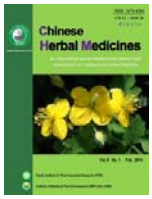

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Original article

Adsorption Properties for Separation of Apigenin from *Viola yedoensis* on LSA-10 Resin

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ABSTRACT

Objective To explore the adsorption properties for the separation of apigenin from *Viola yedoensis* on LSA-10 resin. **Methods** After different types of macroporous resins were optimized, the effects of initial concentration, temperature, pH value, and other factors on resin adsorption were studied, and the kinetics and thermodynamics in the process of the static adsorption of LSA-10 resin for the apigenin separation from *V. yedoensis* were also investigated. **Results** The initial concentration of 4.0 mg/mL, temperature of 50 °C, and pH 5 were suitable for the resin adsorption, the experimental data of adsorption isotherms of LSA-10 resin were validated to fit the Freunclich and Langmuir equation, the adsorption process of apigenin was fitted to the first order adsorption kinetics equation, and the adsorption rate was mainly affected by film diffusion. The thermodynamic parameters such as adsorption enthalpy change ($\Delta H > 0$), adsorption free energy change ($\Delta G < 0$), and adsorption entropy change ($\Delta S > 0$) were investigated. **Conclusion** The adsorption for the separation of apigenin on LSA-10 resin was an entropy-driven spontaneous process of decalescence and entropy increase, which belongs to physical adsorption. LSA-10 resin is suitable for the industrial separation of apigenin from *V. yedoensis*.

Key words

adsorption; apigenin; resin; separation; *Viola yedoensis*

1. Introduction

Viola yedoensis Makino, with the common name known as Philippine violet herb, Yedeon's vilet, viola, purple flower violet, mamyflower gueldemstaedtid herb, etc, is an important Chinese herb from *Viola* L. of Violaceae family (Ren, 1986) that has been reported to contain potential anti-HIV agents. From *V. yedoensis,* five new and three known cyclotides were identified and shown to have anti-HIV activity (Wang et al, 2008). *V. yedoensis* is a perennial herb, its seedlings could be obtained via seed reproduction, and its favorable habitat is grassland. The blooming season is from February to April. This is a wild plant which could grow more than 10 cm in height and be seen everywhere (Bureau of Drug Administration Policy, Ministry of Public Health, 2000). It is reported that the whole plant has the antibacterial, anti-inflammatory, antipyretic, and depurative activities. It is used for the treatment of boils, carbuncles, snakebite, skin disorders, mumps, etc. Recent studies showed that *V. yedoensis* possessed the antiviral activity. Further studies confirmed the extract from *V. yedoensis* could inhibit the replications of herpes simplex virus-1 and enterovirus 71 in human neuroblastoma SK-N-SH

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cell line and strengthen the antiviral immunity through modulating the non-specific immune response (Liao et al, 2010). The extract from *V. yedoensis* was isolated and purified by means of silica gel column chromatography and identified by spectroscopic analyses to determine the structures of compounds. Five compounds were obtained and established from the extract as 6,7-dihydroxycoumarin, β-sitosterol, melissyl alcohol, stearic acid, and methyl palmitate (Yang et al, 2008).

Some researchers proposed that apigenin was one major ingredient of pharmacological effective components in *V. yedoensis*. Xie et al (2003a) reported that seven diverse structural apigenins were isolated from *V. yedoensis* together with luteolin. Moreover, apigenin is a naturally occurring flavone in plant, abundantly present in common fruits and vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts, and some seasonings (Patel et al, 2007). Additionally, the significant progress has been made in studying the biological effects of apigenin at cellular and molecular levels. It was recognized as a bioactive flavonoid shown to possess anti-inflammatory, anti-oxidative, and anticancer properties (Shukla and Gupta, 2010). Therefore, apigenin has received the extensive attention of researchers in recent years.

