportion of water (A) and acetonitrile (B), each containing 0.05% (w/w) trifluoroacetic acid (TFA). The elution system was: 0–10 min, 20–40% of B; 10–15 min, 40% of B; 15–16 min, 40–50% of B; 16–17 min, 50–20% of B; 17–20 min, 20% of B. The flow rate employed was 0.8 ml/min throughout the run, and the retention time of GA was 14.8 min.

The working calibration curve based on GA standard solutions showed good linearity over the range of 0.035–0.60 mg/ml. The regression line for GA was $y = 12405x - 4.5527$ ($R^2 = 1$, $n = 6$), where y is the peak area of GA and x is the concentration of GA (mg/ml).

2.4.2. Determination of concentrations of total flavonoids

An aliquot of 2.0 ml flavonoids solution was added to a 25 ml flask containing 7.5 ml deionized water and 5 ml 0.1 M HOAc/NaAc buffer at pH4.5. Then 5 ml of 0.1 mol/ml aluminum chloride was added. After mixing, an aqueous solution of 70% ethanol was added to the flask and made to the volume. The solution was allowed to stand for 10 min at room temperature, and the absorbance at 416 nm was determined

on a BECKMAN 7400 spectrophotometer. Total flavonoid concentration was calculated using rutin as the calibration standard. A good linear relationship was obtained over the range of 0.0040–0.016 mg/ml, and the regression equation for GA was: $y = 30.361x + 0.0005$ ($R^2 = 0.9999$, $n = 6$), where y is the absorbance at 416 nm, x is the concentration of total flavonoids (mg/ml).

2.5. Static adsorption and desorption tests

The static adsorption tests of licorice extract were performed as follows: preweighed amounts of hydrated adsorbent (equal to 150 mg dry resin) were introduced into an air-tight Erlenmeyer flask. Then, 15 ml of aqueous solution of licorice extract was added into each flask. The flasks were then shaken (100 rpm) in a constant temperature water-bath shaker at 25 °C for 12 h. The selectivity and adsorption capacity of different resins towards GA and LF were evaluated by their adsorption isotherms, fitness to Freundlich equation, and desorption properties obtained from adsorption experiments under different conditions including solution pH, initial solute concentrations and ethanol/water ratios used for desorption. The adsorption kinetics of GA and LF in the selected resin XDA-1 was also studied. Preweighed hydrated adsorbent (equal to 1 g dry resin) was introduced into an Erlenmeyer flask containing 90 ml of aqueous solution of licorice extract. The initial concentration of GA and total flavonoids were 0.73 mg/ml and 0.4 mg/ml, respectively, and with pH5. The flask was shaken in a water-bath shaker. The respective concentrations of GA and total flavonoids in the liquid phase were monitored at equal time intervals till equilibration.

2.6. Dynamic adsorption and desorption tests

Dynamic adsorption experiments were carried out in a glass column(0.5 cm × 30 cm) wet-packed with the selected resin. The bed volume (BV) of the resin was 5 ml. The feed flow rate was 1 BV/h. The GA and the LF in the eluents were monitored by HPLC analysis of the eluted aliquots collected at 5 mL intervals. Breakthrough point was indicated when GA was detected in the eluent (the HPLC limits of detection for GA is about 0.8 ppm). The loading of the sample was stopped. The adsorbate-laden column was washed first with deionized water, and then desorbed with aqueous-ethanol (55:45, v/v) solution. The eluents were concentrated and dried under vacuum before further analyses.

