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Research Progress on the Separation of Alkaloids from Chinese Medicines by Column Chromatography

Yaqin He*, Zhaozeng Chen*, Haibin Qu, Xingchu Gong#

Pharmaceutical Informatics Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China
Email: #gongxingchu@zju.edu.cn

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Abstract

Alkaloids have a variety of bioactivities and great development value in the fields of pharmaceuticals, cosmetics and health food. Column chromatography is a common method for preparing alkaloids. In this paper, the research status of the separation and purification of alkaloids from Chinese medicines by column chromatography is reviewed, and the factors that influence the refining of alkaloids via a macroporous adsorption resin, ion exchange resin and silica gel are summarized. The thermodynamic and kinetic modeling methods for the static adsorption of adsorbents are also reviewed in this paper. It is suggested that the modeling method of the column chromatography process be deeply studied to establish a more stringent quality control method for sampling liquid and to strengthen the online detection of the chromatography process to improve the refining effect of alkaloids.

Keywords

Alkaloid, Column Chromatography, Ion Exchange Resin, Macroporous Adsorbent Resin, Model, Silica Gel

1. Introduction

Alkaloids are widely found in higher plants on land, especially in Caryophyllaceae, Annonaceae, Apocynaceae, Compositae, Berberidaceae, Boraginaceae, Buxaceae and other plants, but less frequently in lower plants and animals. Alkaloids are natural secondary metabolites that are generally synthesized through the biosynthetic amino acid pathway and the mevalonic acid (isoprene) pathway [1].

*These authors contributed equally to this work.

Alkaloids have many bioactivities, such as asthmatic, cough relief, antimicrobial, anti-inflammatory, anti-tumor and other properties [2] [3] [4]. They have a great development value in the fields of pharmaceuticals, health food and cosmetics. The commonly used preparation methods are precipitation, extraction and chromatography. The fillers commonly used in chromatography include macroporous resins, ion exchange resins and silica gels, in addition to the use of alumina. Chromatography is a powerful separation method for natural products including alkaloids. The establishment of chromatographic models is also important to design and optimize the chromatographic processes. In this paper, the research progress in recent years will be summarized from the perspective of different types of chromatographic fillers and modeling methods of the chromatographic process.

2. Macroporous Resins

2.1. Properties of Macroporous Resin

Macroporous resins, as organic polymer adsorbents, generally have a macroporous network structure and large specific surface area. The macroporous resin does not contain exchange groups. The resin adsorbs molecules through van der Waals force, and the molecules are separated and purified after being eluted by a certain eluent. The physical properties of macroporous adsorption resins commonly used in alkaloid refining are summarized in Table 1. Some of their photos are shown in Figure 1. D-101 is opalescent spherical particles with a wide size distribution. The diameter values of some particles are larger than 1mm. Compared with D-101, the particle diameter of HPD-100 or D-151 is smaller and more uniform. 001 × 7 are golden transparent spherical particles, and the particle diameter distribution is relatively narrow.

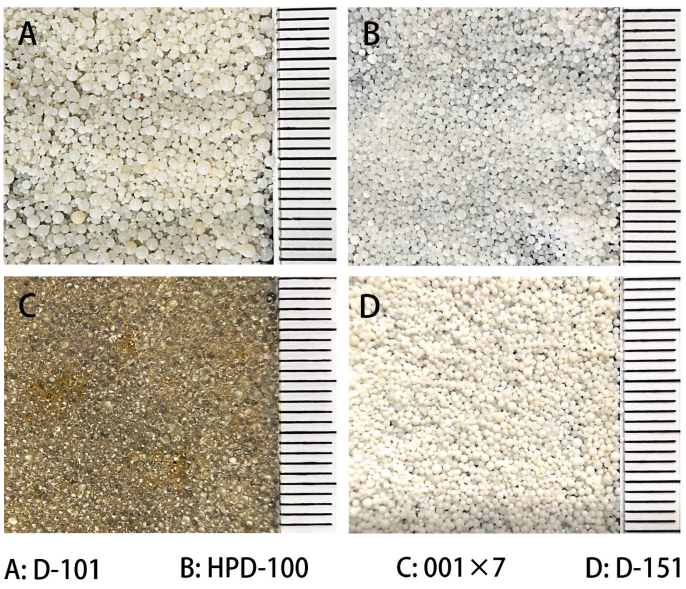

Figure 1. Photos of resins.

Table 1. Physical properties of the macroporous resins used in alkaloid refining.

