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Synthesis and characterization of amino acid-modified adsorption resins and its adsorption properties in the purification of tabersonine from *Voacanga africana* seeds

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Abstract

A series of macroporous adsorption resins anchored valine and glycine were synthesized and identified. The structural characterization indicated that Brunauer–Emmett–Teller (BET) surface area and pore volume of resins decreased after functionalization of amino acid. The adsorption kinetics and adsorption isotherm experiments indicated that the pseudo second-order rate equation was more appropriate for characterizing the kinetic data and the Freundlich model was more suitable for fitting the equilibrium data. And the results also revealed that the maximum adsorption capacity of tabersonine on resins reduced to 14 mg/g from 254 mg/g after the functionalization of valine. Causes for the low adsorption capacity of tabersonine on modified resins were discussed. The modified resins were applied to purify tabersonine extracted from *Voacanga africana* seeds. The results showed that the modified resins were good adsorption to impurities but poor one to tabersonine in the extracts of *Voacanga africana* seeds. And tabersonine was purified efficiently with the purity above 90%. The reusability of the modified resins was also assessed and the modified resins exhibited considerable reusability.

Introduction

Voacanga africana is an evergreen tree, or some would also argue as shrub, which reaches up to the height of 6 meters, with widely spreading low crowns around it. It is

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mainly distributed in rainforests at the West Africa. Generally, its fruits emerge in pairs, spherical shape, containing several brown seeds that are irregular shape.[1] In addition, its bark and root are widely used in traditional medicines. Even so, its seeds are actually the most economically part for the application in medicine industry. The seeds are considered as an important source of annual additional income for harvesters and exporters in West Africa.[2] In order to utilize this seeds efficiently, Koroch et al. have reviewed its chemistry and pharmacological activities.[2] However, there are still very few reports about this seeds. Therefore, more researches are worthy to be carried out for this important African medical plant.

In the past decades, several chemical components have been isolated from different organs of *Voacanga* species.[3-5] Among them, tabersonine, a well known alkaloid, was isolated from *Voacanga* seeds.[1, 6] Its structure and properties have been disclosed in the prior art treatise.[7] It is known to be of indole structure and is related to the molecule of Vinca alkaloids. And its content in *Voacanga* seeds is about 2.0%.[8] The chemical structure of tabersonine is shown in Figure 1. Many researchers have reported that tabersonine plays a significant role in lowering blood pressure and it can be used to semi-synthesize the cerebrally active eburnamine derivatives vincamine and the anti-cancer drug vinpocetine.[9-11] In 1973, Poisson published a patent related to acquisition of tabersonine by repeated extraction and crystallization.[8] However, this method has many disadvantages, such as the use of toxic extraction solvents and the low yield of tabersonine. In 2008, Zhang et al. provided another method to purify tabersonine. Tabersonine in the extracts of *Voacanga* seeds was purified by commercial macroporous adsorption resins (MARs).[12] This is a promising method to obtain tabersonine due to its simple operation and considerable yield. However, this method also has some shortcomings that need to be improved. 10 kg commercial MARs (D101) were required to purify tabersonine from 1 kg *Voacanga* seeds.[13] The dosage of the commercial MARs is the key factor that would increase the cost of industrial production. This problem is mainly because the commercial MARs (D101) lacks of adsorption selectivity toward tabersonine and impurities in the extracts of

Voacanga seeds. Thus, more MARs are needed to increase the numbers of theoretical plate for separating them.

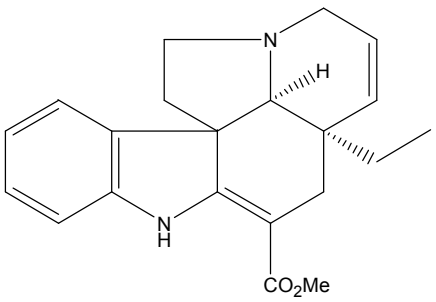

Figure 1. The chemical structure of tabersonine.

Many authors have reported that the commercial MARs exhibits unique adsorption properties and has advantages in purifying natural products.[14-16] However, the adsorption mechanisms of commercial MARs are mainly based on hydrophobic forces such as the Van de Waals force, which may result in low adsorption capacity and selectivity.[17] In recent years, many researchers have showed great interests in modifying MARs with functional groups to obtain new adsorbents with high adsorption capacity and selectivity.[18-21] And these researches showed that the MARs modified by special functional groups such as chloromethyl, amino, and phenylamino can be potentially applied in forming special MARs for purification of target compound. It can be easily found that almost all these researches are concentrating on the improvement of the adsorption capacity of the MARs toward the target compound. However, researches about modifying MARs which are committed to decrease the adsorption capacity of the MARs toward the target compound and to maintain or increase the adsorption capacity of the MARs toward the impurities have rarely been carried out so far. Also, there are no researches that have been carried out to functionalize MARs to purify tabersonine from *Voacanga* seeds.

Considering the importance of this African medical plant and the low efficiency of purification of tabersonine by commercial MARs, the aim of this work was to improve the efficiency of purification of tabersonine. In the present paper, a series of

modified MARs were synthesized by introducing amino acid. The modified MARs exhibited a good ability to adsorb impurities but a poor ability to adsorb tabersonine in the extracts of *Voacanga africana* seeds. Tabersonine could be purified efficiently from the *Voacanga* seeds by modified MARs. The corresponding adsorption behaviors of the modified MARs toward tabersonine were systemically investigated. And the reusability of the modified resins was also assessed.

Experimental

Chemical Reagents, Adsorbents, and Samples

D101, nonpolar styrene co-divinylbenzene copolymer with no functional groups, was purchase from Chemical Factory of Nankai University (Tianjin, China). All the MARs were classified by sieves. The fraction formed by the size between 0.45 and 0.60 mm were used in the experiments. Before the experiments, the resins were pretreated by soaking in ethanol overnight, subsequently washing thoroughly with distilled water. Chloromethyl methyl ether was purchased from Henanwanxiang Technology & Trade Co., Ltd. (Henan, China). The reagents and chemicals (analytic grade unless stated otherwise) were purchased from Sinopharm Chemical Reagent Co., Ltd. and Tianjin Damao Chemical Reagent Co., Ltd (China). Tabersonine (purity > 98%) was purchased from Enjoye C & G Bioengineering Co., Ltd (Xiamen, China).

Synthesis of Adsorbents with Functional Groups

The reaction scheme for the preparation of MARs with chloromethyl groups (PS-Cl) and MARs with amino acid (PS-amino acid) on the basis of D101 is shown in Figure 2. Pretreated D101 copolymer beads (15g) were allowed to swell in dichloromethane (100mL) for 24 h. Then, zinc chloride (15g), sodium chloride (20g) and chloromethyl methyl ether (70mL) were mixed the soaked beads in a 500 mL three-neched round bottomed flask equipped with a mechanical stirrer, a reflux condenser, and a thermometer. The reaction mixture was stirred (100 rpm) at 323 K for 20 h. The synthetic MARs were filtered and washed with distilled water and methanol until there was no white precipitate while an aqueous solution of silver nitrate was added to

the filtrate.[19] The MARs with chloromethyl groups (PS-Cl) were obtained. The chloromethylated beads PS-Cl (6g) were allowed to swell in 15 mL methanol for 24 h. Then an aqueous solution of amino acid (0.05mol) in 125 mL distilled water and pyridine (0.05mol) were added. The mixture was stirred (100 rpm) and refluxed for 20 h. The amino acid linked polymer beads (PS-amino acid) were filtered and then washed with water and methanol, then dried under vacuum.[22]

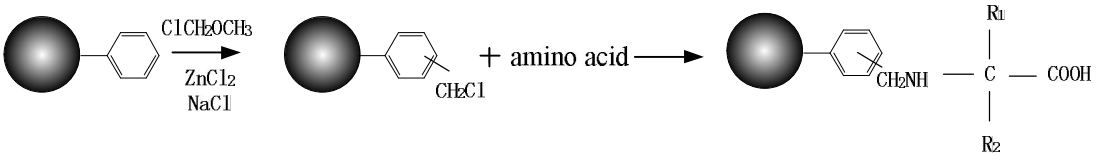

Figure 2. Reaction scheme for the preparation of PS-Cl and PS-amino acid on the basis of D101.

