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Innovative approach for separation and purification of natural products using carbon nanotube-alginate gel beads as a novel stationary phase

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Abstract

Carbon nanotubes(CNT)-alginate gel beads were used as a novel stationary phase for separation and purification of natural products in open glass column. The objective of this study is to find a new packing material which can improve the separation efficiency and resolution in preparative or semi-preparative liquid chromatography. Four types of CNTs were evaluated for their adsorption and desorption capability of various natural compounds. Langmuir and Freundlich isotherms were used to correlate the solute affinity of CNTs towards alkaloid at different temperatures, and the Langmuir isotherm fit better. Static adsorption and desorption tests indicated that multi-walled carbon nanotubes(MWCNT)-1 was suitable to separate alkaloid of *Dature metel L.* MWCNT-1 was then bonded to alginate carrier to form gel beads, which further improved the separation. Under gradient elution, the purity of atropin and anisodamine reached 83.6% (weight of atropin/weight of dry elutent) and 90.5%, respectively, which were 7.9-folds and 11.2-folds to those in control samples. The recovery yields were 88.2% and 89.2%, respectively. Therefore, alginate-supported CNTs gel beads can serve as excellent liquid chromatographic packing material.

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ARTICLE TYPE

1. Introduction

It is one of the most challenging tasks in bioprocess engineering to purify and separate natural products with important biological activity. The objective of the present study is to find a new packing material which has better separation efficiency and resolution in preparative or semi-preparative liquid chromatography. It is required to attain high purity, biological activity, as well as product yield.

Carbon nanotube (CNT), which was first reported by Iigima in 1991[1], possesses unique mechanical properties due to its nanoscale diameter and large specific surface area. Over the decades, CNT has been used in many fields, such as transistors [2], electrodes [3], adsorbing materials [4], biosensors [5] and drug delivery [6,7].Due to their large internal surface, CNTs show a stronger binding ability for molecules compared to other packing material. CNTs can be used to separate different classes of compounds after surface modification. CNTs have been used as stationary phase in gas chromatography (GC) [8,9] and high performance liquid chromatography (HPLC) [10 , 11] for separation and determination of various types of material, such as esters and aromatics [12,13], aliphatic alcohols [14], and gas[15]. But CNTs as a new packing material in purification and separation of natural products has not been reported yet. Our interest in CNTs for purification applications arose from its porosity, high adsorbability, extraordinary strength, and thermal stability.

CNTs are categorized as single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). SWNTs consist of a graphene sheet rolled into a cylinder with a typical diameter on the order of 1 nm. MWNTs consist of concentric cylinders with an interlayer spacing of 3.4 Å and a diameter typically on the order of 10 - 20 nm [16]. In general, the surface of SWCNTs is chemically inert, and SWCNTs are insoluble in aqueous medium. The surface of MWC NTs is more active, and usually reacts with a large number of surface groups, such as carboxyl and oxygen. [17]

To investigate the adsorption and desorption properties of different CNTs towards natural products, two typical compounds were selected from each kind of acidic, alkaline, and neutral natural products, respectively. Then the most effective CNT was chosen to separate suitable natural products. The adsorption kinetic curves of CNTs were studied. Langmuir and Freundlich isotherms were used to describe the interactions between solutes and CNTs at different temperatures, and the equilibrium experimental data were well fitted to Langmuir isotherms. These isotherm models were used to fit the experimental data and explain the adsorption mechanism of test substances on CNTS. This will provide insight for future improvement on separation efficiency and cost reduction. CNTs have better adsorption and desorption capacity of alkaloid than acidic and neutral natural products. Open glass column packed with CNTs was used to perform dynamic tests to optimize the separation process and efficiency. Results show that CNTs are excellent packing material. However, commercial applications of CNTs are challenging due to its high costs. Alginate with its porous structure has been widely used as immobilized material for immobilization of enzyme or bacteria [18]. CNTs combined with alginate support may reduce irreversible adsorption and column pressure. The results of the present investigation can also be applied to larger scale.

2. Materials and Methods

2.1. Materials and Reagents

Dature metel L. was purchased from the herbal medicine market of Shenyang, China. Standard chemicals were purchased from Nanjing Zelang Medicine & Scientific Co., Ltd. (Jiangsu, China). Double-distilled water was used in all experiments. All other reagents were of analytical grade. CNTs were purchased from Department of Chemical Engineering of Tsinghua University in Beijing. Physical and chemical properties of CNTs were stated in table1.

