Journal of Chromatography B, 867 (2008) 253–258

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Separation of chlorogenic acid from honeysuckle crude extracts by macroporous resins

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ARTICLE INFO

Article history:
Received 14 June 2007
Accepted 11 April 2008
Available online 2 May 2008

Keywords:
Adsorption
Chlorogenic acid
Desorption
Macroporous resins
Preparative separation

ABSTRACT

Chlorogenic acid, one of the most bioactive compounds rich in the Chinese medicinal herb honeysuckle, is a natural antioxidant and serves as anti-inflammatory, anti-tumor, anti-mutagenic and anti-carcinogenic agent. An efficient preparative separation process of chlorogenic acid from honeysuckle crude extracts has been developed in the present study. HPD-850 resin offers the best adsorption capacity, and adsorption and desorption ratios for chlorogenic acid among the nine macroporous resins tested, and its adsorption rate at 25 °C fit best to the Langmuir isotherm. The adsorption capacity of HPD-850 resin was found to depend strongly on the pH value of the initial adsorption solution. The dynamic adsorption and desorption experiments have been carried out on a HPD-850 resin packed column to optimize the separation process of chlorogenic acid from honeysuckle crude extracts. After one run treatment with HPD-850 resin, the chlorogenic acid content in the final product was increased 4.46-fold from 11.2% to 50.0%, with a recovery yield of 87.9%. The preparative separation of chlorogenic acid can be easily and efficiently achieved via adsorption and desorption on HPD-850 resin, and the method developed will provide a potential approach for large-scale separation and purification of chlorogenic acid for its wide pharmaceutical use.

1. Introduction

Honeysuckle is the flowers of *Lonicera japonica* Thunb. which has been planted widely in China. It is one of the most famous traditional Chinese medicinal herbs, and usually used to cure common cold and fever [1]. As one of the major bioactive compounds rich in honeysuckle, chlorogenic acid (Fig. 1) can significantly suppress the *N*-nitrosating reaction and inhibit hepatic glucose 6-phosphatase which is a significant factor in the abnormal diabetic state [2], and also serve as antioxidant, anti-inflammatory, anti-tumor, anti-mutagenic and anti-carcinogenic agent [3–6]. The pharmaceutical products containing chlorogenic acid with different purities ranged from 20% to 98% have been widely used as healthcare products, oral liquid & tablets & capsules, herbal injections and so on.

The conventional method for separation of chlorogenic acid from the crude extracts of honeysuckle was performed by solid–liquid extraction or solvent extraction, followed by polyamide chromatography and gel chromatography [7]. The traditional separation process is not effective regarding reagents, energy consumption and labor intensiveness. Recently, preparative high-speed counter-current chromatography and molecular imprint have been developed to efficiently obtain a limited amount of chlorogenic acid with high purity [8,9].

There has been a growing interest in employing macroporous resins to separate bioactive compounds from traditional Chinese herbs because of their unique adsorption properties including ideal pore structure and various surface functional groups available, low operation expense, less solvent consumption and easy regeneration [10–12]. Macroporous resins can be used to selectively adsorb constituents from aqueous solution as well as non-aqueous systems through electrostatic force, hydrogen bonding interaction, complexation, and size sieving action, etc., and they are durable non-polar (polystyrene), middle polar (ester group) and polar (amide, amidocyanogen, acylamino polystyrene) macroporous polymers having a high adsorption capacity. Few studies have been attempted to separate chlorogenic acid from honeysuckle by macroporous resins [13,14], and a relatively low recovery of chlorogenic acid from crude extracts was achieved in the moderate separation process.

In order to achieve efficient large-scale adsorption–desorption separation process of chlorogenic acid, a detailed investigation on suitable macroporous resin and its adsorption properties is needed.