Characterization of MARs

Physical properties of the MARs were determined by N_2 adsorption/desorption isotherms at 77 K using a ASAP 2020 automatic surface area and porosity analyzer. The BET surface area was obtained by the BET method. The pore volume and average pore diameter were obtained by the Barrett–Joyner–Halenda (BJH) method. Surface morphology of the MARs was observed by Field-emission scanning electron microscopy (FEI Sirion 200). FTIR spectral of MARs were obtained on a FTIR spectrophotometer in the 4000-400 cm^{-1} region via the KBr pellet method. The content of chloromethyl in the MARs was determined by an electrochemistry method which was described by Song Lou et al. .[20] A known weight of the dry MARs (0.2000 $\pm$0.0020 g) was heat-dissolving in a nickel crucible at 873 K for 8 h with 0.5 g of melting sodium hydroxide. The melted masses were diluted in distilled water to 250 mL, then, the electric potential of the sample aqueous solution was determined by an electrochemical workstation (RST 5200, Zhengzhou, China). The content of chloromethyl group was calculated from the following equation, with a range of 0.01-100 mmol/g and R^2=0.9997

$$C = \exp(\frac{M - 15.171}{53.431}) \quad (1)$$

$$N = \frac{0.25 \times C}{0.2} \times 1000 \quad (2)$$

where C (mol/L) refers to the concentrations of the sample aqueous solution, M (mV) is the electric potential of the sample aqueous solution, N is the content of chloromethyl group (mmol/g dry adsorbent). The content of amino acid in MARs was calculated according to the reduction of the content of the chloromethyl after the substitution of Cl with amino acid moiety. The content of amino acid in MARs could also be obtained by determining the available content of free –COOH group in PS-amino acid, which could be confirmed by the method described by Valodkar et al..[22] A known weight of the dry MARs (0.5000±0.0020 g) in 30 mL distilled water was refluxed for 12 h in the presence of excess NaOH (10 mL, 0.1N) containing a few drops of phenolphthalein indicator. Upon cooling, the solution was filtered and washed carefully with 2×5 mL portions of water and the filtrate was then titrated with 0.1N HCl. The content of amino acid in the PS-amino acid could be calculated from titer value. The contents for all the functional groups were obtained, considering the mean value of triplicate assays.

Preparation of the extracts of Voacanga seeds

The extracts of *Voacanga* seeds was prepared as follows: the dried powder of seeds (100g) was extracted with 90% methanol (v/v, 200mL×5), refluxing for 1.5 h. The extracts was filtered and concentrated to remove the methanol under reduced pressure. The residue was dissolved in 250 mL 1.0% HCl solution (w/v). After filtering, the extracts was obtained with the concentration of tabersonine was 7.6 mg/mL.

Preparation of the Sample solution of Tabersonine

In this work, the sample solutions of tabersonine were prepared as follows: tabersonine (purity > 98%) with the weight of 0.4000±0.0010 g, 0.3000±0.0010 g, 0.2000±0.0010 g, 0.1000±0.0010 g, 0.0500±0.0010 g, and 0.0200±0.0010 g were dissolved in 50 mL 1.0% HCl (w/v), respectively, and then diluted with 1.0% HCl (w/v) to 100 mL. The concentrations of tabersonine in the obtained sample solutions

were 0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/mL, respectively. In the experiments, tabersonine was dissolved in acidic solution. The ester group in the structure of tabersonine may be hydrolyzed to carboxyl. Thus, the stability of the sample solutions was investigated. The changes of the contents of tabersonine in sample solutions were determined by HPLC. The results showed that the sample solutions were stable within 8 h at room temperature.

Calculation of Adsorption Capacity

The adsorption capacities of the MARs toward tabersonine were calculated according to the following equation:

$$q_e = (C_0 - C_e) \times \frac{V_0}{W} \quad (3)$$

where q_e is the adsorption capacity (mg/g dry resin) toward tabersonine at adsorption equilibrium. C_0 and C_e are the initial and equilibrium concentrations of tabersonine solutions (mg/mL). V_0 is the volume of the tabersonine solutions. W is the weight of dry MARs used (g).

In our experiments, the coefficient of recovery (P) and the relative standard deviation (RSD) were employed to evaluate the accuracy and precision of our experiment methods. The values of P and RSD were 98.8% and 0.38%, respectively, which indicated that the accuracy and precision could satisfy the needs of our experiments.

Adsorption Kinetics

In this paper, we attempted to decrease the adsorption capacity of the modified MARs toward tabersonine and to maintain or increase the adsoption capacity of the modified MARs toward the impurities in *Voacanga* seeds. Considering that the carboxyl groups in modified MARs can not react with protonated tabersonine through acid-base interaction, all the adsorption experiments were carried out in acidic solutions (HCl solution).

The evaluation of kinetics is benefit for the prediction of adsorption time and sample concentration. In this study, the adsorption kinetics curves of tabersonine on the MARs were carried out on the following condition: 25 mL of tabersonine solution (C_0=1.0 mg/mL) was shaken with pretreated MARs (0.2000±0.0020 g dry resin) in a 100 mL stoppered conical flask in SHA-B incubator (100 rpm) at 298, 308 and 318 K, respectively. Subsequently, the concentration of tabersonine in the adsorption solution was determined at different times until equilibrium.

Adsorption Isotherm

The equilibrium adsorption isotherms of tabersonine on the MARs were carried out as follows: solutions (25 mL) with different concentrations of tabersonine (C_0=0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/mL) were contacted with pretreated MARs (0.2000±0.0020 g of dry resin) in conical flasks. The flasks were continually shaken for 6 h (100 rpm) at temperatures of 298, 308 and 318 K, respectively. Then, the concentrations of tabersonine in the adsorption solutions were determined.

Dynamic adsorption and desorption tests (Purification of tabersonine from Voacanga seeds)

Dynamic adsorption and desorption tests were carried out in a glass columns (15mm×200mm) wet-packed with about 10 mL of the selected adsorbents. The weight of MARs was measured to be about 2.9 g. The extracts of *Voacanga* seeds passed through the column at a flow rate of 3 BV/h (1 BV=10 mL). The effluent liquid of the extracts which passed through the column was collected. And the purity of tabersonine in the effluent was determined by HPLC. The adsorption test was terminated until the purity of tabersonine reached the initial purity (37.6%). Then, the columns were washed by de-ionized water (2.5 BV) and then washed by 80% ethanol solution (5 BV). The composition of desorption solution was also determined by HPLC.