Table 1 Physical and chemical properties of tested CNTs (Data was provide by Tsinghua University)

	Diameter (nm)	Length (μ)	Purity (%)	Specific surface area (m^2/g)	Surface group
MWCNT-1	< 8	10-30	> 95	350	carboxyl
MWCNT-2	8-15	10-20	> 97	240	none
SWCNT-1	< 2	5-20	> 99	> 450	carboxyl
SWCNT-2	1-2	0.5-2	> 99	> 450	none
AB-8 resin	Diameter 0.3-1.25 mm		95	480-520	ester

2.2 Apparatus and chromatographic conditions

HPLC analysis was performed using a Waters UPLC system (Waters Company, USA) equipped with a Waters acquit UPLC class pump and Waters PDA Detector. Separation was performed on a DBS HYPERSIL C18 (150 mm × 4.6 mm). The mobile phase consisted of methanol (A) and water (B). It was filtered before use through a 0.45 μm filter (Millipore, Milford, Mass). The mobile phase for separation of *Dature metel L.* alkaloid (atropin and anisodamine) was prepared with 80% double-distilled water contained of 0.1% sodium acetate, 0.02% triethylamine and 20% methanol. Detection wavelength was 220 nm. The mobile phase for determination of polydatin and emodin was 40% methanol and 60% double-distilled water. Detection wavelengths were 330 nm and 254 nm. The mobile phase for determination of obakulactone was 50% acetonitrile and 50% double-distilled water. Detection wavelength was 265 nm. The mobile phase for determination of artemisinin was 80% methanol and 20% double-distilled water. Detection wavelength was 254 nm. The injection volume and the flow rate of mobile phase were 10 μL and 0.6 mL/min, respectively. The column was maintained at 35 °C.

2.3 Ultrasound extraction of Dature metel L. alkaloid

After being washed and dried in shade, *Dature metel L.* was pulverized to homogeneous size by a herbal medicine disintegrator (HX-200A, Yong-Kang Hardware and Medical Instrument Company, China) and then sieved (30 – 40 mesh). For ultrasound extraction, a 250 W, 20 kHz ultrasonic generator (KQ-250DB, Kushan Company of acoustics, China) was used. Alkaloid extraction was performed essentially as described in Ref [19].

2.4 Static adsorption and desorption tests

2.4.1. Adsorption and desorption properties of CNTs

To study adsorption and desorption of natural products on different CNTs, six typical compounds were selected from acidic (emodin and polydatin), alkaline (atropin and anisodamine), and neutral natural products (obakulactone and artemisinin). Standard solutions of compounds were prepared by dissolving 2.5 mg individual compounds into 50 mL of 5% ethanol solution in 100 mL flasks. After adding 1.0 g CNTs to each standard solution, the flask was capped, shaken on a flat-bed orbital shaker (model HZQ-X100, Donglian Company, Harbin, China) for 12 h at 35 ^{o}C. Adsorption kinetics experiments in previous study showed that 12 h was sufficient to achieve adsorption equilibrium. Then, the concentration of each standard compound was analyzed by HPLC. To study desorption recovery, standard solution was then filtered through a 0.2 mm filter to separate CNTs from the solution. CNTs were washed with water and then desorbed by 50 mL ethanol. Macroporous resins have been widely used in purification of natural products. In our study, AB-8 macroporous resins served as positive control. The adsorption and desorption capacities of CNTs and AB-8 were calculated using the following equitions:

Adsorption evaluation:

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

where Q_e was the adsorption capacity at adsorption equilibrium (mg/g); C_0 and C_e were the concentrations of solutes in the solutions before and after adsorption by resin, respectively (mg/mL). V_i was the volume of the initial sample solution (mL) and W was the weight of CNTs (g).

Desorption evaluation:

$$Q_d = \frac{C_d V_d}{W} \quad (2)$$

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

$$R = \frac{C_d V_d}{C_o V_i} \times 100\% \quad (4)$$

where Q_d was the desorption capacity after adsorption equilibrium (mg/g); C_d was the concentration of the solutes in the desorption solution (mg/mL); V_d was the volume of the desorption solution (mL); D was the desorption ratio (%); R was the recovery ratio (%);C_0, C_e and V_i were the same as defined above. In our study, the equations and figures were calculated using Origin software 8.0 (OriginLab, US) and Microsoft excel 2007 (Microsoft Corporation, US).

2.4.2. Adsorption kinetics

The adsorption kinetic curves of standard substance on CNTs were investigated by mixing 50 mL standard substances water solution (concentration 0.6 mg/mL) with 1.0 g CNTs in 100 mL flasks. One gram of CNTs was added into each standard solution in the flask. These flasks were shaken on a flat-bed orbital shaker for 7 h at 35 ^{o}C. During this process, 100 μL of the suspension from each flask was analyzed by HPLC at certain time intervals until equilibration was reached. The optimal CNT was finally determined based on the uptake rates of standard substances onto the adsorbents. The models of adsorption kinetics were correlated with it. The effect of adsorption time was evaluated using the pseudo-first-order equation by Lagergren [20] and the pseudo-second-order equation [21].

