Talanta 82 (2010) 1439–1447

Contents lists available at ScienceDirect

Talanta

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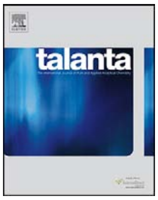

Preparation, characterization, and analytical applications of a novel polymer stationary phase with embedded or grafted carbon fibers

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ARTICLE INFO

Article history:
Received 20 April 2010
Received in revised form 2 July 2010
Accepted 9 July 2010
Available online 17 July 2010

Keywords:
Stationary phase
Multi-walled carbon nanotube
Polystyrene–divinylbenzene–carbon nanotube particle
HPLC

ABSTRACT

A polymer-based chromatographic stationary phase with embedded or grafted multi-walled carbon nanotubes (MWCNTs) has been developed. Three different synthetic methods were utilized to combine the nano-fibers with the substrate of polystyrene–divinylbenzene (PS–DVB). After optimizing the synthetic conditions, this novel polystyrene–divinylbenzene–carbon nanotube (PS–DVB–CNT) stationary phase was characterized by scanning electron microscopy, Raman spectroscopy, thermogravimetric analysis, chemical adsorption and desorption measurement, and mechanical stability test. Compared to PS–DVB particles, PS–DVB–CNT particles have certain improvement in physical and chromatographic performances because the addition of MWCNTs has altered the structures of the particles. The novel stationary phase owns satisfactory resolution, wide pH endurance, and long lifetime, which can be used as an extent to normal HPLC.

1. Introduction

To date, high-performance liquid chromatography (HPLC) has become an indispensable technique for the analysis of samples, the determination of physical constants and the isolation of purified components from complex mixtures in different scientific areas [1–3]. Columns of packed particles are still the most popular devices for liquid chromatographic separations because of their great utility, excellent performance and wide variety. The design of novel stationary phase is a permanent demanding challenge in chromatographic separation science to enable analysis with enhanced selectivity, specificity and speed.

Although new types of stationary phases are continually developed, most columns for reversed phase liquid chromatography (RP-LC) separations are manufactured from silica materials [1–2]. However, silica-based packings are less stable under extreme pH conditions and show some retention abnormalities on old or non-silanol-endcapped reversed phase-columns (RP-column). To overcome these drawbacks, other inorganic materials such as zirconia [4], titania [5] and alumina [6] have been developed as HPLC stationary phase with improved chemical and thermal stabilities. However, they have residual metal hydroxyl and metal-oxo-metal groups and represent amphoteric materials with both anion- and cation-exchange properties. In view of the drawbacks of inorganic RP-columns, effort has been focused on the replacement of inorganic-based RP packings by polymer phases. Organic beaded polymer supports based on polystyrene–divinylbenzene (PS–DVB) [7] and polymethacrylates [8] are mostly investigated and applied polymer phases for RP-HPLC. And yet polymer phases are somewhat less efficient compared to inorganic-based counterparts because of the relatively low rigidity.

In order to overcome the defects of traditional packings, novel composite materials used as chromatographic stationary phases have been developed for years, which maintain the advantages of both organic polymers and inorganic supporters. Coating and coupling techniques are most frequently adopted for the preparation of composite materials, as it is rather difficult to combine macromolecules together. For instance, polymer-coated silica [9] or polymer-grafted silica [10] on pre-formed silica materials and polymer-coated metal oxides [11] are all used as RP-stationary phases. The major advantage of polymer-coated inorganic stationary phases lies in pH-stable layer that may easily be derivatized for various purposes [12]. At the same time, the significant loss of specific surface area that occurs in course of such coating procedures as well as the inhomogeneous distribution of polymer coating represents major drawbacks.

Fortunately, with the development of material science, especially the great advancement of nano-materials, new stationary phases are coming up continually. Carbon-based nano-materials [13–15], especially carbon nanotubes (CNTs) play an important role in analytical chemistry. CNTs can be described as a graphite sheet rolled up into a nanoscale-tube (single-wall carbon nanotubes (SWCNTs)), or with additional graphene tubes around the core of an SWCNT (multi-wall carbon nanotubes (MWCNTs)) [16].

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doi:10.1016/j.talanta.2010.07.019

They have diameters in the range between fractions of nanometers and tens of nanometers, and lengths up to several centimeters with both their ends normally capped by fullerene-like structures. The addition of this nanostructure in traditional materials have impacts on resulting properties, such as tensile strength, modulus, impact resistance, electrical conductivity, thermal stability and mechanical stability [17].

The potential of CNTs has been evaluated in separation techniques with their high thermal and mechanical stability, high surface area available for chemical interactions and the possibility of direct synthesis. The use of CNTs in stationary phases has already been reported as: (i) a novel monolithic stationary phase for μ-HPLC and capillary electrochromatography [18], (ii) a new stationary phase for gas chromatography [19,20], (iii) a solid phase extraction adsorbent for organic [21] and inorganic compounds [22], (iv) a stationary phase in situ grows in microfluidic channels on a microfabricated chip for liquid chromatography [23], gas chromatography [24,25] and electrophoresis [26]. Besides, CNTs are used as the stationary phases of HPLC as well [27–30]. Nevertheless, to the best of our knowledge, all the papers describing the CNT composite stationary phases of HPLC referred to the silica-based stationary phases, regardless of which way was chosen to prepare the Si–CNTs material. Here, we firstly report the preparation of polymer–CNT composite material used as column packings of HPLC and expect to find out some improvements in chromatographic behaviors. Three different synthetic paths have been developed and characterizations were used for comparison with normal polymer packings. Besides, applications of this novel stationary phase are described as well.

2. Experimental

2.1. Reagents

Styrene (ST) (99+%, Lingfeng Chemical Reagent Co. Ltd., Shanghai, China) and divinylbenzene (DVB) (55+%, Zhengguang Chemical Plant, Hangzhou, China) were used after distillation under reduced pressure. MWCNTs (sample purities: 95+%) with the average outer diameter (OD) about 10 nm were supplied by the institute of material physics and microstructure of Zhejiang University, China.

Polyvinylpyrrolidone (PVP, K-30) was purchased from Aldrich (USA). Azobisisobutyronitrile (AIBN) (Shanghai Chemical Reagent Co. Ltd., China) was recrystallized from anhydrous alcohol while benzyl peroxide (BPO) (Lingfeng Chemical Reagent Co. Ltd., Shanghai, China) was recrystallized from chloroform and methanol. Dibutyl phthalate (DBP) was bought from Huipu Chemical Reagent Co. Ltd. (Hangzhou, China). Polyvinyl alcohol (PVA, 1750 ± 50), *n*-butanol, and tetrabutylammonium hydroxide (TBAH) solution (10%, m/m) were all obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Sodium dodecylsulfonate (SDS) was obtained from Beijing Chemical Reagent Co. Ltd. (Beijing, China). Toluene was purchased from Hangzhou Chemical Reagent Co. Ltd. (Hangzhou, China).