HPLC analysis

HPLC analysis was employed to determine the purities of tabersonine purified by different MARs. The samples were analyzed by LC-2010A (Shimadu, Japan). An UV detector operated at 254 nm was applied. The column was Lichrospher C18(4.6mm×250mm, 5 μm). The temperature was 35 ℃. The mobile phase was a 65:35 (v/v) mixture of acetonitrile and ultrapure water. The flow rate was set at 1.0 mL/min. With the content of the standard substance (tabersonine) between 0.0088 mg/mL and 0.0440 mg/mL, the peak area had good linear relationship with the concentration and the equation of linear regression was "Y=1.9313E+8X+148969，R^2=0.9999". Based on the peak areas of tabersonine in HPLC curves, the contents of tabersonine in samples were calculated.

Results and Discussion

Characterization of the MARs

Figure 3 shows the adsorption/desorption isotherms of D101, PS-Cl, PS-glycine and PS-valine. The results showed that the isotherms of D101 and PS-Cl were similar while that of PS-glycine and PS-valine were similar. This result indicated that the properties of MARs have been changed when amino acid was anchored. Physical properties of D101, PS-Cl, PS-glycine and PS-valine were tabulated in Table 1. The BET surface areas of MARs followed the order of PS-Cl > D101 > PS-glycine > PS-valine. This order revealed that the BET surface area of MARs decreased after functionalization of amino acid. The average pore diameter of MARs followed the order of PS-glycine > PS-valine > PS-Cl > D101. It revealed that the average pore diameter of MARs increased after functionalization of amino acid. The pore volume of MARs followed the order of D101 > PS-Cl > PS-glycine > PS-valine. This result revealed that the pore volume of MARs decreased after functionalization of amino acid.

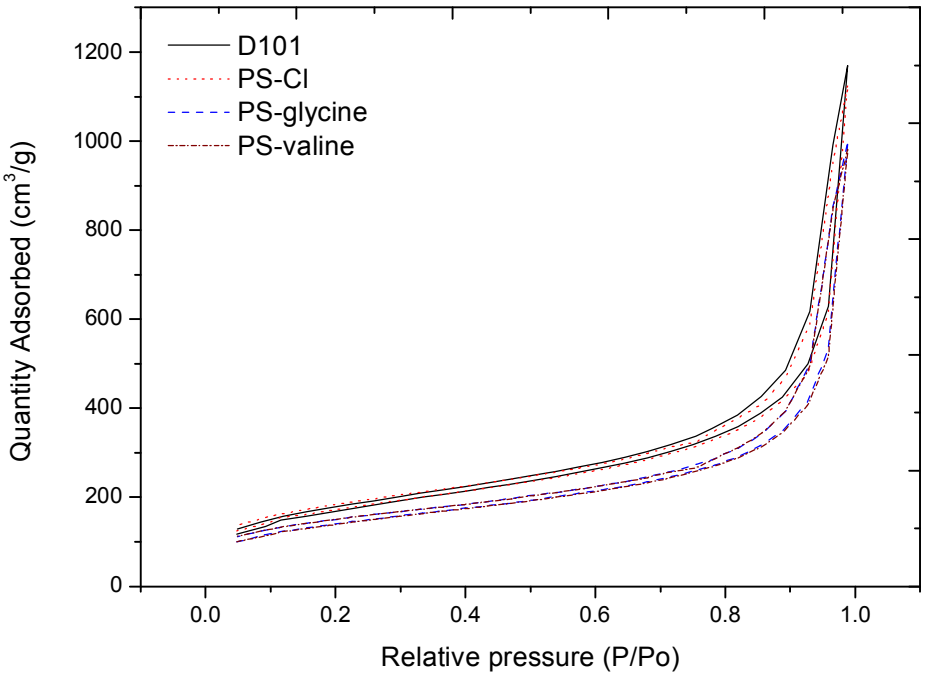

Figure 3. Nitrogen adsorption/desorption isotherms of D101, PS-Cl, PS-glycine and PS-valine.

Table 1. Physical Properties of D101, PS-Cl, PS-glycine and PS-valine.

Resin series	D101	PS-Cl	PS-glycine	PS-valine
BET surface area (m^2/g)	607.5	608.1	499.2	495.6
Average pore diameter (nm)	13.0	13.4	14.5	14.3
Pore volume (cm^3/g)	1.77	1.67	1.52	1.49
Particle size (mm)	0.45-0.60	0.45-0.60	0.45-0.60	0.45-0.60
Fractal dimension *D*	2.608	2.629	2.616	2.617

Fractal geometry is popularly applied in adsorption science. The fractal dimension *D* is the key quantity in fractal geometry. It can be used to measure the surface and structural irregularities of the given solid. The value of *D* can be determined on the basis of the Frenkel-Halsey-Hill (FHH) equation. It can be calculated as follows:[19, 23, 24]

$$\ln q = const - (3 - D)\ln A$$
$$A = RT\ln(p_0 / p) \quad (4)$$

where p_0 and p are the saturation and equilibrium vapor pressures (mmHg), R is the universal gas constant (8.314 J/(mol K)), T is the absolute temperature (K).

The values of D were calculated by the slop of ln q versus ln A and they were shown in Table 1. The values of D for D101, PS-Cl, PS-glycine and PS-valine were 2.608, 2.629, 2.616 and 2.617, respectively. It is reported that the lower limiting value of 2 corresponds to a perfectly regular smooth surface, whereas the upper limiting value of 3 relates to the maximum allowed complexity of the surface.[25] In our experiments, the values of D followed an order of D101 < PS-glycine < PS-valine < PS-Cl. And the values of D between these MARs had small differences. The order of the values D meant that the surface of MARs became relatively irregular after functionalization. The small differences of D indicated that their degrees of regular smooth almost remained constant. This is probably because the amount of functional groups in MARs are less and similar.[26]

Figure 4 shows the SEM characterization of D101 (a) and PS-amino acid (b). Compared image (a) with image (b), it shows that irregular surface structure and large pores were observed on the surface of PS-amino acid (b), while a more uniform surface was observed on the surface of D101 (a). This result was consistent with the data shown in Table 1 (average pore diameter, the fractal dimension D). These differences of the surface may suggest that the amino acid groups existed in the modified MARs.

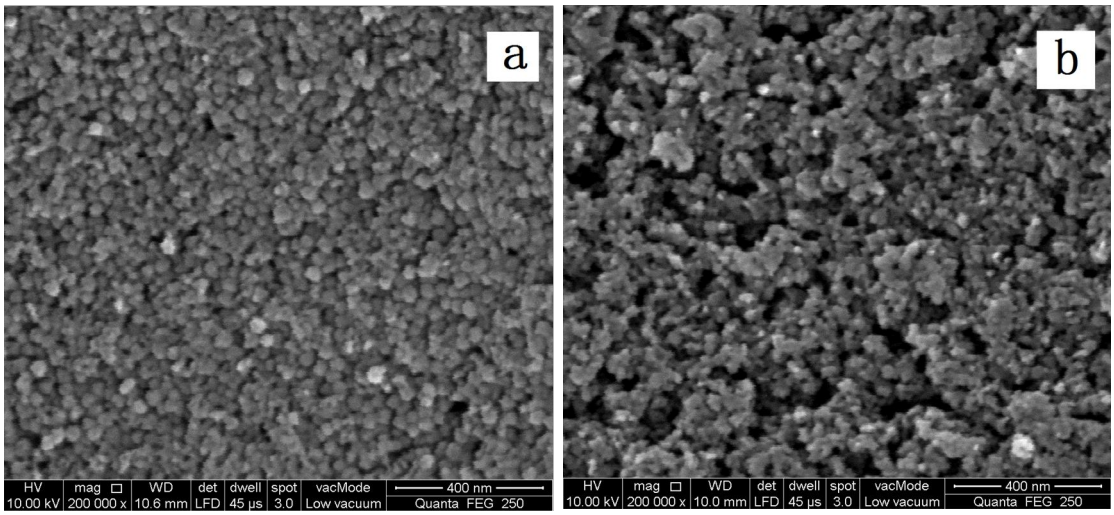

Figure 4. The SEM characterization of MARs: (a) D101, (b) PS-amino acid.

