academicJournals

Vol. 7(24), pp. 1784-1792, 25 June, 2013
DOI: 10.5897/JMPR11.630
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Separation and purification of chlorogenic acid from *Lonicera japonica* Thunb. leaves exact with macroporous resins

Jun Zhang[1], Shujun Zhang[2], Jin Wu[1], Na Li[2] and Junru Wang[2]*

[1]College of Life Sciences, Northwest A and F University, Yangling, Shaanxi 712100, China.
[2]College of Science, Northwest A and F University, Yangling, Shaanxi 712100, China.

Accepted 7 October, 2011

Chlorogenic aicd is a main bioactive constituent in *Lonicera japonica* Thunb. leaves. In this study, a simple method for separation and purification of chlorogenic acid from *L. japonica* leaves was established with macroporous resins. The performance and adsorption characteristics of four macroporous resins had been evaluated and D-101 was affirmed to be the suitable resin for the separation of chlorogenic acid. Dynamic adsorption and desorption tests performed in a column packed with D-101 resin were followed to optimize the process parameters. The final results showed that the highest purity of the chlorogenic acid product was 50.72% with a recovery yield of 54.44% when the parameters were as follows: a sample concentration in solution of 1.01 mg/mL chlorogenic acid, a pH value of 4, a flow rate of 2 bed volume (BV)/h, a sample volume of 5 BV and an elution solvent of 20% (v/v) ethanol.

Key words: Macroporous resins, *Lonicera japonica* Thunb. Leaves, chlorogenic acid, adsorption, desorption ratios.

INTRODUCTION

Lonicera japonica Thunb. (Caprifoliaceae) is an important traditional herb listed in Chinese Pharmacopoeia. It is often used as an anti-inflammatory agent to treat fever, headache, upper respiratory tract infections, urinary disorders, rheumatoid arthritis, and diabetes mellitus (Ubukata et al., 1992; Ko et al., 2006; Kwak et al., 2003). Honeysuckle and flower buds are main commercial resources of chlorogenic acid (CA) (Figure 1), which is a polyphenolic compound and possesses the ability of antioxidant, antidiabetic, antivirus, anticancer and anti-inflammation (Dillard and German, 2000; Sabu et al., 2002; Jin et al., 2005; Nakamura et al., 1997; Jiang et al., 2001). Han et al. (2009) results on phytochemistry showed that, *L. japonica* leaves was also considered a good source of CA.

The conventional method to separate and purify CA from the crude extracts of honeysuckle is solid-liquid extraction or solvent traction, followed by polyamide chromatography and gel chromatography (Li et al., 2005), which is not efficient for high reagent, energy cost and unfavorable results. Alternatively, macroporous resins have been proven to provide a more efficient and economical method to separate bioactive compounds from crude extracts due to their ideal pore structure and various surface functional groups available, low operation expense, less solvent consumption and easy regeneration (Fu et al., 2005). They selectively absorb, recover and concentrate different kinds of compounds based on

*Corresponding author. E-mail: wangjr07@163.com. Tel: +86-29-87092829. Fax: +86-29-87092829.

Table 1. Physical properties of macroporous resins used.

Trade name	Surface area (m^2/g)	Average pore diameter (nm)	Particle diameter (mm)	Polarity	Moisture content (%)
LSA-20	≥630	30	0.3-1.25	Moderate polarity	69.1±0.74
D-101	≥400	9-11	0.3-1.25	Nonpolar	70.6±0.49
LSD-001	——	——	0.3-1.2	Polar	50.6±0.89
AB-8	480-520	12-16	0.3-1.25	Weakpolar	62.6±0.65

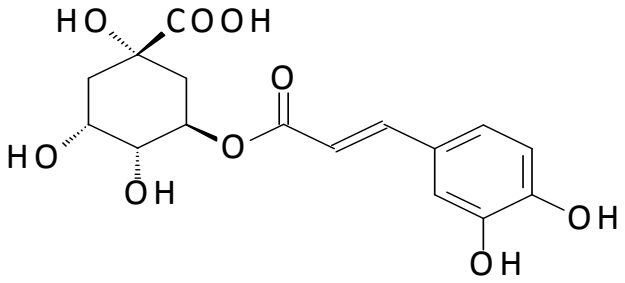

Figure 1. The structure of chlorogenic acid.

differences in molecular weight, polarity or shape of different molecules from the solution. They have been successfully applied for separation of flavonoids, saponins and alkaloids from traditional herbs (Fu et al., 2005; Zhao et al., 2007; Fu et al., 2007). However, few studies have been reported to separate CA from the crude extract of *L. japonica* leaves by macroporous resins. Also, in this study, ethanol is used as work solution, which could provide a safe and potentially important method for CA production.

Therefore, the objective of the present work was to investigate the adsorption and desorption properties of CA on different macroporous resins to select an optimal resin, and to optimize process parameters for the preparative separation of CA from *L. japonica* leaves crude extracts.