The pseudo-first-order equation:

$$\frac{dQt}{dt} = K_1(Qe - Qt) \quad (5)$$

The pseudo-second-order equation:

$$\frac{dQt}{dt} = K_2(Qe - Qt)^2 \quad (6)$$

where Qe and Qt were the adsorption quantity (mg/g) at equilibrium and time t (min), respectively, K_1 and K_2 were the equilibrium adsorption rate constants in the above two models.

2.4.3. Adsorption isotherms

In order to investigate the effect of temperature and initial concentration on alkaloid adsorption, experiments of adsorption isotherm on CNTs were performed. Alkaloid (atropin and anisodamine) solutions with different concentrations (0.1 - 1.2 mg/mL) were freshly prepared in water. 1.0 g CNTs were added to the solutions. The mixture was shaken on the flat-bed orbital shaker for 6 h at the desired temperatures (25 ^{o}C, 30 ^{o}C and 35 ^{o}C). Adsorption isotherm was used to describe the relationship between sorbent and adsorbed material. The adsorption isotherms can be described by the Langmuir and Freundlich theoretical equations [22], [23],[24].

The Langmuir equation was expressed as follows:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_o} = \frac{1}{K_L Q_0} \quad (7)$$

where Q_e (mg/g) was the adsorption capacity at equilibrium; C_e (mg/mL) was the concentration of solute in solution (liquid phase) at equilibrium; K_L was the adsorption equilibrium constant; Q_0 was the empirical constant.

The Freundlich equation was expressed as follows:

$$Q_e = KC_e^{1/n} \quad (8)$$

where K was the Freundlich constant, which was an indicator of adsorption capacity determined by the physical properties of the CNTs such as surface area, average pore diameter and particle diameter, etc., and 1/n was an empirical constant with regard to the magnitude of the adsorption driving force [25].

2.5 Dynamic separation capacities

A glass column (12 mm×350 mm) which had been packed with 5.0 g of MWCNTs was used for dynamic separation experiments for *Dature metel L.* alkaloid. The aim of our study is to establish the method for optimizing the new composite material. Therefore, relatively small columns were used for practical reason. This is a normal size in small scale plot for separation and purification. Once the optimal parameters are determined, larger columns can easily be used for preparation purpose. The purities of atropin and anisodamine in the extracts of alkaloid original samples were 10.6% and 8.1% (w/w). Loading weight of the alkaloid extracts was 1.0 g according to results in section *2.4.1*. The bed volume (BV) of MWCNTs was 40 mL and the packing length was 21.2 cm. The sample solution was loaded into the glass column and flowed through the glass column at a flow rate of 1 mL/min. To evaluate the feed volume of sample solution, 0.5 mL effluent collected at 20 mL intervals were analyzed by HPLC. After adsorption equilibrium was reached, the column was washed with double-distilled water and then successively with ethanol solution of different ratios (10 - 50%) in the amount of 4 - 6 BV. After being analyzed by HPLC, eluent was dried at 50 ^{o}C under reduced pressure using a rotary evaporator and then weighted.

2.6 Dynamic separation capacities of CNT- alginate gel beads

2.6.1 Formation of CNT- alginate gel beads

MWCNT-1 was immobilized on alginate support. Each 1.5 g MWCNT-1was added into 50 mL 1.0% (w/v) sodium alginate, and was mixed under stirring at 120 rpm for 30 min. Beads were formed by injecting the mixture into 0.1 M calcium chloride drop by drop. Beads were incubated in a 0.1 M sodium chloride solution for 2 h [26].

2.6.2 Dynamic separation capacities

Dynamic separation experiments for CNT gel beads were carried out under the same condition described in section 2.5. Glass column (30 mm×600 mm) was wet-packed with 40.0 g of CNT - alginate gel beads which contained 3.6 g MWCNT-1.

3. Results and discussion

3.1. Adsorption properties of CNTs

Two types of MWCNTs and two types of SWCNTs with different physical properties were applied to study adsorption and desorption properties of six standard substances. Atropin and anisodamine are alkaloid. Emodin and polydatin are acidic compounds. Obakulactone and artemisinin are neutral natural products.