Curves (a), (b), (c) and (d) in Figure 5 were the FTIR spectra of D101, PS-Cl, PS-valine and PS-glycine, respectively. In the spectrum of PS-Cl (b), there was an absorption band in the vicinity of 678 cm^{-1} that was the stretching vibrations of the C-Cl bond. The absorption bands at 1264 cm^{-1} was the in-plane bending vibrations adsorption of the =C-H bond of the benzene ring after binary substitution caused by the substitution of hydrogen atoms at the para position of benzene rings by $–CH_2Cl$.[27] In the spectrum of PS-valine (c) and PS- glycine (d), the amine group was disclosed by the adsorption peak appears in 3482 and 3444 cm^{-1}, respectively. In addition, the absorption band in the vicinity of 678 cm^{-1} was still existed in curves (c) and (d). This result indicated that the –Cl groups were not fully substituted by amino acid during the reaction. Based on the electrochemistry method, the content of chloromethyl in the PS-Cl was 1.74 mmol/g and the contents of amino acid in PS-valine and PS- glycine were 1.02 mmol/g and 1.09 mmol/g, respectively. According to the method described by Valodkar et al.,[22] the contents of amino acid in PS-valine and PS- glycine were 0.95 mmol/g and 1.03 mmol/g, respectively, which were close to the result obtained by electrochemistry method.

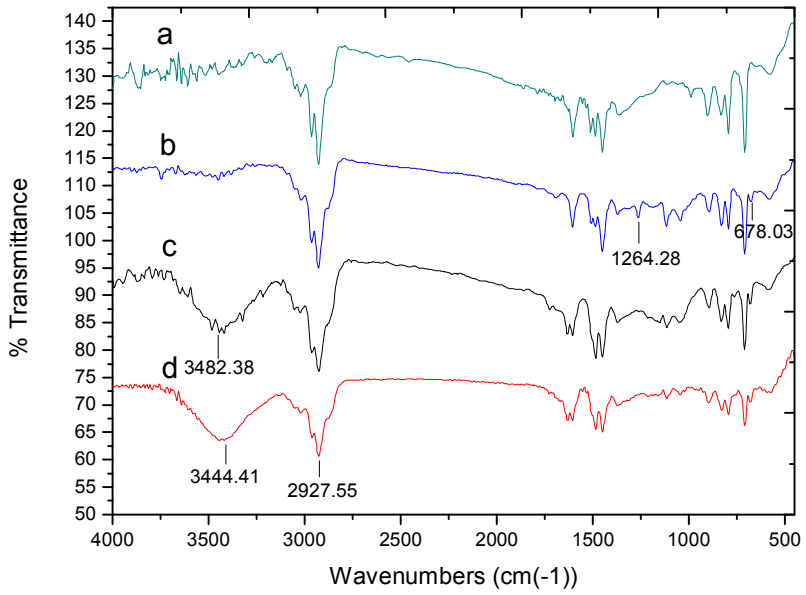

Figure 5. FTIR spectra of adsorbents D101 (a), PS-Cl (b), PS- valine (c) and PS- glycine (d).

Adsorption Kinetics of tabersonine on MARs

Adsorption kinetics of tabersonine on MARs was studied at 298K, 308K, and 318K, respectively. The adsorption kinetics curves were shown in Figure 6. The results indicated that the adsorption capacities of tabersonine on MARs increased sharply in the first 40 min and then became slow until equilibrium and all of the adsorption can reach equilibrium within 150 min. By comparison of curves at different temperatures, it can be concluded that higher temperature leads to lower adsorption capacity of the MARs at equilibrium. Compared with Figure 6a, the adsorption capacities of tabersonine on PS-amino acid (Figure 6c and 6d) were much smaller. The adsorption capacities of tabersonine on PS-amino acid were 9-16 mg/g, which were much smaller than the adsorption capacity of tabersonine on D101 (about 110 mg/g). The PS-amino acid exhibited a poor ability to adsorb tabersonine.

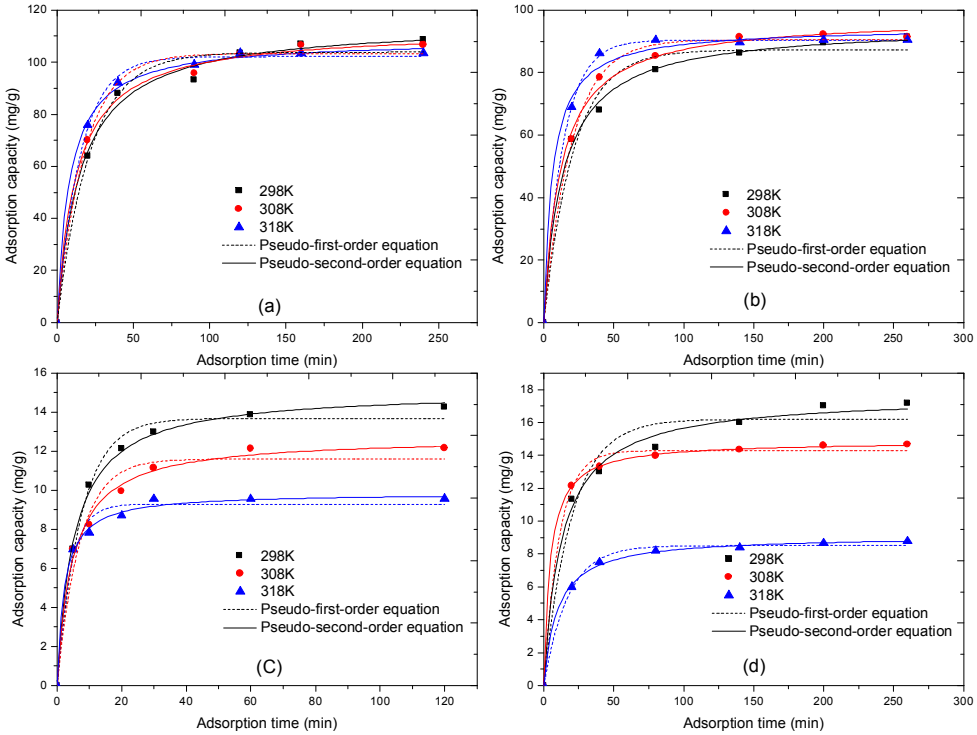

Figure 6. Adsorption kinetics curves of tabersonine on D101 (a), PS-Cl (b), PS-valine (c), and PS-glycine (d) at different temperatures.