All reagents employed for the synthesis were of analytical reagent grade and were purified by normal methods to GB standard of China. HPLC-grade solvents for chromatographic separation were obtained from Tedia Company (Tedia, USA). And the test sample components were all of analytical reagent grade. Model compounds were dissolved in methanol and water. Chromatographic eluents were degassed by ultrasonication for 30 min and filtered through 0.22 μm filtering film before use. Deionized water used was purified by Millipore Simplicity (Millipore, France) to 18.2 MΩ.

2.2. Apparatus and instrumentations

All chromatographic tests were carried out by using a Ultimate 3000 HPLC system (Dionex, USA) including a pump (LPG-3400A), an autosampler (WPS-3000TSL), a thermostat column compartment (TCC-3000) and a variable-wavelength UV detector (VWD-3400). The eluent flow rate was generally 1.0 mL/min and the system temperature of all chromatographic tests was set at 30 °C. Data were collected with Chromeleon 6.80 chromatogram workstation (Dionex, USA). Each injection was repeated at least twice.

The scanning electron microscopy (SEM) images were obtained using a HITACHI S-4700 field emission scanning electron microscope (Hitachi, Japan). The thermogravimetric analysis (TGA) was carried out on DSCQ1000 differential scanning calorimetry (TA, USA). The Raman spectra were taken on Labor Raman HR-800 Raman Spectrometer (Jobin Yvon, France).

2.3. Preparation of MWCNT–COOHs

MWCNT–COOHs were prepared as follows [30–32]: 50 mg MWCNTs were suspended in 200 mL mixture of concentrated H_2SO_4/HNO_3 (3:1, v/v) in a 500 mL flask and sonicated in a water bath for 24 h at 35 °C. The resulting suspension was then diluted with water and washed until the pH value reaches 7. After filtration, the resulting MWCNT–COOHs were dried in vacuum.

2.4. Dispersion polymerization

Monodisperse polystyrene (PS) seed particles were prepared by dispersion polymerization of ST in a mixture of ethanol and water under nitrogen atmosphere using AIBN as an initiator and PVP as stabilizers according to the method reported by Paine et al. [33]. A typical procedure was as follows: 3 g PVP (k-30) and 100 mL mixture of ethanol and water (95:5, v/v) were added to a 250 mL flask, and then a mixture of 0.7 g AIBN and 17.75 g ST was dropped into the flask. The mixture was stirred overnight with a mechanical stirrer at 70 °C under dry nitrogen. After filtration and washing, the seed particles were dispersed in an aqueous solution containing 1% SDS (m/v). The average particle size was about 1.8 μm measured by SEM.

2.5. Synthesis of basic substrate particles

In a 500 mL three neck flask fitted with a mechanical stirrer was placed 2 g seeds prepared above. Then, emulsified solution containing 2 g DBP, 30 mL SDS aqueous solution (0.2%, w/v) was dropped into the flask at 30 °C and stirred overnight. Afterwards, another uniformly mixed solution containing 30 g organic compounds and 250 mL PVA aqueous solution (1%, w/v) was prepared for swelling. The organic compounds were comprised of ST, DVB, toluene (as much as total monomers), initiator BPO (2% of the total monomer, w/w), and surfactant SDS (0.25% of the aqueous solution, w/v). The organic phase was emulsified in the PVA aqueous solution with an ultrasonic disrupter (JY92-II, Scientz Biotechnology Co. Ltd., Ningbo, China) until the particle sizes of the oil drops became at most 0.5 μm. The mixture was poured into the flask for swelling at room temperature for 24 h so that the emulsified organic phase would be absorbed by the seed particles completely. The temperature was then increased to 70 °C under nitrogen atmosphere for another 24 h. Subsequently, the resulting beads were washed successively with hot water and alcohol, and then dried in vacuum. The resulting particle size of PS–DVB was about 5 μm. If the swelling ratio of the beads changed, the final particle size of the polymer would change.

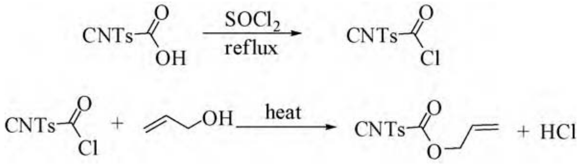

Fig. 1. Esterification of the MWCNTs with allyl alcohol.

2.6. Synthesis of the PS–DVB–CNT stationary phase

There are three methods to synthesize this novel composite material. The first method (method I) was to incorporate MWCNT–COOHs into the emulsified mixture that had been used to synthesize the basic substrate particles directly and poured into the flask for swelling at room temperature. After polymerization, the MWCNTs would be intermingled in the PS–DVB particles to form a kind of PS–DVB–CNT stationary phase.

The second method (method II) for the preparation was named co-polymerization (Fig. 1). The prepared MWCNT–COOHs were refluxed in dichloro sulfoxide at 80 °C for 2–3 h under dry nitrogen to synthesize MWCNT–COCls. Subsequently, the solvent was distilled in about 100 °C oil bath under normal pressure in dry nitrogen to remove the unreacted dichloro sulfoxide. After the flask was cooled to room temperature, allyl alcohol was dropped into the flask slowly and stirred with a magnetic stirrer for several hours at about 50 °C. Then, the product was washed to neutral pH before being dried in vacuum. After the whole process, the MWCNTs had double bonds, which could co-polymerize with ST and DVB. The following steps were the same as method I in which the pretreated MWCNTs were put into the emulsified mixture.

The third method (method III) was named surface graft, which just modified the surface of PS–DVB with MWCNTs (Fig. 2). PS–DVB particles were firstly dispersed in a solvent with a magnetic stirrer at a low stirring speed under dry nitrogen. Then, aluminum chloride was added into the mixture before the reaction temperature changed to the demand. Subsequently, MWCNT–COCls previously dispersed in the same solvent were dropped into the flask and the whole mixture was stirred overnight. When the reaction completed, the product was poured into water and washed to neutral state before dried in vacuum [34–37].

2.7. Column packing procedure

The analytical column (stainless-steel, 150 mm × 4.6 mm I.D.) was slurry packed with the PS–DVB–CNT particles obtained above, by pressing with ethanol as packing solvent under a working pressure of 40 MPa. The volume of solvent passing through the column should be at least 300 mL. The guard column (stainless-steel, 50 mm × 4.6 mm I.D.) and short analytical column (stainless-steel, 100 mm × 4.6 mm I.D.) were slurry packed in the same way.

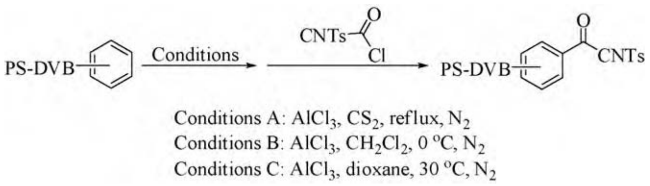

Fig. 2. Surface modification of PS–DVB particles.