MATERIALS AND METHODS

Materials and reagents

L. japonica leaves were collected from Flos Lonicerae GAP Demonstration Bases in Hanzhong City of China and authenticated by Professor Zhaoyang Chang from the College of Life Sciences, Northwest A and F University, where a voucher specimen was deposited. The sample was dried at room temperature, powdered by an herb disintegrator (Shanghai HongJi Instrument Lu., Ltd.), and then sieved (<60 mesh). The CA standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) with the purity not less than 98%. Analytical grade ethanol was purchased from Xi'an Sanpu Fine Chemical Factory. High performance liquid chromatography (HPLC)-grade phosphoric acid, methanol and acetonitrile were purchased from Tianjin Kermel Chemical Reagent Co., Ltd., and deionized water from Hangzhou Wahaha Group Co., Ltd. All solutions and samples were filtered through 0.45 µm membranes (Fisher Scientific) before HPLC.

Adsorbents

Macroporous resins including LSA40, D-101, LSD-001 and AB-8 were supplied from Xi'an Sunresin Technology Co., Ltd.. Their physical properties are summarized in Table 1. In order to ensure the internal pores were thoroughly soaked, pretreated resins were immersed in 95% ethanol for 24 h and then washed by distilled water thoroughly.

The moisture contents of the tested resins were determined as follows: three samples of each kind of resin were weighed, and then placed in a drying oven at 110°C till a constant weight. The moisture contents are shown in Table 1.

High performance liquid chromatography (HPLC) analysis of chologenic acid (CA)

HPLC analysis of CA was performed on Shimadzu LC-20Atvp System (Shimadzu Corp., Kyoto, Japan) equipped with LC-10ATvp binary pump, a SPD-10Avp, a CTO-10Asvp column oven, a SCL-10Avp system controller and an LC (liquid chromatography) solution workstation. A reversed-phase column (Hypersil, 250 × 4.6 mm, 5 µm) (Dalian Elite Analytical Instruments Co., Ltd.) was used at a column temperature of 30°C. The mobile phase consisted of acetonitrile – 0.4% phosphoric acid (13:87, v/v) at a flow rate was 1.0 mL/min. The detection wavelength was set at 273 nm for acquiring chromatograms. All samples were determined in triplicate. The calibration curve for CA is y = 64357.72598x + 22238.94093 and the calibration range is 4×10^{-5}–4×10^{-4} mg (correlation coefficient R^2 = 0.9991).

Preparation of crude extract

Dried powder (200 g) was extracted three times with 2000 mL 70% ethanol at 60°C for 2 h. After filtration, the extracts were combined and concentrated to dryness by removing the ethanol solvent in a rotary evaporator (Shanghai Yarong Instrument Co., China) at 40°C and stored at 4°C. The crude extract could be diluted by deionized water to obtain a different concentration used for the following experiments.

Static adsorption and desorption tests

Static adsorption tests of CA extract on macroporous resins were conducted in 250 mL conical flasks with a lid. Flasks containing a fixed amount of hydrated test resins (equivalent to 1.0 g of dry resin) and 50 mL of crude exact of CA (1.52 mg/mL) were sealed tightly and shaken (150 rpm) in a shaker at 25°C for 24 h. The

Table 2. Adsorption capacities, adsorption and desorption ratios of CA on different macroporous resins.

Name	Q_e (mg/g resin)	E (%)	D (%)
LSA-40	30.6±0.09	64.7±0.09	38.4±0.03
D-101	39.8±0.05	87.4±0.01	74.0±0.02
LSD-001	34.1±0.08	74.8±0.03	60.2±0.03
AB-8	35.2±0.07	78.4±0.02	7.6±0.12

solution after adsorption was analyzed by HPLC. After reaching the adsorption equilibrium, the residual solution was removed. The resins were first washed by deionized water for four times and then desorbed with 50 mL 90% (v/v) aqueous ethanol. The flasks were shaken (150 rpm) at 25°C for 24 h and the desorption solution was analyzed by HPLC. The initial selection of resins was based on their adsorption capacities and ratios of adsorption and desorption.

The adsorption kinetics curve of CA on the preliminarily selected resins was studied according to the aforementioned method. The concentrations of CA in liquid phase were monitored at equal time intervals till equilibration at 25°C.

The equilibrium adsorption isotherms of CA on selected resin were conducted by containing 50 mL sample solution of *L. japonica* leaves crude extracts at different initial concentrations with the pre-weighed hydrated resins (equal to 1.0 g dry resin) and shaking at 25°C for 24 h. Their fitness to Freundlich equations were evaluated (Liu et al., 2004; Li et al., 2008).

Dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments were carried out in a glass column (12 × 150 mm) wet-packed with the selected resin and the BV of the resin was 10 mL. The flow rate of sample solution was 2 BV/h through the glass column and the CA content in the effluent liquid was monitored by HPLC till equilibrium adsorption. The saturation absorption was calculated and the effects of sample pH and flow rate were also determined. The adsorbate-laden column was washed firstly by deionized water with 4 BV and then desorbed with 10 BV 10, 20, 30, 40, 50, 60, 70, 80 and 90% (v/v) ethanol-aqueous solution at the rate of 3 BV/h, respectively. Each part of desorption solutions was concentrated to dryness under vacuum and analysed by HPLC. Dynamic adsorption and desorption tests were repeated three times under optimal conditions, and the purity and recovery of CA were calculated.

Equations

The adsorption capacity, adsorption and desorption ratios of different resins are quantified with the following equations.

Adsorption capacity:

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

Adsorption ratio:

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

Where Q_e is the adsorption capacity at adsorption equilibrium (mg/g resin); E is the adsorption ratio (%); C_0 and C_e are the initial and equilibrium concentrations of CA in the solutions, respectively (mg/mL); V_i is the volume of the initial sample solution added (mL); W is the weight of the tested dry resins (g).

Desorption evaluation

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

where D is the desorption ratio after adsorption equilibrium (%); C_d is the concentration of the solutes in the desorption solutions (mg/mL); V_d is the volume of the desorption solution(mL); C_o, C_e and V_i are the same as those defined previously.

The equilibrium adsorption isotherms of CA on selected resin were evaluated with the following equation.

Freundlich equation:

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

where K_F is the Freundlich constant that indicates the adsorption capacity (mg/g); $1/n$ is an empirical constant related to the magnitude of the adsorption driving force (Jung et al., 2001); Q_e and C_e are the same as those defined previously.

Purity and Recovery of CA were quantified with following equations.

$$P = \frac{W_d}{C_0 \times V_i} \times 100 \quad (5)$$

$$R = \frac{W_d}{(C_0 - Ce) \times V_i} \times 100 \quad (6)$$

where P and R are the purity and recovery yield of CA after treatment of macroporous resin (%), respectively; W_d is the weight of CA in the desorption solution (mg); C_o, C_e and V_i are the same as those defined previously.

RESULT AND DISCUSSION

Adsorption and desorption capacity, desorption ratios

The results of four macroporous resins employed for separation of CA were listed in Table 2. The adsorption capacities and adsorption ratio of CA on AB-8 and D-001 resins were higher than those of other resins, however, the desorption ratio of AB-8 resin was the lowest in all resins.

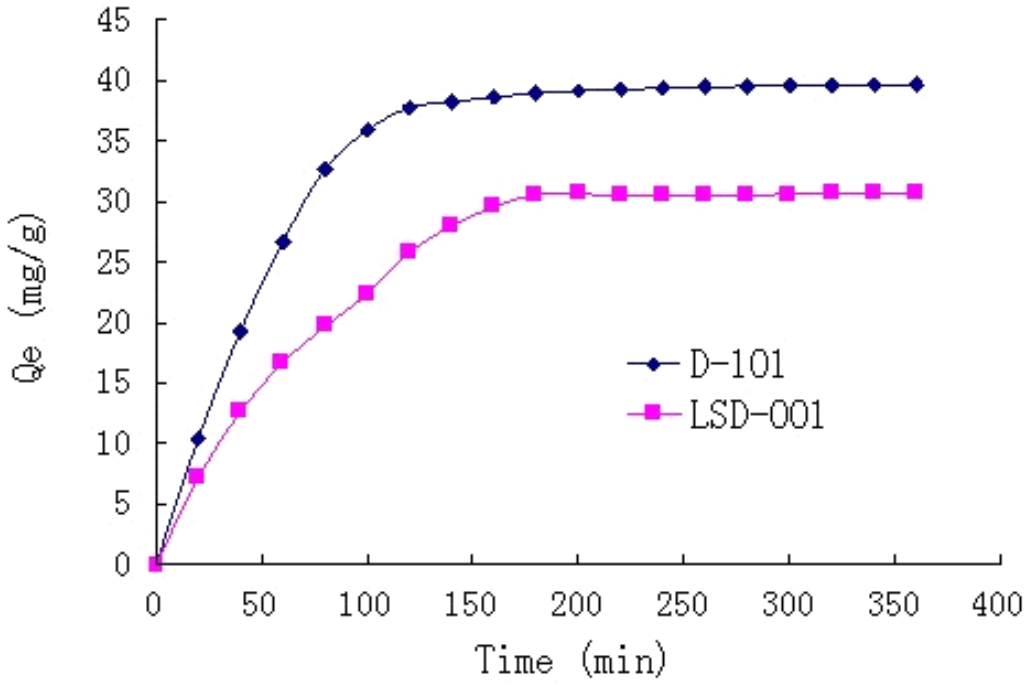

Figure 2. Kinetic curves of adsorption of CA on D-101 and LSD-001 resins.