In previous studies, many extraction techniques have been used for the extraction and isolation of natural bioactive products from diverse plants, including supercritical fluid extraction (Quitain et al, 2006), pressurized fluid extraction (Rostagno et al, 2004), matrix solid phase dispersion (Xiao et al, 2004), ultrasonic extraction (Tian et al, 2002; Achouri et al, 2005), extraction by Soxhlet apparatus, solid phase extraction (Rostagno et al, 2005; Grace et al, 2006), and some new technologies such as microwave assisted extraction, cellulase engineering techniques, and semi-bionic extraction (Xu and Zhu, 2008). Moreover, the researchers focused on exploring the simplest, fastest, most low-cost, and most effective ways for the extraction and isolation of natural bioactive substances from plants, through optimizing all conditions of the procedures to gain the maximum yields of extractives.

Recently, the separation methods based on macroporous resin are popular in pharmaceutical application and have also been used for the separation of bioactive products from natural plants (Fu et al, 2002), However, up to now, few reports were involved in the extraction of apigenin from *V. yedoensis* using the macroporous resin. The objective of the present study is to explore the optimal technological conditions with the features of energy saving and environmental protection for apigenin production in industrial scale through the investigation of the adsorption properties of apigenin in *V. yedoensis* separated by LSA-10 resin.

2. Materials and methods

2.1 Reagents and apparatus

The experimental samples of *Viola yedoensis* Makino were collected from the wild surrounding near to Zhangjiajie, Hunan province, China, and dried at 60 ^{o}C, then ground to 40 mesh fineness, oven dried again till constant weight, sealed up and stored for later use.

Methanol, acetone, trichlormethane, anhydrous ethyl alcohol, 5% HCL, and 5% NaOH were of analytical grade. The apigenin reference substance was supplied by Shanghai Xinma Biological Science and Technology Co., Ltd. (China). The different types of macroporous resins including LSA-7, LSA-10, LSA-20, LSA-21, LSA-40, LSA-58, XDA-1, XDA-8, D941, and HP-10 were supplied by Xi'an Lanxiao Science and Technology Co., Ltd. (China). All the other reagents were also of analytical grade.

The apparatus used in this experiment included the Lamba25 Ultraviolet Spectrophotometer (PerkinElmer, USA), JY98–3DN Ultrasonic Biological Cell Breaker (Japan), AEB–220 and AEL–200 Electronic Balance (Japan), CS101–3D Electric Drying Oven (Japan), BM400 Electric-heated Thermostatic Water Bath, (Yamato), RE540–AW Rotary Evaporator (Japan), and MDF–292AT Ultra Low Temperature Refrigerator, (Sanyo, Japan).

2.2 Preparation and determination of apigenin reference solution

The apigenin reference substance (500 mg) was weighed and dissolved with 70% methanol in 100 mL flask. Two drops of acetic acid solution were added, and the apigenin reference solution with the concentration of 5.0 mg/mL was obtained. The above reference stock solution (10 mL) was diluted with 70% methanol in 50 mL flask. The specific volume of this diluted reference solution was taken to scan at the range of 800–200 nm with the ultraviolet spectrophotometer and the maximum absorbance (*A*) value was obtained at 340 nm. Apigenin reference solution with different gradient concentration was prepared and the corresponding *A* values were determined at 340 nm. Then the standard curve of apigenin was derived from the UV absorbance values of above serial dilutions.

2.3 Preparation of crude V. yedoensis extracts

V. yedoensis powder (500 g) was weighed, added with 500 mL of 80% ethanol, treated with the ultrasonic biological cell breaker for 10 min, and kept under static condition for 30 min. Then the supernatant was extracted from the mixed crude solution and repeated the latter operation for three times. All supernatant was condensed by reduced pressure method to obtain the dried crude extracts, to which 50–100 mL methanol was added. After dissolved completely, the extraction was performed according to the previous study (Dong, 2008). Meanwhile the isolation and purification of apigenin were performed by means of silica gel column chromatography and macroporous resins. The total isolated solution was condensed to 50 mL as the stock solution for further test.

2.4 Macroporous resin screening

Eleven different types of macroporous resins listed in Table 1 were pretreated as the previous study (Lu, 2005).

