Carbon nanoparticles from corn stalk soot and its novel application as stationary phase of hydrophilic interaction chromatography and per aqueous liquid chromatography

Yuanyuan Li, Luan Xu, Tong Chen, Xiaoyan Liu, Zhigang Xu, Haixia Zhang

Abstract

Carbon nanoparticles (CNPs) (6–18 nm in size) were prepared by refluxing corn stalk soot in nitric acid. The obtained acid–oxidized CNPs are soluble in water due to the existence of carboxylic and hydroxyl groups. $^{13}$C NMR measurement shows the CNPs are mainly of sp² and sp³ carbon structure different from CNPs obtained from candle soot and natural gas soot. Furthermore, these CNPs exhibit unique photoluminescence properties. Interestingly, the CNPs might be exploited to immobilize on the surface of porous silica particles as chromatographic stationary phase. The resultant packing material was evaluated by high-performance liquid chromatography, indicating that the new stationary phase could be used in hydrophilic interaction liquid chromatography (HILIC) and per aqueous liquid chromatography (PALC) modes. The separation of five nucleosides, four sulfia compounds and safflower injection was achieved by using the new column in the HILIC and PALC modes, respectively.

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1. Introduction

Nanized carbon materials, which include carbon nanotubes, fullerenes, nanofibers and others, have fascinated researchers and are actively exploring a broad range of applications due to their unique physical, chemical, and mechanical properties [1–3]. For example carbon-based nanomaterials were utilized as sorbents and pollution prevention strategies to identify and address environmental contaminants in environmental study [4–10], biosensors [11–13], ultrahigh-strength engineering fibers [14], quantum wires [15] and so forth. Nanized carbon materials have been mainly prepared through the method of chemical vapor deposition [16–18], laser ablation [19–21], electrooxidation [22]. Recently, carbon nanoparticles (CNPs) were synthesized by oxidation of candle soot [23,24], natural gas soot [25] and paraffin oil soot [26] in nitric acid, which is a very simple and yet effective synthetic method.

Hydrophilic interaction liquid chromatography (HILIC) is a powerful and solid alternative, for normal-phase liquid chromatography and reversed-phase liquid chromatography modes, to separate polar compounds and highly hydrophilic compounds [27]. In HILIC mode, a mixture of water and high percentage of organic modifiers (70–95%) is employed with a polar stationary phase [28–32]. The number of published papers on HILIC has experienced significant growth in the past two or three years. Per aqueous liquid chromatography (PALC) was also called as reversed HILIC. In PALC, mobile phases contain a high percentage of water to separate polar compounds. PALC arouse wide concern as a green LC mode [33–38].

Carbon nanoparticles are very active research topics and have been widely investigated in many fields. Nanized carbon materials are used as stationary phases of chromatography, particularly carbon nanotubes. Nanized carbon-based stationary phases show unique selectivity for solutes in GC [39–45], LC [46–51], and electrochromatographic analyses [52–55]. It may be a good subject for development of HPLC stationary phase. In the present study, CNPs were prepared from corn stalk soot which is a kind of solid waster produced after burning. The obtained CNPs exhibited 6–18 nm size distribution and became water-soluble. Interestingly, the CNPs could be effectively immobilized on the surface of porous silica particles by means of polycondensation of triethoxysilyl groups and condensation between triethoxysilyl groups and silanol groups on the silica surface under basic conditions as chromatographic stationary phase. The separation material can be used in HILIC and per aqueous liquid chromatography (PALC) modes to separate polar compounds and highly hydrophilic compounds which is often challenging due to lack of retention on conventional reversed-phase columns [56–60]. On our resultant CNP-silica column, the
separation of five nucleosides, four sulfa compounds and safflower injection was achieved in HILIC and PALC modes.

2. Experimental

2.1. Reagents and materials

Spherical silica (5 μm particle size, 10 nm pore size, 320 m² g⁻¹ surface area) was purchased from Fuji Sylsyn Chemical (Aichi, Japan). Nitric acid (65%) and salicylic acid were obtained from Tianjin Guangfu Chemical Reagent Co. (Tianjin, China). 3-(Aminopropyl)trimethoxysilane (APTMS) and 3-(chloropropyl)trimethoxysilane (CPTMS) were obtained from Alfa Aesar (Karlsruhe, Germany) and Acros Organics (New Jersey, USA), respectively. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride (EDC) were purchased from GL Biochem Ltd. (Shanghai, China).