This result showed that the selection of proper resins should be based on the correlations between the properties of the resins such as their resin polarities, average pore diameters and surface areas with the chemical features of the absorbed substance. Based on the results of the static adsorption properties, LSD-001 and D-101 resins were further investigated for their adsorption performance toward CA in the following tests.

Adsorption kinetics on D-101 and LSD-001 resins

Adsorption kinetics curves were obtained for CA on D-101 and LSD-001 resins and are shown in Figure 2. A suitable resin must also have high adsorption rates. It was observed that adsorption capacities of both resins increased initially rapidly and then slowed down till adsorption equilibrium. This is perhaps due to the plentiful vacant surface sites initially available for adsorption, but then the remaining vacant surface sites are difficult to occupy because of the repulsive forces between the CA molecules on the resin and the bulk phase (Mall et al., 2005). The adsorption capacities of D-101 showed rapid increases in the beginning 1 h, and little changes after 2 h; whereas, LSD-001 showed slower increases than D-101 in the beginning 1 h and reached adsorption equilibrium after 3 h, which might be attributed to its relatively greater polarity. The results of adsorption capacities, adsorption, desorption ratios and adsorptive rates indicated that the optimal resin for separation of CA is D-101 resin.

Adsorption isotherms on D-101 resin

Langmuir and Freundlich equations are frequently used to evaluate adsorption isotherms in the adsorption process. The Langmuir equation can be used to reveal a monolayer adsorption, whereas the Freundlich equation can be used to describe a monolayer adsorption as well as a multilayer adsorption (Zhang et al., 2008). The initial concentrations of CA in the solutions were 1.52, 1.01, 0.76, 0.61 and 0.51 mg/mL, respectively. As shown in Figure 3, the adsorption capacity increased with the initial concentration and reached the saturation plateau when the initial concentration of CA was 1.01 mg/ml. Thus 1.01 mg/ml CA was used as initial concentration of sample solution in the following tests.

The linear regression line for the Freundlich equation is log Q_e = 0.2826log C_e + 1.7894, with parameter 1/n of0.2826 and K_F of 61.57 (R^2 = 0.97). This result showed that experimental data were fitted to the Freundlich equations. In the Freundlich equation, the adsorption is favorable when the 1/n value is between 0.1 and 1 (Silva et al., 2007), which indicated that D-101 resin is favorable for the separation of CA.

Breakthrough curve

A large surface area and fine pore structure inside the particle like activated carbon of macroporous resins enable them to efficiently absorb organic compounds. However, this capability decreases or disappears when

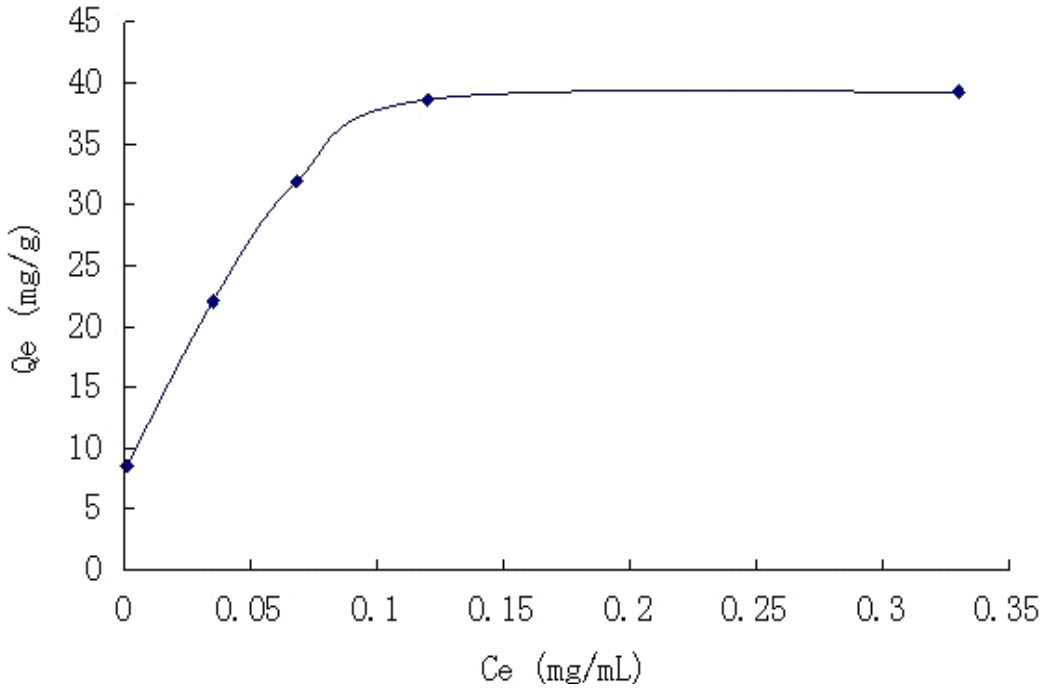

Figure 3. Adsorption isotherms for CA on D-101 resin at 25°C.

