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Zhao Hui[a]; Wang Jun[ab]; Jia Jing[a]; Liu Ji[a]; Ling Xiuquan[a]; Lu Dingqiang[ac]
[a] School of Pharmacy, Nanjing University of Technology, Nanjing, P. R. China [b] College of Biological and Environmental Engineering, Jiangsu University of Science and Technology, Zhenjiang, P. R. China [c] Jiangsu Provincial Institute of Material Medica, Nanjing, P. R. China

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Enrichment and Purification of Total Chlorogenic Acids from Tobacco Waste Extract with Macroporous Resins

Zhao Hui,[1] Wang Jun,[1,2] Jia Jing,[1] Liu Ji,[1] Ling Xiuquan,[1] and Lu Dingqiang[1,3]
[1]*School of Pharmacy, Nanjing University of Technology, Nanjing, P. R. China*
[2]*College of Biological and Environmental Engineering, Jiangsu University of Science and Technology, Zhenjiang, P. R. China*
[3]*Jiangsu Provincial Institute of Material Medica, Nanjing, P. R. China*

In the present study, an evaluation was conducted on the performance and separation characteristics of nine macroporous resins for the enrichment and purification of total chlorogenic acids from tobacco (*Nicotiana tobaccum L.*) waste extracts. Based on the results, XAD-4 offered higher adsorption and desorption capacities for total chlorogenic acids than other resins. To optimize the separation process of total chlorogenic acids, a column packed with XAD-4 resin was used to perform dynamic adsorption and desorption tests. The results show that the highest purity of the total chlorogenic acids product was 89.27% when optimum parameters for the adsorption process packed with the XAD-4 resin were as follows: flow rate 3.6 BV/h, pH 3.0; for desorption: ethanol–water (40:60, v/v) used as eluent, flow rate 3.6 BV/h, respectively. Therefore, the XAD-4 resin revealed a good ability to enrich and purify total chlorogenic acids. The method developed will provide a potential approach for the large-scale separation and purification of chlorogenic acid in pharmaceutical applications as a medical intermediate or a material for traditional Chinese medicine.

Keywords adsorption; desorption; macroporous resins; tobacco waste; total chlorogenic acids (T-CQAs)

INTRODUCTION

Tobacco (*Nicotiana tobaccum* L.) is a cultivated plant of the Solanaceae family with widespread use in the perfume, cosmetic, and pharmaceutical industries (1). One family of biologically active compounds in flue-cured tobacco comprises the chlorogenic acids (2). The chemical structures of chlorogenic acids, including chlorogenic acid (5-CQA), cryptochlorogenic acid (4-CQA), and neochlorogenic acid (3-CQA), are shown in Fig. 1 (3). An interest in polyphenol antioxidants has increased remarkably in the last decade because of their positive effects on different diseases, including cardiovascular, inflammatory, and neurological diseases, as well as cancers (4–10). Pharmaceutical products containing chlorogenic acid with different purities ranging from 20 to 98% are widely used as healthcare products, oral liquids, tablets, and capsules, herbal injections, among others (11).

Solid–liquid extraction or solvent extraction is the conventional method for enriching chlorogenic acid from the crude extracts of honeysuckle or eucommia, followed by polyamide chromatography and gel chromatography (12). However, chromatographic separation is a particularly demanding technique that requires high solvent consumption and an expensive filler, and results in a high-cost process. Alternatively, the adsorption–desorption process on macroporous resins is one of the more efficient and economical methods with a purification effect, and it can be used to recover and concentrate plant secondary metabolites (13–16). Macroporous resins are durable polar, nonpolar, or slightly hydrophilic polymers having a high adsorption capacity with possible recovery of adsorbed molecules, a relatively low cost, and easy regeneration. They are widely used in the field of recovery of natural substances. To date, Zhang et al. (17) have shown a quantitative separation of chlorogenic acid from honeysuckle crude extracts by macroporous resin, making this a cost-effective purification step. Therefore, the general objective of the present work was to assess the use of macroporous resins for the concentration and purification of T-CQAs from tobacco waste extract.