Thymine, cytosine, adenine, inosine, adenosine, theobromine, thiouracil and N-hydroxysuccinimide (NHS) were from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sulfamethazine (SMz), sulfamethoxypyridazine (SMP), sulfurmonomethoxime (SMM) and sulfamethoxazole (SMZ) were obtained from Alfa Aesar (Karlsruhe, Germany). Vitamin B₂ (VB₂), nicotinic acid (VB₃), vitamin B₉ (VB₉), caffeine and clenbuterol were purchased from Sigma (St. Louis, MO, USA). Melamine was obtained from J&K Chemical Ltd. (Beijing, China). Acetonitrile (ACN) of HPLC grade was from Dima Technology (Richmond Hill, ON, Canada). All other reagents were of analytical-reagent grade (Tianjin Chemicals, China) and purified water from a Milli-Q system was used throughout the experiments.

2.2. Synthesis of carbon nanoparticles (CNPs)

Waste soot was collected from a corn stalk burning. CNPs were prepared according to a procedure reported in the literature [23–25,61,62]. Typically, 10 g waste soot was refluxed in 150 mL of 5 M nitric acid for 15 h with stirring. The solution was then cooled and neutralized by NaOH. After centrifugation at 10,000 rpm for 15 min, the reddish-brown supernatant was dialyzed against distilled water for 72 h. Purified CNPs (0.5 g) were obtained.

2.3. Immobilization of CNPs on silica gel

CNPs (0.5 g) were suspended in 130 mL anhydrous DMF and adjusted pH to 4.5 by formic acid. Then the mixture was stirred until it dissolved. The surface carbonyl groups of CNPs were activated by adding 0.1 g of NHS and 0.15 g of EDC in solution. After 30 min, 0.5 mL APTMS was added dropwise. The resulting mixture was stirred at room temperature for 12 h. Subsequently, 2.0 g silica gel was dispersed in the solution. 0.5 mL CPTMS and 20 mL ammonia solution were added to the mixture, then heated under a N₂ atmosphere at 100 °C for 15 h. The obtained mixture was filtered and intensively washed with DMF, water, methanol and acetone, respectively, and then dried under vacuum at 40 °C overnight.

The routes for synthesis of the new separation material are shown in Fig. 1.

2.4. Characterization

The degree of functionalization was determined by elementary analysis, using a Vario EL elemental analysis system (Elementar, Germany) to determine the nitrogen and carbon content. IR spectra during the different stages of the functionalization procedure were obtained on a Nicolet 20 NEXUS 670 FT-IR (Madison, USA) using KBr pellets. The 13C NMR spectra in D₂O were recorded on a Varian INOVA 600 MHz spectrometer by dissolving 50 mg CNPs. Transmission electron microscope (TEM) was carried out by a JEM1200EX (Tokyo, Japan) to show the morphology and size of the CNPs. A ESCALAB210 X-ray photoelectron spectrometer (XPS) was used for compositional analysis and chemical bond determination of CNPs. The photoluminescence spectra were made with a RF-5301 PC spectrophotofluorometer to show the optical property of the CNPs.

2.5. Slurry packing and chromatographic evaluation

The new separation material was slurry-packed into a 150 mm × 4.6 mm I.D. stainless steel column and methanol was used as the packing solvent at 60 MPa pressure. Amino column (150 mm × 4.6 mm, 5 μm particle diameter, 10 mm pore size) was prepared by our laboratory. C18 column (250 mm × 4.6 mm, 5 μm particle diameter, 10 mm pore size) was from Lanzhou Zhongkeantai Corporation (Lanzhou, China). The chromatographic system consisted of a Varian 210 high-performance liquid chromatographic pump (Palo Alto, CA, USA), a Varian 325 UV–vis detector, and a Varian Star chromatographic workstation. The chromatographic evaluations were carried out at room temperature (15 ± 2 °C). The flow rate was 1.0 mL min⁻¹. A set of test probes with a concentration of 20 μg mL⁻¹ was prepared in water/acetonitrile (1/1, v/v). Each measurement was replicated three times.