Name	Polarity	Pore Diameter (nm)	Particle Diameter (mm)	Surface Area ($m^2 \cdot g^{-1}$)	Reference
AB-8	Weakly polar	13 - 14	0.3 - 1.25	480 - 520	[5]
BS-65	Nonpolar	7	0.25 - 0.83	580 - 600	[6]
D-101	Nonpolar	9 - 10	0.25 - 0.83	480 - 520	[6]
D-3520	Nonpolar	8.5 - 9	0.3 - 1.25	480 - 520	[5]
HPD-100	Nonpolar	8.5 - 9	0.3 - 1.25	650 - 700	[7]
NKA-9	Polar	15 - 16.5	0.3 - 1.25	250 - 290	[5]
XAD-4	Nonpolar	5.8	0.49 - 0.69	750	[8]
X-5	Nonpolar	29 - 30	0.3 - 1.25	500 - 600	[9]

As presented in Table 1, the nonpolar or weakly polar macroporous resins are more widely used in the separation and purification of alkaloids than the polar macroporous resins. Table 2 lists the resins used by researchers in China and abroad in the separation of alkaloids. Table 2 shows that many researchers are more likely to use HPD-100, D-101, and AB-8 macroporous resins. The common characteristics of the three resins are that the specific surface area is greater than 480 $m^2 \cdot g^{-1}$, the pore diameter is in the range of 8 to 14 nm and the particle diameter is in the range of 0.25 - 1.25 mm. A larger particle diameter is beneficial to reduce the liquid pressure in the chromatography process.

2.2. Chromatography Process

The basic process of refining the alkaloids of Chinese medicines with a macroporous resin is as follows: resin pretreatment, sample loading, washing, elution, and resin regeneration. Table 2 lists the sample loading, washing, and elution conditions and refining results reported in the literature.

2.2.1. Sample Loading

The main factors affecting the sample loading process are the properties of the loading solution, the loading solution volume, and the flow rate of sample loading. Table 2 shows that most of the work was performed with a water solution. The pH value of the loading solution has a great influence on the adsorption effect [9] [10], and many researchers controlled the pH value of the loading solution to be alkaline [9] [11]. In an alkaline solution, alkaloids often exist in molecular form, which is more favorable for macroporous resin adsorption. In general, a high concentration, large volume, and fast loading speed of the loading solution are more likely to lead to the leakage of alkaloids at the outlet of the chromatographic column. Most researchers controlled the sample loading speed to between 1 and 6 $BV \cdot h^{-1}$ [9] [12]. Some researchers controlled the loading volume according to the leakage of alkaloids in the liquid at the outlet of the chromatographic column [5] [9]. Compared with loading a fixed volume of the sample, this method enabled researchers to make full use of the adsorption capacity of the resin in the column.

Table 2. Study on the separation and purification of alkaloids from traditional Chinese medicine by macroporous resins.

Name of Chinese medicine	Names of alkaloids	Type of resin	Sample loading		Washing			Elution		Purity	Recovery	Reference
			pH of the sample	Flow rate	Type	Flow rate	volume	Type and volume	Flow rate			
Corydalis yanhusuo W.T. Wang	total alkaloids	HPD-100		2 BV·h^{-1}	water	3 BV·h^{-1}	wash to neutral	60% ethanol 5 BV 80% ethanol 1 BV	3 BV·h^{-1}			[12]
Sophora flavescens	matrine, oxymatrine	H103			nothing			30% ethanol-25% ammonia water (115:1) 80% ethanol			matrine: 90.1%, oxymatrine: 85.3%	[30]
Gelsemium elegans Benth. root	total alkaloids	AB-8			water			90% ethanol 4 BV	1 - 2 mL·min^{-1}	5.54%		[26]
Sophora alopecuroides Linn	total alkaloids	D-101	9	5 BV·h^{-1}	nothing			30% ethanol 3 BV 70% ethanol 3 BV	1.0 BV·h^{-1}	66.75%		[9]
Sophora alopecuroides	total alkaloids	D-101	9		nothing			30% ethanol (pH = 9) 210 mL 70% ethanol (pH = 9) 210 mL		over 60%	>97%	[11]
Hyoscyami Semen	total alkaloids	LSA-5B		1.5 BV·h^{-1}	water	1.5 BV·h^{-1}	4 BV	50% ethanol 10 BV	1.5 BV·h^{-1}	4.02%		[13]
Zanthoxylum nitidum (Roxb.) DC	total alkaloids	D-101		4 BV·h^{-1}	nothing			The first time: 95% ethanol 4 BV	2 BV·h^{-1}			[31]
					nothing			The second time: 95% ethanol 3 BV	2 BV·h^{-1}			
				4 BV·h^{-1}	nothing			The first time: 80% ethanol 3 BV	4 BV·h^{-1}	27.67%		
					nothing			The second time: 80% ethanol 2 BV	4 BV·h^{-1}			
Colchicum autumnale L.	total alkaloids	LSA-5B	2.5	4 BV·h^{-1} 1.5 BV·h^{-1}	HCl (pH = 5) water		1 BV 8 BV	50% ethanol 4 BV 70% ethanol 20 BV	2 BV·h^{-1} 1.5 BV·h^{-1}	12.41%	62.27%	[10]
Lotus leaves	total alkaloids	D-101		3 BV·h^{-1}	nothing			90% ethanol 4 BV	1.5 BV·h^{-1}		57.2%	[32]
Peels of Carya cathayensis Sarg.	total alkaloids	NKA-9	7.0	2 BV·h^{-1}	water 2 BV 30% ethanol 2 BV		4 BV	70% ethanol 4 BV	2 BV·h^{-1}	5.27%		[14]
Sophora flavescens	total alkaloids	X-5	10	6 BV·h^{-1}	water	2.5 BV·h^{-1}	5 BV	60% ethanol (pH = 1)	2.5 BV·h^{-1}	39.98%		[19]
Lotus leaves	total alkaloids	HPD-100	5 - 6	2 mL·min^{-1}	water 10 BV 30% methanol 5 BV		15 BV	75% methanol 20 BV				[15]
Macleaya cordata (Willd) R. Br.	rotopine alkaloids	AB-8	8	1 mL·min^{-1}	water		1 BV	90% ethanol 3 BV	1 mL·min^{-1}	>90%		[33]
Lotus leaves	total alkaloids	D-101			water		3 - 5 BV	20% ethanol 2 BV 40% ethanol 2 BV 60% ethanol 2 BV 80% ethanol 2 BV	1.50 BV·h^{-1}	32.56%	62.9%	[34]