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doi:10.1016/j.jchromb.2008.04.016

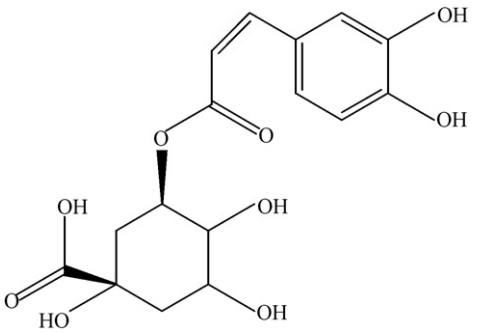

Fig. 1. Chemical structure of chlorogenic acid.

The aim of the current work is to investigate the adsorption and desorption properties of chlorogenic acid on different macroporous resins, and to develop an efficient method for the preparative separation of chlorogenic acid from honeysuckle crude extracts with the optimal resin.

2. Experimental

2.1. Materials and regents

Dry honeysuckle (*Flos Lonicera japonica*) provided by local company in Sichuan province of China was ground into 60 mesh powders by a mortar (selected by sieve), and then was kept at room temperature. The crude honeysuckle extracts were prepared by microwave-assisted extraction with 40 g honeysuckle powder in distilled water (400 ml) using a modified microwave oven for 30 min [15]. Microwave irradiation was set at 700 W, and ran in a circle of 2 s with "power on" with 8 s with "power off" to keep the temperature of the extraction solvent at 60 °C. The extraction solution was filtered and stored at 4 °C in the absence of light for the subsequent experiments.

Analytical-grade ethanol was purchased from Atoz Fine Chemicals Co. Ltd. (Tianjin, China). HPLC-grade methanol was purchased from Concord Tech Co. Ltd. (Tianjin, China). All aqueous solutions were prepared with pure water produced by Milli-Q system (Bedford, MA, USA). Macroporous resins including HPD-300, HPD-450, HPD-500, HPD-700, HPD-750 and HPD-850 were provided by Bonherb Technology Company (Hebei, China), and X-5, AB-8, NKA-II were purchased from Chemical Plant of Nankai University (Tianjin, China). The adsorbent beads were pretreated according to the manufacturer's specifications before experiments. Briefly, the resins were pretreated by 1 M HCl and NaOH solutions successively to remove the monomers and porogenic agents trapped inside the pores during the synthesis process, and then were dried at 60 °C under vacuum. A given amount of macroporous resins was soaked with 95% ethanol for 12 h and subsequently washed by pure water thoroughly before use. The moisture content of these resins was determined as follows: three samples of each kind of macroporous resins were weighed, and then placed in a drying oven at 110 °C until the mass did not change. Their physical properties are listed in Table 1.

2.2. Analysis

Quantitative analysis of chlorogenic acid concentration was carried out by HPLC on Agilent 1100 HPLC system composed of a quaternary pump with a degasser, a thermostatted column compartment, a variable wavelength detector, an autosampler and 1100 ChemStation software. Sample analyses were performed on an Alltech C18 column (250 mm × 4.6 mm I.D., 5 μm) fitted with an Alltech C18 guard cartridge (8 mm × 4.6 mm I.D., 5 μm) at a column temperature of 25 °C. The mobile phase was methanol–water–acetic acid (20:78.4:1.6, v/v/v) at a flow rate of 1 ml/min, and the effluent was monitored at 327 nm by UV detector. The reference standard of chlorogenic acid was supplied by National Institute for the Control of Pharmaceutical and Biological Product with the purity no less than 98%. All samples were centrifuged at 4250 × *g* for 5 min, and filtered through 0.45 μm membrane before HPLC analysis.

2.3. Static adsorption and desorption tests

All macroporous resins were screened through static adsorption tests which were performed as follows: the hydrated resin of 1 g dry mass was put into an Erlenmeyer flask and 20 ml of aqueous solution of honeysuckle extracts (1.11 mg/ml chlorogenic acid) was added. The flask was shaken on an incubation shaker (120 rpm) at 25 °C for 24 h to reach adsorption equilibrium. The solution after adsorption was analyzed by HPLC. After adsorption equilibrium was reached, the resin was first washed by pure water (160 ml) and then desorbed with 20 ml 95% ethanol (v/v) in the flask on an incubation shaker (120 rpm) at 25 °C for 4 h. The desorption solution was analyzed by HPLC.