In order to better illustrate the adsorption mechanisms of tabersonine on MARs, two kinetics models (pseudo-first-order and pseudo-second-order kinetics models) were employed to fit the experimental data. The model which performed best was selected on the basis of the linear regression correlation coefficient values (R^2) and the average absolute relative deviation (*AARD*). The kinetics equations are presented as follows:

Equation of pseudo-first-order kinetics model:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \qquad (5)$$

Equation of pseudo-second-order kinetics model:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^{\,2}} + \frac{1}{q_e} t \qquad (6)$$

where q_e and q_t are the adsorption capacity of tabersonine on the MARs at equilibrium and at any time t (mg/g dry resin), respectively. The parameters k_1 (1/min) and k_2

(g/(mg • min) are the rate constants of the pseudo-first-order and pseudo-second-order models for the adsorption process, respectively.

In the calculations, the adjustable parameters are obtained by fitting the model against the experimental data, and the average absolute relative deviation (*AARD*) between the experimental adsorption capacities and calculated values was employed to evaluate the models. The equation of *AARD* is shown as follows:

$$AARD = \frac{1}{N} \times \sum_{i=1}^{N} \frac{\left| q_{cal} - q_{\exp} \right|}{q_{\exp}} \times 100\% \quad (7)$$

where N is the number of experimental data points. The q_{cal} and q_{exp} are the calculated and experimental values, respectively.

The kinetic data in Figure 6 were fitted by these two models. It was found that the pseudo second-order kinetic equation was more appropriate for the adsorption. The values of kinetic parameters were shown in Table 2. The calculated results of the pseudo-first-order rate equation showed that the values of R^2 were almost less than 0.99 for all MARs, showing the bad quality of linearization. In contrast, the values of R^2 for the pseudo-second-order rate equation were all larger than 0.99. In addition, the values of *AARD* for the pseudo-second-order rate equation were almost smaller than that for the pseudo-first-order rate equation. Thus, the adsorption of tabersonine on D101, PS-Cl, PS-valine and PS-glycine fitted the pseudo-second-order model better. The calculated q_e decreased with increasing of temperature, accordant with the phenomenon discussed above.

Table 2. Values of Kinetic Parameters for Adsorption of Tabersonine on MARs.

Adsorbent	Temperature (K)	pseudo-first-order equation				pseudo-second-order equation			
		k_1 (1/min)	q_e (mg/g)	R^2	AARD(%)	k_2 (g/mg min)	q_e (mg/g)	R^2	AARD(%)
D101	298	0.0454	103.8	0.9844	3.18	0.000536	116.	0.9943	2.66
	308	0.0556	103.3	0.9899	2.86	0.000679	113.6	0.9971	1.44
	318	0.0652	102.1	0.9971	1.83	0.001390	107.5	0.9986	1.13
PS-Cl	298	0.047	87.2	0.9797	5.19	0.00068	97.0	0.9973	1.65
	308	0.057	90.5	0.9967	1.56	0.00105	96.1	0.9959	2.03
	318	0.073	90.4	0.9998	0.425	0.00278	92.5	0.9924	2.8
PS-valine	298	0.134	13.6	0.9919	3.18	0.0126	15.1	0.9970	2.18
	308	0.146	11.6	0.9662	7.23	0.0166	12.7	0.9933	3.16
	318	0.243	9.2	0.9814	4.75	0.0457	9.8	0.9955	2.06
PS- glycine	298	0.0504	16.1	0.9677	6.36	0.00445	17.6	0.9928	3.01
	308	0.0901	14.2	0.9948	2.13	0.0147	14.8	0.9998	0.33
	318	0.0583	8.5	0.9957	2.23	0.0113	9.1	0.9986	1.15

Adsorption Isotherms of tabersonine on the MARs

The equilibrium adsorption isotherms of tabersonine on MARs were carried out at 298K, 308K and 318K, respectively. The results were shown in Figure 7. It is obvious that the adsorption capacities of all MARs increased with increasing concentration of tabersonine, while decreased with increasing of temperature. Compared the adsorption capacities between Figure 7a, 7c and 7d, it can be concluded that the maximum adsorption capacity of tabersonine on MARs decreased significantly after modification.

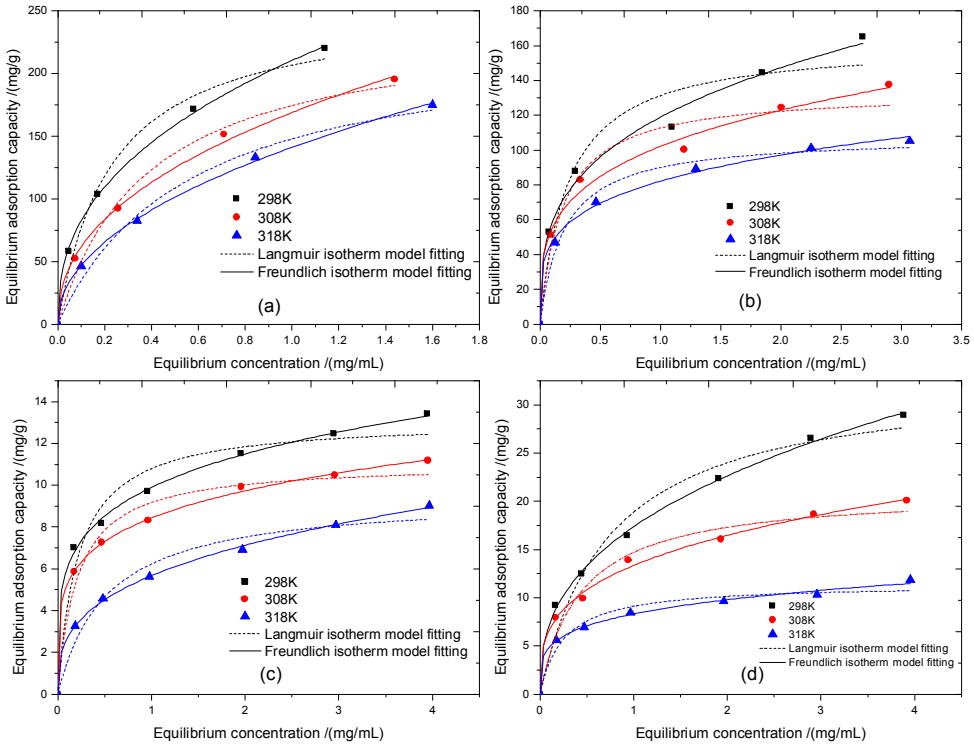

Figure 7. Adsorption isotherms of tabersonine on D101 (a), PS-Cl (b), PS-valine (c), and PS-glycine (d) at different temperatures.

In order to understand the adsorption mechanism of tabersonine on MARs, two isotherm equations had been employed to explain the process of adsorption equilibrium. In the present paper, the kinetic curves were fitted with the isotherm models of Langmuir and Freundlich. And the results were shown in Table 3.

Table 3. Langmuir and Freundlich Isotherm Parameters for the Adsorption Process of Tabersonine on MARs at 298 K, 308 K and 318 K.

