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PAPER

Effect of multi-walled carbon nanotubes incorporation into benzyl methacrylate monolithic columns in capillary liquid chromatography

Ahmad Aqel,*[a] Kareem Yusuf,[b] Zeid A. Al-Othman,[ab] A. Yacine Badjah-Hadj-Ahmed[b] and Abdulrahman A. Alwarthan[ab]

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This work describes the preparation of polymer based monolithic materials and their use as stationary phases in capillary liquid chromatography. Multi-walled carbon nanotubes (MWCNT) were incorporated into a mixture containing benzyl methacrylate (BMA) and ethylene dimethacrylate (EDMA) as co-monomers. The optimized porogenic mixture was a ternary solution composed of cyclohexanol, 1,4-butandiol and butanol which resulted in a stable and homogeneous suspension. Six capillary columns with increasing amounts of MWCNT, from 0 to 0.4 mg mL^{-1}, were prepared by thermal polymerization in 0.32 mm (i.d.) and 150 mm length fused silica tubing. The chromatographic evaluation showed that the synthesized monolithic beds were mechanically stable while their porosity and permeability increased with the MWCNT content. The prepared capillary columns were tested for the separation of mixtures of ketones and phenols at an optimum flow rate of 2 μL min^{-1}. The results showed that incorporation of MWCNT slightly affected the retention while it enhanced the column efficiency by increasing the column efficiency by a factor of up to 9. This effect corresponded also to an improved resolution and full separation of the solutes.

1. Introduction

During the last few years, great effort has been made to improve, modify and enhance the separation efficiency of stationary phases used in separation methods and especially in chromatographic techniques.[1–4] Current improvements in chromatography technology mainly focus on the development of new stationary phases.

Due to their novel chemical, physical and electrical properties, nanomaterials with at least one dimension of about 1 to 100 nm have received much attention. A series of nanoparticles including silica,[5–8] gold,[8–11] titanium dioxide,[12] zirconia[13] and fullerenes,[14,15] have been used as stationary phases that achieved higher selectivity and separation efficiency either in gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) or capillary electrochromatography (CEC). Nanomaterials are versatile and can play various roles in separation techniques; they can be used as modifiers, stabilizers or stationary phases. Using nanoparticles for chromatography has proved to be advantageous in terms of chemical stability, selectivity and separation efficiency.[6,10,16,17]

Carbon nanotubes (CNT) have been of great interest to researchers since their discovery because of their unique properties such as high surface area and excellent stability.[18–22] CNT is one form of carbon, with nanometer-sized diameter and micrometer-sized length (where the length to diameter ratio generally exceeds 1000).[23] The atoms are arranged in hexagons, the same arrangement as in graphite. The structure of CNT consists of an enrolled cylindrical graphitic sheet (called graphene) rolled up into a seamless cylinder with a diameter of the order of one nanometer. CNT is considered to be a material lying in between fullerenes and graphite as a quite new member of carbon allotropes.[24] The two main types of CNT are the single-walled (SWCNT) and multi-walled (MWCNT) carbon nanotubes. SWCNT can be considered to be formed by the rolling of a single layer of graphite into a seamless cylinder.[25–27] MWCNT was the first CNT type discovered by Iijima in 1991.[28] It can be considered to be a collection of concentric SWCNT with different diameters, consisting of multiple layers of graphite rolled in on themselves to form a tube shape. The length and diameter of these structures differ a lot from those of SWCNT and, of course, their properties are also very different.[29]

MWCNT were used in a GC packed column and their ability to separate aromatic hydrocarbons, alkanes, halogenated hydrocarbons, alcohols, ketones, esters, and ethers was investigated.[30] Open tubular GC columns were easily fabricated by self-assembly of a CNT film directly bonded onto the capillary surface to obtain a stable stationary phase; MWCNT exhibited

[a]*King Abdullah Institute for Nanotechnology, College of Science, King Saud University, P.O. Box 2454, Riyadh 11451, Saudi Arabia, Kingdom of Saudi Arabia. E-mail: ahmad3qel@yahoo.com; Fax: +966 14675992; Tel: +966 14674198*

[b]*Chemistry Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Kingdom of Saudi Arabia*

greater selectivity and higher separation efficiency than SWCNT toward alkanes.[31,32] An application of SWCNT as stationary phase in a microfabricated GC capillary column was also reported.[33] In another interesting research, SWCNT were linked end to end to form a network structure on the inner surface of the capillary tubing; the results reported that SWCNT assist ionic liquids by enhancing the separation of alkanes, alcohols, aromatic compounds, ketones and racemates.[34,35] Separation of alkanes and aromatic hydrocarbons was achieved using derivatized MWCNT synthesized into home-made packed glass columns.[36]