The preliminary choice of these resins was evaluated by their adsorption capacity, and the ratios of adsorption and desorption. The adsorption property of HPD-850 resin selected from the preliminary experiments was investigated at different pH values of adsorption solution. The equilibrium adsorption isotherms of chlorogenic acid on HPD-850 resin were studied by containing 20 ml sample solution of honeysuckle crude extracts at different initial concentrations with the preweighed hydrated resins (equal to 1 g dry resin), and then shaking at 25 °C for 24 h, and their fitness to Freundlich and Langmuir equations were evaluated.

The adsorption kinetics of chlorogenic acid on HPD-850 resin was also studied. Preweighed hydrated adsorbent (equal to 5 g dry resin) was introduced into an Erlenmeyer flask containing 100 ml of aqueous solution of honeysuckle crude extracts. The initial concentration of chlorogenic acid was 1.11 mg/ml at pH 3, and the flask was shaken (120 rpm) at 25 °C. The concentration of chlorogenic acid in liquid phase was monitored at certain time intervals till equilibration.

2.4. Dynamic adsorption and desorption

Dynamic adsorption and desorption experiments were carried out on glass columns (20 mm × 300 mm) wet-packed with HPD-850 resin, and the bed volume (BV) of the resin was 40 ml (equal to 15 g of dry resin). Sample solution containing various levels of chlorogenic acid (pH 3.0, adjusted by 3 M HCl) flowed through the glass column at a flow rate of 2 ml/min to test breakthrough point, and the chlorogenic acid in the eluents was monitored by HPLC analysis of the eluted aliquots collected at 5 ml intervals by a BSZ-100 auto-fractional collector (Shanghai, China). While adsorption equilibrium, the adsorbate-laden column was eluted by with ethanol–water (70:30, v/v) solution at a flow rate of 1 ml/min, and the concentration of chlorogenic acid in the desorption solution was determined by HPLC.

2.5. Calculation of adsorption capacity, adsorption and desorption ratios

The capacity of adsorption, and adsorption and desorption ratios are calculated as follows.

Table 1
Physical properties of macroporous resins

Name	Surface area (m^2/g)	Average pore diameter (Å)	Moisture content (%)	Polarity
NKA-II	160–200	145–155	38.6	Polar
HPD-500	500–550	100–120	52.9	Polar
HPD-850	1100–1300	85–95	71.1	Polar
AB-8	480–520	130–140	65.7	Moderately polar
HPD-450	500–550	90–110	71.4	Moderately polar
HPD-750	650–700	85–90	58.8	Moderately polar
X-5	500–600	290–300	62.4	Non-polar
HPD-700	650–700	85–90	60.8	Non-polar
HPD-300	800–870	50–55	70.3	Non-polar

Adsorption capacity:

$$Q_e = \frac{V_0(C_0 - C_e)}{W} \quad (1)$$

Adsorption ratio:

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

where Q_e is the adsorption capacity, which represents the mass of adsorbate adsorbed on 1 g of dry resin at adsorption equilibrium; E is the adsorption ratio, which means percentage of total adsorbate being adsorbed at adsorption equilibrium; C_0 and C_e is the initial and equilibrium concentration of chlorogenic acid in the solutions, respectively; V_0 is the initial volume of solution added into the flask, and W is the weight of the dry resin.
Desorption ratio:

$$D = \frac{C_d V_d}{(C_0 - C_e)V_0} \quad (3)$$

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_0 are the same as those defined above.

3. Results and discussion

3.1. Selection of macroporous resins suitable for separation of chlorogenic acid

Nine macroporous resins with different physical properties were employed for separation of chlorogenic acid, and the results were listed in Table 2. The adsorption and desorption ratios of chlorogenic acid on HPD-850 resins were higher than those of other resins because that the HPD-850 resin with higher polarity and larger surface area showed stronger adsorption capacity to polar substance chlorogenic acid. Similar observation was also found in luteolin separation from pigeonpea leaves by macroporous resins [12]. Therefore, HPD-850 was picked out for the further study of adsorption process of chlorogenic acid.