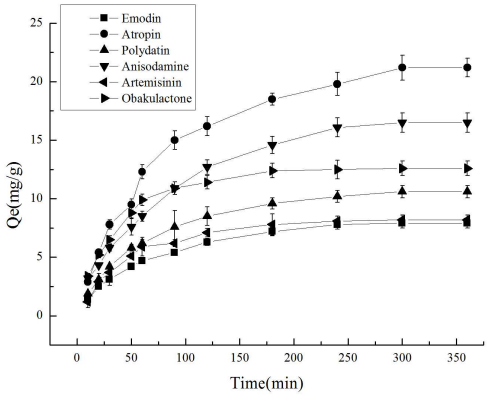

Fig. 1. Adsorption kinetics on MWCNT-1

Four types of CNTs were compared in terms of their adsorption and desorption capacities as well as desorption ratios (Table 2). CNTs have better adsorbability for all the substance than AB-8 macroporous resins. Desorption capacities of most types of CNTs are higher than macroporous resins. SWCNTs showed higher adsorbability for some of the compounds than other CNTs, however, their desorption capacities were relatively low. MWCNT-1 revealed considerably higher desorption ratios than other CNTs and AB-8. Because SWCNTs are much more expensive than MWCNTs, MWCNT-1 is chosen for further experiments.

Table 2 Adsorption capacities (Qe), desorption capacities (Qd) and desorption ratios (D) of standard substances on different CNTs.

		(1)	(2)	(3)	(4)	(5)	(6)
MWCNT-1	Qe(mg/g)	21.2	16.5	12.6	8.2	7.9	10.6
	Qd(mg/g)	19.3	13.6	10.2	6.4	5.2	8.2
	D (%)	91	82.4	81	78	65.8	77.4
MWCNT-2	Qe(mg/g)	19.5	18.4	13.1	8.1	7.5	10.3
	Qd(mg/g)	17.6	13.4	10.5	6.5	4.9	8.4
	D (%)	90.3	72.8	80.2	80.2	65.3	81.6
SWCNT-1	Qe(mg/g)	22.1	17.2	11.8	7.2	7.5	12.3
	Qd(mg/g)	14.8	11.6	9.4	5.2	5.5	7.9
	D (%)	67	67.4	79.7	72.2	73.3	64.2
SWCNT-2	Qe(mg/g)	25.4	18.9	12.9	8.0	7.4	10.5
	Qd(mg/g)	16.9	7.9	10.2	6.2	5.6	8.5
	D (%)	66.5	41.8	79.1	77.5	75.7	81
AB-8	Qe(mg/g)	18.6	15.2	9.8	7.8	7.2	10.1
	Qd(mg/g)	14.2	8.9	7.9	5.1	5.2	7.6
	D (%)	76.3	58.6	80.6	65.4	72.2	75.2

* (1) Atropin; (2) Anisodamine,(3) Obakulactone, (4) Artemisinin,(5) Emodin,(6) Polydatin

3.2 Adsorption kinetics of MWCNT-1

The adsorption kinetics was measured on MWCNT-1. As shown in Fig. 1, the adsorption capacities increased with adsorption time for all six compounds. During the initial 90 mins, the adsorption capacities were considerably enhanced, and then increased slowly,

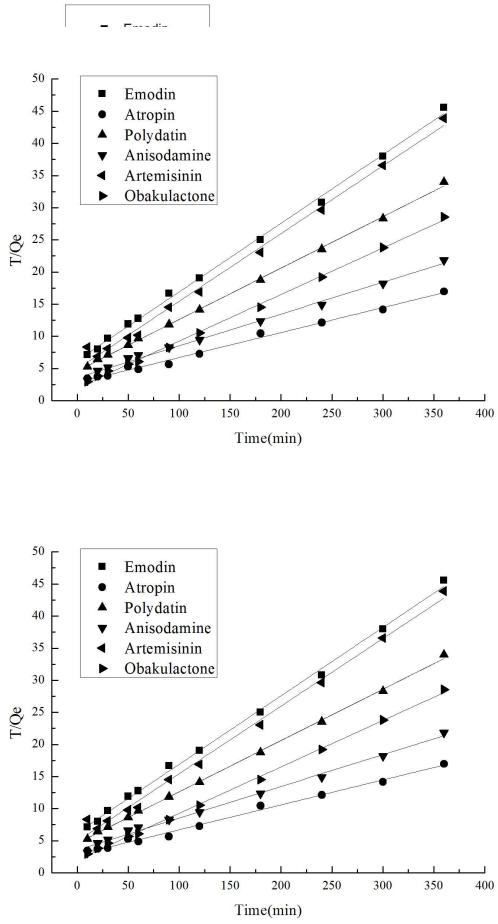

Fig.2. Pseudo-first-order simulation curve and pseudo-second-order simulation curve on MWCNT-1

and reached a plateau. Finally, the adsorption capacities reached equilibrium in 180 mins for obakulactone and artemisinin, in 240 mins for polydatin, anisodamine and emodin, and in 300 mins for atropin. Therefore, 300 mins was sufficient to reach adsorption equilibrium over the entire system. MWCNT-1 possessed better adsorption capacity to separate alkaloid compared to other compounds. Hence, alkaloid was chosen for further experiments.