3. Results and discussion

3.1. Synthesis and characterization of CNPs

Corn stalk soot is black in color and completely insoluble in water. After being refluxed in nitric acid, the reddish-brown supernatant was obtained. It suggests that a part of the carbon particles becomes very small and their surfaces are introduced −OH and −CO₂H groups during the refluxing processes, thus making the particles become negatively charged and hydrophilic. The formation of CNPs was first confirmed by TEM measurement. Fig. 2a shows a typical TEM image of the overall morphology of the CNPs which were mostly of spherical shape and have a size distribution from
6 to 18 nm. As shown in Fig. 2b by the HRTEM imaging technique, various lattice planes can be clearly identified in addition to what appears to be amorphous CNPs which may be attributable to the diffraction planes of diamond-like (sp³) carbon and graphitic (sp²) carbon [25]. These structural assignments are further supported by ¹³C NMR measurements (Fig. 3). First, below 50 ppm sp³ carbon peaks can be seen. Second, within the range of 50–100 ppm only three featureless small peaks appear, this most possible led from aliphatic (sp³) carbons connected within the CNPs. Third, lots of peaks between 100 and 150 ppm are sp² carbon peaks. Compared with previously reported [23–25] carbon nanoparticles obtained from candle soot and natural gas soot, saturated sp³ carbon atoms were observed and a peak at 114 ppm attributed to terminal C=C bonds was not observed in the present study, which indicated that the terminal C=C bonds have most likely been oxidized into carboxylic/carbonyl moieties. Fourth, the broad peaks between 170 and 180 ppm arise from carboxylic/carbonyl carbons within the CNPs. That is, the CNPs are mainly of sp² and sp³ carbon structure.

A FT-IR spectrogram of the CNPs (Fig. 4a) was also measured. Broad absorption bands at 3421 and 1608 cm⁻¹ were attributed to O–H and C=O vibrational stretching of the carboxylic moiety. The peak at 1380 cm⁻¹ was the symmetric stretching of the carboxylate. A strong peak at 1130 cm⁻¹ belonged to the absorption of C–O.

Elemental analysis of the CNPs is given in Table 1. The CNPs had a low carbon and high oxygen content. Nitric acid oxidation induces doping of nitrogen and oxygen atom into the carbon particle and produces –OH and –CO₂H groups on the carbon nanoparticle surfaces, which made them water-soluble.

XPS compositional analysis results was as follows: C1s: 284.7 eV (C=C), 288.2 eV (C=O); N1s: 399.6 eV (C–N), 406.8 eV (N=N); O1s: 531.0 eV (C–O/N=O), 532.1 eV (C=O/N=O), 535.6 eV (COO–). These XPS data show that CNPs are mainly composed of graphitic carbon (sp²) and oxygen/nitrogen-bonded carbon. This is well-matched with bonding structures obtained from the above compositional analysis.

Additionally, the optical property of the prepared nanoparticles was investigated by photoluminescence spectra. These CNPs exhibit unique photoluminescence property. Line in Fig. 5 indicated the CNPs exhibited an emission peak at 548 nm when the excitation wavelength was fixed at 486 nm. The surface passivation of CNPs can lead to significant fluorescence. However, the exact mechanism is not yet clear.

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**Table 1**

<table>
<thead>
<tr>
<th>Elemental content (%)</th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
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<tr>
<td>The CNPs</td>
<td>28.14</td>
<td>1.14</td>
<td>3.18</td>
</tr>
<tr>
<td>CNPs – silica gel</td>
<td>8.26</td>
<td>1.36</td>
<td>1.29</td>
</tr>
</tbody>
</table>

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**Fig. 2.** (a) TEM image of the CNPs, (b) HRTEM image of the CNPs, (c) TEM image after immobilization of CNPs on silica gel surfaces.

**Fig. 3.** ¹³C NMR spectrum of CNPs in D₂O.

**Fig. 4.** FT-IR spectra of (a) CNPs and (b) the new separation material after immobilization of CNPs on silica gel surfaces.
3.2. Immobilization of CNPs on silica gel [43–45,48,63]

After immobilization of CNPs on silica gel surfaces, the corresponding TEM images are presented in Fig. 2c. As can be seen, Fig. 2c reveals a distinctly different surface with many particles and shows a coating layer due to the presence of the CNPs on silica gel. Further evidence for the presence of the CNPs on silica gel surface came from elemental analysis given in Table 1. The result showed that the obtained material had a high carbon content of 8.26%. FT-IR spectrum of the new material was measured (Fig. 4b). The absorption peaks at 1722 (υ(C=O)) and 1675 (υ(N-H)) cm\(^{-1}\) appeared. The band at 1536 and 1386 cm\(^{-1}\) belonged to the stretching of the carboxylate within the CNPs on silica gel surfaces, which implied that silica gel surfaces existed residual –CO\(_2\)H groups. Other peaks were 3436 (υ(O-H, N-H)), 1000–1200 (υ(Si-O-Si)) cm\(^{-1}\), respectively. The result indicated that the CNPs had been immobilized on silica gel surfaces.

3.3. Column characterization

3.3.1. Retention properties in the HILIC mode

(1) The effect of water content on retention.