In 2005, China produced approximately 1840.5 million kg tobacco leaves amounting to 36.5% of the total output worldwide, making it the largest tobacco-producing country in the world. Massive quantities of tobacco waste are produced annually by the cigarette-manufacturing industry. Because tobacco waste contains nicotine, which is highly toxic, its disposal has become a serious ecological problem. However, tobacco waste is considered a good source of numerous bioactive substances. Yang et al. (18) have shown a method for the synthetic utilization of tobacco waste by obtaining solanesol from the by-product, instead of degrading or discarding it. Thus, enrichment and

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Address correspondence to Lu Dingqiang, School of Pharmacy, Nanjing University of Technology, Nanjing 210009, China. E-mail: ludingqiang@njut.edu.cn

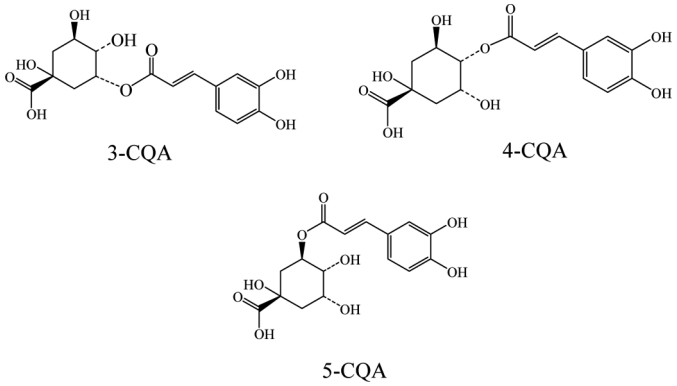

FIG. 1. Chemical structure of chlorogenic acids in tobacco waste extracts.

purification of T-CQAs through adsorption are a novel approach in tobacco waste disposal.

In this study, adsorption and desorption of T-CQAs on nine widely employed macroporous resins were investigated in order to select a suitable resin for chromatographic purification of T-CQAs from tobacco waste extracts. In addition, the adsorption process parameters based on the chosen resin were optimized.

EXPERIMENTAL

Materials and Reagents

Tobacco waste was kindly provided by the Jiangsu Tobacco Company Nanjing Branch in China, and authenticated by Professor Dingqiang Lu from the College of Life Science and Pharmaceutical Engineering in the Nanjing University of Technology. The sample was dried at 60°C, powdered by an herb disintegrator (Qinzhou Sanyang Package Equipment Co., Ltd.), and then sieved (60 mesh).

The 3-CQA and 4-CQA standards were purchased from Chendu Biopurify Phytochemicals (Chendu, China), while 5-CQA standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Distilled water was purchased from Hangzhou Wahaha Group Co., Ltd. Acetonitrile and acetic acid were HPLC grade, and ethanol and ammonium acetate were analytical grade.

Adsorbents

Macroporous resins including Amberlite XAD-4, XAD-16, XAD-1180, and XAD-7HP were purchased from Rohm and Haas (Philadelphia, PA, USA); Diaion HP20 and Diaion HP2MGL were purchased from Mitsubishi Chemical Corporation (Tokyo, Japan); and XDA-1, D942, and LSA-700, which were used as adsorbents, were purchased from Sunresin Technology Ltd. (Xi'an, China). The manufacturer information regarding the characteristics of the resins is reported in Table 1. Necessary preconditioning of the adsorbents was realized by an extensive wash with abundant distilled water to remove salts and impurities, followed by drying at 60°C for 24 h and posterior cooling in desiccators. The dried resin was immersed in ethanol for 12 h. The ethanol was then replaced by distilled water through washing.

Determination of Moisture Content of Resins

The dried resins were leached with 95% ethanol for 24 h to ensure that the internal pores were thoroughly soaked and washed five times with deionized water. Three samples of each kind of macroporous resins were weighed after pumping filtration, and then dried at 60°C in a drying oven, until the mass ceased to change. These were then weighed, and the moisture content was calculated using Equation (1). The moisture contents of the resins are shown in Table 2.

$$M = \frac{w_1 - w_2}{w_1} \quad (1)$$

where M is the ratio of the moisture content of resin (%), w_1 is the weight of hydrated resin (g), and w_2 is the weight of dried resin (g).