2.7. Sorption and desorption capacity

The following equations are used to quantify the sorption and desorption capacities:

Sorption evaluation

$$Q_e = (C_0 - C_e)\frac{V_i}{W} \quad (1)$$

Table 2
The effect of pH value of the solution of licorice extracts on the adsorption ratio of four kinds of macroporous resins towards glycyrrhizic acid (initial concentration, 0.723 mg/ml)

pH Value	Adsorption ratio (%)			
	XDA-1	LSA-20	D101	LSA-10
5	99.92	93.66	88.69	87.35
6	98.95	78.21	69.84	60.58
7	97.73	65.36	52.21	44.64
8	94.61	56.11	47.99	37.89

$$E = \frac{(C_0 - C_e)}{C_0}100\% \quad (2)$$

where Q_e is the adsorption capacity at adsorption equilibrium (mg/g resin); E is the adsorption ratio (%), which is the per cent of the mass of total adsorbate being adsorbed after reaching equilibrium; C_0 and C_e are the initial and equilibrium concentrations of solutes in the solutions, respectively (mg/ml); V_i is the volume of the initial feed solution(ml) and W is the weight of the dry adsorbent (g).

Desorption evaluation

$$D = \frac{C_d V_d}{(C_0 - C_e)V_i}100\% \quad (3)$$

where D is the desorption ratio (%); C_d is the concentration of the solutes in the desorption solutions(mg/ml); V_d is the volume of the desorption solution; C_0, C_e and V_i are the same as those defined above.

3. Results and discussion

3.1. Effect of initial solution pH

The pH dependence of adsorption is of importance since the pH determines the extent of ionization of GA and flavonoids molecules, thereby affecting their adsorption affinity. As shown in Tables 2 and 3, for all the resins studied, the adsorption capacities and adsorption ratio of XDA-1 towards both GA and LF are the highest. A decrease in pH value results in an increase in adsorption capacity and adsorption ratio for both GA and LF. The observation of high Q_e and adsorption ratio at low pH value suggests that hydrogen bonding plays an important role in the adsorption/desorption pro-

Table 3
The effect of the pH value of the solution of licorice extracts on the adsorption ratio of four kinds of macroporous resins towards licorice flavonoids (initial concentration, 0.366 mg/ml)

pH Value	Adsorption ratio (%)			
	XDA-1	LSA-20	D101	LSA-10
5	93.28	90.73	83.54	79.89
6	90.85	87.53	80.82	76.25
7	89.62	77.49	76.78	73.47
8	83.72	69.10	67.61	59.01

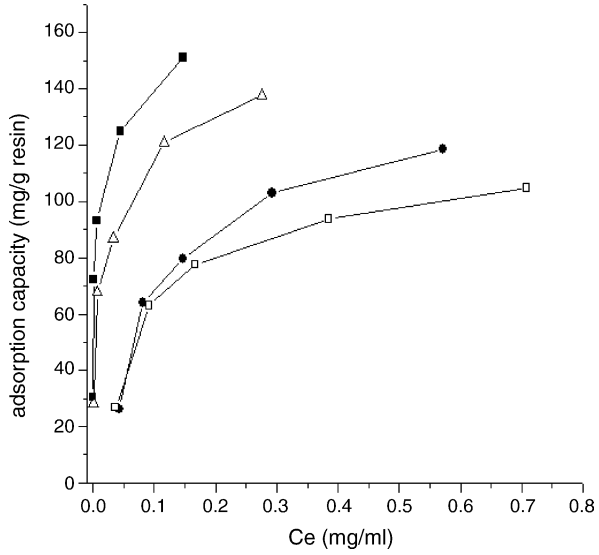

Fig. 1. Adsorption isotherm at 25 °C for glycyrrhizic acid on XDA-1 (■), LSA-20 (△), D101 (●), LSA-10 (□).