Table 2
Results of adsorption capacity (Q_e), adsorption ratio (E) and desorption ratio (D) of chlorogenic acid for different resins examined

Name	Q_e (mg/g resin)	E (%)	D (%)
NKA-II	8.44	37.9 ± 2.3	51.2 ± 1.9
HPD-500	17.90	80.4 ± 3.1	71.4 ± 2.5
HPD-850	20.03	90.0 ± 1.4	95.8 ± 0.7
AB-8	7.86	35.3 ± 2.6	43.6 ± 3.1
HPD-450	10.66	47.9 ± 2.1	48.6 ± 2.4
HPD-750	11.40	51.2 ± 1.8	72.8 ± 3.5
X-5	5.74	22.4 ± 1.9	54.9 ± 2.2
HPD-700	8.37	37.6 ± 1.9	78.7 ± 1.4
HPD-300	17.96	80.7 ± 2.1	77.4 ± 1.5

Initial concentration of chlorogenic acid in adsorption solution: 1.11 mg/ml; desorption solution: 95 % (v/v) ethanol; adsorption and desorption temperature: 25 °C; shaking speed: 120 rpm.

3.2. Effect of the pH value of sample solution

The most important parameter influencing the sorption capacity is the initial pH of adsorption solution [16]. The initial pH of adsorption solution is related to the adsorption mechanisms onto the adsorbent surface from water and reflects the nature of the physicochemical interaction of chlorogenic acid in solution and the adsorptive sites of adsorbent. As shown in Fig. 2, the adsorption ratio increased greatly for chlorogenic acid with the decrease of pH value. There is no obvious change on adsorption ratio of HPD-850 when pH value was lower than 3. At lower pH values, the surface of the resin would be surrounded by the hydronium ions which enhance the interaction of the unionized phenolic hydroxyl groups of chlorogenic acid with the macroporous resin by greater attractive forces. At higher pH values, the adsorption of chlorogenic acid on HPD-850 resin was probably caused by electrostatic interactions due to ionization of chlorogenic acid with the increase of pH. In addition, pH value of 3 was found to be favorable for the stability of chlorogenic acid in water solution in previous study [17]. Therefore, the pH value of the solution was adjusted to 3 for all later experiments.

3.3. Adsorption isotherms

The Langmuir and Freundlich equations are the most popular ones frequently used in description of the experimental data of adsorption isotherms because of their relative simplicity and

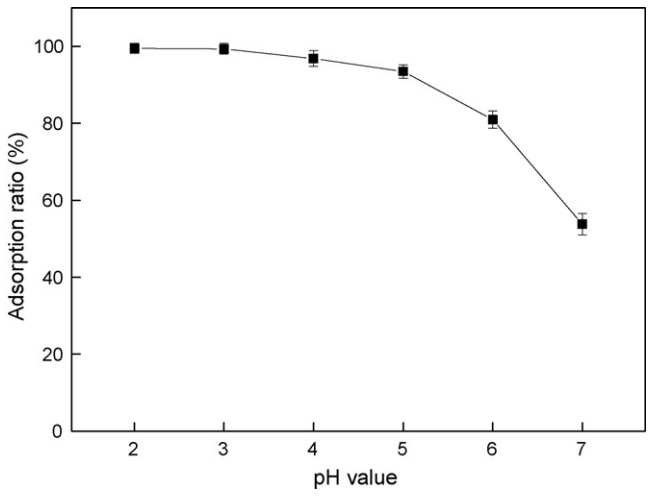

Fig. 2. Effect of pH value on the adsorption capacity of chlorogenic acid on HPD-850 resin. Initial concentration of chlorogenic acid in adsorption solution: 1.06 mg/ml; adsorption temperature: 25 °C; shake speed: 120 rpm.