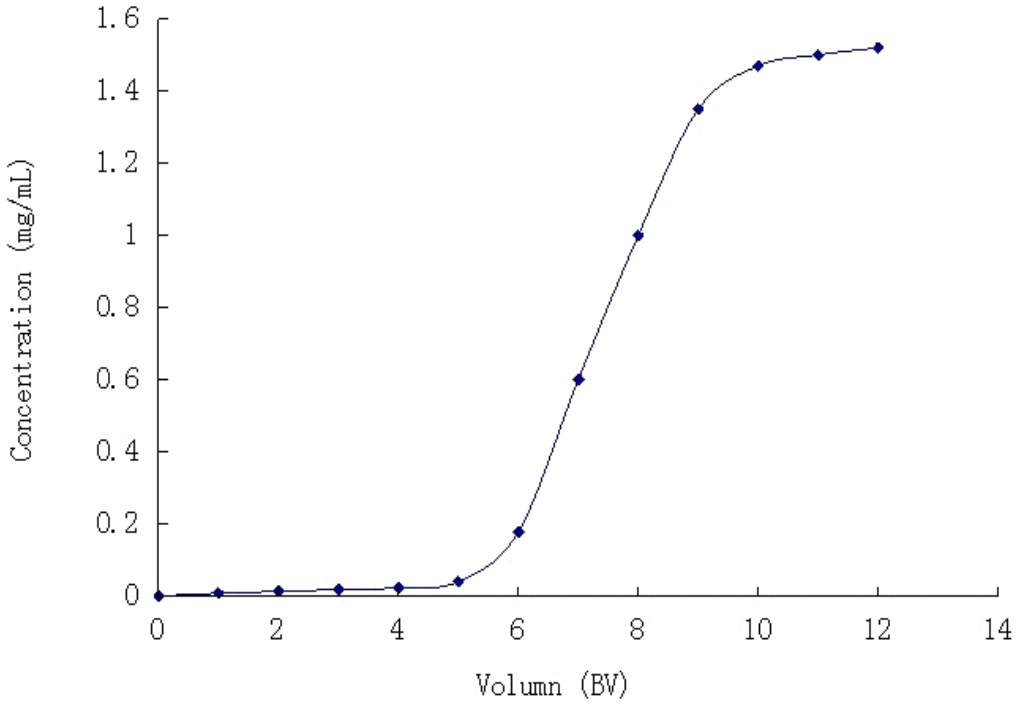

Figure 4. Breakthrough curves of CA on column packed with D-101 resin.

the breakthrough point was reached (Zhao et al., 2010). Thus breakthrough volume is important to calculate the quantity of resins and the processing volume of sample solution. Breakthrough point was defined at which exit solution concentration reached to 1% of the inlet concentration (Jung et al., 2001). As shown in Figure 4, the breakthrough volume of CA on D-101 resin at the rate of 2 BV/h was approximate 50 mL (5 BV). Therefore, the

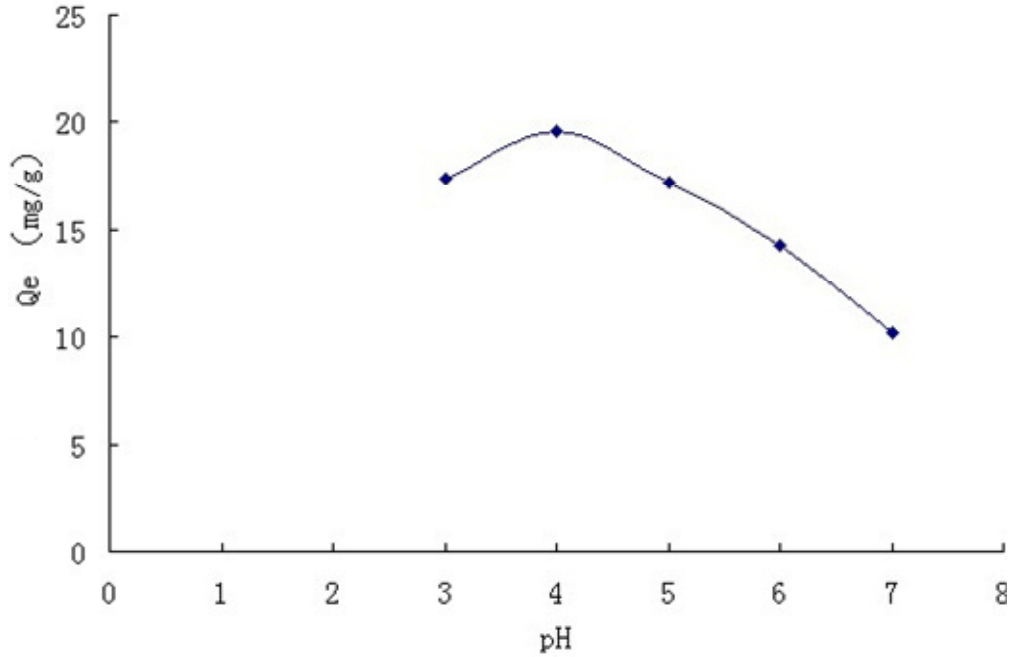

Figure 5. Effect of pH value on dynamic adsorption capacity of CA on column packed with D-101 resin.