In order to elucidate the mechanism of adsorption and to determine the rate of the adsorption process, the adsorption kinetics data for six compounds were analyzed with pseudo-first-order and pseudo-second-order equations. As shown in Table 3, in both cases the regression of data was linear and the R^2 (correlation coefficient) was higher than 0.98. The second-order equation fit the experimental data better ($R^2 \geqslant 0.9927$) for all the cases, in comparison to the first-order equation ($R^2 \geqslant 0.9828$) in terms of the correlation coefficients. Pseudo-first-order and pseudo-second order regression curve are shown in Fig. 2.

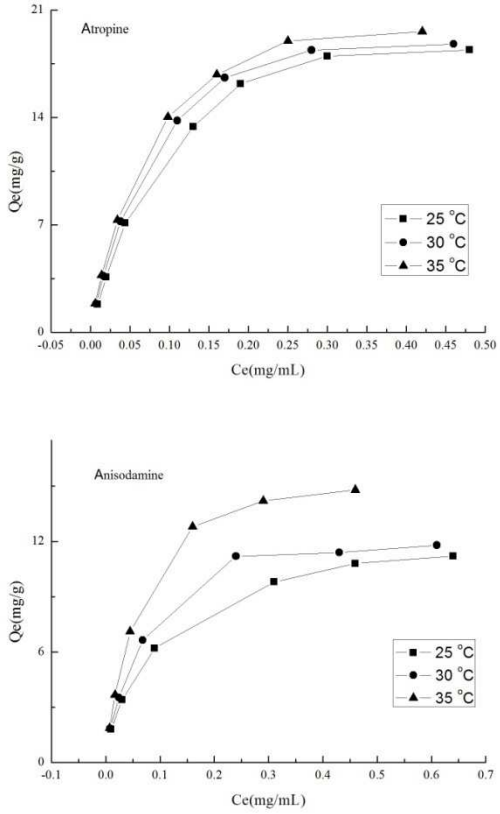

Fig.3. Adsorption isotherms on MWCNT-1 at 25, 30 and 35 °C for atropine and anisodamine.

Table 3 Kinetic parameters for the adsorption of six standard substances on MWCNT-1

Compound	Pseudo-first-order kinetic model			Pseudo-second-order kinetic model		
	Qe(mg/g)	K_1 (min^{-1})	R^2	Qe(mg/g)	K_2 ($g/mg \times min^{-1}$)	R^2
Atropin	20.457	0.015	0.993	25.760	0.001	0.993
Anisodamine	17.063	0.011	1.000	20.218	0.001	0.994
Obakulactone	12.254	0.028	0.988	13.814	0.003	0.998
Polydatin	10.208	0.017	0.990	12.513	0.001	0.999
Artemisinin	8.003	0.021	0.988	9.501	0.002	0.994
Emodin	7.650	0.017	0.983	9.376	0.002	0.998