Li *et al.*[16] incorporated SWCNT into an organic polymer used as a monolithic stationary phase for micro-HPLC. Pretreated SWCNT was dispersed in 2-propanol and used as a porogen in the preparation of the monolithic column by employing vinylbenzyl chloride (VBC) as a monomer and ethylene glycol dimethacrylate (EDMA) as a cross-linker. A control monolithic column without incorporated SWCNT was prepared by an identical procedure. The performance of the monolithic column indicated that SWCNTs could enhance the chromatographic retention of small neutral molecules due to the hydrophobic interactions between SWCNT and analytes. A packed column with MWCNT was used in reversed-phase liquid chromatography (LC), and the interaction properties of MWCNT as a stationary phase in HPLC were investigated.[37] As a chiral stationary phase, mixing of SWCNT with cellulose trisphenylcarbamate could increase the enantioselectivity of this polysaccharide chiral selector.[38] Stronger interactions with the aromatic groups have been shown when SWCNT were bonded to 3-aminopropyl silica gel and used as a stationary phase in HPLC for the separation of polycyclic aromatic hydrocarbons (PAHs).[39] On the other hand, functionalized SWCNT linked to an aminopropyl silica surface were used to separate PCB isomers and terpenic compounds in HPLC.[40]

In addition, CNT have also been used in CE and CEC. It has been shown that functionalized CNT added to the background electrolyte could adsorb and/or interact with analytes. This effect improved the peak shape of homologues and structural isomers of caffeine and theobromine, increased the electrophoretic separation of purine and pyrimidine bases but altered the resolution of DNA fragments.[41] CE was also used for the chiral separation of clenbuterol and ephedrines after modification of CNT by a chiral selector.[42] Non-covalent solubilization of CNT can be accomplished using surfactant molecules. The separation of flavonoids and phenolic acids has been achieved[43] and the resolution of aromatic compounds has been improved using surfactant-coated SWCNT.[44]

Acid treated SWCNT and MWCNT were coated into fused-silica capillaries and used for CEC applications; these capillaries were applied for baseline separation of a mixture of seven nitrogen-containing aromatic compounds[45] and for the electrophoretic determination of different pharmaceutical compounds.[46] The columns were very stable and reproducible, and the results gave good CEC resolution, capillary efficiency and retention factors. Excellent performance of CEC was reported for mixing of SWCNT with an organic monolithic polymer as stationary phase.[47] Chiral stationary phases based on bovine serum albumin conjugated CNT and vertically aligned CNT nanostructured pillars were also synthesized and evaluated.[48,49]

As mentioned in all these works, the presence of CNT in the separation media used for chromatographic analytical methods could provide several advantages: higher resolution, improved separation efficiency and selectivity, enhanced chromatographic retention, reduced analysis time, better repeatability and chemical stability. However, CNT are insoluble in most common solvents, this poor solubility is the main obstacle of their using. As mentioned above, many efforts have been made to overcome this barrier, and various strategies including surface modifications and functionalization approaches have been reported in the literature.

The aim of this study was to investigate the influence of MWCNT on chromatographic separation properties of a monolithic polymeric stationary phase. This work presents a simple way to prepare and maintain a uniform matrix in the capillary column. For this purpose, a stable suspension was prepared by mixing MWCNT in a suitable solvent and using the suspension as a porogen for the preparation of monolithic polymeric material. The fabricated columns were characterized and their performance evaluated for the separation of different model compounds.

2. Experimental

2.1. Chemicals and columns

All chemicals used such as formic acid, acetone, acetophenone, butyrophenone, aminophenol, nitrophenol, chlorophenol were of analytical grade and purchased from BDH (Lutterworth, UK). Multi-walled carbon nanotubes were provided from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China) with the following characteristics: 10–50 μm length, less than 8 nm outer diameter, 2–5 nm inner diameter and 95% purity.

HPLC grade acetonitrile and methanol were purchased from BDH. Purified water used throughout all experiments was prepared on a Milli-Q system (Advantage with Elix, Millipore S.A.S. 67120 Molsheim, France), then filtered with 0.2 μm nylon membrane filter Whatman (Maidstone, UK). Always before use, the mixed mobile phases were filtered using a vacuum glass filtration system through the same nylon membrane filters and degassed ultrasonically for 30 min.

Fused silica tubing (0.32 mm i.d.) was purchased from Restek (Bellefonte, USA). The chemicals used for monolithic materials preparation in this work were purchased from Aldrich (Steinheim, Germany) as follows: 3-(trimethoxysilyl)propyl methacrylate (TMSM) 98%, ethylene dimethacrylate (EDMA) 98% used as crosslinker, benzyl methacrylate (BMA) 98% as monomer and 2,2′-azobisisobutyronitrile (AIBN) as thermal initiator. Toluene, hydrochloric acid, sodium hydroxide, cychlohexanol, butanol and 1,4-butandiol were acquired from BDH. All chemicals were used without further purification.

2.2. Preparation of capillary monolithic columns

Prior to the polymerization, the fused-silica capillaries (150 × 0.32 mm i.d.) were first rinsed with 1.0 M NaOH solution for 30 min and left in the same solution for 2 h, then rinsed with water and dried with air for 10 min two times for each. The columns were then flushed with 1.0 M HCl for 30 min and dried with air for 5 min; after that the capillaries were rinsed with toluene for

10 min then flushed with a 20% 3-(trimethoxysilyl)propyl methacrylate solution in toluene for 10 min and left in the same solution for 4 h, then rinsed with toluene for 10 min and dried with air for 5 min.