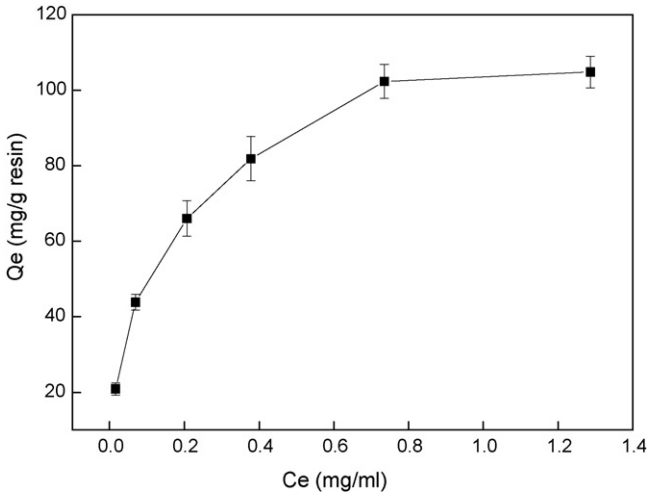

Fig. 3. Adsorption isotherm of chlorogenic acid on HPD-850. Initial concentrations of chlorogenic acid in adsorption solutions: 1.06, 2.26, 3.51, 4.47, 5.85 and 6.53 mg/ml; adsorption temperature: 25 °C; shake speed: 120 rpm.

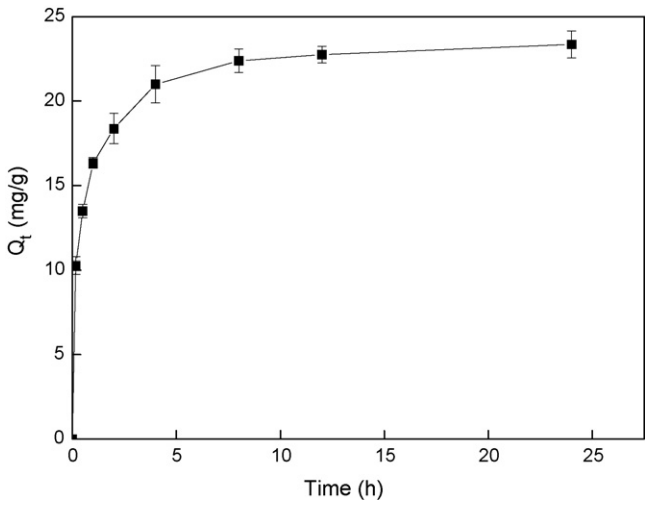

Fig. 4. Adsorption kinetics of chlorogenic acid on HPD-850 at 25 °C. Initial concentration of chlorogenic acid in adsorption solution: 1.11 mg/ml; adsorption temperature: 25 °C; shake speed: 120 rpm.

reasonable accuracy [18]. The Langmuir equation can be used to describe a monolayer adsorption, whereas the Freundlich equation can be used to describe a monolayer adsorption as well as a multilayer adsorption.

Freundlich equation:

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

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

Langmuir equation:

$$Q_e = \frac{Q_0 K_L C_e}{1 + K_L C_e} \quad (5)$$

where K_L is the adsorption equilibrium constant, Q_0 is the theoretical maximum adsorption capacity (mg/g-resin).

The equilibrium adsorption isotherm of chlorogenic acid on HPD-850 was constructed at 25 °C, and the initial concentrations of chlorogenic acid were 1.06, 2.26, 3.51, 4.47, 5.85 and 6.53 mg/ml, respectively. As shown in Fig. 3, the adsorption capacity increased with the initial concentration, and reached the saturation plateau when the initial concentration of chlorogenic acid reached 5.85 mg/ml. All model parameters and correlation factor (R^2) were listed in Table 3. Langmuir equation described the better adsorption behavior of chlorogenic acid on HPD-850 because the correlation coefficient (0.996) of Langmuir equation was higher than that (0.972) of Freundlich equation. The theoretical maximum adsorption capacity Q_0 determined from the Langmuir equation was 114.16 mg/g, and the constant K_L, an indicator of the stability of the combination between adsorbate and adsorbent surface, was 8.80 ml/mg. In the Freundlich equation, the adsorption takes place easily when the $1/n$ value is between 0.1 and 0.5, and it is not easy to happen if $1/n$ value is above 1 [19]. In Table 3, the $1/n$ value was 0.376, which indicated that HPD-850 resin is favorable for the separation of chlorogenic acid.