3. Results and discussion

3.1. Surface characterization of the composite particles

PS–DVB–CNT particles were taken into consideration because they are suitable for LC, with great stability against many oxidizing reagents; high rigidity and wide tolerance of pH values. Particle size and morphology were determined by SEM, which showed different synthetic methods resulted in different morphologies. The sample was coated with a thin conducting layer of gold by sputter coating before sampling. From the SEM photography of PS–DVB (Fig. 3(a)), it can be seen that the particles are monodisperse, uniform and spherical. The average diameter was calculated with the following equation:

$$\overline{d} = \sum_{i=1}^{n} \frac{d_i}{n} \tag{1}$$

The average diameter $d = 5.0\ \mu\text{m}$ (obtained from measurements for three times and 30 particles were taken as samples each time). From other SEM images with high magnification factor, the particles of pure PS–DVB looks smoother than PS–DVB–CNT particles for there are no MWCNTs added. The addition of MWCNTs changed the macroscopic color of the stationary phase from white to grey. The more MWCNTs were added, the darker the color would be. However, the basic morphology, size and uniformity of the stationary phase were not changed (Fig. 3).

If PS–DVB–CNT particles were prepared following method I, the added MWCNT–COOHs could be easily seen on the surface of the composite particles (Fig. 3(c)). The particles looks uniform but rougher compared to PS–DVB particles. If the amount of MWCNT–COOHs exceeds the monomers' by 2%, conglomeration of MWCNT–COOHs happens (Fig. 3(d)), leading to the heterogeneity of the stationary phase. It will take a lot of time to remove the extra MWCNT–COOHs from the stationary phase by waterpower sedimentation before slurry packing. Anyway, the recommended amount of MWCNT–COOHs by method I is below 1% of total monomers (w/w), because only a short time is needed to disperse these MWCNT–COOHs uniformly.

In method II, although the content is the same, there are less MWCNTs on the surface of the stationary phase (Fig. 3(e)). That is to say, most MWCNTs are packed inside the particles after co-polymerization. And after oxidation and esterification, the MWCNTs are difficult to be adsorbed on the surface of the stationary phase, but they become easier to be dispersed in the emulsified mixture by an ultrasonic disrupter. The recommended amount of MWCNTs is also below 1% to prevent conglomeration.

The method III is much more difficult compared to the other two methods because the surface graft between two macromolecules is not easy to realize. Different reaction conditions had been attempted (Fig. 2), among which conditions B were adopted, as the PS–DVB particles were slightly compromised in CS_2 and severe conglomerations of MWCNTs occurred in dioxane. The amount of MWCNTs affects the syntheses as well, because the redundant MWCNTs will conglomerate in bundles on the surface of the particles. After comparisons of 1, 3 and 5% MWCNTs in the monomers (w/w) (Fig. 3(f–h)), it is found that the amount should be controlled under 1%, in which MWCNTs can disperse uniformly in the mixture. When the amount increased to 5%, MWCNTs all conglomerate on the surface (Fig. 3(h)).

3.2. Mechanical stability evaluations

The main disadvantage of polymer-based stationary phase is the weaker mechanical stability compared to silica-based packings. Mechanical instability of HPLC packing materials at elevated pres-

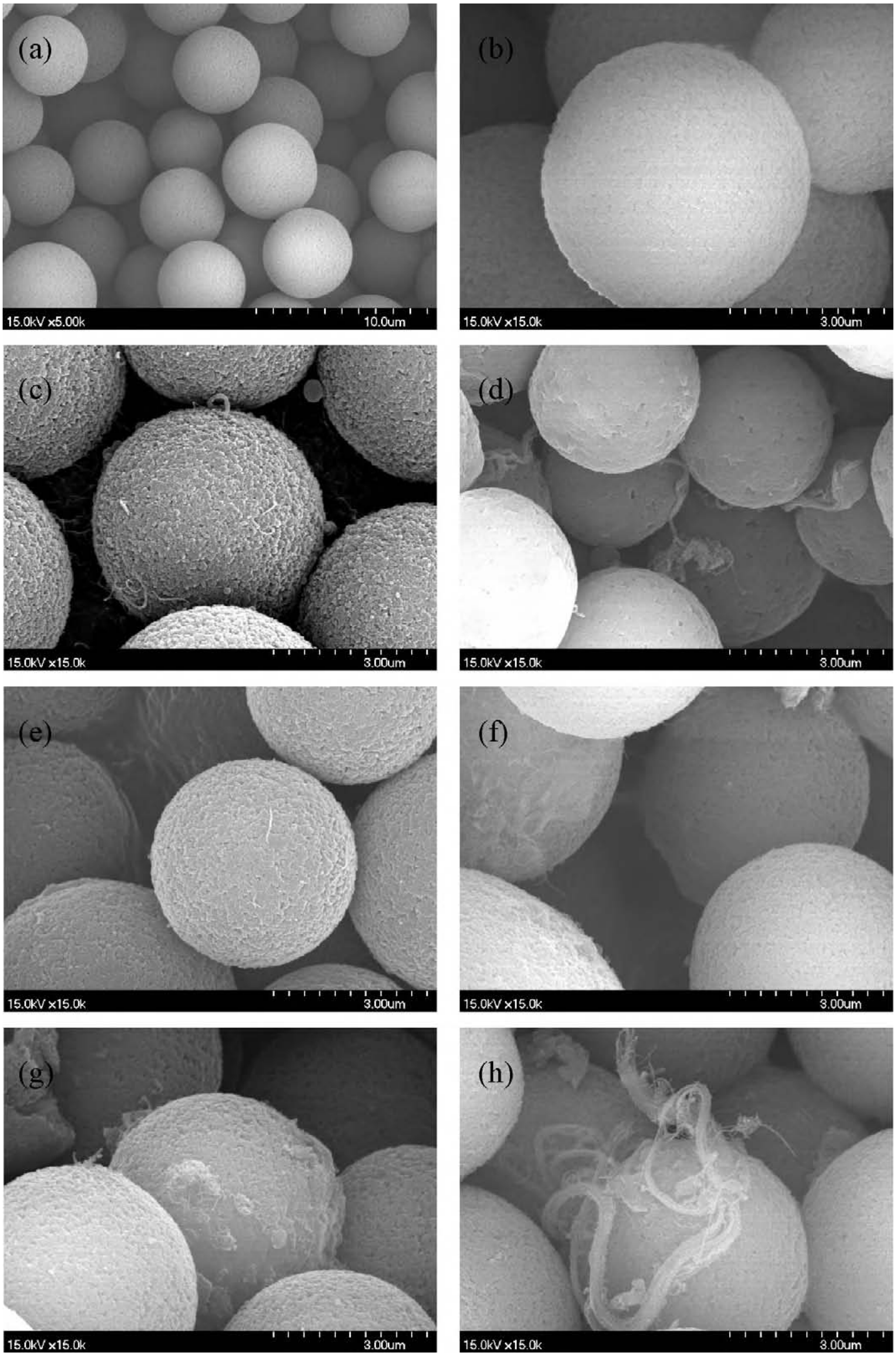

Fig. 3. SEM images of (a) PS–DVB, (b) PS–DVB, (c) PS–DVB–CNT (0.5% MWCNTs, method I), (d) PS–DVB–CNT (5% MWCNTs, method I), (e) PS–DVB–CNT (1% MWCNTs, method II), (f) PS–DVB–CNT (1% MWCNTs, method III), (g) PS–DVB–CNT (3% MWCNTs, method III), (h) PS–DVB–CNT (5% MWCNTs, method III).