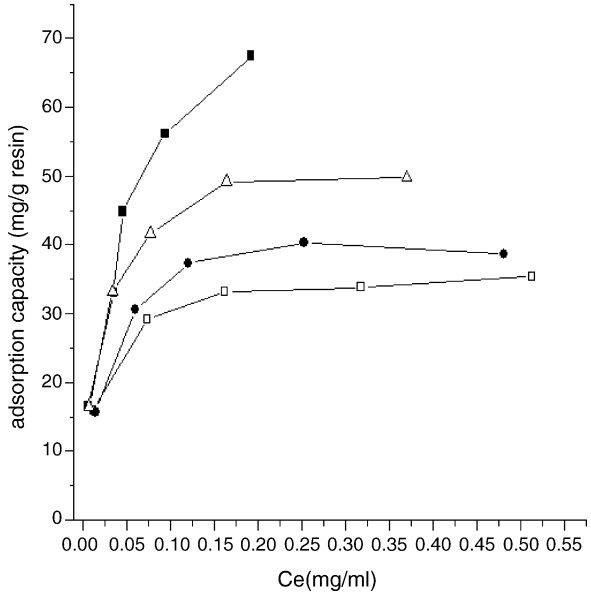

Fig. 2. Adsorption isotherm at 25 °C for licorice flavonoids on XDA-1 (■), LSA-20 (△), D101 (●), LSA-10 (□).

cesses for both XDA-1 and LSA-10. Both resins have polar functional groups such as acylamino and phenolic hydroxyl groups on the surface. At higher pHs, the hydrogen bonding interactions are reduced because the phenolic hydroxyl groups in flavonoids and the carboxyl groups in glycyrrhizic acid both dissociate to form H^+ and their corresponding anions. Such ionization processes also reduced their adsorption on non-polar resins D101 and LSA-20. Thus, the pH of the solution was adjusted to 5 for all the later experiments.

3.2. Adsorption isotherms

Equilibrium adsorption isotherms were obtained by contacting 15 ml of aqueous solution of licorice extract at different concentrations with the resins in a shaker bath controlled at 25 °C. The C_0 of GA in the solutions were 0.305, 0.723, 0.942, 1.322, and 1.757 mg/ml, respectively. The C_0 of total flavonoids in the corresponding solutions were 0.172, 0.366, 0.494, 0.656, and 0.867 mg/ml, respectively.

As can be seen from Figs. 1 and 2, for both GA and LF, the adsorption reached the saturation plateau when the initial concentration of GA was 1.322 mg/ml and the corresponding initial concentration of LF was 0.656 mg/ml. Thus, the concentrations of GA and LF in the feed solution were selected at 1.3 and 0.7 mg/ml, respectively.

The experimental data were fitted to the Freundlich Eq. (4) to describe how solutes interact with the adsorbents:

$$Q_e = K_F C_e^{1/n} \quad (4)$$

where K_F is the Freundlich constant that is an indicator of adsorption capacity, and $1/n$ is an empirical constant related to the magnitude of the adsorption driving force [19].

A linearized form of Eq. (4) can be written as:

$$\lg Q_e = \lg K_F + \left(\frac{1}{n}\right) \lg C_e \quad (5)$$

Freundlich isotherms can be plotted using Eq. (5). The K_F and $1/n$ values can be obtained from the intercept and slope, respectively, in the linear regression line from a plot of lg Q_e versus lg C_e. The Freundlich parameters are summarized in Table 4. Among the four kinds of resins, the isothermal adsorption equation of XDA-1 correlates the best with Freundlich equation. The correlation coefficients for LF and GA are 0.9744 and 0.9937, respectively. Compared with other resins, K_F of both GA and LF on XDA-1 are high, indicating its high adsorption capacity towards both species. Moreover, its $1/n$ value for LF is the largest and $1/n$ value for GA is the smallest compared with those of other resins. Differences in

Table 4
Freundlich adsorption parameters of LF and GA on four kinds of macroporous resins at 25 °C

		XDA-1	LSA-20	D101	LSA-10
LF	Freundlich equation	$Q_e = 145.8C_e^{0.4259}$	$Q_e = 78.0C_e^{0.2887}$	$Q_e = 56.1C_e^{0.2656}$	$Q_e = 44.9C_e^{0.214}$
	Correlation coefficient R^2	0.9744	0.911	0.8371	0.9018
	$1/n$	0.4259	0.2887	0.2656	0.214
GA	Freundlich equation	$Q_e = 191.8C_e^{0.1347}$	$Q_e = 217.2C_e^{0.2774}$	$Q_e = 190.1C_e^{0.5372}$	$Q_e = 141.8C_e^{0.4312}$
	Correlation coefficient R^2	0.9937	0.9595	0.8539	0.8667
	$1/n$	0.1347	0.2774	0.5372	0.4312