TABLE 1
Physical properties of the test macroporous resins

Trade name	Polarity	Particle diameter (mm)	Surface area (m^2/g)	Average pore diameter (Å)
XAD-4	Weak polar	0.64	750	100
XAD-16	Nonpolar	0.7	800	150
XAD-1180	Nonpolar	0.53	700	400
XAD-7HP	Polar	0.56	500	450
HP20	Weak polar	0.5	600	200
HP2MGL	Middle polar	0.5	500	200
D942	Polar	0.9	1600	200
LSA-700	Polar	0.8	1500	150
XDA-1	Polar	0.7	1200	100

TABLE 2
Moisture content of resins

Resin	Moisture content (%)
XAD-4	55.87
XAD-16	67.41
XAD-1180	61.67
XAD-7HP	67.29
HP20	59.56
HP2MGL	50.92
D942	53.99
LSA-700	79.24
XDA-1	59.91

Analysis of T-CQAs by HPLC

The concentrations of 3-CQA, 4-CQA, and 5-CQA were simultaneously determined by performing a reversed-phase high-performance liquid chromatography (RP-HPLC) according to the reference literature (3). RP-HPLC separation was performed on an Alltima C_{18} (250 × 4.6 mm, 5 μm) column (Alltech, Deerfield, IL, USA). The mobile phase contained an acetonitrile:ammonium acetate buffer (pH 4.5) (5:95, v/v). The flow rate was 1 mL/min. The UV detection with the column was maintained at 30°C, which was detected at 327 nm. All solutions were filtered through a 0.45 μm filter before injection. All samples were determined in triplicate.

Preparation of Crude Extract

One thousand grams of oil-free tobacco waste was extracted three times with 5,000 mL 75% ethanol at 30°C for 4 h. After filtration, the crude extracts were combined and evaporated to eliminate the alcoholic fraction using an R-200 rotary evaporator (BÜCHI, Flawil, Switzerland) under reduced pressure (0.01 MPa, 35–45°C), then stored at 4°C. This tobacco waste crude extract could dilute by deionized water to obtain a different concentration which was used for the next set of experiments.

Static Adsorption and Desorption Tests

The static adsorption tests of tobacco waste crude extract were carried out as follows: 0.5 g hydrated test resins and 50 mL sample solution of tobacco waste crude extract (T-CQAs concentration of 0.56 mg/mL, pH 3) were added into a flask with a lid. The mixture was continuously stirred at 150 rpm for 24 h and kept at 30°C by thermostatic bath. The adsorption solution was analyzed by HPLC. The desorption process was performed as follows: after reaching adsorption equilibrium, the resin was first washed by deionized water and then desorbed with 50 mL ethanol–water (40:60, v/v) solution. The flasks were shaken (150 rpm) for 24 h at 30°C. The desorption solution was analyzed by HPLC. Candidate resins were selected in terms of their adsorption capacities, adsorption ratios, and desorption ratios.

The adsorption kinetics of T-CQAs on the preliminarily selected resins was studied according to the method described above. The concentrations of T-CQAs in the liquid phase were monitored by HPLC at equal time intervals until equilibration at 25, 30, and 35°C, respectively. The optimum resin was finally determined based on the adsorption speed.

The adsorption–desorption properties of the selected XAD-4 resin under different conditions, including pH of the sample solution and initial concentration, were also evaluated.

Dynamic Adsorption and Desorption Tests

Dynamic adsorption and desorption experiments were performed in the glass columns (16 × 500 mm) wet-packed with XAD-4 resin. The bed volume (BV) of resin was 50 mL, and the packed length of the resin bed was 25 cm. A sample solution flowed through the glass column at a certain flow rate, and the T-CQAs content in the effluent liquid was monitored by HPLC analysis. While in an adsorptive equilibrium, the adsorbate-laden columns were washed first by deionized water, then eluted by ethanol–water (40:60, v/v) solution. The concentrations of T-CQAs in the desorption solution were determined by HPLC.