Continued

Coptis chinensis Franch	total alkaloids	AB-8			water	1 BV·h^{-1}	2 BV	40% ethanol 2 BV	1 BV·h^{-1}	80%		[21]
Fritillaria hupehensis Hsiao et K.C.H sia	total alkaloids	D-101	9	2 BV·h^{-1}	water	2 BV·h^{-1}	8 BV	50% ethanol 4 BV	2 BV·h^{-1}	74.20%		[35]
Plumula nelumbinis	neferine	LSA-5B		2 BV·h^{-1}	water	2 BV·h^{-1}		50% ethanol	2 BV·h^{-1}	3.50%	91.62%	[28]
Macleaya cordata (Willd) R. Br.	total alkaloids	AB-8	7 - 8	2 - 3 mL·min^{-1}	water	3 - 4 mL·min^{-1}	4 BV	90% ethanol 2 - 3 BV	2 - 3 mL·min^{-1}	>90%	91.24%	[36]
Lotus leaves	aporphine alkaloids	HPD-100			water	7.5 mL·min^{-1}	5 BV	70% methanol 5 BV 80% methanol 10 BV 95% methanol 10 BV	7.5 mL·min^{-1}	nuciferine: 68.52% N-nornuciferine: 44.01% O-nornuciferine: 7.61%		[16]
Gelsemium elegans (Gardn. & Champ.) Benth.	total alkaloids	HPD-800	11		water		20 mL	30% ethanol 15 mL 50% ethanol 15 mL 70% ethanol 15 mL 95% ethanol 15 mL		95.32%		[24]
Ephedra	ephedrine	FXD-1	10 - 11	3.0 mL·min^{-1}	nothing			0.08 mol/L oxalic acid	3.0 mL·min^{-1}	91.20%	99.3%	[17]
Corydalis yanhusuo W.T. Wang	total alkaloids	NKA-9			water	2 BV·h^{-1}	5 BV	70% ethanol 12 BV	1.5 BV·h^{-1}	>50%		[37]
Lateral Root of Aconitum carmichaelii	diterpenoid alkaloids	HPD-110			water		50 L	30% ethanol 120 L 50% ethanol 120 L 95% ethanol 100 L				[25]
Nitraria sibirica leaves	total alkaloids	HPD-450			water			50% ethanol		18.08%		[38]
Chelidonium majus	chelidonine	D101		6 BV·h^{-1}	water	6 BV·h^{-1}	2 BV	30% ethanol 5 BV 80% ethanol 14 BV	6 BV·h^{-1}	37.81%	80.77%	[39]
Stephania cepharantha Hayata	total alkaloids	D101			nothing			ethanol-water-triethylamine (30:65:5) ethanol-water-formic acid (70:25:5) 95% ethanol 10 BV (in total)		3.4%		[40]
Huperzia serrata	huperzine-A and huperzine-B	SP850	9.0	5 BV·h^{-1}	water		6 BV	10% ethanol 3 BV 70% ethanol 6 BV pure ethanol 3 BV	5 BV·h^{-1}	huperzine-A: 2.03% huperzine-B: 0.91%	huperzine-A: 90.1% huperzine-B: 93.2%	[29]
Fritillaria cirrhosa	total alkaloids	H103	7.0	4 BV·h^{-1}	water	2 BV·h^{-1}	8 BV	10% ethanol 4 BV 90% ethanol 6 BV	2 BV·h^{-1}		94.43%	[27]
Sophora alopecuroides	matrine, oxymatrine, and sophoridine	AB-8	10	2 BV·h^{-1}	water			80% ethanol 5 BV	2 BV·h^{-1}	matrine: 22.22% oxymatrine: 21.44% sophoridine: 28.02%	matrine: 69.4% oxymatrine: 78.3% sophoridine: 72.6%	[7]