3.4. Adsorption kinetics

Adsorption kinetic curve was obtained for chlorogenic acid on HPD-850. As shown in Fig. 4, the adsorption capacity increased with the extension of adsorption time, and reached equilibrium at pH 3.0 in 4 h. The adsorption behavior is consistent with Langmuir monomolecular layer adsorption theory. In order to determine the rate of the adsorption process, the adsorption kinetics data were analyzed with pseudo-first-order equation and pseudo-second-order equation [20,21].

The pseudo-first-order equation:

$$\frac{dQ_t}{dt} = K_1(Q_e - Q_t) \quad (6)$$

where Q_t is the amount adsorbed on the adsorbent at time t, and K_1 is the rate constant of pseudo-first-order. By integrating Eq. (6) at the boundary condition of $Q_t = 0$ at $t = 0$ and $Q_t = Q_t$ at time t, the Eq. (6) became Eq. (7).

$$\ln(Q_e - Q_t) = \ln Q_e - K_1 t \quad (7)$$

The pseudo-second-order equation:

$$\frac{dQ_t}{dt} = K_2(Q_e - Q_t)^2 \quad (8)$$

where K_2 is the rate constant of pseudo-second-order adsorption. By integrating Eq. (8) at the boundary condition of $Q_t = 0$ at $t = 0$ and $Q_t = Q_t$ at time t, the Eq. (8) became Eq. (9).

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t \quad (9)$$

Table 3
Freundlich and Langmuir adsorption isotherm parameters for chlorogenic acid on HPD-850 at 25 °C

	Linear equation	Q_0 (mg/g)	K_L (ml/mg)	R^2
Langmuir equation	$C_e/Q_e = 0.00880C_e + 0.00086$	114.16	8.802	0.996
	Linear equation	K_F (mg/g)	$1/n$	R^2
Freundlich equation	$\ln Q_e = 4.708 + 0.367 \ln C_e$	110.78	0.376	0.972

Table 4
Adsorption kinetics parameters for chlorogenic acid on HPD-850

Kinetic equation	Parameters		
	Q_e (mg/g)	k_1 (h)	R^2
Pseudo-first-order	11.60	0.279	0.906
	Q_e (mg/g)	k_2 (mg/g/h)	R^2
Pseudo-second-order	23.70	0.097	0.999
	k_i (mg/g/h$^{0.5}$)	C	R^2
Intraparticle diffusion	2.71	12.82	0.766

As shown in Table 4, the second-order equation ($R^2 = 0.999$) described the experimental data more accurate in comparison with the first-order equation ($R^2 = 0.906$) in consideration of equilibrium sorption capacities and correlation coefficients since Q_e of the first-order equation deviated significantly from the experimental value.

The adsorption kinetic data were further fitted to the linear intraparticle diffusion equation to determine whether intraparticle diffusion is rate-limiting step [22].

The intraparticle diffusion equation:

$$Q_t = k_i t^{0.5} + C \quad (10)$$

where k_i and C are the intraparticle diffusion rate constant.

As shown in Table 4 and Fig. 5, the adsorption data were not fit to the intraparticle diffusion equation. The results indicated that the adsorption process was controlled by two or more rate-limiting steps such as external diffusion, boundary layer diffusion and intraparticle diffusion.

3.5. Static desorption on HPD-850

As seen in Fig. 6, the desorption ratio of chlorogenic acid from HPD-850 resin increased with the increase of ethanol concentration and reached the maximum desorption ratio when using ethanol at a concentration of 70%. When ethanol concentration in desorption solution was over 70%, the desorption ratio of chlorogenic acid from HPD-850 resin did not increase more. The result indicated that both hydrophobic interaction and hydrogen bonding might exist between the HPD-850 resin and chlorogenic acid molecule. The purity of chlorogenic acid slightly decreased with increase of ethanol concentration because more impurities with less polarity were desorbed at higher ethanol concentrations. Therefore, ethanol–water (70:30, v/v) solution was selected as the appropriate desorption solution and was used in the dynamic desorption process.