The monomer mixture was prepared as follows (*v*%): 15% benzyl methacrylate, 15% ethylene dimethacrylate and 1% AIBN initiator. The porogenic mixture was 69% of the total solution and was prepared as follows (*v*%): 20% cyclohexanol, 40% butanol and 40% 1,4-butandiol. More than 20 solvents were tested and optimized in order to allow a stable suspension of the MWCNT in the monomer mixture. The best result was obtained with a mixture of cyclohexanol, butanol and 1,4-butandiol. Six columns were prepared with different MWCNT contents as described in Table 1; the monolithic column without incorporated MWCNT was prepared using the same procedure.

The monomer mixture and the porogenic solvents were mixed into an homogenous solution then sonicated and purged with nitrogen gas for 5 min. While maintaining a uniform reaction mixture, the capillary columns were then immediately filled with the reactant solution and both ends were plugged with a piece of rubber. The polymerization was performed at 70 °C for 20 h. After the polymerization, the seals were removed; the resulting columns were connected to an HPLC pump and thoroughly washed with acetonitrile to remove the unreacted materials and porogenic solvents.

2.3. Porosity and bed permeability

The total column porosity (ε_T) is considered to be an important parameter for column evaluation. The flow method was used to evaluate ε_T in this study; it is based on the retention volume determination of an unretained marker (uracil was used in this work) and the geometrical volume of the empty column (since it can be considered to be a long cylindrical tube), after correction for extra-column volume contributions, depending on the tubes used for connection.[50]

Another factor which should be studied is the permeability of the column. The permeability (K^0) of a porous medium is a measure of its capacity to transmit a fluid driven by an imposed pressure drop across the column. Darcy's law links the solvent viscosity, pressure drop and ε_T to K^0.[51] Also, the Hagen–Poiseuille equation[52] gives the pressure drop in a fluid flowing through a long cylindrical pipe; this physical law has been used to calculate the average diameter of monolith channels (macropores). The average velocity of mobile phase over the channel section (v) is obtained from integration of the momentum transport equation that is derived on the basis of the Navier–Stokes equation.[52]

Table 1 Multi walled carbon nanotubes content of the prepared capillary columns. Porosities ε_T, permeabilities K^0 and average diameter of the monolith channels R using acetonitrile as mobile phase

Column	CNT/mg mL^{-1}	CNT (%)	ε_T	K^0/m^2	R/μm
C_1	0.0	0	0.79	2.43×10^{-14}	0.44
C_2	0.5	0.05	0.71	1.05×10^{-14}	0.29
C_3	1.0	0.1	0.66	6.45×10^{-15}	0.23
C_4	2.0	0.2	0.63	4.51×10^{-15}	0.19
C_5	3.0	0.3	0.61	3.36×10^{-15}	0.16
C_6	4.0	0.4	0.60	2.63×10^{-15}	0.15

2.4. Characterization of the monolithic columns

After chromatographic experiments were performed, the monolith rods in the tubes were washed and cut into small pieces then dried. The dried columns and monolith materials were subjected to optical microscopy, scanning and transmission electron microscope (SEM and TEM), Fourier transform infrared (FT-IR) spectroscopy, specific surface area and thermogravimetric analysis (TGA) characterization.

The optical microscope images were obtained using a Micromaster Fisher Scientific optical microscope (G2009-A 702-042, China) with typically 100-fold magnification. The pore properties and microscopic morphology of the polymers were characterized by a Jeol (JSM-6380LA) analytical scanning electron microscope (Japan) at 5 kV after the monolith samples were sputtered with platinum. TEM images of the prepared materials were obtained using a Jeol JEM-2100F 200 kV transmission electron microscope. 0.01 g of the incorporated solid sample mixture was dispersed in pure ethanol by sonication, and 0.1 mL of this suspension was placed on a copper grid. The ethanol was then evaporated prior to the TEM analysis.

The FT-IR spectra were recorded on a Thermo Nicolet 6700 FT-IR spectrophotometer (USA). The synthesized porous monolith was crushed; the powder was then immersed in 1 mL of acetonitrile/water (50 : 50, *v*/*v*) and shaken for 10 min to remove any soluble compounds, the latter step was repeated twice. After vacuum drying, the monolith powder was thoroughly mixed with KBr in an approximate ratio of 1 : 20 and pressed into a pellet. FT-IR spectra were then recorded at a resolution of 4 cm^{-1} over the full mid-IR range (400–4000 cm^{-1}). TGA was conducted for both pure and incorporated monolithic materials prepared in the same way with a Mettler Toledo TGA/DSC 1 Star System (Switzerland). Each sample was heated from 25 to 400 °C with a heating rate of 10 °C min^{-1}.

Adsorption–desorption isotherms of liquid nitrogen were measured to obtain information about surface area of the polymer monolithic materials using a Nova surface area analyzer (Quantachrome, Boynton Beach, FL, USA) at −196 °C; the monolithic samples were ground and degassed at 300 °C prior to the measurements.