Continued

Aconiti kusnezoffii radix	aconitine, mesaconitine, hypaconitine, benzoylaconine, benzoylmesaconine, and benzoylhypaconine	NKA-II	6	1 BV·h^{-1}	water 2.8 BV 35% ethanol 2.8 BV	1 BV·h^{-1}	5.6 BV	95% ethanol (pH = 2) 3.3 BV	1 BV·h^{-1}	total alkaloids: 60.3%	75.8%	[18]
Dicranostigma leptopodum (Maxim.) Fedde	total alkaloids	D101	6 - 7	1 BV·h^{-1}	water		4 BV	70% ethanol 10 BV	2 BV·h^{-1}	(65.92 ± 1.33)%		[41]
Camellia ptilophylla	theobromine	XAD-16		0.75 BV·h^{-1}	water		8 BV	20% ethanol		74%		[22]
Toad venom	crude alkaloids	D101			water			5% ethanol				[23]
Pepper	capsaicin	SKP-10-4300	8	1.0 BV·h^{-1}	water		2 BV	20% ethanol 2 BV 45% ethanol 2 BV 45% ethanol-55% sodium hydroxide solution (1%, w/w) 8 BV	1.0 BV·h^{-1}	92%	85%	[20]
Folium isatidis	indigotin and indirubin	D3520		1 BV·h^{-1}	water			50% ethanol 13/3 BV pure ethanol 16/3 BV	3 BV·h^{-1}	indigotin: 4.73% indirubin: 8.99%		[5]
Sophora flavescens	matrine and oxymatrine	BS-65	10	2 mL·min^{-1}	water			ether 2.5 BV 50% ethanol 1.5 BV	2 mL·min^{-1}	matrine: 67.2% oxymatrine: 66.8%	matrine: 90.3% oxymatrine: 86.9%	[6]
Macleaya cordata (Willd) R. Br.	chelerythrine and sanguinarine	methyl acrylate-co-divinylbenzene macroporous adsorbents		0.5 BV·h^{-1}	water			60% ethanol 2 BV 80% ethanol (including 8% acetic acid) 3 BV	0.5 BV·h^{-1}	chelerythrine: 92.8% sanguinarine: 96.1%	nearly 90%	[8]

Ps: in the case of no special instructions, the proportions in this table are volume ratios.

2.2.2. Washing

There was a washing step after the sample loading in most of the literature. The goal of washing is not only to remove impurities but also to minimize the loss of the target alkaloids. Considering that water is cheap and has a good washing ability for polar substances, such as sugars and salts, researchers chose water to wash impurities in most of the literature. From **Table 2**, we can see that the volume of water used to wash was generally between 2 - 8 BV [10] [13], and the washing speed was generally between 1 - 6 BV·h^{-1} [12] [13]. There is also a study in which an ethanol solution with a low concentration was used for washing, and the concentration of the ethanol solution was below 35% [14].

2.2.3. Elution

The main factors affecting the elution effect include the composition of the eluent, the amount of the eluent, and the flow rate of the eluent. Researchers often used an ethanol solution for elution, and a few used ether [6], a methanol solution [15] [16], an acid solution [8] [17] [18] [19] or a sodium hydroxide solution [20]. According to **Table 2**, researchers used an ethanol solution of which the concentration was between 50% and 90% in most of the literature. However, an

ethanol solution with a low concentration (<40%) was also used as an eluent in a few studies [21] [22] [23]. To separate various alkaloids, using ethanol solutions with different concentrations for multiple elutions can be considered [20] [24] [25]. The desorption of target alkaloids, the desorption of impurities, and the consumption of eluent should be considered in the optimization of the elution volume. In **Table 2**, the elution volume was generally 4-12 BV [9] [12] [26]. In the optimization of the elution flow rate, the consumption of eluent, time, and column working pressure should be considered. In **Table 2**, the elution speed was mostly between 1 and 5 $BV \cdot h^{-1}$, generally not higher than the washing speed [7] [18] [20] [27].

2.2.4. Refining Results

In **Table 2**, the recoveries of alkaloids from macroporous resin column chromatography can often exceed 90% [11] [28]. This observation indicates that the optimal adsorption and elution conditions can reduce the loss of alkaloids in the chromatography process. The purities of some alkaloids obtained by chromatography reached 95% [8] [24], and the purities of some alkaloids were less than 5% [13] [29]. These results show that the Chinese medicine system is complex. High-purity alkaloids may not be obtained by macroporous resin column chromatography alone because the working pressure of macroporous resin chromatography is not high and the processing capacity is also large. Macroporous resin chromatography can be used as a preliminary purification, and then, further purification can be carried out through crystallization and other methods.

3. Ion Exchange Resin

3.1. Properties of Ion Exchange Resins

Ion exchange resins are organic macromolecular adsorbents with ion exchange groups and a network structure [42]. Ion exchange resins commonly used for alkaloid separation are strongly acidic or weakly acidic cation exchange resins with styrene or acrylic acid macroporous backbone structure, and their properties are listed in **Table 3**. Some of their photos are shown in **Figure 1**. Among them, the 001 × 7 strong acidic cation exchange resin is most commonly used in alkaloid refining.