Calculation of Adsorption and Desorption Capacities, Desorption Ratio

The following equations were used to quantify the capacities of adsorption and desorption, as well as the ratio of desorption.

Adsorption Evaluation

$$q_e = (C_0 - C_e)\frac{V_i}{(1 - M) \times W} \tag{2}$$

where q_e is the adsorption capacity at adsorption equilibrium (mg/g dry resin); C_0 and C_e are the initial and equilibrium concentrations of T-CQAs in the solution, respectively (mg/mL); V_i is the volume of the initial sample solution (mL); M is the ratio of moisture content of resin (%); and W is the weight of hydrated test resin (g).

Desorption Evaluations

$$q_d = \frac{C_d \times V_d}{(1 - M) \times W} \tag{3}$$

$$D = \frac{C_d \times V_d}{(C_0 - C_e)V_i} \times 100 \quad (4)$$

where q_d is the desorption capacity after adsorption equilibrium (mg/g dry resin); C_d is the concentration of T-CQAs in the desorption solution (mg/mL); V_d is the volume of the desorption solution (mL); D is the desorption ratio (%); C_0, C_e, V_i, W, and M are the same as described above.

RESULTS AND DISCUSSION

Adsorption and Desorption Capacities, Desorption Ratio

The adsorption capacities of T-CQAs on LSA-700, D942, XDA-1, and XAD-4 resins were considerably higher than those of other resins, as shown in Fig. 2. However, the desorption capacity and desorption ratio of LSA-700 and D942 resin were the lowest in all resins. After full consideration of the static adsorption properties, XDA-1 and XAD-4 were further evaluated for their adsorption performance toward T-CQAs in the following tests.

Based on the results, there are close correlations between the capabilities of the resins and the chemical features of the adsorbed substance, that is, resins with a higher polarity and surface area exhibited stronger adsorption abilities to polar substances. The selection of proper resins should be in accordance with the resin polarities, as well as their average pore diameters and surface areas, among other factors.

Adsorption Kinetics on XDA-1 and XAD-4 Resins

The use of static adsorption capacity and desorption ratio is not enough to assess the performance of an adsorbent. A suitable resin must also have high adsorption rates. The adsorption kinetic curves obtained for T-CQAs on XAD-4 and XDA-1 resins in the temperature range of 25–35°C are revealed in Figs. 3 and 4, respectively. It was observed that both resins showed increasing adsorption capacities with the extension of adsorption time. The adsorption capacities of XAD-4 showed rapid increases in the beginning 3 h, and little changes after 6 h. However, XDA-1 showed slower increases than XAD-4 in the beginning 3 h, which might be attributable to its relatively greater polarity. In particular, the mobility of T-CQAs adsorption increased, and the proportion of the adsorbed T-CQAs decreased with increasing temperature on XAD-4 resin. In the comprehensive consideration of the adsorption capacity and desorption ratio, XAD-4 resin was seem to be fit to separating T-CQAs from tobacco waste extract.

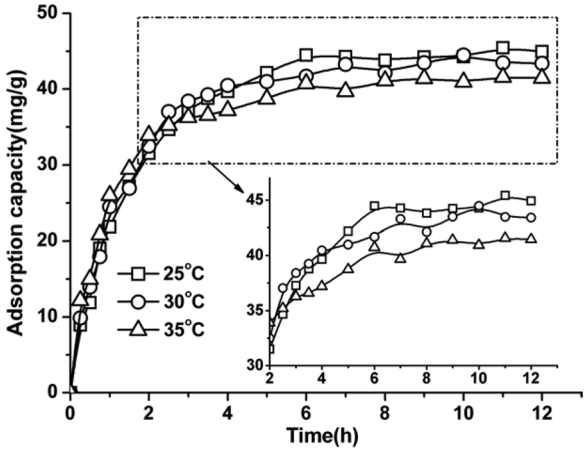

FIG. 3. Adsorption kinetic curves for T-CQAs on XAD-4 resin.