Mobile phase strength in the eluent is probably the factor that has the largest effect on retention of polar compounds, because hydrophilic interaction is enhanced by decreasing the polarity of the eluent. In order to investigate the HILIC properties of the new column, seven polar compounds (VB\(_2\), VB\(_3\), clenbuterol, VB\(_6\), melamine, thiourea and theobromine) were used as test probes. As shown in Fig. 6a, the retention of all test compounds gradually increased with increasing %ACN from 50 to 80%, and then dramatically increased as the %ACN was increased above 80%, which showed characteristics typical of HILIC. Hence, retention at high levels of organic solvents reflected the hydrophilic nature of the new phase.

(2) The effect of buffer pH on retention.

Mobile phase pH also plays an important role in affecting selectivity by influencing solute ionization and polarity of the stationary phases in HILIC. The effect of mobile phase pH was investigated by adjusting the pH of ammonium formate aqueous solutions to 6.3, 5.7, 4.6, 3.6 and 3.0 before mixing with ACN while keeping the concentration of ammonium formate constant at 10 mM. For VB\(_6\), thiourea and theobromine, the retention time only fluctuated slightly on the new column in the pH range used. The retention time of VB\(_3\) (pK\(_a\) ∼ 3.0), thiourea and clenbuterol (pK\(_a\) ∼ 9.7) had a dramatic decrease with pH, which was due to decrease of electronic interaction and ion-exchange interaction, respectively. Carboxyl groups present at the surfaces of the CNPs have been associated with these electronic interactions and ion-exchange effect.

(3) The effect of ionic strength of mobile phase on retention.

Ionic strength of mobile phase can also have influence on the retention of polar compounds in HILIC. In this study, we selected four compounds (theobromine, thiourea, VB\(_3\), and VB\(_6\)) as test probes to investigate the effect of ionic strength on retention time at pH 6.3 with ammonium acetate concentration at 5 mM, 10 mM and 15 mM, respectively (Table 2). For theobromine and thiourea, the retention time remained essentially unchanged. For VB\(_3\), the retention time increased with an increase in salt concentration. The reason could be that the negatively charged material is repelling the acidic probe electrostatically at the pH of 6.3. Higher salt concentrations may have weakened any electrostatic repulsion, leading to the stronger retention. For VB\(_6\), the retention time decreased with the increase in salt concentration which was due to ion-exchange interaction between the new material and VB\(_6\) at pH 6.3. Higher salt concentrations could weaken ion-exchange effect, leading to the weaker retention.

![Fig. 6](image_url)

**Table 2**

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Theobromine</th>
<th>Thiourea</th>
<th>VB(_3)</th>
<th>VB(_6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.74</td>
<td>2.74</td>
<td>6.80</td>
<td>5.45</td>
</tr>
<tr>
<td>10</td>
<td>2.75</td>
<td>2.79</td>
<td>10.98</td>
<td>5.25</td>
</tr>
<tr>
<td>15</td>
<td>2.70</td>
<td>2.78</td>
<td>12.83</td>
<td>4.75</td>
</tr>
</tbody>
</table>

* Mobile phase: ACN/ammonium acetate solution (85/15, v/v); pH=6.3.
3.3.2. Retention properties in the PALC mode

(1) The effect of water content on retention.

Five polar compounds including melamine, VB₆, caffeine, theobromine and thiourea were selected as test probes to investigate the PALC properties of the new stationary phase. The retention time of five test probes were plotted against the volume fraction of water in the eluent as shown in Fig. 6b. Above 50% water the retention times were observed to be proportional to the water content in the eluent, which exhibited typical PALC behaviors on the new phase. The retention time of all five compounds slightly increased as the water content increased from 50 to 90%, and then increased dramatically when the water content further increased to 100%. It showed that the retention of the test probes was extremely sensitive to any small variation of ACN content in water-rich eluents. The possible reason might be that the surfaces of the new phase were significantly saturated with water and ACN, resulting in slight decrease of the retention factors of compounds with the water content of 90–60% in mobile phase. But within the ACN concentration ranges of 0–10%, small variation of ACN content could lead to the drastic composition change of the adsorbed eluent multilayer onto the new stationary phase. So the retention factors were strongly sensitive to little change of mobile phase with the bulk water concentration [34,37,64].

(2) The effect of buffer pH on retention.

The effect of mobile phase pH was also investigated in order to obtain more insight into the retention mechanism in PALC mode. It was found that the retention of salicylic acid experienced the most dramatic decrease from pH 3.1 to 6.3. The possible reason was that the carbonate groups of the stationary phase were gradually ionized and acquired more negative charge when pH was increased, generating electrostatic repulsion with negative salicylic acid (pKa ~ 2.8). For caffeine, theobromine, adenosine and cytosine, the stationary phase acquired more negative charge as the pH was increased, generating ion-exchange effect with basic compounds, thus leading to a little increase in retention.