Table 1 Physical characteristics of macroporous resins

Resin	Polarities	Pore diameter / mm
LSA-20	nonpolar	0.32–1.05
D101	nonpolar	0.25–0.84
HP-10	nonpolar	0.28–1.15
LSA-21	moderate polarity	0.25–1.20
LSA-10	moderate polarity	0.20–1.30
LSA-40	moderate polarity	0.31–1.28
XDA-1	polar	0.31–1.26
XDA-8	polar	0.32–1.25
LSA-7	polar	0.34–1.35
XDA-7	high specific surface area active	0.36–1.29
LSA-58	high specific surface area active	0.32–1.31

Pretreated macroporous resin (1 g) of each type was taken to 50 mL conical beaker and added with 15 mL apigenin reference solution to keep static adsorption for 24 h. After filtration, the concentration of apigenin solution was determined by UV method. The adsorption quantity is calculated as follows:

$$Q_e = (C_0 - C_e) \times V_i / W$$

where Q_e is the adsorption quantity at adsorption equilibrium (mg/g resin); C_0 and C_e (mg/mL) are the initial and equilibrium concentration of solutes in the solutions respectively; V_i is the volume of initial feed solution (mL) and W is the weight of the dry adsorbent (g)

The adsorption rate is calculated as the following adsorption equation:

$$E = (C_0 - C_e) / C_0$$

where E is the adsorption ratio, which is the percent of the total adsorbate mass adsorbed after reaching equilibrium; C_0 and C_e are the same as described above

The desorption rate is calculated as the desorption evaluation:

$$D = C_d \times V_d / [(C_0 - C_e) \times V_i]$$

where D is the desorption rate; C_d is the concentration of the solutes in the desorption solutions (mg/mL); V_d is the volume of desorption solution; C_0, C_e, and V_i are the same as above

2.5 Effect of initial solution concentration on adsorption capacity

Each pretreated resin (3 g) was added into five tubes contained 10 mL crude *V. yedoensis* extract solutions with the apigenin concentration of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/mL, respectively. Then all the tubes with different apigenin concentration were put into the electric-heated thermostatic water bath at 50 °C, and the contents of each tested solution were determined every hour. The adsorption quantity of apigenin resin was calculated as the adsorption equation mentioned above.

2.6 Effect of temperature on adsorption capacity

Every pretreated resin (3 g) was added into 12 tubes contained 10 mL crude *V. yedoensis* extract solutions with the same apigenin concentration of 2.0 mg/mL. Then the tubes were kept statically in the electric-heated thermostatic water bath at 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, and 80 °C, respectively. After 12 h, the corresponding A values of the tested solutions (n = 4) were determined at 340 nm. The adsorption quantity was calculated as the adsorption equation described above.

2.7 Effect of pH value on adsorption capacity

Each pretreated resin (3 g) was added into 12 tubes contained 10 mL crude *V. yedoensis* extract solutions with the same apigenin concentration of 4.0 mg/mL. The pH values of the 12 tubes were adjusted to 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0, respectively. Then all the tubes were kept statically in the electric-heated thermostatic water bath at 50 °C. After 12 h, the corresponding A values of the tested solutions (n = 4) were determined at 340 nm. The adsorption quantity was calculated as the adsorption equation described above.

2.8 Adsorption isotherms

Each pretreated resin (3 g) was added into eight tubes contained 10 mL crude *V. yedoensis* extract solutions with the apigenin concentration of 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/mL, respectively. Then all the tubes with different apigenin concentration were put into the electric-heated thermostatic water bath at 50 °C for 12 h. The adsorption quantity of apigenin on LSA-10 resin was calculated as the adsorption equation described above.

2.9 Dynamic adsorption and desorption tests of LSA-10 resin

Five portions of pretreated resin (20 g each) were put into five columns respectively, The flow speeds of the initial apigenin extract solutions were controlled at 0.5, 1, 1.5, 2, and 3 BV/h, respectively. The adsorption quantities were determined correspondently. The effluent solution was collected till that the apigenin content of influent solution was the same as the effluent solution. Then the resin columns were eluting by the 70% methanol at the speeds of 1, 2, 3, 4, and 5 BV/h, respectively. The apigenin contents in the effluent eluting solution were determined respectively.

3. Results and discussion

3.1 Apigenin reference curve

The maximum A value of apigenin reference substance was obtained at 340 nm by the ultraviolet spectrophotometer. The different A values of the concentration gradient of apigenin reference substance were also determined, and the apigenin reference curve was established, from which the regression equation was calculated as $y = 0.0651\ x - 0.0002$, $r^2 = 0.9998$ and used for quantification of apigenin.