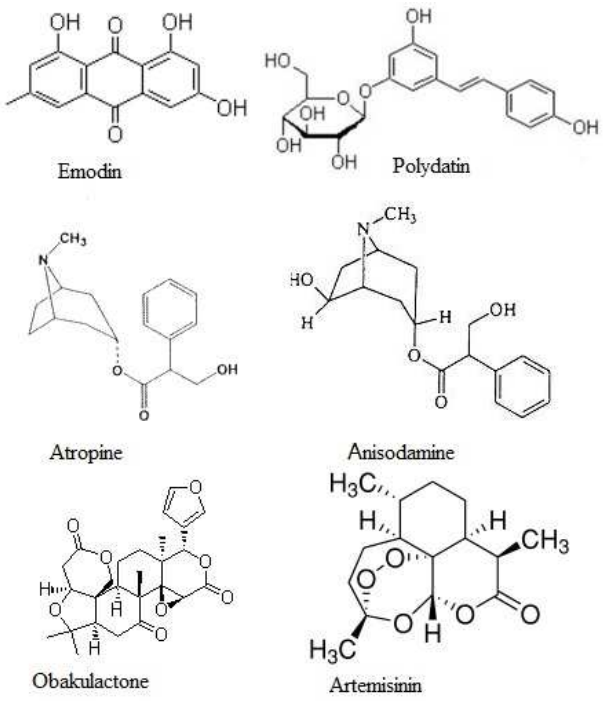

Fig.4. Structures of the standard substances used in Section 2.4.1

The results indicated that the adsorption of natural products can be fit to the pseudo-second-order kinetic model. It can be speculated that the adsorption process might be controlled by two or more rate-limiting steps such as external diffusion, boundary layer diffusion and intraparticle diffusion. Meanwhile, due to its surface functional group, the rate-limiting step may be chemical sorption or chemisorption involving valency forces through sharing or exchange of electrons between sorbent and sorbate. Furthermore, MWCNT-1 has better adsorption properties towards alkaloid compounds (atropin and anisodamine) after comparing the model equilibrium. Therefore alkaloid was used in further experiments. MWCNT-1 was used in column separation, which has carboxyl group on the surface. Therefore, it works like cation exchange resin. The amine groups of atropin and anisodamine are more likely to be attracted to the surface of MWCNT-1. Structures of the compounds were shown in Fig.4. The other compounds are harder to bind on the stationary phase.

3.3. Adsorption isotherms

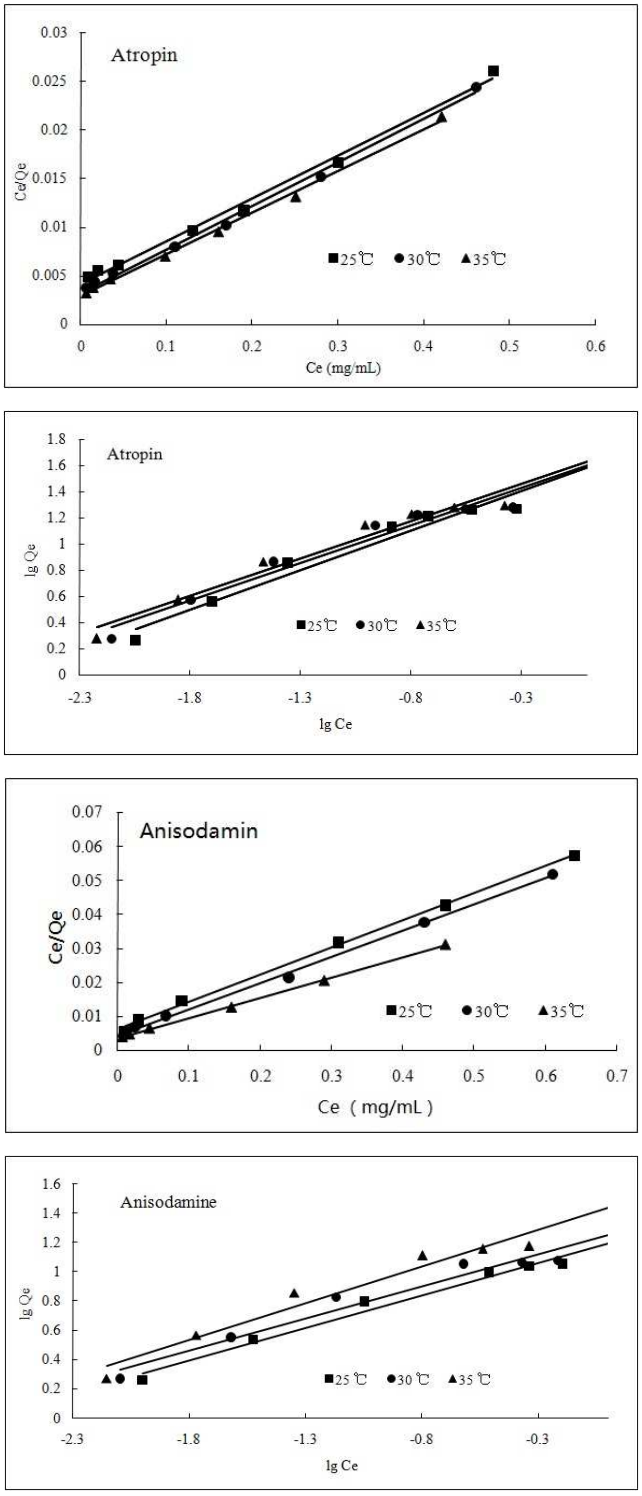

Fig.5. Linear correlation of Atropin and Anisodamine on MWCNT-1 at 25, 30 and 35 ^{o}C on the basis of the Langmuir and Freundlich models.