(3) The effect of ionic strength of mobile phase on retention.

Table 3 shows the effect of ammonium acetate concentrations on the retention of salicylic acid, caffeine, theobromine, adenosine and cytosine with 98% water in the mobile phase. The retention time decreased with an increase in salt concentration for all test probes including acid and basic compounds. As mentioned previously, the retention of solutes is mainly due to hydrophobic interaction in PALC mode. Higher salt concentration could drive more solvated salt ions into the water layer, leading to the more ACN composition of the adsorbed eluent multilayer onto the new stationary phase. This would result in a decrease in hydrophobic interactions and lower retention of the solutes.

Additionally, the retention of four nucleosides (adenine, cytosine, adenosine and thymine) depending on the ACN content in mobile phase exhibited “U-shaped” curves with a minimum of retention for the 60/40 water–ACN mixture on the new column (Fig. 6c). Similar “U-shaped” curves have been also observed with a great many of stationary phases and mobile phase conditions [65–68]. U-shaped elution curves indicate mixed retention effects.

3.3.3. Applications

To demonstrate the separation ability and special selectivity of the new stationary phase, the separation of small polar compounds that were difficult to retain and separate by RPLC was achieved.
in the HILIC or PALC mode. Additionally, in order to assess HILIC process on the new stationary phase, amino and C18 silica columns were used to compare in this study.

1) The separation of five nucleosides.

Five nucleosides were separated on the three columns under HILIC conditions, as shown in Fig. 7. It could be seen that all the components were well resolved and the nucleosides displayed stronger retention on the new column than on amino column, but were difficult to retain on C18 column in HILIC mode. The elution order of adenine varied from column to column, indicating different selectivity of the new phase. It was reasonable to assume that surface carboxylic groups on the new phase might interact with the basic adenine, thus leading to stronger retention and greater tailing for adenine. In order to further understand the interactions on the new phase, aniline and phenol compounds were used for further evaluation. The peak retention factors \( k \) and asymmetry factors \( A_5 \) of three phenol compounds with 100% water in mobile phase were 4-aminophenol \( (3.4, 0.96) \), phenol \( (4.9, 1.3) \) and 4-nitrophenol \( (5.9, 1.5) \), respectively. However, \( A_5 \) of three aniline compounds, aniline \( (k = 5.2, A_5 = 1.2) \), 2-nitroaniline \( (k = 15.2, A_5 = 3.0) \) and 2,4-dinitroaniline \( (k = 20.8, A_5 = 2.5) \), increased significantly under the same condition. It indicated that there was a significant ion-exchange effect for basic compounds on the new phase.

We compared the peak efficiency in the PALC (100% water) and HILIC (82%ACN) conditions at a flow rate of 1.0 mL min\(^{-1}\). The results are shown in Table 4. In contrast to PALC, under HILIC conditions high efficiencies were achieved for all solutes. However, all peaks were very broad and have tailing in PALC conditions as can be seen in Fig. 7c.

2) The separation of four sulfa compounds.

The separation chromatogram of four sulfa compounds is shown in Fig. 8 in PALC mode. Four sulfa compounds were retained and separated in water-rich eluents on the new phase.

3) The separation of safflower injection.

To demonstrate the special selectivity of the new phase, safflower injection was used for further evaluation. Safflower has been widely used in China for thousands of years and is a popular medicine for the treatment of cardiovascular and hypertension disease because of its reputation for facilitating blood circulation and dispersing blood stasis. Safflower injection was separated on the three columns in HILIC mode. On C18 column, the components in safflower injection are not retained due to their hydrophilic character. A dozen compounds were well resolved and showed good selectivity on the new phase and amino column, as shown in Fig. 9.

4. Conclusion

In this work, the CNPs were prepared by oxidation of corn stalk soot with nitric acid. The resultant CNPs with 6–18 nm in size are soluble in water due to existence of hydroxyl and carboxylic groups. The CNPs also exhibit fluorescence property. Furthermore, the CNPs have been successfully immobilized on the surface of porous silica particles by a simple and efficient method. The obtained stationary phase can provide the similar retention for polar compounds in HILIC and PALC modes and exhibit U-shape retention pattern. The new stationary phase showed good separation selectivity for polar compounds and hydrophilic compounds. The CNPs chemically immobilized on matrix could be very useful separation materials. Therefore, the utility of the CNPs in various practical applications will be explored in the future (e.g., fluorescence property).

Acknowledgments

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References