3.2. Ion Exchange Resin Chromatography Separation Process

The main steps for the purification of alkaloids by ion exchange resins are the same as those of macroporous resins, which also include pretreatment, sampling, washing, elution and regeneration. The sampling, washing, elution steps and purification effects for certain research works are listed in **Table 4**.

3.2.1. Sample Loading, Washing and Elution

Compared with macroporous resins, the characteristics of a sample solution treated by an ion exchange resin include two points. First, the pH value of the sample solution is mostly less than 3, which means that the alkaloids are in the

form of salts when they are loaded. Second, a higher proportion of ethanol is allowed in the sample solvent, which means that the eluate of a macroporous resin may again be refined by the ion exchange resin. In Table 4, the sample loading speeds are mostly controlled at 2 - 4 mL·min^{-1} or 4 - 6 BV·h^{-1}. The higher sample loading speed may be related to the larger particle size of ion exchange resins.

After sample loading, the ion exchange resin can be washed with water first. The washing speeds listed in Table 4 shall not be lower than the sample loading speeds.

Table 3. Performance parameters of ion exchange resins.

Resin model	Maternal model	Structure	Functional group	Appearance	Exchange capacity/(mmol·g^{-1})	Particle size range/mm	Reference
001 × 7	styrene	gel	$-SO_3H$	brown to tan globular granules	≥4.5	0.40 - 0.70	[43] [44]
001 × 2.5	styrene	gel	$-SO_3H$	brown-yellow globular granules	≥4.30	0.400 - 1.250	[45]
D-151	acrylic acid	macropore	−COOH	milky opaque globular particles	≥9.50	0.315 - 1.25	[46]
D-152	acrylic acid	macropore	−COOH	milky opaque globular particles	≥9.00	0.315 - 1.25	[46] [47]

Table 4. Study on the separation of alkaloids by ion exchange resin chromatography.

Chinese medicines	Alkaloids	Resin Type	Loading Process		Washing Process		Elution Process			Purity	Recovery	Reference
			pH Value of Sample Solution	Sample Flow Rate	Detergent	Volume of Detergent	Eluent Composition	Elution Flow Rate	Volume of Eluate			
Cynoglossum amabile	total alkaloids	001×7	2	6 BV·h^{-1}	water	5 BV	1 mol·L^{-1} Sodium chloride solution	6 BV·h^{-1}	26 BV			[50]
Corydalis hendersonii	total alkaloids	001×7			water	6 BV	70% ethanol - 5% ammonium hydroxide		6 BV	>45%		[51]
Motherwort	total alkaloids	001×7	2		water		5% ammonium hydroxide 70% ethanol 5% ammonium hydroxide					[49]
Uncaria rhynchophylla	total alkaloids	001×7		6 BV·h^{-1}	water		5% Sodium chloride solution - 50% ethanol	8 BV·h^{-1}	10 BV	47.60%	89.9%	[48]
Sini powder	total alkaloids	001×7	2				4% ammonium hydroxide - ethanol		40 mL	0.373%		[52]
Ephedra and Hindu datura flower	total alkaloids	D-151	11	4.0 mL·min^{-1}	water	300 mL	0.08 mol·L^{-1} hydrochloric acid	4.0 mL·min^{-1}	750 mL		76.14%	[53]

It can be seen from Table 4 that the eluent for refining alkaloids by ion exchange resin chromatography is usually an alcohol solution, ammonium hydroxide, sodium chloride solution, acid added solution, etc. The concentration of ethanol solution is generally more than 50%. A comparison of macroporous resins and ion exchange resins on the adsorption and desorption of alkaloids are shown in Figure 2.

Wang *et al.* [48] used the 001 × 7 ion exchange resin to separate and purify the total alkaloids of Uncaria and found that the 50% ethanol solution of 5% sodium chloride had a high elution rate for the total alkaloids. Peng *et al.* [49] found that 5% ammonia and 70% ethanol could wash out part of Leonurus alkaloids. In the literature, the range of elution velocities is 6 - 8 $BV \cdot h^{-1}$. In the literature, there are examples of not only a fixed elution volume but also the determination of the high performance liquid chromatography (HPLC) [50] and precipitation reactions [49].

3.2.2. Refining Results

From Table 4, it can be seen that the purity of alkaloids separated by ion exchange resin chromatography is between 0.3% and 78%, and the recovery of alkaloids is between 76% and 92%. Compared with macroporous resins, the purity of total alkaloids from ion exchange resins is not high.