sures can lead to particle fracturing or compression deformation, which in turn results in higher column back pressures, column clogging, particle percolation or ununiformity of the stationary phase in a packed column. According to the Van Deemter equation, the ununiformity of the packing material in a chromatographic column will cause multipath effect and longitudinal diffusion during separation, resulting in the reduced theoretical plate number and poor column efficiency. Thus, one of the main purposes to add MWCNTs into the PS–DVB column is to increase the rigidity of the polymer-based packing.

To assess the mechanical stability of PS–DVB and PS–DVB–CNT particles, a series of experiments were performed in

Table 1
Porosity characteristics of PS–DVB–CNT particles.

Contents of MWCNTs	Surface areas (m^2/g)[a]	Pore volumes (cm^3/g)[b]	Pore sizes (Å)[c]	Pore size distribution
0% MWCNTs (method I)	212.5787	0.6789	39.8620	Micropores, mesopores
0.5% MWCNT–COOHs (method I)	102.2572	0.1881	76.6220	Mesopores, macropores
5% MWCNT–COOHs (method I)	13.2217	0.02908	150.7240	Macropores

[a] Calculated by Brunauer–Emmett–Teller (BET) method.
[b] Referred to Barret–Joyner–Halendar (BJH) desorption cumulative volume of pores.
[c] Referred to BJH desorption average pore diameter.

4.6 mm × 50 mm steel columns, which were initially slurry packed with ethanol. These columns were then subjected to increasing pressures with a constant flow rate pump with methanol and water as the eluent. At elevated pressures, columns containing beds of weak materials compact or crush resulting in a restriction of the flow. Pressure was manually recorded at each flow rate.

As illustrated in Fig. 4, PS–DVB–CNT columns incorporated with different contents of MWCNTs were compared to PS–DVB stationary phase under increasing column pressures up to 40 MPa—the upper limit of conventional HPLC. Six packing materials were tested among which five columns exhibited similar behaviors while the last one displayed quite differently. The PS–DVB–CNT columns with 1% MWCNTs (method II and method III) were almost overlapped with the PS–DVB packed column, indicating that the co-polymerized and surface grafted MWCNTs slightly changed the structure and porosity of the packing material and these two methods had not much effect in increasing the mechanical stability of this material. However, the column containing 0.5% MWCNT–COOHs (method I) remained linear below 2.3 mL/min while the column containing 1% MWCNT–COOHs (method I) were below 2.7 mL/min, indicating that the addition of MWCNT–COOHs can increase the mechanical stability of the particles. In addition, the last curve that represents the column containing 5% MWCNT–COOHs (method I) was quite linear till the flow velocity exceeds 6.0 mL/min, which meant this packing material had excellent mechanical stability under elevated pressures.

In order to get more information, the porous properties of three representative materials were characterized with chemical adsorption and desorption method and calculated by Brunauer–Emmett–Teller (BET) method (Table 1). When the proportion of MWCNTs increases, the surface areas and pore volumes will decrease, which shows striking contrasts to the pore sizes. This may be due to the addition of MWCNTs that fill in the pores of the packing materials and lead to reduced surface areas and increased average pore sizes. Most micropores and mesopores were filled with MWCNTs and as a result the mechanical stability was greatly improved (Fig. 4). More MWCNTs were added, smaller pore volumes would be, such as the packing material with 5% MWCNT–COOHs (method I). This material was quite rigid under elevated pressure because it was almost non-porous. However, the separation on this material was not so satisfactory and the resolution and recovery were all severely reduced.

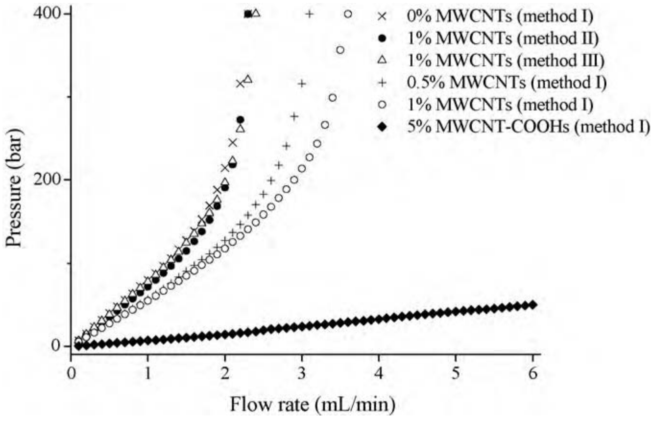

Fig. 4. Pressure *versus* flow velocity for columns containing PS–DVB and PS–DVB–CNT packings with methanol–water (70:30, v/v) as eluent at ambient temperature.

Table 2
Elemental analysis of PS–DVB–CNT particles obtained from different synthetic methods.

MWCNTs added	Proportion of carbon (%)	Proportion of hydrogen (%)
0% MWCNTs (method I)	90.3556	8.4492
1% MWCNTs (method I)	90.4125	8.5340
5% MWCNTs (method I)	90.7197	8.2978

3.3. Chemical composition of the stationary phase

Information on the composition of the stationary phase was gained by combining the results from Raman spectra, elemental analysis, and TGA. The Raman spectra was carried out to characterize the chemical composition before and after the incorporation of MWCNTs. Fig. 5 shows the Raman spectra of PS–DVB particles, PS–DVB–CNT particles and MWCNTs in the spectral ranges from 800 to 1800 cm^{-1}. A broad band at 1500–1600 cm^{-1} known as "G" band, associated with the tangential vibration mode (TM) and another one called "D" band at 1300–1400 cm^{-1} assigned to disorder state in graphite compounds and/or defects in nanotubes are located in the spectra of MWCNTs. The Raman intensity rises at the wavenumber of 1580 and 1350 cm^{-1} in PS–DVB–CNT particles rather than the PS–DVB particles, indicating that the incorporation of MWCNTs caused the spectral changes though the signals were not as strong as MWCNTs itself.