2.5. HPLC modification and conditions

All chromatographic analyses were performed on a Shimadzu HPLC system (Kyoto, Japan) including a pump (LC-6A), a Rheodyne 7125 manual injector, a UV detector (SPD-6A) and a C-R6A integrator. The detector was set at different wavelengths according to the type of analyzed compounds. Acetonitrile/water solutions with or without acid additives at different ratios were used as the mobile phase. All solutions were filtered through 0.2 μm nylon membrane filter (Whatman, England) prior to use. All experiments were carried out at room temperature.

The HPLC system was successfully modified for using microcolumns. The detector was equipped with a homemade UV cell corresponding to a 2 cm path length with 320 μm i.d. and 1.6 μL volume. A simple system was constructed for splitting both mobile phase flow and sample injected volume; it was controlled by a custom-built adjustable flow splitter based upon a T-derivation piece connected between the injector and the column. In

this configuration, both column and split flow rates could be adjusted by changing the inner diameter or length of the restrictor made of PEEK tubing (Varian, Palo Alto, USA). In the present work, the selected splitting ratio was 1 : 200 and the actual injection volume was fixed at 5 nL.

3. Results and discussion

3.1. Preparation and optimization of the monolithic columns

Six monolithic columns have been prepared by incorporation of increasing MWCNT amounts as summarized in Table 1. The composition percentages of the polymerization mixture were fixed for the six columns as mentioned in Section 2.2. The polymerization time was set at 15 h; it corresponded to the maximum conversion of monomeric precursors.

In order to select the most suitable polymerization medium, a series of more than 20 solvents was tested. The best liquid mixture should allow a good compromise between carbon nanotubes suspension (to maintain a uniform polymer matrix) and a good solubility of both monomer and crosslinker. The optimum porogenic mixture was a ternary solution composed of cyclohexanol, 1,4-butandiol and butanol (20/40/40%, *v*/*v*/*v*). By using this ternary mixture, the MWCNT were well dispersed and the suspension was homogenous and stable and no precipitation was observed for about 2 h after mixing, as can be seen in Fig. 1.

The composition range of MWCNT content was set based on preliminary experiments that have been carried out in order to evaluate the upper limit of the composition. These experiments showed that with MWCNT contents above 4.0 mg mL^{-1} (or 0.4%, wt/wt), the corresponding capillary columns operated at a backpressure exceeding 5000 psi. According to these preliminary studies, MWCNT content was set in the range between 0.5 and 4.0 mg mL^{-1}. C_1 has been prepared without incorporated nanotubes as control column.

3.2. Characterization of the monolithic columns

3.2.1. Hydrodynamic properties and porosity. In order to evaluate the mechanical stability and permeability of the monolithic materials, the prepared monolithic columns were tested. The influence of the mobile phase flow rate on the column backpressure was investigated using acetonitrile at different flow rates. Fig. 2 shows this effect through all the prepared columns. An excellent linear dependence of inlet pressure *versus* the flow rate for all columns was indicated by a regression factor *R* better than 0.997 for all measured curves.

All prepared columns exhibit backpressure less than 1600 psi (11 MPa) at 1 μL min^{-1} up to less than 4500 psi (31 MPa) at 10 μL min^{-1} acetonitrile flow rate. In comparison with a recently published work, Chambers *et al.*[17] prepared glycidyl polymethacrylate monolithic capillary columns with 0.1 mm i.d. which showed backpressures in the range of 8–17 MPa. Their results showed that adding 0.25% of CNT to the monolith induced a slight increase of columns backpressure.

On the other hand, the relationship between MWCNT contents and column backpressure remains linear over all the studied flow rate range, with a correlation coefficient higher than 0.983. This effect means that the monolith channels radius decreased due to a layer of MWCNT, even in a column with only 0.05% of MWCNT in the porogenic mixture.

Numerical values for the permeability of the prepared monolithic capillary columns were determined using acetonitrile as mobile phase at a volumetric flow rate of 5 μL min^{-1} at room temperature. The pressure drops were measured and the permeabilities were calculated and are summarized in Table 1 for the six columns. The Hagen–Poiseuille's law was also used to estimate the average diameter of the monolith channels (macropores) that ranged from 0.15 to 0.44 μm for all the prepared columns. The determined values at a flow rate of 5 μL min^{-1} for the six columns are presented in Table 1 which shows that higher permeabilities correspond to larger average pore diameters. As shown in Fig. 2, the good linear response between backpressure and flow rate, observed for all columns, clearly demonstrates that the prepared monoliths were mechanically stable. This linear response is in accordance with both Darcy's and Hagen–Poiseuille's laws.[52,53]

For determination of the total porosity of all prepared monolithic columns, uracil was injected as non-retained marker with acetonitrile/water (50/50, *v*/*v*) as mobile phase, the calculated values being reported in Table 1. It was found that the total porosity of the prepared columns ranged from 60% for C_6 column corresponding to the highest MWCNT content to

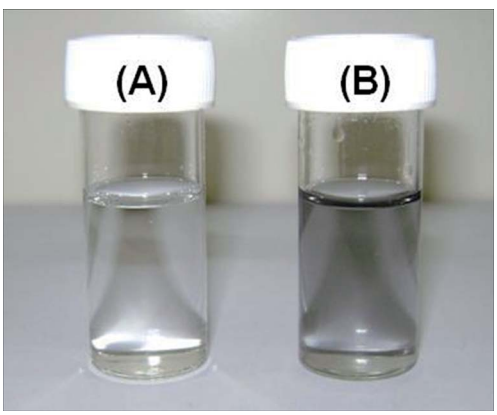

Fig. 1 Porogenic mixture (composed of: cyclohexanol, 1,4-butandiol and butanol, 20/40/40% *v*/*v*/*v*), (A) without MWCNT, (B): with incorporated 1.0 mg mL^{-1} MWCNT.