4. Silica Gel

4.1. Properties of Silica Gel for Column Chromatography

The silica gel for column chromatography is generally a transparent or milky-white

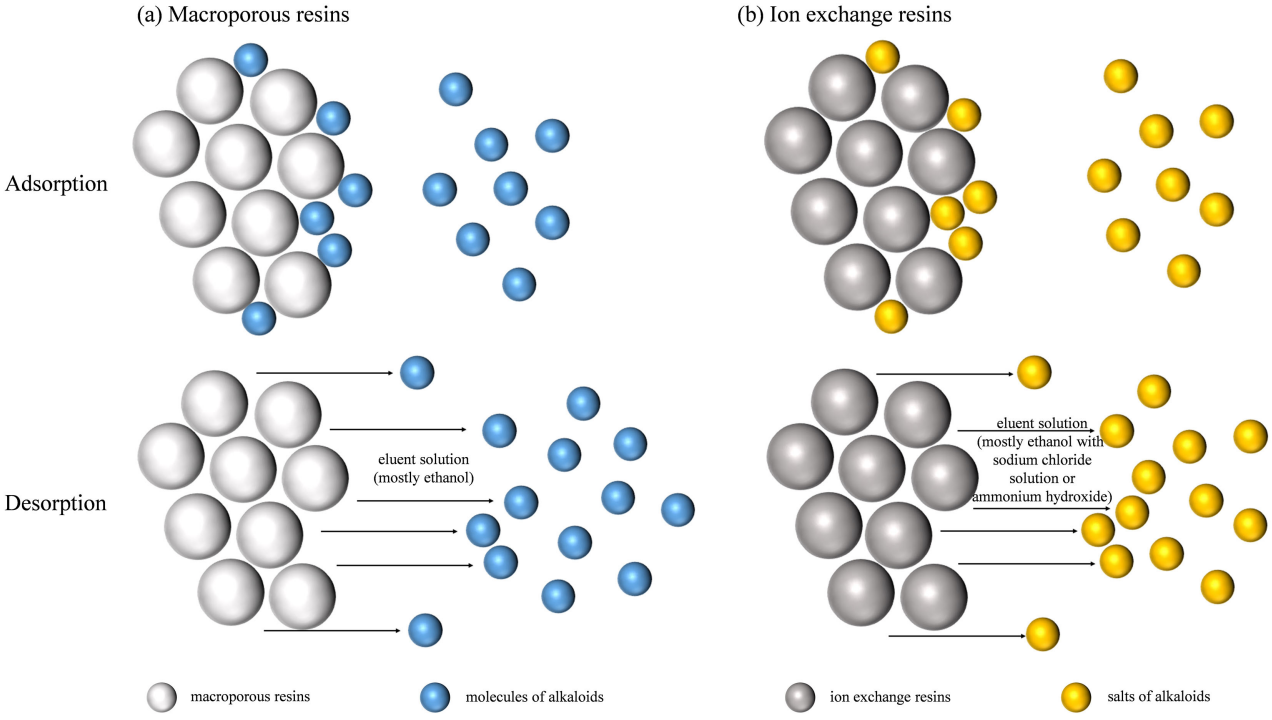

Figure 2. Comparison of macroporous resins and ion exchange resins on the adsorption and desorption of alkaloids.

granular solid with a microporous structure, which has the advantages of a high adsorption performance, stable physical and chemical properties, high mechanical strength and renewable use. In general, the van der Waals force is used to adsorb organic molecules from the solution, and the differences in the adsorption capacity for different molecules are used for separation from the silica gel during elution. At present, there are many reports about silica gel being used to refine the alkaloids of Chinese medicines, as seen in Table 5. It can be seen from the table that most silica gel used for column chromatography is 200 - 300 mesh, and its particle size range is 45 - 75 μm. The smaller particle size is beneficial to increase the specific surface area for adsorption and separation effects, but it will also increase the pressure in the chromatography process.

4.2. Separation Technology of Silica Gel Chromatography

4.2.1. Loading and Elution

When silica gel is used as an adsorbent, in addition to the wet method, the dry method is also often used. The operation of wet sampling is more convenient, but dry sampling can solve the problem of low solubility of the components to be separated in the sample solution.

For a positive silica gel, the eluant includes chloroform methanol [54] [55], methanol water [56], methanol [57] [58], etc., and sometimes ammonium hydroxide [59] [60] are added to adjust the pH value of the eluant. For a reversed phase silica gel, the eluant is generally an organic solvent water solution. Compared with macroporous resins and ion exchange resins, gradient elution is commonly used when silica gel is used as the adsorbent. Gradient elution is more complex than isoelution, but it is beneficial to obtain high-purity alkaloids.

The amount of eluent and the elution time should be considered when choosing the elution flow rate. The elution flow rate is usually 0.5 - 1 $BV \cdot h^{-1}$, which is substantially slower than that of macroporous adsorption resins and ion exchange resins. The main reason is that the particle size of silica gel is obviously smaller than that of the commonly used macroporous adsorption resin or ion exchange resin, and the operating pressure will be high when the flow rate is large.

4.2.2. Refining Results

It can be seen from Table 5 that the purity of the products obtained from silica gel separation of alkaloids is mostly over 90%, sometimes even close to 100%. Compared with ion exchange resins and macroporous resins, the purity of the product is high. The overall recovery in Table 5 is not high, which may be due to the sacrifice of the recovery to obtain high-purity target alkaloids. If the purity of the alkaloids is required to be high, macroporous adsorption resins or ion exchange resins can be used for a crude separation, and then, silica gel can be used for refining.