Effect of Sample Solution pH

The pH influences the extent of the solute ionization, thus affecting the affinity of the solutes and solutions. As shown in Fig. 5, the adsorption capacities of T-CQAs

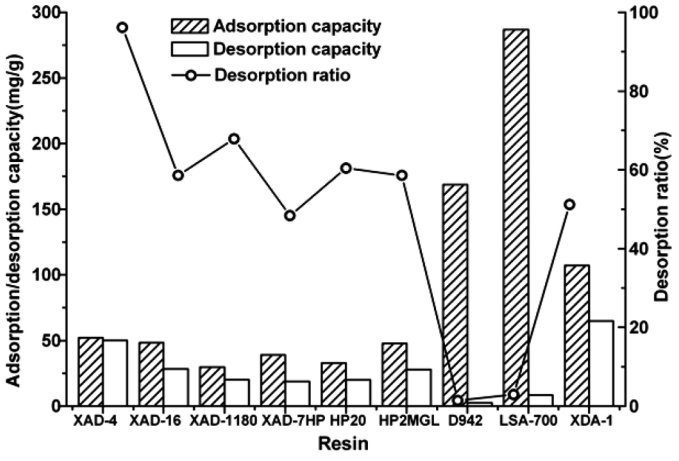

FIG. 2. Adsorption and desorption capacities and desorption ratio of T-CQAs on different resins.

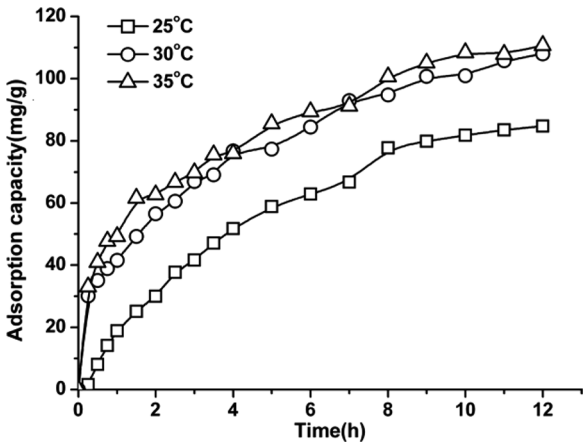

FIG. 4. Adsorption kinetic curves for T-CQAs on XDA-1 resin.

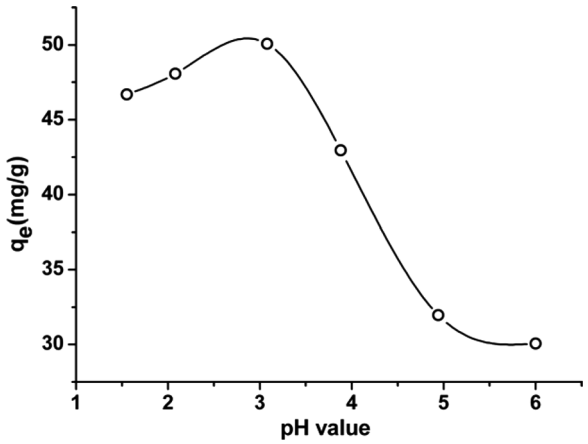

FIG. 5. Effect of different pH values of sample solution on the adsorption capacities of T-CQAs on XAD-4 resin.

on XAD-4 resin at different pH values were changed significantly. For T-CQAs, the highest adsorption capacity appeared at pH 3.0, and then decreased with the increase of the pH value. Hydrogen bonding may play an important role in the adsorption process on XAD-4 resin. At a higher pH value, the hydrogen bonding interactions were reduced because the phenolic hydroxyl groups in T-CQAs dissociated to H^+ and their corresponding anions, thus resulting in the lower adsorption capacity. Hence, the pH value of the sample solution was adjusted to 3.0 for the following tests.