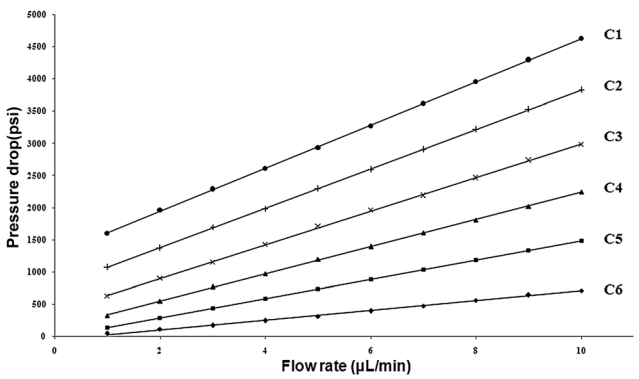

Fig. 2 Graph illustrating plots of pressure drop *versus* flow velocity for each column using acetonitrile as mobile phase, temperature: 24 °C.

79% for C_1 column that had the lowest content. This trend confirmed the high influence of the carbon nanotubes on the column porosity, in spite of its relatively low content, as it was previously reported.[17]

3.2.2. Monoliths morphology. Morphology of the monolithic bed is considered to be one of the key factors affecting the separation capability of the capillary column. To obtain high efficiency and stability, homogeneity and rigidity of the polymer bed are needed.[54] Therefore, it is important to investigate and control the column monolith morphology governing these parameters. The morphology and surface property of the monoliths were evaluated in order to characterize the stationary phases. Several experiments including optical microscopy, SEM, TEM, FT-IR spectroscopy, specific surface area measurement and TGA were carried out.

Representative optical micrographs, SEM and TEM images of these monolithic columns are shown in Fig. 3. SEM pictures demonstrate that the procedure for synthesis renders permeable monoliths with a uniform structure and porosity, completely bonded to the inner capillary walls. As shown in the figure pictures of the C_1, C_2 and C_3 columns, the formed monoliths were well attached to the surface of the capillaries. Cross section of intact and homogeneous column beds can also be seen in Fig. 3 (a)–(c). The microglobules which appear in the figures have an approximate diameter in the range 1–2 μm. On the other hand, the values of the macropore diameters estimated from SEM images are in good agreement with the calculated average values given in Table 1. Optical microscopy examinations (at magnification of 100 times) confirmed that the continuous beds were homogeneous and also closely bonded to the capillary walls, indicating that the synthesis was made properly.

TEM was used to characterize the MWCNT structure; the multiwalled carbon nanotubes bundles can be clearly observed in Fig. 3 (k) and (l). These micrographs confirmed that the MWCNT structure was not damaged after polymerization. It can be clearly seen in this figure that the average external diameter of the MWCNT is less than 8 nm, as stated by the manufacturer. As was established by Chambers *et al.*, the carbon nanotubes clearly cross through the pores of the monolithic polymeric bed.[17]

For further characterization, the monoliths were examined by FT-IR spectroscopy to identify the organic functional groups of the polymeric phase. The monolith FT-IR bands show the presence of the main groups corresponding to the ester functional group of the methacrylate (1730 and 1147 cm^{-1}), as well as aromatic C=C at 1457 cm^{-1} and C–H bending and stretching frequencies. On the other hand, the absence of bands at 1650 and 3090 cm^{-1}, corresponding to stretching of C=C and =C–H bonds, confirms completion of the polymerization reaction. The observed results demonstrated that the proposed polymerization conditions were suitable to produce a homogenous and continuous monolith resulting from the reaction of EDMA and BMA co-monomers.

The specific surface area of the polymeric material corresponding to columns C_1 and C_3 was measured using liquid nitrogen physisorption according to the BET method; the obtained values were 2.897 and 4.185 $m^2\ g^{-1}$, respectively. As expected, this result confirmed that incorporation of MWCNT to the polymer induced a increase of its specific surface area.

Typical TGA data shown in Fig. 4 indicated that the porous poly(benzylmethacrylate) monolith of the C_1 column did not undergo any significant thermal degradation below 230 °C while the degradation occurred above 270 °C for the C_3 column monolith. Both monoliths showed a relatively high degree of thermal stability. This excellent thermal behavior enables these monolithic columns to operate routinely at temperatures up to 200 °C, without observing any deterioration of their properties. When the temperature was raised, the two curves rapidly dropped and stabilized over 380 °C, indicating a complete degradation of the monoliths.

It should be noted that the thermal stability of the porous monolith is an important characteristic when used either in CEC or GC, because of the high voltages and temperatures involved in these techniques.