Due to its better adsorption properties, MWCNT-1 was selected to investigate the adsorption capacity and to characterize the adsorption behaviour of atropin and anisodamine. As shown in Fig. 3, equilibrium adsorption isotherms were investigated at 25 ^{o}C, 30 ^{o}C and 35 ^{o}C by using six different initial concentrations, ranging from 0.1 to 1.4 mg/mL and 0.1 to 1.2 mg/mL. An increase in the adsorption capacities was observed with a rise of

C_e corresponding to the C_0 ranges of 0.1 - 1.2 mg/mL and 0.1 - 0.8 mg/mL, respectively. After the initial concentration of atropin reached 1.2 mg/mL, increase of adsorption capacities slowed down. After the initial concentration of anisodamine reached 0.8 mg/mL, the extraction yield gently increased to a maximum at last. Thus, the concentration of 1.2 mg/mL for atropin and 0.8 mg/mL for anisodamine were chosen as the initial concentrations in the present study.

The adsorption process is characterized by the distribution of the solute molecules between the adsorbent and the liquid phase. The adsorption of solutes on adsorbent particles has been extensively studied. In summary: The adsorption of a solute on a sorbent may follow a three-step process:(1) external diffusion or boundary layer diffusion and surface adsorption during which the solute molecules diffuse rapidly from the solution to the external surface of sorbent particles; (2) gradual diffusion of the solutes to the sorption sites within the internal structure of the sorbent; and (3) rapid uptake of the solutes by the sorption sites [27].

Langmuir and Freundlich models were used to describe how solutes interact with sorbents. The adsorption capacity of MWCNT-1 was compared in terms of the Langmuir and Freundlich parameters obtained from the linearized equations using *Qe* and *Ce* values at 25, 30, and 35 ^{o}C. The Langmuir isotherm is well known to describe the adsorption of a solute from a solution. This model assumes monolayer adsorption with a homogeneous distribution of adsorption energies and without mutual interaction between adsorbed molecules. A linearized form of the equation is described, and the linear regression line can be generated from a plot of *Ce*/*Qe* versus *Ce*. The experimental data was statistically analyzed and R^2-value was obtained.

The Freundlich model is an empirical equation, used for physical and chemical adsorption in nonideal adsorption systems. It assumes a heterogeneous distribution among the adsorption sites at different energies. It is a two parameters model widely employed for many different adsorbate/adsorbent systems for liquid and gas phase adsorption [28]. The Langmuir and Freundlich parameters at different temperatures were summarized in Table 4. The correlation coefficients of Langmuir model for both atropin and anisodamine were higher than that of Freundlich model, indicating that Langmuir model was suitable describing the tested adsorption system.

Fig. 5 showed the results of model-fitting to the experimental data for both atropin and anisodamine. As shown in Fig. 3, the adsorption capacity at equilibrium improved with increasing temperatures at the same initial concentration. Therefore, 35 ^{o}C was the optimal temperature and was used in the following tests.

Table 4 Langmuir and Freundlich parameters of atropine and anisodamin on MWCNT-1 at different temperatures

Compound	Temp. (^{o}C)	Langmuir equation	R^2	Freundlich equtaion	R^2	1/n
Atropin	25	Ce/Qe=0.04Ce +0.004	0.994	$Qe=38.168Ce^{0.602}$	0.955	0.602
	30	Ce/Qe=0.045Ce +0.0033	0.996	$Qe=39.500Ce^{0.574}$	0.953	0.574
	35	Ce/Qe=0.043Ce +0.003	0.997	$Qe=42.737Ce^{0.572}$	0.959	0.572
Anisodamine	25	Ce/Qe=0.080Ce +0.006	0.998	$Qe=15.492Ce^{0.444}$	0.980	0.444
	30	Ce/Qe=0.077Ce +0.004	0.998	$Qe=17.555Ce^{0.436}$	0.956	0.436
	35	Ce/Qe=0.060Ce+ 0.003	0.999	$Qe=26.984Ce^{0.500}$	0.956	0.500

3.4. Dynamic adsorption and desorption of MWCNT-1

Separation process was investigated for alkaloids of *Dature metel L.* containing atropin and anisodamine. Under the chromatographic condition given in Section 2.2., chromatographic peak of atropine was observed at a retention time of 6.148 min and peak of anisodamine was observed at the 14.711 min in HPLC (Fig.6 supplementary/supporting information). Most of atropin and anisodamine absorbed by CNTs were eluted between 20% and 50% ethanol–water solution. The highest contents of atropin were eluted in the 20% ethanol–water solution, and the highest contents of anisodamine were eluted in 50% ethanol–water solution. Furthermore, in gradient elution, impurities accounted for 98.6% in water and 92.8% in 10% ethanol phase, and only very small amounts of atropin and anisodamine were detected in the eluent. Considering both purification and recovery yields of the two compounds together, the column was gradient eluted as follows: 4 BV of water and 4 BV of 10% ethanol for removal of impurities，6 BV of 20% ethanol for atropine elution , 4 BV of 30% ethanol and 4 BV of 40% ethanol for the removal of other impurities sequencely, and ending at 6 BV of 50% ethanol for anisodamine elution. The total elution volume was approximately 30 BV (1200 mL), and the flow rate was 10 mL/min. Therefore the separation time was 2 hours. The separation time is no longer than that of standard reversed phase liquid chromatography, such as macroporous resins. Under gradient elution, the purity of atropin and anisodamine reached 83.6% (weight of atropin/weight of dry elutent) and 90.5%, respectively, which were 7.9-folds and 11.2-folds to those in control samples. The recovery yields were 88.2% and 89.2%, respectively. The recovery yields were 90.3% and 91.3%. By comparison, most of the impurity was removed from the sample and the relative peak areas of atropin and anisodamine increased significantly after the separation. The results demonstrated a good selectivity of the adsorption/desorption process for enrichment and separation of atropin and anisodamine from the alkaloids of *Dature metel L.* CNT can be recognized as a graphite plane rolled up into a cylinder with the surface consisting of regular hexagons. Analytes may be drawn onto the nanotube surface or channels between nanotube bundles due to their surface tension and capillary effects