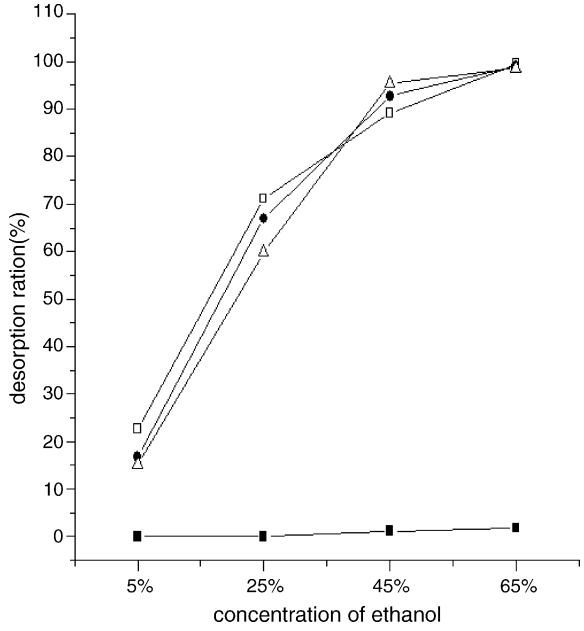

Fig. 3. Static desorption ratio of glycyrrhizic acid on XDA-1 (■), LSA-20 (△), D101 (●), and LSA-10 (□) resins at 60 °C. Desorption was by elution with different concentrations of aqueous ethanol solution.

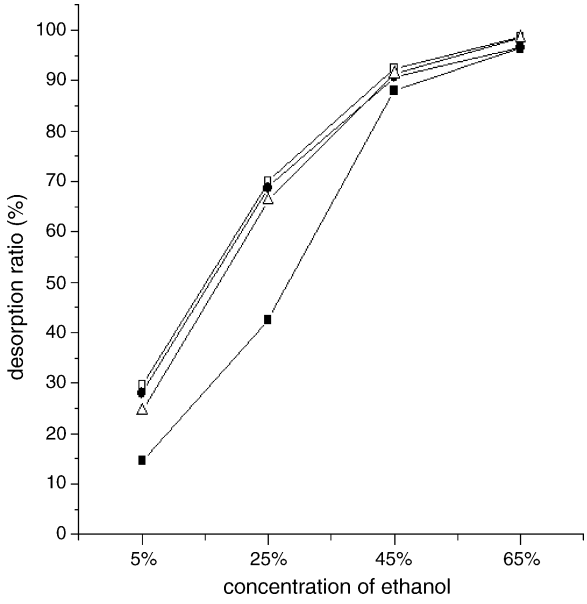

Fig. 4. Static desorption ratio of licorice flavonoids on XDA-1 (■), LSA-20 (△), D101 (●), and LSA-10 (□) resins at 60 °C. Desorption was by elution with different concentrations of aqueous ethanol solution.

the *1/n* value indicate that the affinity of XDA-1 for LF is the highest among all resin types. On the other hand, the driving force for adsorption by XDA-1 is the smallest among all resins, i.e., its adsorption towards GA is the weakest. The data thus illustrate that XDA-1 is the resin of choice for separating GA and LF.

3.3. Static desorption

The adsorption process was conducted by the procedure described in Section 2.5. After adsorption equilibrium was reached, the adsorbates were desorbed for 6 h in shakers at 60 °C using 15 ml of 5, 25, 45, and 65% aqueous ethanol solutions, respectively.

Figs. 3 and 4 show that with the increase of the ethanol concentration, the desorption ratios of both LF and GA increase. GA is highly polar because of the presence of three carboxyl groups, resulting in its highly adsorptive interactions with the XDA-1 resin. It could not be eluted even by 65% of aqueous ethanol solution. In contrast, the desorption ratio of LF on XDA-1 by 65% ethanol was 96.4%. As a result, GA and LF were well separated on the resin. No such separation was achievable on other resins.