Adsorbent	Temperature (K)	Langmuir				Freundlich			
		q_m (mg/g)	K_L (mL/mg)	R^2	AARD(%)	K_F ((mg/g)(mL/mg)$^{1/n}$)	1/n	R^2	AARD(%)
D101	298	254.2	0.23	0.9814	12.33	210.4	0.406	0.9991	2.80
	308	241.2	0.38	0.9867	5.37	168.8	0.437	0.9972	3.58
	318	231.0	0.56	0.9864	8.86	141.0	0.477	0.9989	3.12
PS-Cl	298	161.8	0.233	0.9830	8.13	118.9	0.507	0.9959	3.53
	308	133.8	0.188	0.9814	9.96	102.1	0.437	0.9934	4.01
	318	107.9	0.199	0.9810	8.33	82.1	0.414	0.9909	4.35
PS-valine	298	13.1	0.519	0.9667	7.31	9.7	0.224	0.9991	1.13
	308	11.1	0.215	0.9782	5.51	8.3	0.213	0.9989	1.17
	318	9.5	0.205	0.9680	7.85	5.6	0.334	0.9989	1.21
PS-glycine	298	32.9	0.287	0.9735	8.7	17.4	0.383	0.9987	1.75
	308	21.0	0.213	0.9742	7.83	13.3	0.303	0.9956	2.48
	318	11.4	0.21	0.9663	7.25	8.3	0.234	0.9957	1.95

The Langmuir isotherm equation and Freundlich isotherm equation are represented as equation (8) and (9), respectively:

$$C_e / q_e = K_L / q_m + C_e / q_m \quad (8)$$

$$\lg q_e = (1/n) \lg C_e + \lg K_F \quad (9)$$

where q_e and q_m are the equilibrium and maximum adsorption capacity (mg/g dry resin), respectively. C_e is the equilibrium concentration of tabersonine solution (mg/mL). K_L is the parameter related to the adsorption energy (mL/mg). K_F reflects the adsorption capacity of MARs ((mg/g)(mL/mg)$^{1/n}$). The parameter n represents the adsorption affinity of the adsorbent for MARs.

The equilibrium data in Figure 7 were fitted by these two isotherm models. It was

found that the Freundlich model was more appropriate for describing the experimental data. The corresponding parameters of Langmuir model and Freundlich model were calculated and tabulated in Table 3. The values of R^2 and *AARD* were accordant with the results of the data fitting in Figure 7. The q_m calculated from Langmuir model indicated that the maximum adsorption capacities of tabersonine on D101, PS-Cl, PS-valine and PS-glycine were about 254 mg/g, 161 mg/g, 14 mg/g, and 34 mg/g, respectively. The values of q_m followed the order of D101 > PS-Cl > PS-glycine > PS-valine. The PS-amino acid exhibited a poor ability to adsorb tabersonine.

Adsorption Thermodynamics of tabersonine on MARs

In order to obtain in-depth information on inherent energetic changes associated with the adsorption process. Adsorption thermodynamics of tabersonine on MARs were investigated. K_L, the Langmuir isotherm equilibrium constant, can be used to examine thermodynamic parameters. The free energy of adsorption (ΔG^0) is related to the equilibrium constant. Enthalpy (ΔH^0) and entropy (ΔS^0) changes are calculated based on the Van't Hoff equation. Thermodynamic parameters such as ΔG^0, ΔH^0, and ΔS^0 associated with the adsorption process can be estimated using the following equations:

$$K = M / K_L$$
$$\Delta G^0 = -RT \ln K \quad (10)$$
$$\ln K = -\Delta H^0 / RT + \Delta S^0 / R$$

where M is the molecular weight of tabersonine (336.42 g/mol) and T is the absolute temperature (K). The plot of ln K versus $1/T$ gave a straight line, and the values of ΔH^0 and ΔS^0 were calculated from the slope and intercept, respectively.

As shown in Table 4, the values of ΔG^0 were all negative, which indicated that the adsorption processes were spontaneous. The value of ΔH^0 for D101 was negative, indicating that the adsorption process was exothermic. The positive values of ΔH^0 for PS-Cl, PS-valine and PS-glycine indicated that the adsorption processes were endothermic in nature. The value of ΔS^0 for D101 was negative, which indicated that the randomness decreased in the adsorption process. The positive values of ΔS^0 for

PS-Cl, PS-valine and PS-glycine indicated that randomness at the solid-liquid interface increased during the adsorption process.[28] In section *Characterization of the MARs*, the results showed that the surface of MARs became relatively irregular after functionalization. The surface of D101 was more regular and tabersonine molecules were arranged from the irregular state to the regular state at the solid-liquid interface during the adsorption process. Thus, the value of ΔS^0 for D101 was negative. For PS-Cl, PS-valine and PS-glycine, their surface became relatively irregular after functionalization. Thus, the values of ΔS^0 for them were positive.

Table 4. Thermodynamics Parameters for the Adsorption Process of Tabersonine on MARs at Different Temperatures.

	ΔG^0(kJ/mol)				
Adsorbent	298K	308K	318K	ΔH^0(kJ/mol)	ΔS^0(J/(mol K))
D101	-18.0	-17.4	-16.9	-34.9	-57
PS-Cl	-18.0	-19.2	-19.7	6.5	81
PS-valine	-17.1	-18.2	-18.9	9.6	97
PS- glycine	-17.5	-18.8	-19.4	12.2	100

Reasons for the low adsorption capacity of tabersonine on the PS-amino acid

The effective interactions between adsorbate and adsorbent include van de Waals force, hydrogen bonding, hydrophobic interaction, and electrostatic interaction.[19] D101 is a nonpolar styrene-co-divinylbenzene copolymer with no functional groups. The π-π interaction between the phenyl rings of D101 and tabersonine was maybe one of the main driving forces for the adsorption. Compared with D101, PS-Cl possessed a chloromethyl group, which had hardly any effect on the adsorption of tabersonine. However, by introducing a chlormethyl group into D101, the steric resistance increased so that adsorption capacities for tabersonine slightly decreased. When amino acid was introduced into the MARs, the steric resistance was further increased and adsorption capacities were correspondingly decreased. In section

Characterization of the MARs, the structural characterization revealed that the BET surface area and pore volume of MARs decreased after functionalization of amino acid. Such changes of the physical properties could also result in the low adsorption capacities for PS-amino acid. More important, the modified MARs contained carboxyl groups. Tabersonine is an alkaloid and it was dissolved in HCl solution. Tabersonine was protonated and can not react with carboxyl group on the adsorbent matrix through acid-base interaction. Therefore, the adsorption capacity of tabersonine on modified MARs was low in the acidic pH conditions.

Dynamic adsorption and desorption tests (Purification of tabersonine from Voacanga seeds)

The dynamic test is important for judging the efficiency of purification of tabersonine by PS-amino acid. Figure 8 presents the continuous column adsorption curves of D101 and PS-amino acid columns toward tabersonine. The initial concentration of tabersonine in the extracts was 7.6 mg/mL. In this study, the residual concentration of tabersonine in the effluent was recorded until it reached the initial concentration. The results showed that the residual concentration of tabersonine in the effluent of D101, PS-valine and PS-glycine reached the initial concentration at the volume of effluent were 8.5 BV, 0.6 BV and 0.7 BV, respectively. This result meant that the dynamic adsorption capacities of tabersonine on D101, PS-valine and PS-glycine were about 222.3 mg/g, 15.7 mg/g and 18.3 mg/g, respectively. The result confirmed that PS-amino acid has low adsorption capacity toward tabersonine.