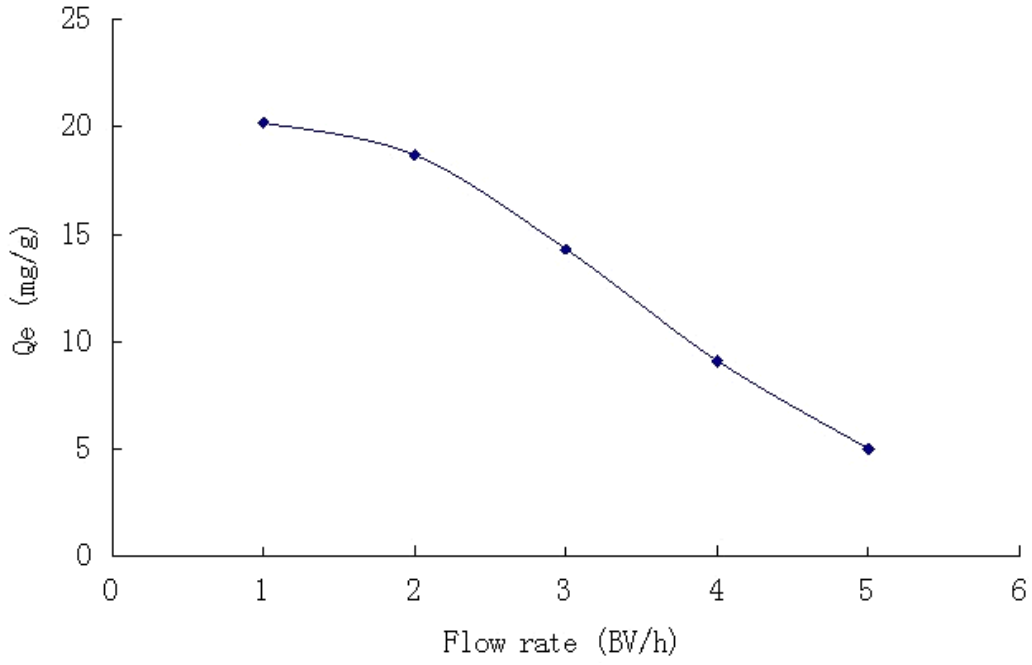

Figure 6. Effect of flow rate on dynamic adsorption capacity of CA on column packed with D-101 resin.

saturation absorption is 76 mg.

Effect of initial solution pH

The pH of the sample solution significantly affected the adsorption capacity of CA on D-101 resin. As shown in Figure 5, the adsorption capacity initially increased to reach its peak value at pH 4, and then decreased gradually. Macroporous resins at lower pH values are favorable for the adsorption of acid materials. However, too low pH values may lead to hydrolysis of Hydroxyl