5. Modeling Method

Modeling is important for the chromatography process optimization. In general,

Table 5. Study on the separation of alkaloids by silica gel chromatography.

Chinese medicines	Alkaloids	Adsorbent		Elution Process		Purity	Recovery	Reference
		Type	Mesh/Particle Size	Eluent Composition	Elution Method			
Lindera aggregata	several fractions of aconite alkaloids	C18-reversed silica gel		methanol - water	gradient elution			[56]
Clausena anisum-olens	8 fractions of aconite alkaloids	silica gel		petroleum ether - ethyl acetate ethyl acetate ethyl acetate - methanol methanol	gradient elution			[61]
Angelica dahurica	20 fractions of aconite alkaloids	RP-C18-reversed silica gel		methanol - water	gradient elution			[58]
Rauvolfia yunnanensis	2 fractions of aconite alkaloids	silica gel		chloroform - methanol	gradient elution			[54]
Herba Aconiti	heteratisine, hordenine, talatisamine	silica gel		cyclohexane - acetone	gradient elution	all >98%		[58]
Semen holarrhenae	total alkaloids (containing three alkaloid monomers)	silica gel	200 - 300 mesh	chloroform - methanol	gradient elution	chemical compound 1: 89.23%, chemical compound 2: 94.89%, chemical compound 3: 62.64%		[55]
Fritillary	4 fractions of aconite alkaloids	silica gel	200 - 300 mesh	petroleum ether - acetone	gradient elution			[62]
	suchengbeisine	silica gel	200 - 300 mesh	chloroform - methanol	gradient elution			
	verticinone-N-oxide	silica gel	200 - 300 mesh	chloroform - methanol	gradient elution			
Corydalis saxicola	total alkaloids	silica gel	200 - 300 mesh	chloroform - methanol	gradient elution			[63]
Aconitum taipeicum	total alkaloids	silica gel		chloroform - methanol	gradient elution			[64]
Semen plantaginis	7 fractions of aconite alkaloids	silica gel		chloroform - methanol - ammonium hydroxide	isocratic elution			[59]
Catharanthus roseus	vincristine, vincaleukoblastinum	silica gel		carrene - methanol	gradient elution	vincristine: 97.26%, vincaleukoblastinum: 94.18%	vincristine: 61.12%, vincaleukoblastinum: 58.50%	[65]
Sophora alopecuroide	N-oxysophocarpine, oxymatrine	silica gel	200 - 300 mesh	chloroform – methanol - ammonium hydroxide	isocratic elution	>99%		[60]
	sophoridine	silica gel	200 - 300 mesh	Acetone - methanol	isocratic elution	95.80%		
Sophora alopecuroide	matrine, oxymatrine	silica gel	300 - 400 mesh	chloroform, chloroform - methanol	isocratic elution	matrine: >95%, oxymatrine: >95%	matrine: 22.2%, oxymatrine: 42.6%	[66]

it is possible to obtain the global optimal conditions only by establishing the model first.

5.1. Modeling Based on Statistics

Dr. Yu suggested adopting a design of experiments in research on pharmaceutical processes [67]. Some researchers adopt the Taguchi design to study the technological parameters of column chromatography [9] [12] [14] [35]. This design requires a few experiments, but the obtained data can only be modeled by linear equations. The Box-Behnken design [20], by contrast, enables researchers to model second-order polynomials with quadratic terms and interaction terms, although the design requires more experiments. The second-order polynomials modeled are beneficial to obtain the optimum conditions in the research scope after optimization. The model form is generally as shown in Formula (1).

$$Y = a_0 + \sum_{i=1}^{m} a_i X_i + \sum_{i=1}^{m} a_{ii} X_i^2 + \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} a_{ij} X_i X_j \quad (1)$$

where Y is the response variable; a_0 is a constant; a_i, a_{ii}, and a_{ij} are the linear, quadratic, and cross-product coefficients, respectively; X_i and X_j are different parameters; and m is the number of parameters.

5.2. Thermodynamic Model of Static Adsorption

In static adsorption, the adsorption capacity of a macroporous resin for alkaloids can be evaluated by adsorption isotherms. There may be many forms of adsorption isotherms. Certain examples are listed in **Table 6**. The Langmuir and Freundlich models are widely used in the study of alkaloid chromatography [6] [7] [18] [27] [39]. Because of the complexity of the adsorption principle, the above two models are often used by researchers at the same time to compare and choose the better one according to the determination coefficient (R^2) [6] [18] [39].

5.3. Kinetic Model of Static Adsorption

The static adsorption rate is an important index when optimizing the resin for refining alkaloids. The results can be fitted by using a variety of models, as seen in **Table 7**.

The pseudo-first-order and pseudo-second-order kinetic models are the most commonly used empirical models for alkaloid adsorption [73] [74] [75]. The pseudo-first-order kinetic model is more accurate in fitting the initial stage of adsorption when the initial concentration of adsorbate is high, while the pseudo-second-order kinetic model is suitable for fitting the subsequent stage of adsorption when the initial concentration is low, and the pseudo-nth-order model dynamics is the generalization result of these two models. The mixed order model combines the pseudo-first-order kinetic model and the pseudo-second-order kinetic model, so it can be used to fit the whole adsorption process of any initial concentration.