3.2 Resin screening through static adsorption and desorption rates

Table 2 shows that LSA-10 resin had the maximum mean value of adsorption ratio and desorption ratio among all the resins, thus indicated that the LSA-10 resin had the optimal static adsorption properties. Therefore, the LSA-10 resin was screened to isolate and purify the apigenin from *V. yedoensis*.

Table 2 Comparison of static adsorption and desorption properties of different resins

Resin	Adsorbance quantity / ($mg \cdot g^{-1}$)	Adsorption ratios / %	Desorption ratios / %
LSA-20	58.52	70.3	90.8
D101	48.20	57.9	86.3
HP-10	52.53	63.1	72.1
LSA-21	71.10	85.4	83.5
LSA-10	75.17	90.3	91.3
LSA-40	74.59	89.6	86.7
XDA-1	76.67	92.1	78.2
XDA-8	75.42	90.6	82.3
LSA-7	74.68	89.7	81.4
XDA-7	71.51	85.9	86.6
LSA-58	77.59	93.2	79.8

3.3 Effect of initial concentration on adsorption capacity

As shown in Figure 1, the apigenin adsorption quantity of LSA-10 resin was accelerating in a linear relationship with the time at the initial stage. Afterward, the adsorption pattern was eased promptly, and finally the adsorption reached the saturation plateau. The adsorption rate was closely related to the initial concentration of apigenin. The results also revealed that it took about 1 h to reach the adsorption equilibrium when the initial concentration of apigenin was 8.56 mg/mL, and about 6 h with 0.5 mg/mL, suggesting that the lower the initial concentration of apigenin was, the longer the adsorption time reached the equilibrium. Thus, the concentration of apigenin in the initial feed solution was selected at 4.0 mg/mL.

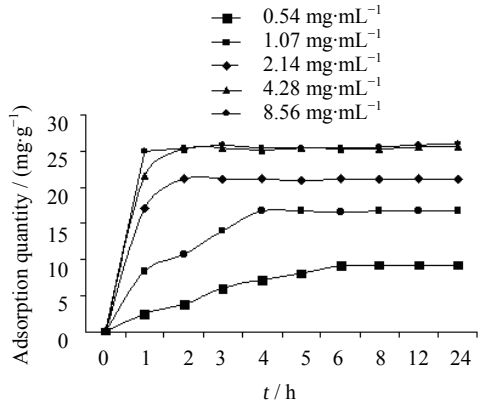

Figure 1 Effect of initial concentration on adsorption capacity

3.4 Effect of temperature on adsorption capacity

The results showed that the apigenin adsorption quantity of LSA-10 resin was elevating as the temperature increasing within the range of 30–50 °C and reached the maximum at 50 °C. Then it maintained at almost the same level when temperature was increasing (Figure 2).

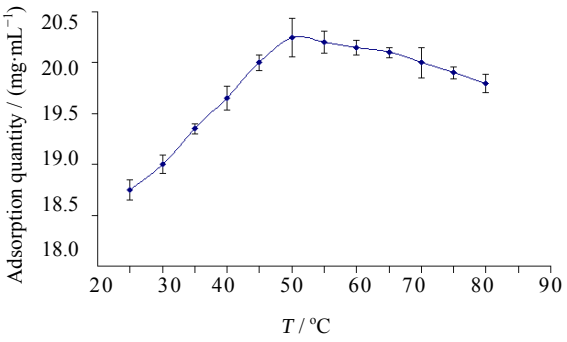

Figure 2 Effect of temperature on adsorption capacity

3.5 Effect of pH value on adsorption capacity

The results showed that the optimal adsorbance efficiency was obtained within the pH range of 4–5 (Figure 3), because the apigenin formed the hydrogen bond that was beneficial to adsorption. As the pH value was increasing, the adsorption rate obviously decreased (Figure 3), which was caused by protonation or dissolution action, which made the apigenin transform into ionic forms, such caused obvious decrease of hydrogen bond adsorption. But there were still some adsorption quantities in resin that probably caused by the action of van der Waals force.