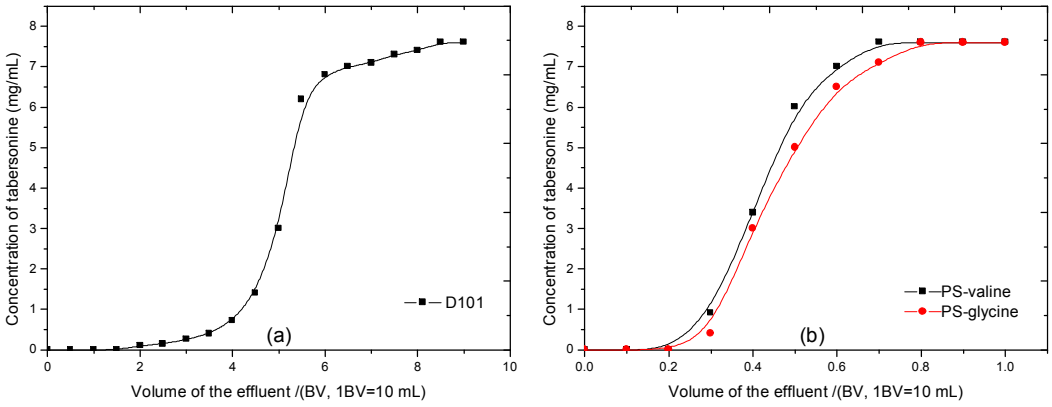

Figure 8. The residual concentration of tabersonine in the effluent of D101 (a) and PS-amino acid (b).

The aim of this work was to obtain high purity of tabersonine. The investigation of the purity of tabersonine in the effluent is more important to evaluate the performance of PS-amino acid. Figure 9 shows the purities of tabersonine at different volumes of effluent. In this study, the breakthrough point was set to be that the purity of tabersonine was 90% and the leaking volumes of extracts on PS-valine and PS-glycine were 2.8 BV and 2.3 BV, respectively. This result revealed that high purity of tabersonine (>90%) could be obtained in the effluent and impurities were adsorbed on PS-amino acid. In our experiments, 250 mL extracts were obtained from 100 g *Voacanga* seeds. Thus, the required dosages of PS-valine and PS-glycine, which were used to purify tabersonine from 1 kg of *Voacanga* seeds, were about 268 g and 326 g, respectively. And this result also revealed that impurities from 3.73 g and 3.06 g seeds could be sufficiently adsorbed on 1.0 g PS-valine and 1.0 g PS-glycine, respectively. These data indirectly revealed the adsorption capacities of impurities on PS-valine and PS-glycine. The curve of D101 in Figure 9 showed that the purity of tabersonine had a little decrease after adsorption. This result indicated that the adsorption ability of tabersonine on D101 is slightly stronger than that of impurities. The commercial MARs D101 showed no apparent adsorption selectivity toward tabersonine and impurities in the extracts. Thus, a large number of D101 were required to separate them.

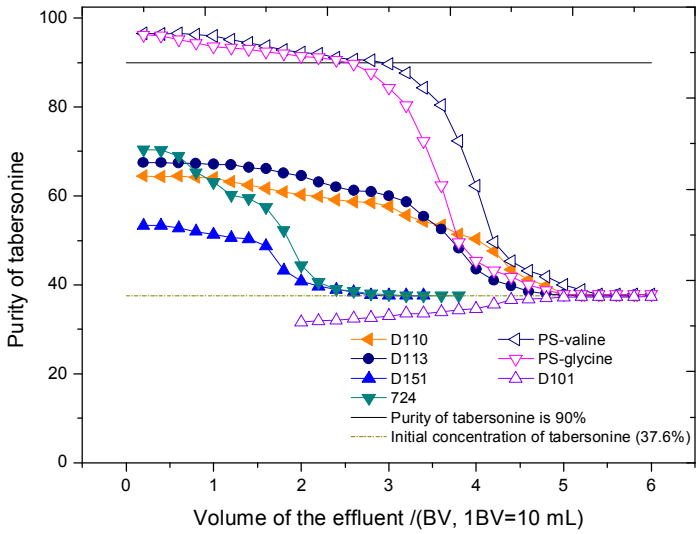

Figure 9. The purity of tabersonine in the effulent.

The desorption tests of removing impurities on PS-amino acid were also investigated. The result revealed that impurities could be desorbed by washing with 5 BV 80% ethanol (v/v). This result suggested that PS-amino acid could be regenerated using 5 BV 80% ethanol.

Surface morphology of tabersonine adsorbed on PS-amino acid (tabersonine solution with concentration was 7.6 mg/mL passed through the PS-amino acid column), and impurities adsorbed on PS-amino acid (the extracts of *Voacanga* seeds passed through the PS-amino acid column, and then washed with de-ionized water) were observed by SEM and the results were shown in Figure 10a and 10b, respectively. Compared Figure 4b with Figure 10, it can be seen that the adsorbent surface became dense and pores decreased after adsorption of tabersonine or impurities. This result proved that the adsorbates were adsorbed into its pores. Compared Figure 10a with Figure 10b, it is clear that pores in Figure 10b decreased more remarkably. This result revealed that the adsorption capacity of impurities on PS-amino acid was higher than that of tabersonine. PS-amino acid exhibited a good ability to adsorb impurities but a poor

ability to adsorb tabersonine in the extracts of *Voacanga* seeds.

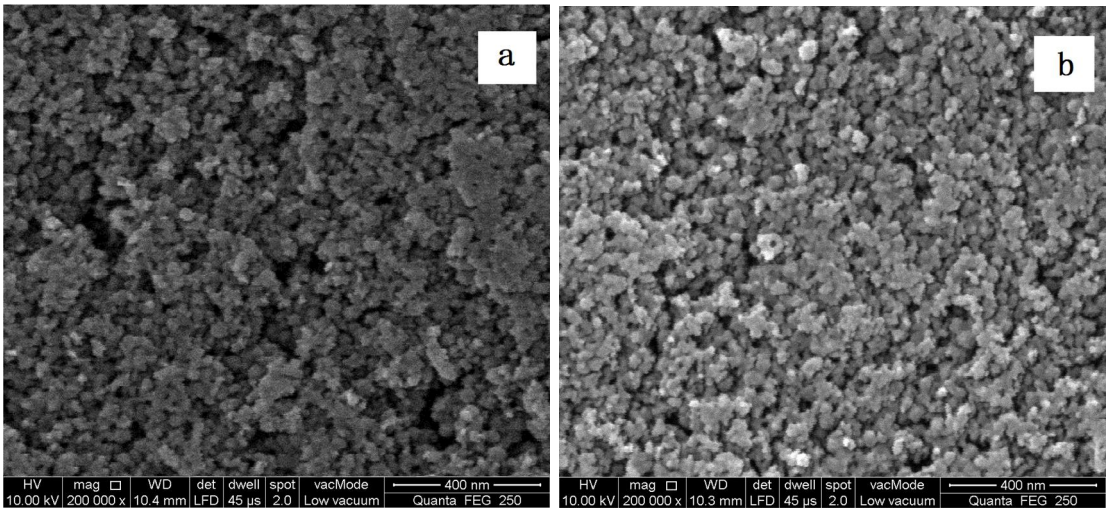

Figure 10. The SEM characterization of MARs: (a) tabersonine absorbed on PS-amino acid and impurities absorbed on PS-amino acid (b).