3.3. Separation and efficiency of monolithic columns

Several parameters such as morphology of the monolithic bed, its specific surface area, accessibility of the surface and chemical structure may affect the column efficiency which is considered to be a key factor for column evaluation. The efficiencies of the columns fabricated in this study were calculated at room temperature and their dependence on the mobile phase flow rate was investigated. Ketonic and phenolic compounds including acetone, acetophenone, butyrophenone, aminophenol, nitrophenol and chlorophenol were tested as model solutes to check and evaluate the efficiency of the prepared columns. The effect of flow rate and composition of the mobile phase on the retention time of each analyte (t_R, min), its width at half peak ($w_{0.5}$, min), the number of theoretical plates (N) and the resolution between two neighboring peaks (R_s) were studied in all cases.[55] The sample injection volume used was 5 nL. The obtained results expressed in terms of retention time (t_R), plate height (H) and resolution (R_s) are presented in Table 2.

3.3.1. Separation of ketonic compounds. The prepared capillary columns were tested for the separation of a mixture of ketones (acetone, acetophenone and butyrophenone) using different experimental conditions. As an example, Fig. 5(A) shows the separation of the three components on C_1 and C_3 columns at a flow rate of 1 μL min^{-1} and a detection wavelength of 254 nm using a binary acetonitrile/water (50 : 50, *v*/*v*) mixture with 1% formic acid mobile phase. While incomplete separation was obtained on column C_1, the C_3 column allowed a full separation of the three solutes in approximately the same run time. This result shows that incorporation of MWCNT to the stationary phase does not greatly affect the retention time of the ketonic compounds, because of the hydrophobic character of the MWCNT while the model solutes are quite polar.

On the other hand, incorporating MWCNT into the monolithic material obviously showed a marked enhancement of the column efficiency by increasing the plate number by a factor between 5 and 9. This fact was observed by measuring the number of theoretical plates at different flow rates, as can be seen in Table 2.

The best enhancement was obtained for acetophenone injected on C_1 column at 1 μL min^{-1} which corresponded to a column efficiency of 1244 plates m^{-1}, compared with 10 839 plates m^{-1} for the C_3 column using the same chromatographic conditions.

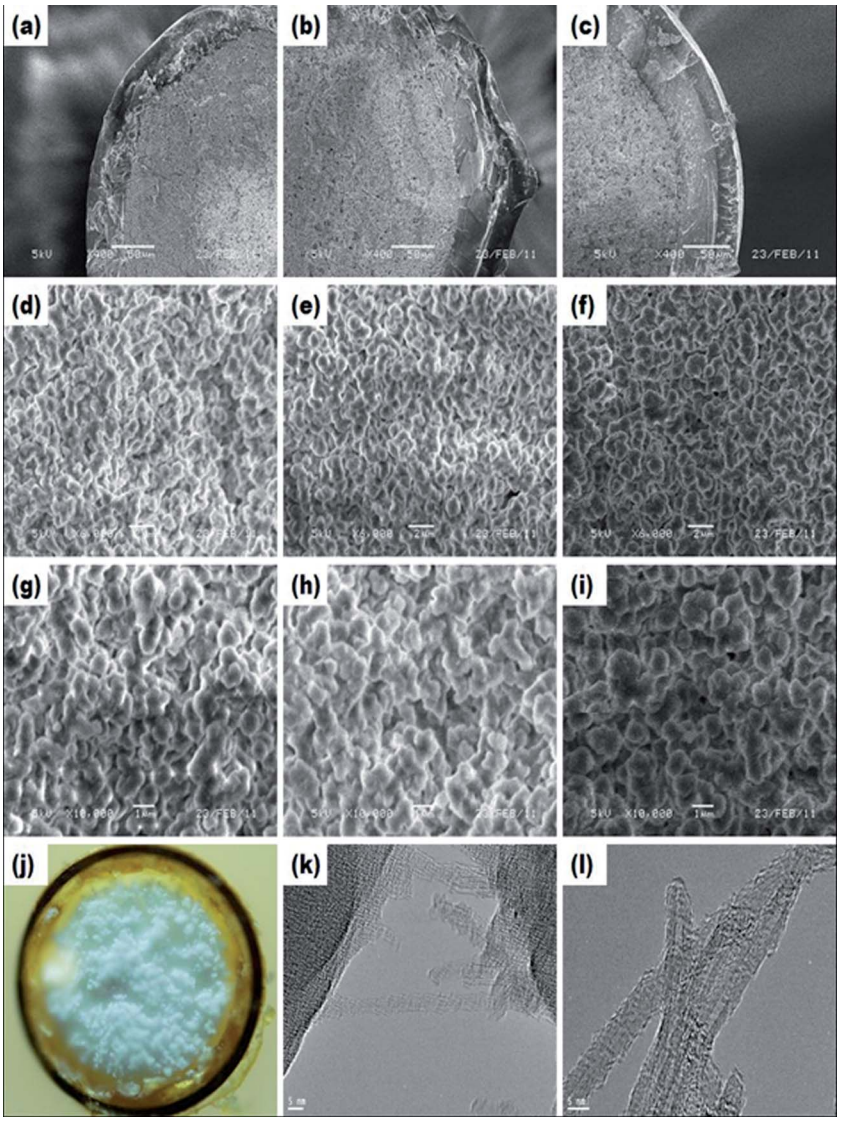

Fig. 3 (a–c) Cross-section SEM images of C_1, C_2 and C_3 columns respectively. (d–i) Bulk region SEM images of C_1, C_2 and C_3 columns respectively. (j) Optical micrograph (with a 100× magnification) of C_1 column. (k and l) TEM images showing the MWCNT structure for C_3 column monolith.