Effect of Sample Solution Concentration

The effect of the initial concentration of the solution also affected the adsorption process of T-CQAs from tobacco waste extract. The sample solutions of different initial concentration were adjusted by adding water. As shown in Fig. 6, under the conditions of low concentration, the adsorption capacity will raise with the increase of the concentration of T-CQAs. The highest adsorption capacity of the resin was obtained when T-CQAs concentration in tobacco waste extract solution was 0.56 mg/mL, and then decreased with the increase of T-CQAs concentration. The reason of this phenomenon perhaps can be interpreted by the complexity of the sample solution system, because there are many factors which affect the adsorption behavior of resin, such as impurities, the pH value, and so on. For example, with the increase of T-CQAs concentration, the concentration of impurities as protein and pigment in sample solution also increased, then lots of impurities will block the pores of XAD-4 resin, and at the same times the adsorption capacity of XAD-4 resin will get decreased. Moreover, a pH value decrease with the increase of T-CQAs concentration, which also decrease the adsorption capacity of XAD-4 resin (see Fig. 5). Hence, the maximum adsorption capacity emerged when T-CQAs concentration in the sample solution was adjusted to approximately 0.56 mg/mL.

Effect of Concentration of Ethanol Solution on the Ratio of Desorption

Different concentrations of ethanol solutions were used to perform desorption tests in order to choose a proper desorption solution. As seen in Fig. 7, initially, with the increase of ethanol concentration, the desorption ratios of T-CQAs increased accordingly and reached their peak value at the 40% concentration, then decreased with the concentration of ethanol. At the 40% ethanol concentration, the desorption ratio of T-CQAs was 97.14%, and the relative content was the highest compared with those at other ethanol concentrations. Therefore, ethanol–water (40:60, v/v) solution was selected as the appropriate desorption solution and was used in the following tests.

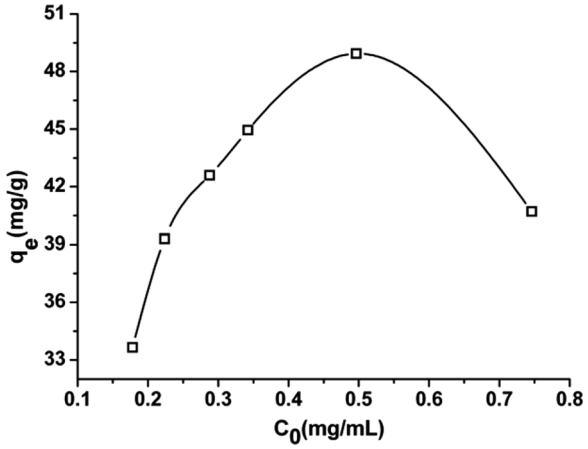

FIG. 6. Effect of initial concentration of sample solution on the adsorption capacities of T-CQAs on XAD-4 resin.

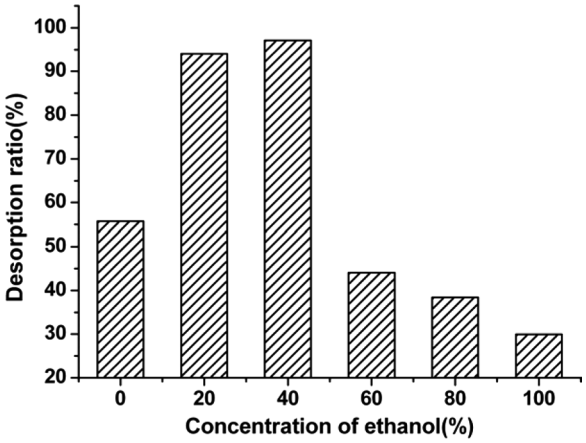

FIG. 7. Effect of concentration of ethanol solution on the desorption ratio of T-CQAs on XAD-4 resin.