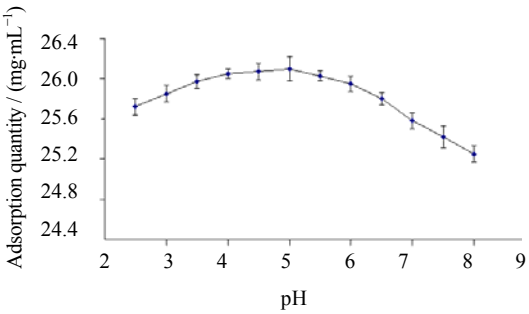

Figure 3 Effect of pH value on adsorption capacity

3.6 Adsorption isotherm

Equilibrium adsorption isotherm was obtained by contacting 10 mL of crude *V. yedoensis* extract solution with the LSA-10 resin in a bath controlled at 25 °C. The initial concentration of apigenin in the solutions was 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 mg/mL, respectively. As shown in Figure 4, for apigenin, the adsorption reached the saturation plateau when the initial concentration of apigenin was 4.0 mg/mL. Thus, the optimal apigenin concentration of the initial feed solution was validated at 4.0 mg/mL.

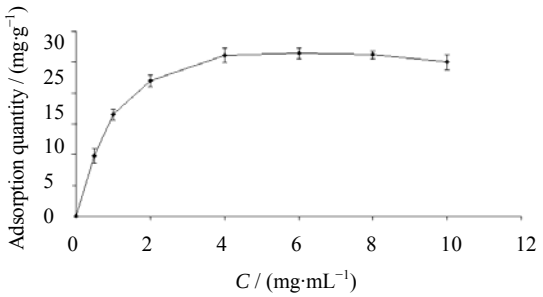

Figure 4 Adsorption isotherm at 25 °C for apigenin on LSA-10 resin

The experimental data were fitted to the Langmuir equation to describe how solutes interact with the adsorbent of LSA-10 resin.

$$q = K_1 \cdot q_m \cdot C_f / (1+K_1 \cdot C_f)$$

where q is the adsorption quantity (mg/g), K_1 is the Langmuir adsorption constant, q_m is the saturation adsorption quantity or maximum adsorption quantity (mg/g), C_f is the apigenin concentration (mg/mL) when reached the equilibrium.

Langmuir isotherm could be plotted using the Langmuir equation, which could be expressed as the linear equation: $C_f / q = 0.038\ C_f + 0.0034$. The correlation coefficient for LSA-10 resin derived from this equation is $r = 0.9972$ ($P < 0.05$). According to this linear equation, the theoretical maximum saturation adsorption quantity could be calculated as $q_m = 26.32$ mg/g, without significantly statistical difference comparing with the experimental maximum saturation adsorption quantity [(25.96 ± 0.72) mg/g], the Langmuir constant $K_1 = 11.1765$, which suggested that the apigenin adsorption on LSA-10 resin was consistent with the Langmuir model, and further indicated the apigenin adsorption on LSA-10 resin belonged to monolayer adsorption .

The experimental data were also fitted to the Freundlich equation to validate how solutes interacted with the adsorbent of LSA-10 resin:

$$1 - \frac{q_t}{q} = kc_f^{\frac{1}{n}}$$

where q is adsorption quantity (mg/g), q_t (mg/g) is the adsorption quantity at the specific time t, C_f is the apigenin concentration of equilibrium (mg/mL), k is equilibrium adsorption coefficient; n is the specific constant.

Freundlich isotherm of apigenin on LSA-10 resin could be plotted using the Freundlich equation that could be expressed as follows:

$$\lg \frac{q - q_t}{q} = 0.6372 \lg c_f - 0.64$$

The correlation coefficient for LSA-10 resin derived from this equation is $r = 0.9950$ ($P < 0.05$), which revealed that the apigenin adsorption on LSA-10 resin was also consistent with the Freundlich model. Further analysis revealed that when $n = 1.5694$ (> 1), the apigenin adsorption on LSA-10 resin was the preferential monolayer adsorption on inhomogeneous surface. Furthermore, the experimental real saturation adsorption quantity of apigenin on LSA-10 resin is almost equal to the theoretical maximum value calculated using Langmuir model or Freundlich model.