3.4. Adsorption kinetics on XDA-1

Adsorption kinetics curves were obtained for GA and LF on XDA-1. As can be seen from Figs. 5 and 6, the adsorption capacity increases with adsorption time, reaching equilibrium in about 4.0 h for both GA and LF. The adsorption rate that is the slope of the tangent at different time on the kinetics curve decrease rapidly, and then levels off after 200 min for both GA and FL. The adsorption behavior is consistent with Langmuir monomolecular layer adsorption theory. The fast initial step is likely due to the occurrence of adsorption in the easily accessible mesopores of the particles, proceeding with low mass transfer in the bulk solution. The later slower uptake, on the other hand, is indicative of processes with high intraparticle mass transfer resistance.

3.5. Dynamic adsorption on XDA-1

Table 5 summarizes results from dynamic adsorption. The highest adsorption capacity is observed when the initial

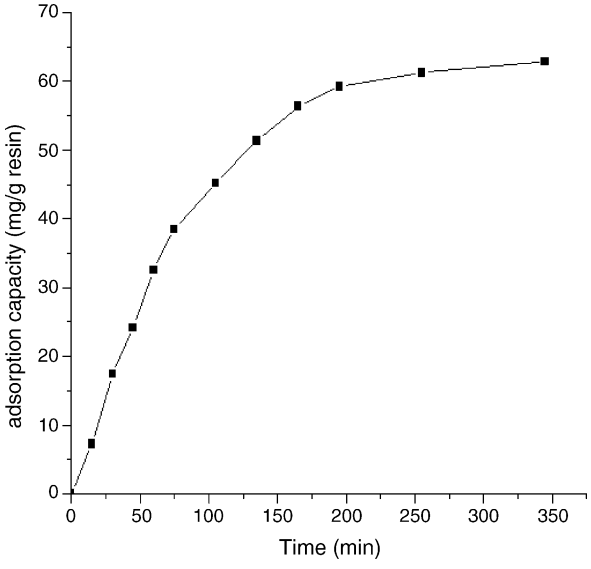

Fig. 5. Adsorption kinetics curve for GA on XDA-1.

Table 5
Breakthrough volume and mass of GA and LF adsorbed on XDA-1 at different feed concentrations under dynamic adsorption conditions

C_{0GA} (mg/ml)	C_{0LF} (mg/ml)	Breakthrough volume (ml)	Mass of GA adsorbed (mg)	Mass of LF adsorbed (mg)
1.08	0.542	150	162	81
1.5	0.75	158	237	118
1.85	0.946	85	157	80

concentration of GA and LF are 1.5 and 0.75 mg/ml, respectively. When the feed concentration was low, the amount of the adsorbate relative to active sites is low, and adsorption increased proportionally with increasing concentration of the GA and LF. However, with further increase of feed concentration, the amount of other impurities also increased, and the active site-to-absorbate ratio reduced. These species would compete for active sites on the resins with GA and LF, each in their own concentration-dependent rate, resulting in the observed lower adsorption of the target solute. Furthermore, the lower diffusivity of GA and LF into the micropores of the resins at high feed concentrations also affected the adsorption of GA and LF.

3.6. Dynamic desorption on XDA-1

Fig. 7 illustrates the HPLC profile of the raw feed solution. Peaks of GA and individual flavnoids (the flavnoids with retention times of 5.3, 7.4, and 8.1 min correspond to liquiritin, licuraside, and isoliquiritin, respectively [20]) are marked. Fig. 8 shows the HPLC profile of the desorbed licorice solution after XAD-1 adsorption. As shown, GA is completely removed from the extract while the major licorice flavonoids peaks remain in the sample. The desorbed solution was freeze-dried after removing ethanol. The flavonoids purity, defined as the wt.% of flavonoids in the DGL powder, and the estimated recovery of total flavonoids are 21.9 and 74.8%, respectively, comparing to 2.86 and 64.9%, respectively from conventional acid precipitation method.