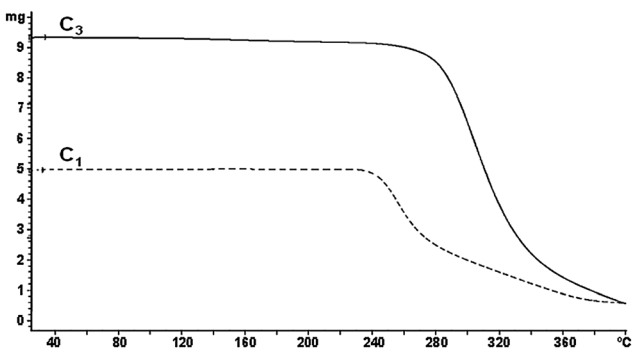

Fig. 4 Thermogram of C_1 and C_3 monolithic polymers. The samples were heated from 25 to 400 °C with a heating rate of 10 °C min^{-1}.

Meanwhile, the highest performance was observed for the C_3 column with 11 123 plates m^{-1} for acetophenone using a flow rate of 2 μL min^{-1}. On the other hand, the C_2 column with a MWCNT content of 0.5 mg mL^{-1} gave a poor separation, while for columns C_4, C_5 and C_6 both backpressure and retention were excessive. Therefore, C_3 corresponded to an optimum content of MWCNT, so it was compared with the C_1 column which did not include nanotubes.

The effect of incorporating MWCNT into monolith material on the separation of the model compounds was also examined. Comparison of the two chromatograms in Fig. 5(A) obviously shows a noticeable influence on their separation. Indeed, the peak shape of the three ketones was improved while the results reported in Table 2 indicated that the resolution increased by a factor higher than 2.5. In a previous study, monolithic glycidyl polymethacrylate columns exhibited no separation of alkylbenzenes, while addition of 0.25% of CNT greatly improved their efficiency which reached 44 000 plates m^{-1} using 0.1 mm i.d. capillaries.[17]

3.3.2. Separation of phenolic compounds. The prepared columns were also used for separation of three phenolic

Table 2 Columns efficiency and separation parameters expressed in terms of t_R, H and R_s

	Column without CNT (C_1)			Column with CNT (C_3)			
Ketones							
At 1 μL min^{-1}	t_R/min	H/mm	R_s	t_R/min	H/mm	R_s	H_{C1}/H_{C3}
Acetone	15.36	0.716	—	15.41	0.092	—	7.78
Acetophenone	22.04	0.804	1.25	22.25	0.092	3.67	8.74
Butyrophenone	33.60	0.862	1.39	33.99	0.103	4.07	8.37
At 1.5 μL min^{-1}							
Acetone	11.37	0.604	—	10.28	0.092	—	6.57
Acetophenone	16.30	0.636	1.39	14.70	0.101	3.48	6.30
Butyrophenone	24.90	0.733	1.54	23.33	0.112	4.24	6.54
At 2 μL min^{-1}							
Acetone	8.91	0.575	—	7.75	0.091	—	6.32
Acetophenone	12.99	0.641	1.46	11.27	0.090	3.77	7.12
Butyrophenone	19.30	0.653	1.49	16.95	0.114	3.83	5.73
Phenols							
At 1 μL min^{-1}							
Aminophenol	10.32	0.366	—	14.12	0.136	—	2.69
Nitrophenol	13.97	0.448	1.44	19.42	0.140	2.61	3.20
Chlorophenol	17.91	0.485	1.10	24.75	0.143	1.96	3.39
At 1.5 μL min^{-1}							
Aminophenol	8.23	0.323	—	9.45	0.148	—	2.18
Nitrophenol	10.38	0.642	1.01	14.25	0.147	3.24	4.37
Chlorophenol	14.25	0.587	1.23	16.69	0.140	1.28	4.19
At 2 μL min^{-1}							
Aminophenol	6.88	0.322	—	7.02	0.137	—	2.35
Nitrophenol	9.47	0.590	1.43	10.92	0.145	3.53	4.07
Chlorophenol	11.96	0.613	0.92	12.63	0.137	1.19	4.47

compounds (*i.e.*, aminophenol, nitrophenol and chlorophenol). As an example, chromatograms are shown in Fig. 5(B). The three substituted phenols were partially separated in the C_1 column with a resolution of less than 1.5 over all the studied flow rate range. Using the same chromatographic conditions (254 nm detection wavelength using acetonitrile/water (50 : 50, *v*/*v*) with