Breakthrough Curve on XAD-4 Resin

The breakthrough curves on XAD-4 resin were obtained for T-CQAs based on the volume of effluent liquid and the concentration of solute herein. Macroporous resins have a large surface area and fine pore structures inside the particle-like activated carbon. With this porous characteristic, they can effectively adsorb organic compounds from aqueous solutions. When the adsorption reaches the breakthrough point, the adsorption effect decreases and even disappears. Thus, it is important to set up the breakthrough point in order to calculate the resin quantity, the processing volume of the sample solution, and the proper sample flow rate.

As shown in Fig. 8, the best adsorption performance was obtained at the lowest flow rate of 3.6 BV/h, which is likely due to better compound diffusion in sample solutions. An even lower flow rate prolonged the working period. Therefore, 3.6 BV/h was selected as the best sample flow rate for further experiments. Under this condition, the processing volume of the sample solution on XAD-4 resin was approximately 12 BV.

Dynamic Desorption Curve on XAD-4 Resin

The dynamic desorption curves (see Fig. 9) using XAD-4 resin were obtained based on the volume of desorption solution and the T-CQAs concentration in the desorption solution.

It is important to choose a proper flow rate to desorb T-CQAs from the resin effectively. The chromatograms of the test samples before and after treatment with XAD-4 resin are shown in Fig. 10. In Table 3, the purity of T-CQAs is shown to be nearly equal. In the dynamic desorption test, the desorption performance at 2.4 BV/h was the best. However, at this desorption flow rate, the working time was too long. Therefore, 3.6 BV/h was chosen as the optimal desorption flow rate on account of the short working time and the highest desorption ratio. Approximately 2.5 BV of the desorption solution could completely desorb T-CQAs from XAD-4 resin when the flow rate was 3.6 BV/h.

TABLE 3
Effect of different flow rates on the desorption capacities of T-CQA on XAD-4 resin

Flow rate (BV/h)	Desorption capacity (mg)	Purity (%)
2.4	169.21	89.27
3.6	165.01	88.74
4.8	161.84	88.19

The following equation was used to quantify the recovery yield of T-CQAs:

$$Y = \left[\frac{C_d \times V_d}{(C_0 - C_a) \times V_p}\right] \times 100 \quad (5)$$

where Y is the recovery yield of T-CQAs (%); C_a is the concentration of T-CQAs in the effluent liquid (mg/mL); V_p is the processing volume of the sample solution (mL); and C_0, C_d and V_d are the same as described above.

After treatment with XAD-4 resin, the purity of T-CQAs reached 89.27%, which was a 106-fold increase to those in tobacco waste extracts. The recovery yield was 65.03%.

The chromatograms of the test samples before and after treatment with XAD-4 resin are shown in Fig. 9. By comparison, it can be seen that some impurities were removed, and the relative peak area of T-CQAs obviously increased after the separation on XAD-4 resin.

The optimum parameters for the preparative separation of T-CQAs with XAD-4 macroporous resin were confirmed as follows: for adsorption: concentration of T-CQAs in sample solution: 0.49 mg/mL, processing volume: 12 BV,

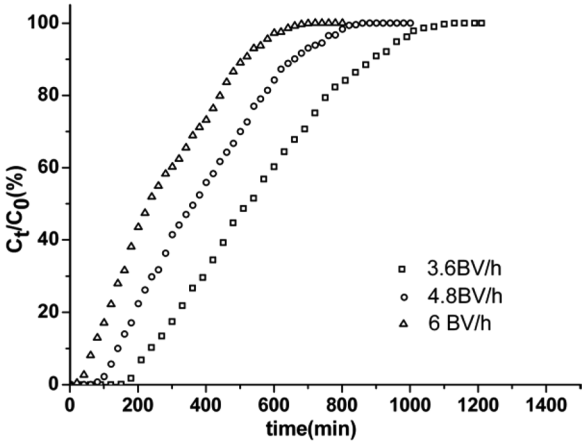

FIG. 8. Breakthrough curves of T-CQAs on column packed with XAD-4 resin at different flow rates.