Afterwards, the remaining GA was eluted off from the column by washing continuously with 80% aqueous ethanol solution containing 1% NaOH. The HPLC-UV purity

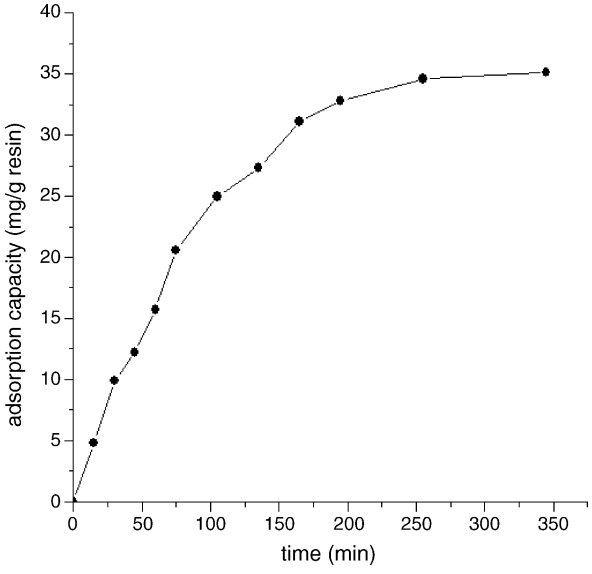

Fig. 6. Adsorption kinetics curve for LF on XDA-1.

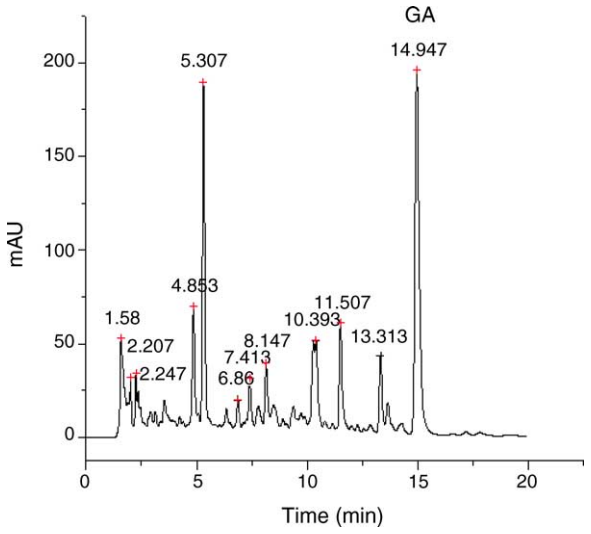

Fig. 7. HPLC profile of the feed solution of licorice extract monitored at 254 nm.

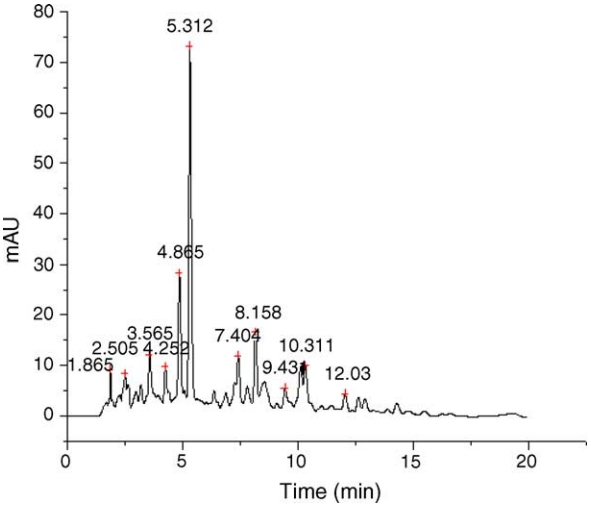

Fig. 8. HPLC profile of the DGL solution desorbed from XDA-1 by 45% aqueous ethanol solution and monitored at 254 nm.

of GA was 65.6%, and the mass recovery of GA was 52%.