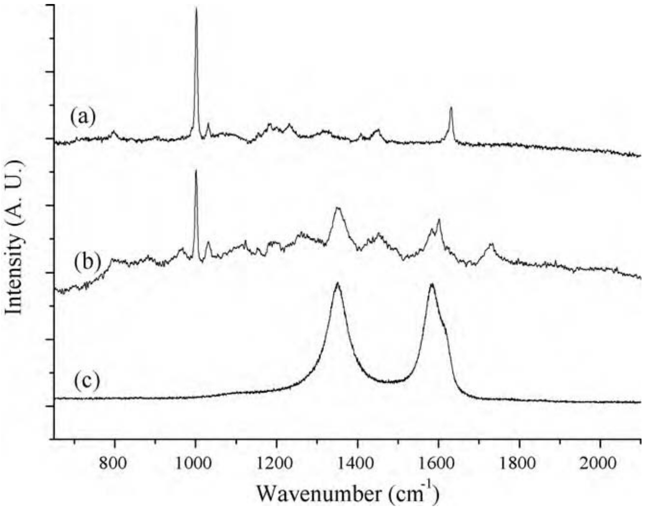

Fig. 5. Raman spectra ($\lambda_{exc.} = 514$ nm) of (a) PS–DVB particles, (b) PS–DVB–CNT particles (5% MWCNT–COOHs, method I) and (c) MWCNT–COOHs.

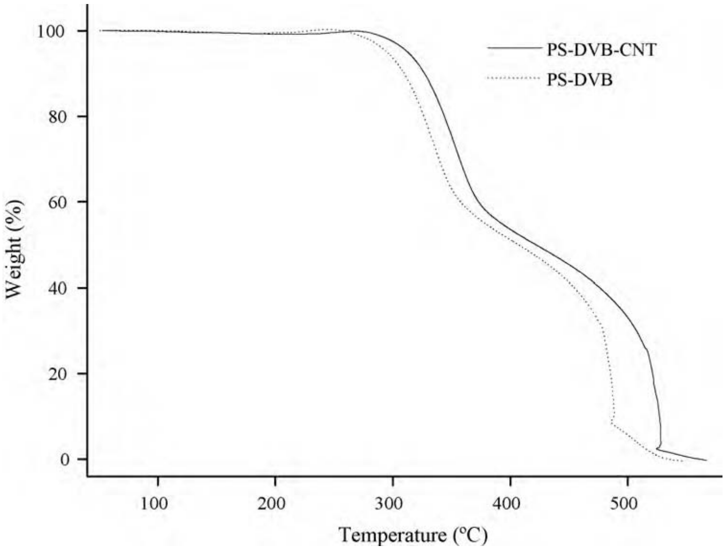

Fig. 6. Thermogravimetric analysis of PS–DVB–CNT (5% MWCNTs, method I) and PS–DVB composite particles. Conducted in nitrogen atmosphere: heating rate: 10 °C min^{-1}.

The elemental analysis (Table 2) shows the contents of MWCNTs in the stationary phase following method I. The proportion of carbon in the composite particles increases with the addition of MWCNTs. Because of the losses of MWCNTs in the synthetic procedures and the small amounts of MWCNTs used, the increment is so unconspicuous. The more MWCNTs are utilized, the greater the loss percentages would be, indicating that superabundant MWCNTs would lead to conglomerations during the polymerization procedures. Anyway, a better method should be established to embed more MWCNTs in the composite material.

In addition, the thermal decomposition behaviors of the novel PS–DVB–CNT particles have slightly changed with the addition of MWCNTs as studied by TGA (Fig. 6). The dotted line represents the data of PS–DVB particles and the solid line exhibits the PS–DVB–CNT particles with 5% MWCNTs embedded following method I. From the plots, it can be seen that the total weight of this two particles both stay the same under 200 °C. Then, different decomposition behaviors represent different thermal stability, indicating that the addition of MWCNTs slightly improves the thermal endurance. However, the improvement is so inconspicuous resulting from the small amount of MWCNTs added. If the content of MWCNTs could be increased, the thermal stability would improve.

3.4. Retention behaviors of organic compounds on different columns packed with PS–DVB–CNT and PS–DVB particles

In order to test the chromatographic behaviors of synthesized PS–DVB–CNT and PS–DVB columns, experiments were performed for the data of retention times, including neutral, acidic and alkaline compounds. Three columns (containing 0, 0.5, and 1% MWCNTs (method I)) have been tested in our work under two types of mobile phases (90% methanol and 75% methanol in water (v/v)), respectively. Similar trends of retention times on columns containing different amount of MWCNTs displayed under these two mobile phases. As Table 3 shows, the retention times of most

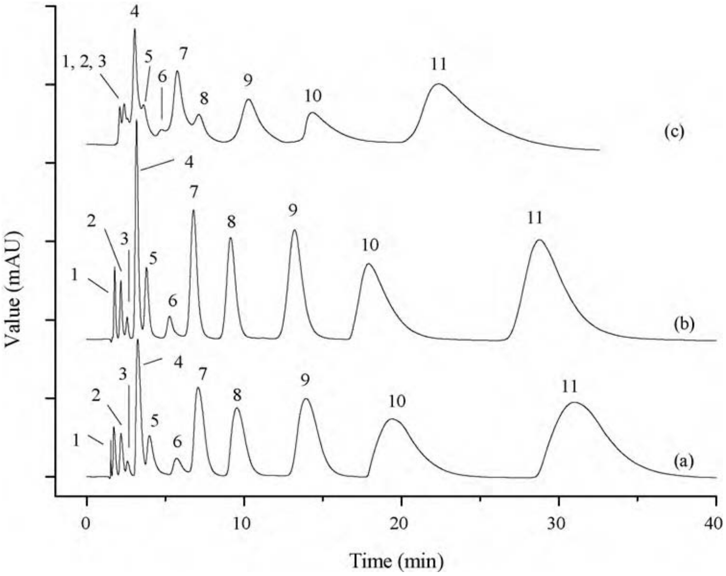

Fig. 7. Separation of eleven organic compounds on (a) PS–DVB column, (b) PS–DVB–CNT columns (1% MWCNTs, method I) and (c) PS–DVB–CNT column (5% MWCNTs, method I). Column: PS–DVB and PS–DVB–CNT particles packed in 4.6 mm × 150 mm stainless-steel column. Mobile phase: methanol:H_2O = 90:10 (v/v). Detection: UV detector with 254 nm. Flow rate: 1.0 mL/min. Peak identification: (1) resorcinol; (2) sulfadimidine; (3) benzylalcohol; (4) aniline; (5) *p*-toluidine; (6) 2-naphthol; (7) *p*-methoxybenzaldehyde; (8) anisole; (9) *N*,*N*-dimethylaniline; (10) 1,3,5-trimethylbenzene; (11) 2-methoxynaphthalene.

Table 3

Retention times of different organic compounds on three polymer columns containing 0, 0.5 and 1% MWCNTs (method I), which is packed in 4.6 mm × 150 mm stainless-steel columns with a flow rate of 1.0 ml/min and a UV detector of 254 nm.