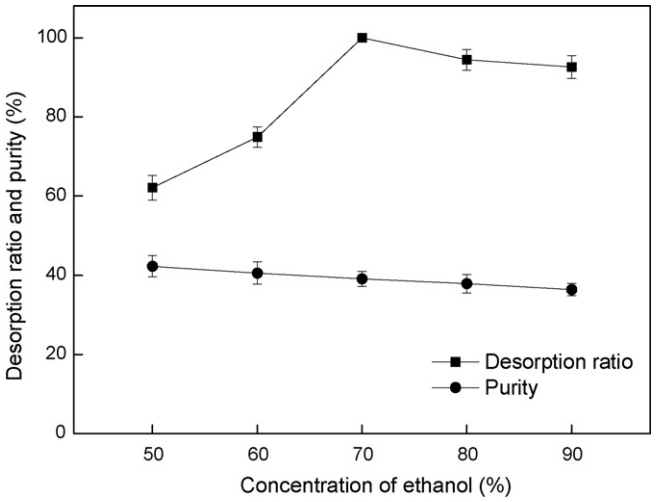

Fig. 6. Effect of ethanol concentration on the desorption ratio and purity of chlorogenic acid on HPD-850. Initial concentration of chlorogenic acid in adsorption solution: 1.06 mg/ml; adsorption and desorption temperature: 25 °C; shake speed: 120 rpm.

Table 5
Breakthrough volume and mass of chlorogenic acid (CHA) adsorbed on HPD-850 resin at different feed concentrations under dynamic adsorption conditions

Initial concentration (mg/ml)	Breakthrough point (ml)	Mass of CHA adsorbed (mg)
0.988	366 ± 27	361.61 ± 26.68
2.061	352 ± 19	725.47 ± 39.20
2.967	239 ± 12	709.11 ± 35.60
4.044	174 ± 7	703.66 ± 28.31
5.056	138 ± 5	697.73 ± 25.28

3.6. Dynamic adsorption and desorption

The results of dynamic adsorption were summarized in Table 5. The highest adsorption capacity was observed when the initial concentration of chlorogenic acid was 2.06 mg/ml. The adsorption

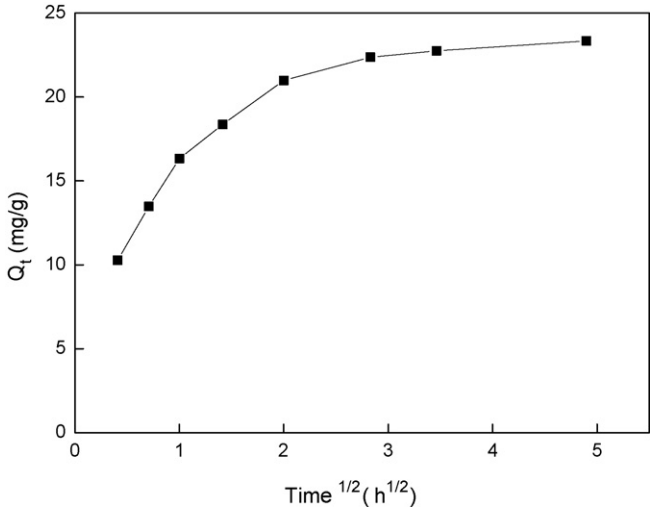

Fig. 5. Plot of Q_t vs. $t^{1/2}$ in intraparticle diffusion equation.