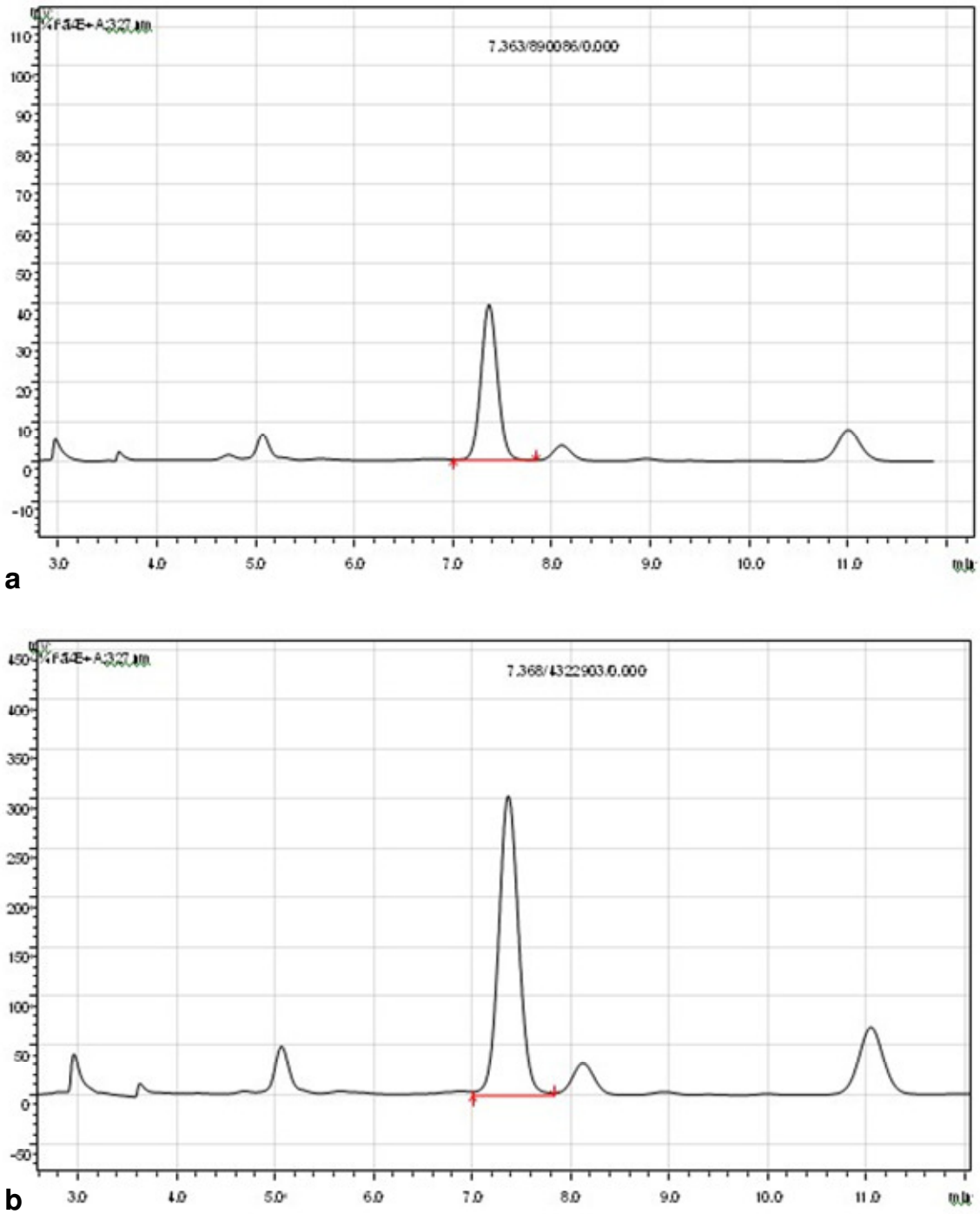

Figure 7. Chromatograms of sample solution before (a) and after (b) separation on a column packed with D-101 resin.

groups. Hence, suitable pH value for the following tests was adjusted to 4.

Effect of flow rate

As shown in Figure 6, the adsorption capacity declined with the increase of flow rate and the best adsorption performance was obtained at the lowest flow rate of 1 BV/h, which is likely due to better intraparticle diffusion of sample solutions on the resin. Since there is small difference between the adsorption capacity of resin at the flow rate of 1 and 2 BV/h and a low flow rate prolonged the working period. Therefore, 2 BV/h was selected as

Table 3. Desorption ratio of different concentration of ethanol and purity and recovery for CA.

Concentration of ethanol (%)	D (%)	P (%)	R (%)
10	61.19±0.41	41.95±0.35	45.92±0.37
20	72.48±0.37	50.72±0.27	54.44±0.32
30	75.05±0.46	38.13±0.24	56.21±0.27
40	69.97±0.44	38.95±0.32	52.44±0.31
50	69.82±0.44	36.76±0.30	52.38±0.36
60	80.29±0.34	29.05±0.43	60.23±0.43
70	77.22±0.33	18.46±0.40	57.91±0.35
80	80.79±0.43	17.54±0.36	60.59±0.42
90	81.18±0.29	19.64±0.32	60.84±0.54

the best sample flow rate for further experiments.

Dynamic desorption tests on D-101 resin

The dynamic desorption tests were repeated for 3 times and the results of different concentrations of ethanol solutions used to perform desorption solution tests were summarized in Table 3. It can be seen that under the elution of 20% (v/v) ethanol, the purity of CA is notably higher than other eluent. While there are smaller differences between the values of desorption ratio and recovery ratio of different eluents. Thus, 20% (v/v) ethanol was favorable for the desorption of CA on D-101 resin. The chromatograms of sample solutions before and after treatment with D-101 resin were shown in Figure 7. By comparison, some impurities were removed and the relative peak area of CA increased obviously after the separation on D-101 resin. After treatment with D-101 resin, the content of CA increased 50.72%, which was 133-fold to that of crude exact. The recovery yield is 54.44% and the RSD for recovery yield was 0.32%.

Conclusion

In this study, the separation and purification of CA from crude extracts of *L. japonica* leaves with macroporous resin were obtained successfully. For static adsorption and desorption tests, D-101 resin was selected among four macroporous resin with different polarities for the separation of CA because of its relative high adsorption and desorption ratios and adsorption rate. For dynamic adsorption and desorption ests, certain process parameters including solution volume, initial CA concentration, pH value, flow rate and concentration of eluents were optimized. After the treatment of D-101 resin under optimized parameters, the purity of CA reached 50.72% with a recovery yield of 54.44%.