Samples	90% methanol[a]						75% methanol[b]					
	0% MWCNT		0.5% MWCNT		1.0% MWCNT		0% MWCNT		0.5% MWCNT		1% MWCNT	
	Time (min)	k'[c]	Time (min)	k'	Time (min)	k'	Time (min)	k'	Time (min)	k'	Time (min)	k'
m-Phenylene diamine	2.06	0.33	2.04	0.32	2.01	0.31	2.38	0.54	2.38	0.55	2.30	0.50
p-Toluene sulfonamide	2.07	0.34	2.04	0.32	1.98	0.29	2.91	0.88	2.80	0.82	2.76	0.80
o-Phenylendiamine	2.41	0.55	2.38	0.55	2.33	0.52	3.11	1.01	3.05	0.98	2.94	0.92
Acetanilide	2.60	0.68	2.53	0.64	2.45	0.60	4.06	1.62	3.90	1.53	3.77	1.46
o-Toluidine	4.23	1.73	4.09	1.66	3.99	1.61	8.78	4.66	8.63	4.60	8.05	4.26
m-Chloroaniline	4.49	1.90	4.40	1.86	4.35	1.84	11.91	6.68	11.84	6.69	10.35	5.76
N-Methylaniline	6.25	3.03	6.02	2.91	5.83	2.81	16.23	9.47	15.68	9.18	14.35	8.38
Sulfadimidine	2.23	0.44	2.21	0.44	2.14	0.40	3.33	1.15	3.31	1.15	3.11	1.03
Pyridine	2.81	0.81	2.79	0.81	2.77	0.81	3.83	1.47	3.57	1.32	3.55	1.32
Diphenylamino urea	2.93	0.89	2.91	0.89	2.83	0.85	7.42	3.79	7.35	3.77	6.60	3.31
Quinoline	6.14	2.96	5.82	2.78	5.77	2.77	13.91	7.97	13.13	7.53	12.23	6.99
Indole	5.79	2.74	5.35	2.47	5.29	2.46	16.73	9.79	16.18	9.51	14.15	8.25
p-Hydroxybenzaldehyde	2.66	0.72	2.58	0.68	2.51	0.64	4.05	1.61	3.97	1.58	3.63	1.37
p-Methoxybenzaldehyde	7.09	3.57	6.97	3.53	6.49	3.24	17.38	10.21	16.44	9.68	15.31	9.01
Nitrobenzene	7.70	3.97	7.07	3.59	6.83	3.46	21.83	13.08	19.85	11.89	19.57	11.79
4-Chloro-nitrobenzene	12.98	7.37	12.39	7.05	12.20	6.97	50.77	31.75	46.05	28.90	43.88	27.68
Benzene	7.38	3.76	6.91	3.49	6.84	3.47	22.15	13.29	20.49	12.31	19.55	11.78
Toluene	11.42	6.37	10.73	5.97	9.02	4.90	43.20	26.87	36.75	22.86	34.48	21.54
1,4-Dimethoxybenzene	12.23	6.89	11.89	6.72	11.10	6.25	38.66	23.94	37.17	23.14	32.01	19.92
1,3-Dimethoxybenzene	12.42	7.01	11.72	6.61	11.63	6.60	45.07	28.08	42.41	26.54	36.67	22.97
Resorcinol	1.79	0.15	1.76	0.14	1.78	0.16	2.09	0.35	1.95	0.27	2.03	0.33
3,4-Xylenol	3.77	1.43	3.65	1.37	3.69	1.41	9.27	4.98	8.34	4.42	8.39	4.48
2,4-Xylenol	3.94	1.54	3.77	1.45	3.79	1.48	10.91	6.04	9.26	5.01	9.91	5.48
2-Naphthol	5.57	2.59	5.11	2.32	5.17	3.38	17.97	10.59	14.47	8.40	15.45	9.10
1-Naphthol	6.05	2.90	5.78	2.75	5.93	2.88	21.45	12.84	17.39	10.29	18.39	11.02
Benzylalcohol	2.64	0.70	2.50	0.62	2.56	0.67	4.31	1.78	3.88	1.52	4.05	1.65
Ethylene glycol phenyl ether	3.08	0.99	2.96	0.92	3.03	0.98	5.75	2.71	5.20	2.38	5.35	2.50
p-Nitrobenzaldehyde	3.54	1.28	3.31	1.15	3.45	1.25	7.67	3.95	6.65	3.32	6.91	3.52
Benzaldehyde	6.17	2.98	5.73	2.72	5.99	2.92	13.91	7.97	11.59	6.53	11.88	6.76
Acetophenone	5.77	2.72	5.51	2.58	5.57	2.64	14.42	8.30	11.91	6.73	12.92	7.44

[a] Mobile phase composition is MeOH:H_2O = 90:10 (v/v).

[b] Mobile phase composition is MeOH:H_2O = 75:25 (v/v).

[c] k' refers to the capacity ratio.

organic compounds, especially alkaline and neutral compounds reduce as well as the capacity ratios (k') when the content of MWCNTs increase, which may be caused by the structural changes of PS–DVB–CNT particles. With the addition of MWCNTs, the micropores and mesopores of PS–DVB–CNT particles are filled with carbon fibers, resulting in decreased surface areas and pore volumes (Table 1). Thus, the volume of the mobile phase reduce but the volume of the stationary phase is on the contrary, leading to a smaller phase ratio (φ). Moreover, the smaller surface areas lead to decreasing partition coefficients (K) between stationary phase and mobile phase. From the data of capacity ratios (k'), it can be inferred that the decrements of partition coefficients (K) are greater than the phase ratio (φ), so the retention times decrease.

However, most acidic compounds and some neutral compounds have an inflexion in the retention times, which decrease immediately with the addition of MWCNTs and increase when the contents of MWCNTs rise. This may be attributed to the oxygen atoms on carboxyl groups of MWCNTs after pretreatment, which have strong binding forces with other oxygen atoms in the compounds. With higher contents of MWCNTs and carboxyl groups, the combination force strengthens till it exceeds the impact of partition coefficient (K). Thus, there is a flexion on the retention time. Of course, there are also linkages between carboxyl groups and other compounds like amines, which are weaker than the changes of partition coefficients (K), so the retention times have degressive trends in the mass.