3.7 Kinetic analysis of adsorption

The adsorption process contained four steps: (1) external diffusion of adsorbate in solution, (2) liquid film diffusion of adsorbate at the liquid film surface of adsorbent, (3) porous diffusion of adsorbate in particles of adsorbent or particle diffusion, (4) adsorption and desorption of adsorbate on the adsorbent surface. The step (1) was completed rapidly through stirring, and the step (4) was completed promptly. Therefore, the adsorption of adsorbate on the adsorbent surface was mainly controlled by the liquid film diffusion or porous diffusion, thus the Lagergren kinetic rate equation on adsorption reaction and Kannan intra-particle diffusion equation were applied to analyzing the controlling of the adsorption rate.

The Lagergren kinetic rate equation on adsorption reaction is fitted in terms:

$$\ln [1 - F] = kt$$

where $F = q_t / q$, q_t is the adsorption quantity after t time, q is the adsorption quantity when reached equilibrium, k is the adsorption coefficient at temperature T

The apigenin adsorption kinetic equation of LSA-10 resin was expressed as $y = 0.4601\ x - 0.2393$, $r = 0.9987$ ($P < 0.05$). Further analysis revealed that when the adsorption process was controlled by the liquid film diffusion, there existed better linear relation between $\ln [1 - F]$ and t. But when the temperature was higher than 50 °C, the adsorption was deviated from linear equation, which suggested that the adsorption process was not fully controlled by the liquid film diffusion under this condition. Kannan and Sundaram (2001) described the intra particle diffusion model as follows:

$$q_t = kt^{1/2} + c$$

where q_t (mg/g) is the adsorption quantity at various time (t). k is the intra particle diffusion rate constant (mg·min$^{1/2}$/g).

The kinetic equation was fitted to the experimental data and obtained the intra particle diffusion linear equation of LSA-10 resin as $y = -4.8169\ x + 6.94$, $r = 0.7082$, which showed the linear relationship was not significance ($P > 0.05$) and validated that the apigenin adsorption process of LSA-10 resin was mainly controlled by the liquid film diffusion.

3.8 Adsorption thermodynamics on LSA-10 resin

The thermodynamic parameters for the adsorption equilibrium of LSA-10 included the adsorption enthalpy change (ΔH), the adsorption free energy change (ΔG), and the adsorption entropy change (ΔS).

When the adsorption quantity is unchanged, the ΔH is equivalent adsorption enthalpy change, which could be calculated according to Van't Hoff equation:

$\ln (C_f^{-1}) = \ln k + (-\Delta H \cdot R / T)$, which could be written as

$$\ln C_f = \Delta H / (R \cdot T) - \ln k$$

where C_f (mg/mL) is the solute concentration of equilibrium at thermodynamic temperature T (K), R is the ideal gas constant [8.314 J/(mol·K)], k is equilibrium constant, ΔH is the standard equivalent adsorption enthalpy change (kJ/mol)

The experimental data were fitted to this equation, the

Van't Hoff equation of LSA-10 could be expressed as y = 1.1859 x − 4.2317, the correlation coefficient r = 0.9952 (P < 0.05). From the slope of the linear equation, it could be calculated out the ΔH = 9.86 (Table 3).

The ΔG was calculated according to Gibbs equation:

$$\Delta G = -RT \int_0^X Q_e \frac{d_x}{x}$$

where X indicates the mole fraction of solute in solution, Q_e is substituted for adsorption isotherm equation or Freundlich adsorption equation, R is ideal gas constant [8.314 J/(mol·K)]. The ΔG values calculated from experimental data were listed in Table 3

The ΔS on LSA-10 resin was calculated according to Gibbs-Helmholtz equation:

$\Delta S = (\Delta H - \Delta G) / T$

where T is the absolute temperature. The ΔS values calculated from our study are listed together with ΔH and ΔG values in Table 3

Table 3 ΔG, ΔH, and ΔS values of apigenin adsorption thermodynamics on LSA-10 resin

T / K	ΔG / (kJ·mol^{-1})	ΔH / (kJ·mol^{-1})	ΔS / (J·mol^{-1}·K^{-1})
303	−4.0	9.86	45.74
313	−4.1	9.86	44.60
323	−4.2	9.86	43.53
333	−4.3	9.86	42.52

As shown in Table 3, the apigenin adsorption on LSA-10 resin was a spontaneous process (ΔG < 0), and companied with the heat absorbing reaction (ΔH > 0), the ΔS values were positive value (ΔS > 0). With the temperature increasing, the absolute value was also elevated that indicated the adsorption trend was increasing. The analysis results also coincided with the experimental results that the adsorption quantity was increased as the temperature was elevated.