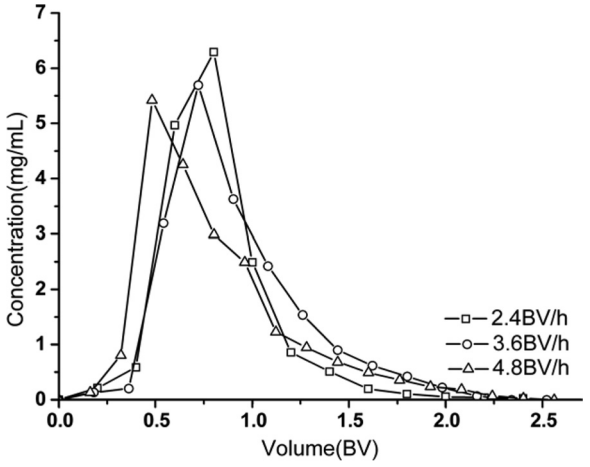

FIG. 9. Dynamic desorption curves of T-CQAs on column packed with XAD-4 resin at different desorption flow rates.

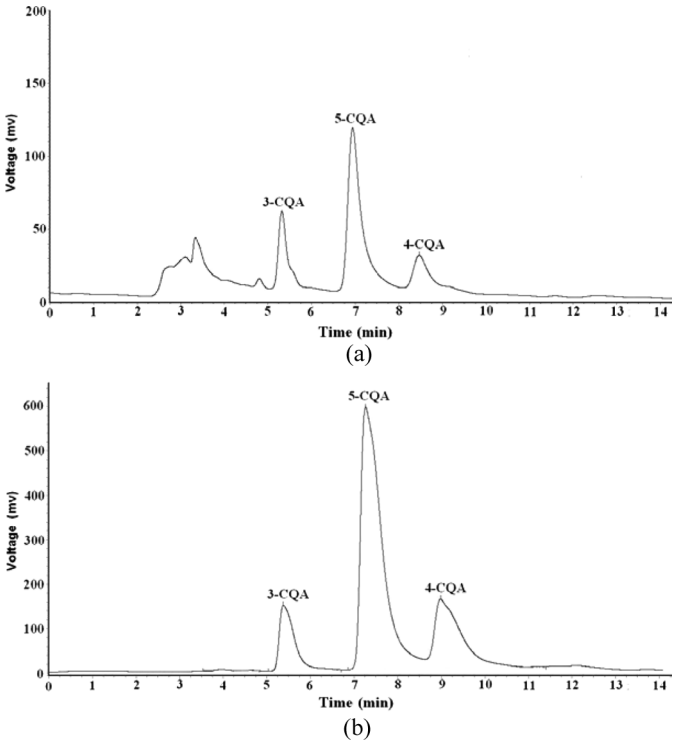

FIG. 10. Chromatograms of sample solution before (a) and after (b) separation on a column packed with XAD-4 resin.

flow rate: 3.6 BV/h, pH 3.0; for desorption: ethanol–water (40:60, v/v), 2.5 BV as eluent, flow rate: 3.6 BV/h.

CONCLUSIONS

In this study, the adsorption and desorption identities of nine different macroporous resins were investigated by static adsorption and desorption of T-CQAs. In addition, the separation process of each selected macroporous resin from the tobacco waste extracts was tested by dynamic adsorption and desorption experiments. XAD-4 resin was selected because of the favorable adsorption and desorption capacities, and the highest adsorption rate from nine different resins. To ensure the most effective separation with the optimal XAD-4 resin, certain parameters such as sample solution T-CQAs concentration, pH value, and concentration of washing solution were optimized. Furthermore, the processes of dynamic adsorption and desorption were conducted to ensure optimal separation parameters, such as solution volume and flow rate. Column packed with XAD-4 resin to enrich and purify T-CQAs, the purity of the T-CQAs product was 89.27%, which is 106-fold higher than those in tobacco wastes. The recovery yield was 65.03%. In conclusion, this adsorption–desorption method is useful in the enrichment and purification of T-CQAs from tobacco waste extract because of its low cost, high efficiency, and procedural simplicity.

ACKNOWLEDGEMENTS

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