Table 6. Common adsorption isotherm equations.

Name	Form	Meaning of Parameter	Applicability	Reference
Langmuir	$q_e = \frac{q_m C_e}{K_L + C_e}$	q_e: the adsorption capacity at adsorption equilibrium (mg/g - resin) q_m: the theoretical maximum adsorption capacity (mg/g-resin) K_L: the Langmuir constant C_e: the equilibrium concentration in liquid phase	Monolayer adsorption on uniform surface	[18]
Freundlich	$q_e = K_F C_e^{1/n}$	K_F: the Freundlich constant $1/n$: an empirical constant	Monolayer adsorption on a heterogeneous surface	[39]
Henry	$q_e = K_H C_e$	K_H: the Henry constant	The amount of adsorption accounts for less than 10% of the amount of adsorption forming the monolayer	[68]
Redlich–Peterson	$q_e = \frac{K_R C_e}{1 + a_R C_e^g}$	K_R: the Redlich-Peterson constant a_R: an empirical constant g: an empirical constant that is between 0 and 1	Monolayer adsorption	[69]

Table 7. Common static adsorption kinetic equations.

Name	Form	Meaning of Parameter	Reference
Pseudo-nth-order model	$n = 1$, $\frac{dq_t}{dt} = k_1(q_e - q_t)$ (Pseudo-first-order model) $n = 2$, $\frac{dq_t}{dt} = k_2(q_e - q_t)^2$ (Pseudo-second-order model) n take other values, $\frac{dq_t}{dt} = k_n(q_e - q_t)^n$	k_1 : pseudo-first-order rate constant (h^{-1}) k_2 : pseudo-second-order rate constant ($g \cdot mg^{-1} \cdot h^{-1}$) k_n : pseudo-nth-order rate constant ($g^{n-1} \cdot mg^{1-n} \cdot h^{-1}$) t: adsorption time (h) q_t : adsorption capacity at time t ($mg \cdot L^{-1}$) q_e : equilibrium adsorption capacity ($mg \cdot L^{-1}$) n: number of active sites occupied by adsorbed ions/molecules	[70]
Mixed-order model	$\frac{dq_t}{dt} = k_1'(q_e - q) + k_2'(q_e - q)^2$	k_1' : pseudo-first-order rate constant of mixed-order model (h^{-1}) k_2' : pseudo-second-order rate constant of mixed-order model ($g \cdot mg^{-1} \cdot h^{-1}$)	[71]
Elovich equation	$\frac{dq_t}{dt} = \alpha e^{-\beta q_t}$	α : initial desorption rate constant ($mg \cdot g^{-1} \cdot h^{-1}$) β : desorption rate constant ($g \cdot mg^{-1}$)	[72]

6. Conclusions

In conclusion, there are many studies on the purification of alkaloids by chromatography. The most commonly used adsorbents are macroporous adsorption resins, ion exchange resins and silica gel. In the separation and purification of alkaloids, a nonpolar macroporous resin is often used. The purity of alkaloids from an ion exchange resin and macroporous resin is not high, but the purity of alkaloids from silica gel refining is high. Compared with silica gel, macroporous resins and ion exchange resins are cheaper and have a lower operating pressure, so they are more suitable for the preliminary separation of alkaloids.

The authors think that future research can be carried out in the following directions:

Firstly, the modeling method of the column chromatography process needs to

be further studied. The dynamic adsorption process is usually described by the general rate model [76]. Xu *et al.* [77] used the model to simulate the chromatography of a simulation system containing Puerarin and Daidzein, and the results were in good agreement with the experimental values. It is difficult to describe the phenomenon of competitive adsorption because of the complexity of the components in Chinese medicine extract. To date, there has been no research report related to the general rate model of alkaloid chromatography. Therefore, it is still necessary to develop effective modeling methods to describe the chromatography process of Chinese medicine extracts.

Secondly, stricter quality control methods should be established. There are differences in the content of components in different batches of Chinese medicine extract solution, which affect the effect of chromatography. However, there is no research focus on the influence of the quality change in the sample solution on the chromatography effect so far. To control the consistency between different batches of alkaloids, it is suggested to set the quality standard of the sample solution. Pan *et al.* [78] established a quantitative model of process parameters, raw material properties and evaluation indexes of column chromatography eluent and then calculated the quality standards of raw materials according to the requirements of the evaluation indexes. This idea can be used for reference to establish the quality standard of a sample solution for alkaloid column chromatography.

Thirdly, online detection of chromatography processes should be strengthened. At present, in academia, spectral technologies combined with multivariate statistical methods are often used to detect the content of indicators/major components in Chinese medicines or to detect the process trajectory [79] [80] [81]. This approach has the advantage of real-time online quantitative detection. However, it has not been reported that it can be used in the chromatography of alkaloids, so it needs further development.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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