3.5. Dynamic adsorption and desorption of CNT- alginate gel beads

Table 5 Comparison of AB-8 macroporous resins and CNT- alginate gel beads

	CNT- alginate gel beads	AB-8
Cost [RMB yuan] to separate 1 g alkaloids of *Dature metel L.*	15	10
Time [hour]	2	3
Organic solvent (ethanol) used [mL]	200	400
Reusability [times]	3	2
Purity of atropin	83.6%	75.6%
Purity of anisodamine	90.5%	81.2%
Recovery yield of atropin	88.2%	77.8%
Recovery yield of anisodamine	89.2%	73.5%

Separation process of CNT- alginate gel beads for alkaloids was slightly different from MWCNT-1. Most of atropin and anisodamine were eluted within the ranges of concentration of ethanol from 20% to 40%. The highest contents of atropin were in the 20% ethanol–water solution, and the highest contents of anisodamine were in 40% ethanol–water solution. Furthermore, in gradient elution, impurities account for 95.4% in water and 89.2% in 10% of ethanol phase, and less than 5% of atropin and anisodamine were detected in the eluent. Gradient elution was carried out as follows: 5 BV of 10% ethanol for removal of impurities, 5 BV of 20% ethanol for atropine elution, 4 BV of 30% ethanol for the removal of other impurities, and ending at 6 BV of 40% ethanol for anisodamine. Under gradient elution, the purity of atropin and anisodamine reached 83.6% (weight of atropin/weight of dry elutent) and 90.5%, respectively, which were 7.9-folds and 11.2-folds to those in control samples. The recovery yields were 88.2% and 89.2%, respectively. Though the separation capacity was not as good as MWCNT-1, most of the impurity was still removed from the sample and the relative peak areas of atropin and anisodamine increased after the separation. The results still demonstrated a good selectivity of the adsorption/desorption process for enrichment and separation of atropin and anisodamine from the alkaloids of *Dature metel L.* Taking into consideration of the cost, CNT-alginate gel beads were better separation material.

CNT- alginate gel beads contained 9 wt% MWCNT-1. In our experiments, 40.0 g beads (containing 3.6 g MWCNT-1) were used to separate 1.0 g alkaloids. According to section 3.4, the same amount of MWCNT-1 could only separate approximately 0.7 g alkaloids. Alginate gel beads alone were used to separate alkaloids as control. Results showed that gel had no separation effects of alkaloids by itself. The better performance could also be explained by that CNTs combined with alginate support may reduce irreversible adsorption and column pressure. In our study, AB-8 macroporous resins, one of the most commonly used stationary phase for reversed phase liquid chromatography, were used as positive control. Table 5 showed that better separation can be achieved with CNT-alginate gel beads. Although CNT-alginate beads are slightly more expensive, the overall performance is more favorable.

Conclusion

Herein a novel stationary phase was reported. Adsorption and desorption capacities of CNTs towards six typical compounds of natural products were evaluated. MWCNT-1 was more effective for the purification of natural products. It has better adsorption and desorption capacity of alkaloids than AB-8 resin. Langmuir and Freundlich isotherms were used to correlate the solute affinity of CNTs towards alkaloid at different temperatures, and the Langmuir isotherm fit better. Static adsorption and desorption tests indicated that MWCNT-1 was suitable to separate alkaloid of *Dature metel L.*. MWCNT-1 was then bonded to alginate carrier to form gel beads, which further improved the separation. Therefore, alginate-supported CNTs gel beads can serve as excellent liquid chromatographic packing material.

Notes and references

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A new packing material is studied in preparative or semi-preparative liquid chromatography with high separation efficiency and quality.