ABBREVIATIONS

CA, Chlorogenic acid; **HPLC,** high performance liquid chromatography; **BV,** bed volume.

REFERENCES

Dillard CJ, German JB (2000). Phytochemicals: Nutraceuticals and human health. J. Sci. Food Agric. 80:1744-1756.

Fu BQ, Liu J, Li H, Li L, Lee FSC, Wang XR (2005). The application of macroporous resins in the separation of licorice flavonoids and glycyrrhizic acid. J. Chromatogr. A. 1089:18-24.

Fu YJ, Zu YG, Liu W, Hou CL, Chen LY, Li SM, Shi XG, Tong MH (2007). Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. J. Chromatogr. A 1139:206-213.

Han SH，Zhang Y，Huo AL，Yang CHF, wang JR (2009). Studies on the chemical constituents of the stems and leaves of *Lonicera japonica* Thunb．Acta Agric. Boreali-OCidentalis Sin. 18(5):363-364.

Jiang Y, Satoh K, Watanabe S, Kusama K, Sakagami H (2001). Inhibition of chlorogenic acid-induced cytotoxicity by $CoCl_2$. Anticancer Res. 21:3349-3353.

Jin UH, Lee JY, Kang SK, Kim JK, Park WH, Kim JG, Moon SK, Kim CH (2005). A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type and strong matrix metalloproteinase-9 inhibitor: Isolation and identification from methanol extract of euonymus alatus. Life Sci. 77:2760-2769.

Jung MW, Ahn KH, Lee Y, Kim KP, Paeng IR, Rhee JS, Park JT, Paeng KJ (2001). Evaluation on the adsorption capabilities of new chemically modified polymeric adsorbents with protoporphyrin ix. J. Chromatogr. A. 917:87-93.

Ko HC, Wei BL, Chiou WF (2006). The effect of medicinal plants used in chinese folk medicine on rantes secretion by virus-infected human epithelial cells. J. Ethnopharmacol. 107:205-210.

Kwak WJ, Han CK, Chang HW, Kim HP, Kang SS, Son KH (2003). Loniceroside c, an antiinflammatory saponin from *Lonicera japonica*. Chem. Pharm. Bull. 51:333-335.

Li H, Lee JH, Ha JM (2008). Effective purification of ginsenosides from cultured wild ginseng roots, red ginseng, and white ginseng with macroporous resins. J. Microbiol. Biotechnol., 18: 1789-1791.

Li H, Liu Y, Zhang Z, Liao H, Nie L, Yao S (2005). Separation and purification of chlorogenic acid by molecularly imprinted polymer monolithic stationary phase. J. Chromatogr. A, 1098: 66-74.

Liu XM, Xiao GS, Chen WD, Xu YJ, Wu JJ (2004). Quantification and purification of mulberry anthocyanins with macroporous resins. J. Biomed. Biotechnol. 5:326-331.

Mall ID, Srivastava VC, Agarwal NK, Mishra IM (2005). Removal of congo red from aqueous solution by bagasse fly ash and activated carbon: Kinetic study and equilibrium isotherm analyses. Chemosphere 61:492-501.

Nakamura T, Nakazawa Y, Onizuka S, Satoh S, Chiba A, Sekihashi K, Miura A, Yasugahira N, Sasaki YF (1997). Antimutagenicity of tochu tea (an aqueous extract of *Eucommia ulmoides* leaves) .1. The clastogen-suppressing effects of tochu tea in cho cells and mice. Mutat. Res./Gen. Tox. En. 388:7-20.

Sabu MC, Kuttan R (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol. 81:155-160.

Silva EM, Pompeu DR, Larondelle Y, Rogez H (2007). Optimisation of the adsorption of polyphenols from inga edulis leaves on macroporous resins using an experimental design methodology. Sep. Purif. Technol. 53:274-280.

Ubukata K, Nakagami S, Nitta A, Yamane A, Kawakami S, Sugiura M, Konno M (1992). Rapid detection of the meca gene in methicillin-resistant staphylococci by enzymatic detection of polymerase chain-reaction products. J. Clin. Microbiol. 30:1728-1733.

Zhang B, Yang RY, Zhao Y, Liu CZ (2008). Separation of chlorogenic acid from honeysuckle crude extracts by macroporous resins. J. Chromatogr. B. 867:253-258.

Zhao H, Wang J, Jia J, Liu J, Ling XQ, Lu DQ (2010). Enrichment and purification of total chlorogenic acids from tobacco waste extract with macroporous resins. Sep. Sci. Technol. 45:794-800.

Zhao Y, Chen B, Yao SZ (2007). Separation of 20(s)-protopanaxdiol type ginsenosides and 20(s)-protopanaxtriol type ginsenosides with the help of macroporous resin adsorption and microwave assisted desorption. Sep. Purif. Technol. 52:533-538.