3.9 Dynamic adsorption and desorption on LSA-10 resin

The results of dynamic adsorption for apigenin on LSA-10 resin at different flow speeds under the conditions of 50 °C and pH 5 were shown in Table 4. As seen from Table 4, when the concentration of initial feed solution was the same as 4.0 mg/mL, the total time taken by dynamic adsorption was decreased as the flow speed increased. Comprehensive analysis indicated that the flow speed of feed solution at 1.5 BV/h was the optimal suitable for the dynamic adsorption of apigenin on LSA-10 resin.

Table 4 Dynamic adsorption for apigenin on LSA-10 resin under conditions of 50 °C and pH 5

Apigenin / (mg·mL^{-1})	Flow speed / (BV·h^{-1})	Adsorption time / min	Breakthrough volume / mL
4.0	0.5	190	800
4.0	1.0	170	910
4.0	1.5	155	1000
4.0	2.0	140	1110
4.0	3.0	110	1320

The experimental results of the dynamic desorption for apigenin on LSA-10 resin at different flow speeds under the conditions of 50 °C and pH 5 are shown in Table 5. As shown in Table 5, the different flow speeds of eluting solution (70% methanol) had different breakthrough elution volumes and different total dynamic desorption time. Comprehensive analysis showed the eluting flow speed at 3 BV/h was optimal for the dynamic desorption of apigenin on LSA-10 resin. Further analysis of the experimental results showed the apigenin production ratios could reach more than 95%, when the flow speeds for the dynamic adsorption and desorption were at 1.5 and 3 BV/h, respectively.

Table 5 Dynamic adsorption for apigenin on LSA-10 resin under conditions of 50 °C and pH 5

Eluted solution	Flow speed / (BV·h^{-1})	Desorption time / min	Breakthrough volume / mL
70% methanol	1.0	110	600
	2.0	80	620
	3.0	55	650
	4.0	65	780
	5.0	60	900

4. Conclusion

In general, the LSA-10 resin is screened from 11 different types of macroporous resins, and is optimal for the isolation and purification of apigenin from *V. yedoensis*. The results showed that the initial concentration of 4.0 mg/mL, temperature of 50 °C, pH 5 are suitable for the resin adsorption, and the experimental data of adsorption isotherms of LSA-10 resin are validated to fit the Freunclich and Langmuir equation, it is also observed that the adsorption process of apigenin is fitted to the first order adsorption kinetics equation, and the rate of adsorption is mainly affected by film diffusion. The ΔS values for apigenin on LSA-10 resin are above zero (ΔS > 0) that indicates the adsorption is an entropy driving process. The total entropy change ΔS_t is the sum of solute adsorption, which companies with ΔS value or degree of freedom decreasing, and solvent desorption, which leads to ΔS value or degree of freedom increasing (Xie et al, 2003b). Moreover, the results suggest that the adsorption for apigenin on LSA-10 resin surface is dominant, the ΔS value increase caused by water molecule desorption is greater than the ΔS value decrease caused by the adsorption for apigenin. Therefore, the adsorption entropy change ΔS is positive value. From the results of our study, the variation scopes of ΔH and ΔG are smaller as well as the ΔG values varied little with the temperature that indicates the adsorption for apigenin on LSA-10 resin belongs to physical adsorption, which is validated by the experimental results of dynamic adsorption and desorption. Thus the isolation and the purification of apigenin from *V. yedoensis* with the LSA-10 resin are energy-saving and beneficial to environment protection. Finally, the LSA-10 resin has the optimal adsorption properties among the 11 macroporous resins,

which is suitable for the isolation and purification of apigenin from *V. yedoensis.*

References

Achouri A, Boye JI, Belanger D, 2005. Soybean isoflavones: Efficacy of extraction conditions and effect of food type on extractability. *Food Res Int* 38(10): 1199-1204.