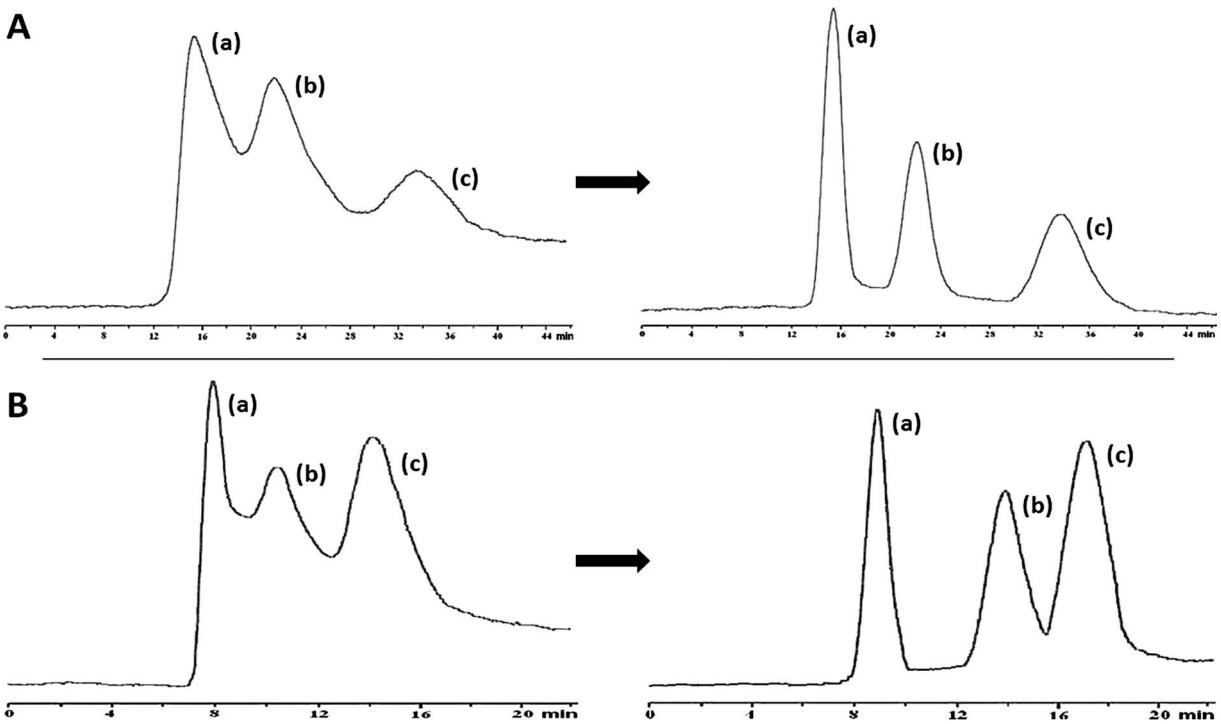

Fig. 5 Chromatograms on columns C_1 (left) and C_3 (right) of: (A) ketones with a binary acetonitrile/water (50 : 50, *v*/*v*) with 1% formic acid mobile phase at 1 μL min^{-1}, where: (a) acetone, (b) acetophenone and (c) butyrophenone, (B) phenols using acetonitrile/water (50 : 50, *v*/*v*) with 1% formic acid mobile phase at 1.5 μL min^{-1}, where: (a) aminophenol, (b) nitrophenol and (c) chlorophenol.

1% formic acid as mobile phase), their separation was complete on the C_3 column at slightly higher retention times with better resolutions (R_s >1.5).

The plate height H was then calculated for each phenol derivative at different flow rates. The corresponding values are shown in Table 2. For the three phenolic compounds, the height equivalent to a theoretical plate was clearly reduced by incorporating MWCNT to the monoliths in the investigated flow rate range. As it was observed for the separation of ketones, incorporation of carbon nanotubes to the monolith induced an improvement of column efficiency, but in a very limited range with an optimum value corresponding to 1.0 mg mL^{-1} for the C_3 column.

4. Conclusion

Few studies have been published on the effect of carbon nanotubes on column performance in LC. The present work demonstrated the potential of incorporating such nanomaterials to improve the stationary phase efficiency. Monolithic capillary columns were prepared by single step *in situ* free radical polymerization of benzyl methacrylate in fused silica tubing using different compositions. The effect of MWCNT incorporation into the polymerization mixture was investigated in the range 0.05 to 0.4%. The procedure proved to be rapid, simple and efficient; it needs only small quantities of solvents and reagents. The prepared monoliths were characterized by optical microscopy, SEM, TEM, FT-IR, TGA and specific surface area measurements. Their porosity and permeability were also determined and compared with the morphology parameters obtained from the micrographs. The capillary columns were successfully applied to achieve reproducible separation of ketones and phenols as model compounds with good separation efficiency. The obtained chromatographic performances were satisfactory and confirmed that capillary columns offer several advantages over packed conventional columns in liquid chromatography.

The retention behaviour of ketonic and phenolic model solutes, as well as the column efficiency of the prepared columns, was studied. Incorporating MWCNT obviously showed an improvement of the column performance for all solutes, corresponding to an increase of efficiency by a factor 6 to 9 for ketonic compounds and 2 to 4 for phenolic solutes. In addition, the presence of carbon nanotubes induced a notable increase of the chromatographic resolution between peaks about 2 to 3 times for C_3 column which contained 0.1% of carbon nanotubes.

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