Particularly, benzaldehyde derivatives have different variations due to the functional groups. The carboxyl group of benzaldehyde itself will form conjugation with benzene ring, which donates negative charge to oxygen atom resulting in similar properties of acidic

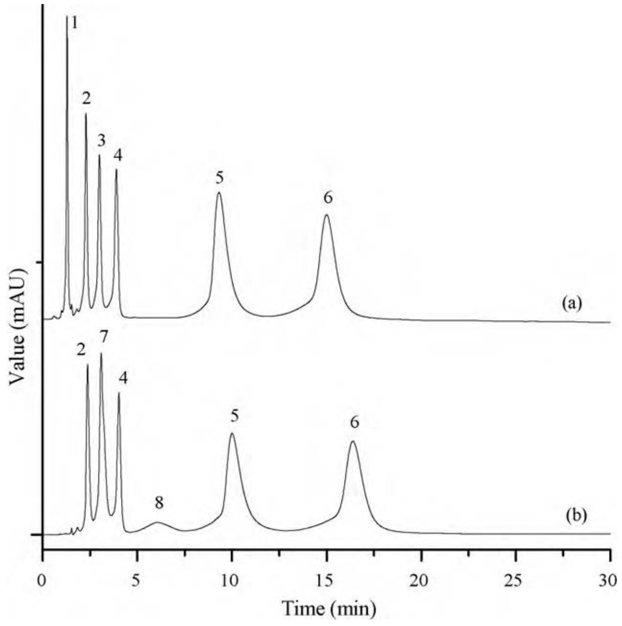

Fig. 8. Chromatogram of six amines under different pH values. Stationary phase: PS–DVB–CNT containing 0.5% MWCNTs (method I). Column: PS–DVB–CNT particles packed in 4.6 mm × 150 mm stainless-steel column. Mobile phase (a) methanol:ammonia solution = 75:25 (v/v) (pH ≈ 9.0); (b) methanol:H_2O = 75:25 (v/v) (pH ≈ 7.0). Detection: UV detector with 254 nm. Flow rate: 1.0 mL/min. Peak identification: (1) sulfadimidine; (2) *m*-phenylene diamine; (3) *o*-phenylendiamine; (4) acetanilide; (5) benzidine; (6) indole; (7) mixture of sulfadimidine and *o*-phenylendiamine; (8) impurity.

compounds. When benzaldehyde is substituted, the phenomenon depends on the substituted groups. When it is substituted with electron-withdrawing groups like nitro groups, the charge density of benzene ring drops off, which lead to similar result as acidic compounds, while the situation is just opposite to the electron-donating groups substituted benzaldehydes (hydroxyl or alkoxyl groups), which have similar phenomenon as neutral compounds.

3.5. HPLC applications of PS–DVB–CNT stationary phase

In order to evaluate the resolution and column efficiency of PS–DVB–CNT stationary phase, acidic, neutral and alkaline compounds were all used as model compounds on three representative columns with different contents of MWCNTs. Fig. 7 shows the chromatograms of eleven organic compounds on PS–DVB–CNT columns containing 0, 1 and 5% MWCNTs (method I), respectively. Due to the structural changes of the stationary phase after the incorporation of MWCNTs, the retention times of 11 organic compounds change on different columns, which accordance with the results of Section 3.4. When 1% MWCNTs has been embedded in the stationary phase, the resolutions, peak shapes and column efficiency are all improved compared to PS–DVB column. However, when the content of MWCNTs is increased to 5%, the resolutions and plate numbers both reduce, indicating a worse column efficiency, which is contrary to expectation. That is to say, superabundant MWCNTs will lead to rather small surface areas as well as the column capacity, though the rigidity is improved. There is a balance between the surface area and the rigidity of the particles. In our research, 1% MWCNTs (method I) is suitable for chromatographic separation.

Usually, high concentration of water will be used to refresh the column when acid solution, alkali solution or salt solution has been utilized as eluent. One of the merits of this novel stationary phase is that it is compatible with different mobile phases, from 100% common organic solvents to 100% water. Besides, mobile phases with different pH values range from 1 to 14 can be used as well. After having been eluted by NaOH solution (pH 14) at a flow rate of 1 mL/min for 8 h, this column still has excellent efficiency and can be used to analyze six amides under alkali mobile phase (Fig. 8). As sulfadimidine and *o*-phenylendiamine cannot be separated under neutral eluent, it is simple and convenient to adjust the pH value of the mobile phase to realize the separation.

As we know, normal HPLC is not able to separate all kinds of organic compounds, such as polar organic compounds that are quite important in medicine and pharmacology. However, the addition of ion-pair reagent makes it possible to analyze polar organic molecules and inorganic ions by liquid chromatography. The counter ions used as ion-pair reagents are usually strong hydrophilic, such as alkyl ammoniums and alkyl sulfonic acids, owning strong binding forces with the reverse stationary phase. Once the ion-pair reagents are combined with the stationary phase, it is rather difficult to wash them down. The columns get compromised quickly, especially for silica-based stationary phases. To our