Bureau of Drug Administration Policy, Ministry of Public Health, People's Republic of China, 2000. *Current Practical Materia Medica*. People's Medical Publishing House: Beijing.

Dong AW, 2008. Study on extraction separation and purification of apigenin from *Viola yedoensis* Makino. Changsha: Hunan Agricultural University.

Fu DH, Liu AL, Deng NK, Zhou T, Xiao JD, 2002. Study on the adsorption behavior and application of DM-130 resin to purifying glycyrrhizic acid. *Nat Prod Res Dev* 14: 60-64.

Grace PB, Teale P, 2006. Purification of the crude solution from Helix pomatia for use as beta-glucuronidase and aryl sulfatase in phytoestrogen assays. *J Chromatogr B* 832(1): 158-161.

Kannan N, Sundaram M, 2001. Kinetics and mechanism of removal of methylene blue by adsorption on various carbons-a comparative study. *Dyes Pigm* 51: 25-40.

Liao HF, Lu MC, Chang HC, Wei CC, Kao CH, Chen ZH, Huang CC, Li C, 2010. Effects of herbal medicinal formulas on suppressing viral replication and modulating immune responses. *Am J Chin Med* 38: 173-190.

Lu Y H, 2005. *Extraction and Isolation Technology of active ingredients of Traditional Chinese Medicine.* Chemical Indrustry Press: Beijing.

Patel D, Shukla S, Gupta S, 2007. Apigenin and cancer chemo-prevention: Progress potential and promise. *Int J Oncol* 30: 233-245.

Quitain AT, Oro K, Katoh S, Moriyoshi T, 2006. Recovery of oil components of okara by ethanol-modified supercritical carbon dioxide extraction. *Biores Tech* 97: 1509-1514.

Ren RA, 1986. *Science for Identifying Chinese Materia Medica*. Shanghai Science and Technology Press: Shanghai.

Rostagno MA, Palma M, Barroso CG, 2004. Pressurized liquid extraction of isoflavones from soybeans. *Anal Chim Acta* 522: 169-177.

Rostagno MA, Palma M, Barroso CG, 2005. Solid-phase extraction of soy isoflavones. *J Chromatogr A* 1076(2): 110-117.

Shukla S, Gupta S, 2010. Apigenin: A Promising Molecule for Cancer Prevention. *Pharm Res* 27(6): 962-978.

Tian FF, Zhu YX, Xie FM, Long H, 2002. Analysis of isoflavones in natural sources and nutritional supplements by liguid chromatography and multi-channel electrochemical detection. *J Liq Chromatogr Rel Techn* 25: 475-485.

Wang CKL, Colgrave ML, Gustafson KR, Ireland DC, Goransson U, Craik DJ, 2008. Anti-HIV cyclotides from the Chinese medicinal herb *Viola yedoensis. J Nat Prod*, 71: 47-52.

Xiao HB, Krucker M, Albert K, Liang XM, 2004. Determination and identification of isoflavonoids in *Radix astragali* by matrix solid-phase dispersion extraction and high-performance liquid chromatography with photodiode array and mass spectrometric detection. *J Chromatogr A* 1032: 117-124.

Xie C, Veitch NC, Houghton PJ, Simmonds MS, 2003a. Flavone C-glycosides from *Viola yedoensis* Makino. *Chem Pharmaceut Bull* (Tokyo) 10: 1204-1027.

Xie ZF, He XC, Xia JH, Yan Q, 2003b. Adsorption thermodynamics and dynamics of polyamide to picric acid. *Chem Res* 4: 53-56.

Xu ZL, Zhu Y, 2008. Advances on the application of extraction technologies of Chinese traditional medicines. *J Yunan Univ Trad Chin Med* 5: 66-70.

Yang PP, Yan FL, Liang YB, 2008. Study on chemical constituents of *Viola yedoensis*. *J Xinxiang Med Coll* 2: 185-187.