4. Conclusion

In this study, the performance and separation characteristics of four widely used macroporous resins for the preparation of deglycyrrhizinated, flavonoids enriched licorice (DGL, have been critically evaluated. Among the four resins investigated, XDA-1 provides the best fit to Freundlich isotherm. It offers the highest adsorption capacity for GA and LF because of its higher surface area, optimum average pore diameter and appropriate surface functional polarity. The adsorption affinity of XDA-1 towards LF is the highest while its affinity towards GA is the lowest among all four resins. XAD-1 thus provides the best separation power for the two classes of compounds in licorice. The adsorption rate curves indicate that the adsorption on XDA-1 fits the Langmuir monomolecular layer adsorption theory. DGL can be obtained in high yield and high purity by resin adsorption followed by desorption with 45% ethanol aqueous solution. Compared to the conventional acid precipitation method, the adsorption method is advantageous because of its procedural simplicity, low cost, high efficiency, and ease in upscaling.

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References

[1] J. Bruneton, Pharmacognosy, Phytochemistry, Medicinal Plants, Lavoisier Publishing, Paris, 1995.
[2] Y. Fujisawa, M. Sakamoto, M. Matsushita, T. Fujita, K. Nishioka, Microbiol. Immunol. 44 (2000) 799.
[3] A.R. Dehpour, M.E. Zolfaghari, T. Samadian, F. Kobarfard, M. Faizi, M. Assari, Int. J Pharm. 119 (1995) 133.
[4] I. Akihiko, H. Norio, K. Kazuhiro, H. Hideki, K. Akinori, K. Toru, Y. Harumasa, F. Hideyuki, K. Takenobu, Int. Hepatol. Commun. 50 (1997) 233.
[5] T. Utsunomiya, M. Kobayashi, R.B. Pollard, F. Suzuki, Antimicrob. Agents Chemother. 41 (1997) 551.
[6] J. Cinatl, B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, H.W. Doerr, Lancet 361 (2003) 2045.
[7] H. Sasaki, M. Takei, M. Kobayashi, R.B. Pollard, F. Suzuki, Pathobiology 70 (2002) 229.
[8] B.R. Walker, C.R. Edwards, Endocrin. Metab. Clin. 23 (1994) 359.
[9] J. Heikens, E. Fliers, E. Endert, M. Ackermans, G. van Montfrans, Neth. J Med. 47 (1995) 230.
[10] M.M. Rafi, B.C. Vastano, N. Zhu, C.T. Ho, G. Ghai, R.T. Rosen, M.A. Gallo, R.S. DiPaola, J Agric. Food. Chem. 50 (2002) 677.
[11] T. Fukai, A. Marumo, K. Kaitou, T. Kanda, T. Nomura, Life Sci. 71 (2002) 1449.
[12] H. Haraguchi, H. Ishikawa, K. Mizutani, Y. Tamura, T. Kinoshita, Bioorg. Med. Chem. 6 (1998) 339.
[13] L. Glick, Lancet 340 (1982) 817.
[14] S.K. Das, V. Das, A.D. Gulati, V.P. Singh, J Assoc. Phys. Ind. 37 (1989) 647.
[15] A. Bennett, T. Clark-Wibberley, J.E. Wright, J Pharm. Pharmacol. 32 (1980) 151.
[16] D.H. Fu, A.L. Liu, N.K. Deng, T.J. Zhou, Nat. Prod. Res. Dev. 14 (2002) 60.
[17] D.S. Grzegorczyk, G. Carta, Chem. Eng. Sci. 51 (1996) 807.
[18] R.S. Juang, J.Y. Shiau, J Hazard. Mater. B 70 (1999) 171.
[19] M.W. Jung, K.H. Ahn, Y.H. Lee, K.J. Paeng, J Chromatogr. A 917 (2001) 87.
[20] B.Q. Fu, H. Li, X.R. Wang, F. S.C. Lee (submitted to J. Agric. Food Chem.).