The adsorption behaviors of tabersonine on commercial weak acid MARs

In this paper, the modified MARs contained carboxyl groups. Some properties of modified MARs are similar to the commercial weak acid MARs. In order to understand the differences between modified MARs and commercial weak acid MARs, four kinds of commercial weak acid MARs with carboxyl groups (D110, D113, D151, 724) have been applied to purify tabersonine from *Voacanga* seeds. The adsorption capacities of tabersonine on these commercial MARs were investigated and the results were shown in Figure 11. It can be seen that the adsorption capacities of tabersonine on these commercial weak acid resins were lower than that on modified MARs. This is probably because the commercial weak acid MARs contains more carboxyl groups, which can not react with protonated tabersonine through acid-base interaction.

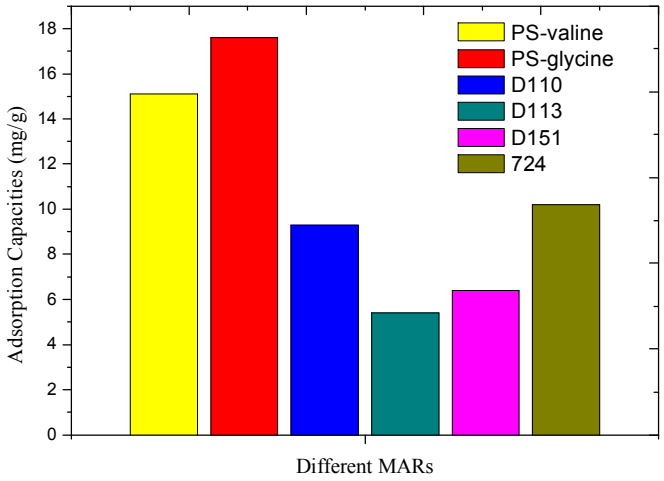

Figure 11. Adsorption capacities of tabersonine on different weak acid MARs.

The dynamic test of these commercial weak acid MARs was also investigated. The purity of tabersonine in the effluent was recorded and shown in Figure 9. It can be seen that the purities of tabersonine purified by commercial weak acid MARs were much lower than that purified by modified MARs. This result indicated that the commercial weak acid MARs showed low adsorption selectivity toward tabersonine and impurities in the extracts of *Voacanga* seeds. The efficiency of purification of tabersonine by these commercial MARs was low. Although the content of carboxyl group or the synthetic cost of the commercial weak acid MARs is superior to the modified MARs synthesized in this article, the low adsorption selectivity of the commercial weak acid MARs leads to the high cost of purification. Compared with the commercial weak acid MARs, the modified MARs synthesized in this paper was a good MARs to purify tabersonine.

The modified MARs showed high adsorption selectivity toward tabersonine and impurities in the extracts of *Voacanga* seeds. This is probably because the modified MARs contained amino acid groups and featured special structures. For the components of impurities in the extracts were complex, the adsorption mechanism of impurities on modified MARs was not fully understood. Further investigation is

proceeding to clarify the adsorption mechanism of impurities on modified MARs.

Recycling ability of the MARs

The PS-valine and PS-glycine were repeatedly used five times for the continuous adsorption and desorption of extracts of *Voacanga* seeds. After the adsorption, 50 mL of 80% of ethanol (v/v) was applied as the desorption reagent. The leaking volumes (purity of tabersonine > 90%) of the extracts on PS-amino acid for every time were shown in Figure 12. After reusing the PS-amino acid five times, the leaking volumes of the extracts on PS-valine and PS-glycine decreased to approximately 2.4 BV and 2.0 BV, respectively. The results revealed that the PS-amino acid exhibited considerable recyclability.

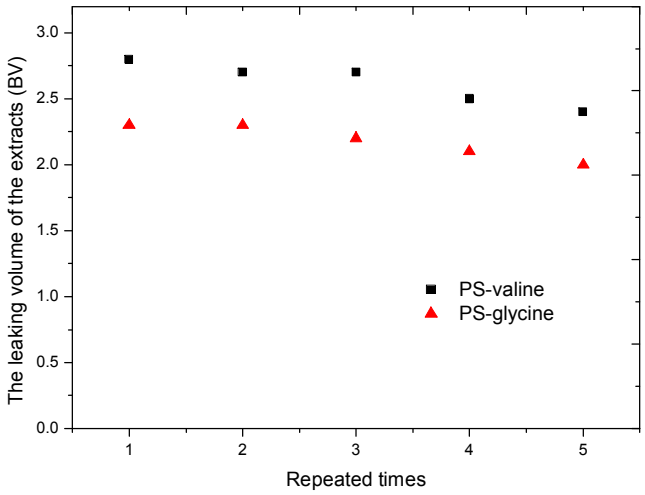

Figure 12. Effect of the regeneration cycles on the leaking volume of the extracts on PS-amino acid.

Conclusion

In summary, high purity of tabersonine was obtained by using MARs functionalized with amino acid. The adsorption behaviors of tabersonine on MARs were investigated. The results indicated that the pseudo second-order rate equation was more appropriate for characterizing the kinetic data and the Freundlich model was more suitable for fitting the equilibrium data. And the results also revealed that the maximum

adsorption capacity of MARs towards tabersonine reduced to 14 mg/g from 254 mg/g after functionalization of valine. Reasons for the low adsorption capacity of tabersonine on PS-amino acid were discussed. The results of dynamic adsorption and desorption tests, SEM images, and HPLC analysis revealed that the PS-amino acid had high adsorption selectivity toward tabersonine and impurities in the extracts of *Voacanga* seeds, and they were feasible to purify tabersonine from *Voacanga africana* seeds. Tabersonine was purified efficiently with the purity above 90%. Further investigation is proceeding to clarify the adsorption mechanism of impurities on modified MARs. The reusability of the PS-amino acid was investigated and the PS-amino acid exhibited considerable reusability. This work provided a promising method to purify tabersonine.

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Synthesis and characterization of amino acid-modified adsorption resins and its adsorption properties in the purification of tabersonine from *Voacanga africana* seeds

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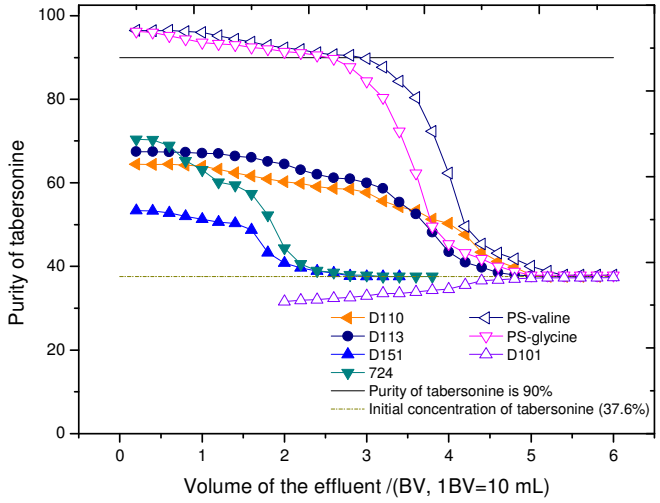

The modified MARs were good adsorption to impurities but poor one to tabersonine in the extracts of *Voacanga africana* seeds