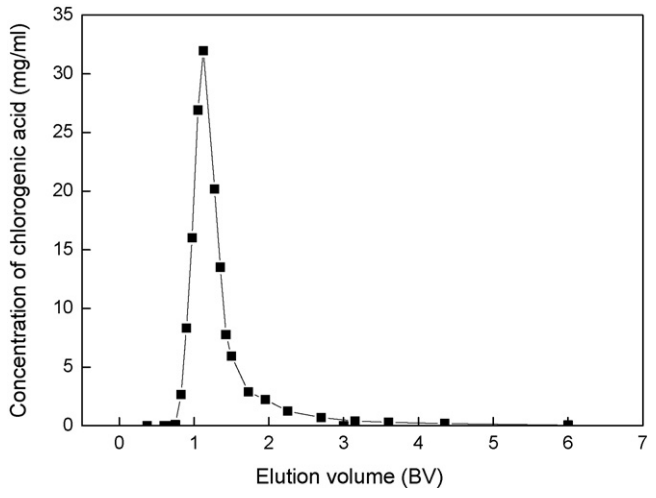

Fig. 7. Dynamic desorption curve of chlorogenic acid on a column packed with HPD-850 at 25 °C. Desorption solution: 70 % (v/v) ethanol; desorption flow rate: 1 ml/min.

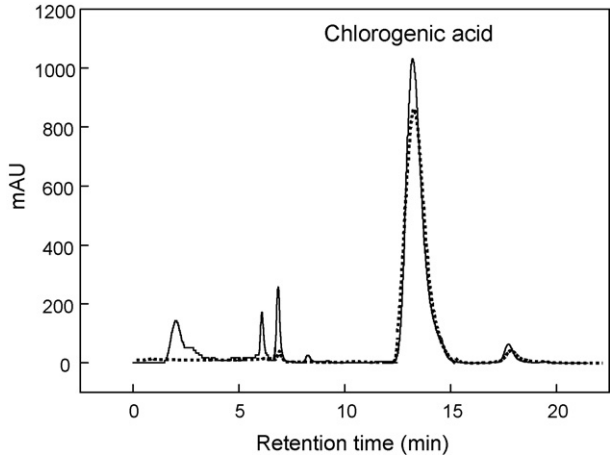

Fig. 8. Chromatograms of sample solution before (solid line) and after (dot line) separation on a column packed with HPD-850 resin. HPLC column: Alltech C18 (250 mm × 4.6 mm I.D., 5 μm); mobile phase: linear elution of methanol–water–acetic acid (20:78.4:1.6, v/v/v); flow rate: 1.0 ml/min; UV detection wavelength: 327 nm.

capacities decreased slightly at higher initial feed concentrations due to competition to active sites of HPD-850 resin by impurities in the crude extracts and limitation of diffusivity of chlorogenic acid into the micropores of HPD-850. Similar result was also observed in the separation of licorice flavonoids and glycyrrhizic acid by XDA-1 resin [10].

The dynamic desorption curve on HPD-850 was obtained based on the volume of desorption solution and the concentration of solute herein. As shown in Fig. 7, approximately 6 BV of desorption solution desorbed chlorogenic acid completely from HPD-850 resin at a flow rate of 1 ml/min. The chromatograms of the tested samples before and after treatment with HPD-850 resin were shown in Fig. 8. By comparison, it can be seen that some impurities in the crude extracts were removed and the relative peak area of chlorogenic acid increased after the separation on HPD-850 resin. The desorption solution was dried under vacuum. The dried product was weighed and the contents of chlorogenic acid were calculated. After treatment with HPD-850, the content of chlorogenic acid reached 50.0% in the product, which was 4.46-fold higher than that in honeysuckle crude extracts, and the recovery yield of chlorogenic acid was 87.9%. The HPD-850 resin was regenerated by flowing 400 ml NaOH solution (0.5 M) through the glass column at a flow rate of 2 ml/min, and subsequently washed thoroughly by pure water before its reuse. The HPD-850 exhibited excellent reusability property, and no remarkable change was observed on the separation performance of chlorogenic acid during 10 successive separation cycles.

4. Conclusion

The preparative separation process of chlorogenic acid with macroporous resin has been successfully developed in this study. Among the nine macroporous resins tested, HPD-850 offers the best separation efficiency for chlorogenic acid because of its high surface area and appropriate surface functional polarity, and its adsorption data at 25 °C fit better to the Langmuir isotherm. The adsorption kinetic data fit well to the second-order equation and two or more rate-limiting steps were found to influence the adsorption process. Using the HPD-850 resin at optimal conditions, the chlorogenic acid content in the product was increased 4.46-fold from 11.2% to 50.0% with a recovery yield of 87.9%.

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