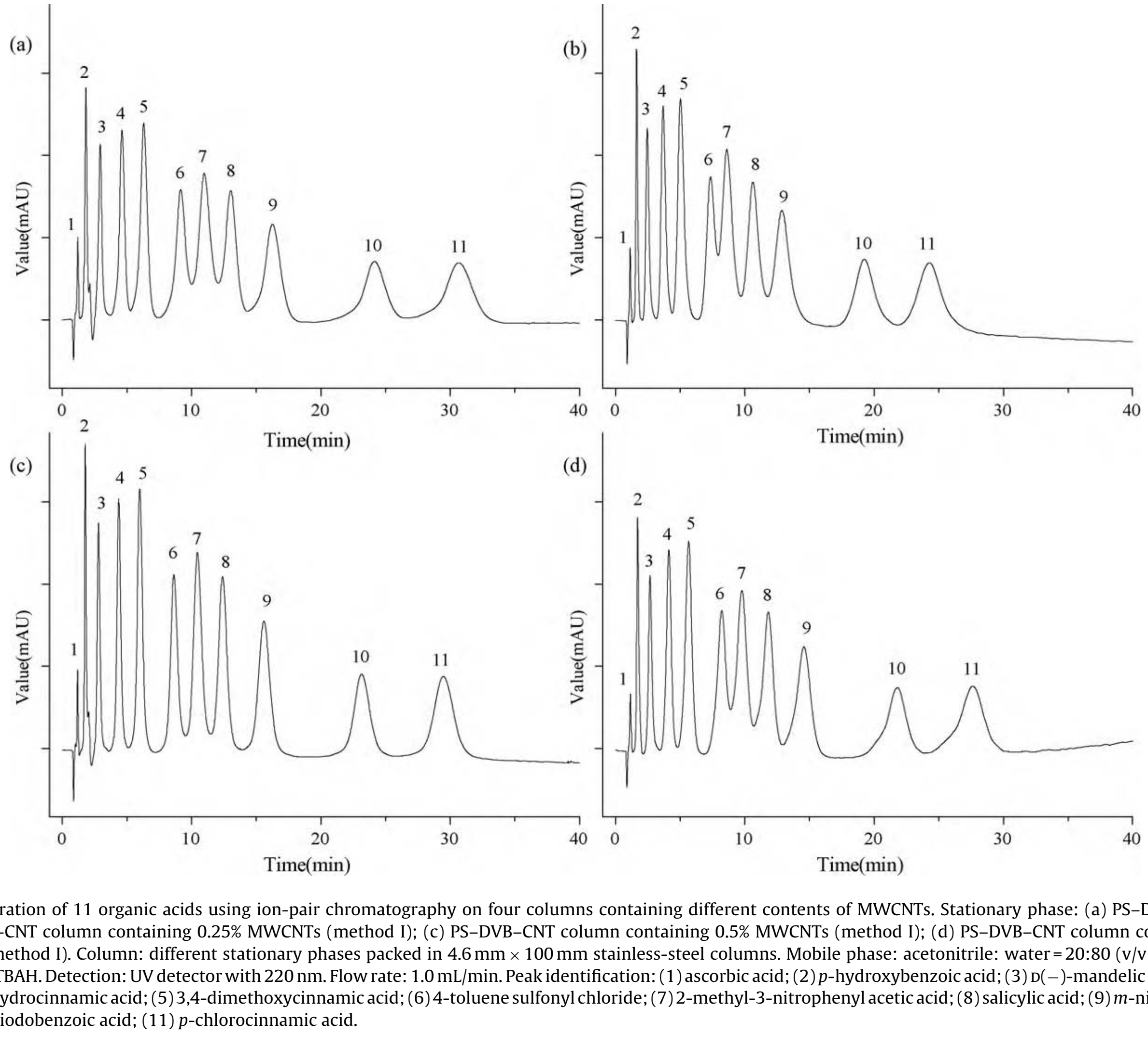

Fig. 9. Separation of 11 organic acids using ion-pair chromatography on four columns containing different contents of MWCNTs. Stationary phase: (a) PS–DVB column; (b) PS–DVB–CNT column containing 0.25% MWCNTs (method I); (c) PS–DVB–CNT column containing 0.5% MWCNTs (method I); (d) PS–DVB–CNT column containing 1% MWCNTs (method I). Column: different stationary phases packed in 4.6 mm × 100 mm stainless-steel columns. Mobile phase: acetonitrile: water = 20:80 (v/v), containing 5.0 mmol/L TBAH. Detection: UV detector with 220 nm. Flow rate: 1.0 mL/min. Peak identification: (1) ascorbic acid; (2) *p*-hydroxybenzoic acid; (3) D(−)-mandelic acid; (4) 3,4-dimethoxyhydrocinnamic acid; (5) 3,4-dimethoxycinnamic acid; (6) 4-toluene sulfonyl chloride; (7) 2-methyl-3-nitrophenyl acetic acid; (8) salicylic acid; (9) *m*-nitrocinnamic acid; (10) *p*-iodobenzoic acid; (11) *p*-chlorocinnamic acid.

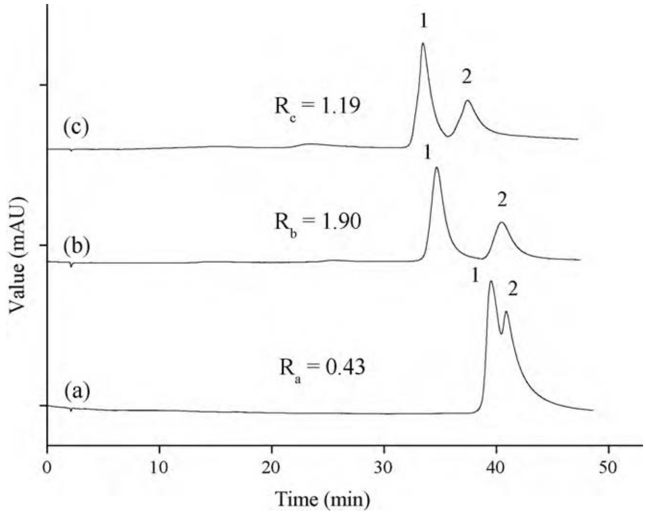

Fig. 10. Separation of two organic compounds on (a) PS–DVB column, (b) PS–DVB–CNT columns (0.5% MWCNTs, method I) and (c) PS–DVB–CNT column (1% MWCNTs, method I). Column: PS–DVB and PS–DVB–CNT particles packed in 4.6 mm × 150 mm stainless-steel column. Mobile phase: methanol: H_2O = 90:10 (v/v). Detection: UV detector with 254 nm. Flow rate: 1.0 mL/min. Peak identification: (1) 2-methoxy naphthalene; (2) *N*-(*p*-toluenesulfonyl) indole.

column, different kinds of eluent can be adopted to regenerate the column and remove the counter ions. After the treatment, the column has quite a long lifetime and marvelous reproducibility, the column efficiency remains the same after more than 800 injections. PS–DVB–CNT columns with different content of MWCNTs were used to separate 11 organic acids with TBAH as the counter ion (Fig. 9). From curves (a) to (d), it can be found that (c) displays the best base line, resolution, peak shapes and plate numbers. The PS–DVB–CNT particles with 0.5% MWCNTs is suitable for ion-pair chromatography as an extent of HPLC.

Besides, as the PS–DVB–CNT column has different retention behavior from the traditional PS–DVB column, it can be used to separate different compounds that are difficult to separate on PS–DVB columns. Fig. 10 shows the separation of 2-methoxy naphthalene and *N*-(*p*-toluenesulfonyl) indole on three columns containing different amount of MWCNTs. These two compounds have quite strong combination with the RP-stationary phases. Although 90% of methanol in water (v/v) has been tried as the eluent, the retention times are still longer than 30 min. On PS–DVB column (Fig. 10a), the two compounds cannot be separated. When PS–DVB–CNT column (0.5% MWCNTs) is used, the retention time of 2-methoxy naphthalene changes greatly and the resolution is higher than 1.50 (Fig. 10b), meaning that these two compounds have been separated. If the content of MWCNTs increases, the resolution declines again (Fig. 10c). Thus, the PS–DVB–CNT (0.5% MWCNTs) column can be used to analyze these two compounds. Furthermore, all the PS–DVB–CNT columns can be used as a supplement of traditional PS–DVB columns.

4. Conclusion

The synthesis of this novel stationary phase was based on classical swelling and polymerization method. As both MWCNTs and PS–DVB are macromolecules, the combination was not so easy to realize. We developed three different synthetic methods to combine MWCNTs with PS–DVB particles. After characterization and comparison, the first method was considered to be the best choice for the synthesis of PS–DVB–CNT particles. The results show that the beads have the uniformity in particle size and monodispersion in particle conglomeration. On the other hand, the amount of MWCNTs added plays an important role in the chromatographic performance of the stationary phase. 0.5–1% MWCNTs can offer the best results for chromatographic separations. Although higher content of MWCNTs improves the mechanical stability, it will cause conglomeration during preparation and the column efficiency is affected as well. This stationary phase has a wide pH tolerance range and a long lifetime in mobile phase suppression chromatography and ion-pair chromatography.

Acknowledgements

This research was financially supported by National Natural Science Foundation of China (Nos. 20775070, J0830413), Zhejiang Provincial Natural Science Foundation of China (Nos. R4080124, Y4090104), and Zhejiang Qianjiang Project of Science and Technology for Competent People (No. 2008R10028).

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