Tritium label in studying sorption of humic substances by carbon-based nanomaterials

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ARTICLE INFORMATION

ABSTRACT

Sorption capacity of single-walled carbon nanotubes, detonated nanodiamonds and graphene to humic substances was studied by radiotracer method. Tritium labeled brown coal humic acids and fulvic acids separated from Suwannee River were used as sorbates. Adsorption isotherms were described by Langmuir equation. It was found that, for all tested carbon-based nanomaterials, adsorption of coal humic acids is higher than of river fulvic acids. Adsorption capacity of nanomaterials in attitude to humic substances was changed in the order, nanodiamonds < single-walled nanotubes < graphene. Composites of humic substances with carbon-based nanomaterials were subjected to dynamic light scattering analysis.

1. Introduction

Humic substances (HS) are chemically heterogeneous class of polymeric organic compounds that are distributed in the environment. They are also the most important source of organic carbon in both aquatic and terrestrial environments and play a key role in nature. Structural model proposed by Kleinhempel in 1970 (Figure 1) [1] illustrates the complexity of HS that impeded study chemistry of these materials.
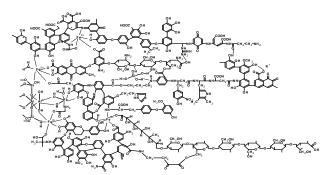

Figure 1. Structural model of humic substances proposed by Kleinhempel [1].

Common classification of HS is based on its solubility. Thus, HS are subdivided into four groups. They are (*i*) humic acids that are soluble in alkalis; (*ii*) hymatomelanic acids that are soluble in alcohols; (*iii*) fulvic acids that are soluble in aqueous in wide range of concentrations and (*iv*) humin, which is insoluble fraction of natural organic matter. An overview of important milestones in aquatic humic chemistry, analytical methods and structural models are comprehensively discussed in review [2].

The modern science of HS is focused on either obtaining new soils-like materials or the development of methods for analysis of HS and their application in environmental science. All these fields are unified by one goal, which is the development of new materials with certain properties. To achieve this goal, a number of researchers investigate the interaction of HS with different colloids and nanoparticles [3-6]. In this aspect, carbon-based nanomaterianls should be taken into critical account. The contributions of carbon-based nanomaterials to a broad range of environmental applications: sorbents, high-flux membranes, depth filters, antimicrobial agents, environmental sensors, renewable energy technologies, and pollution prevention strategies assessed in review [7]. Now days, carbon-based nanomaterials particularly carbon nanotubes are tested as a unique substrate for adsorption of biomolecules including peptides and proteins. Furthermore, these materials are considered as being introduced in biological systems. In review [8] single-walled carbon nanotubes were also tested for removal of contaminants in drinking water. Therefore, modern developments of chemistry of nano-materials pointed us at the study of sorption ability of carbon-based nanomaterials to HS and to determine properties of formed complexes.

Three types of carbon-based nanomaterials with different hydrophobic properties were under the test. They are graphene, single-walled carbon nanotubes (SWNT) and detonated nanodiamonds (ND). Sorption capacity of carbon-based nanomaterials was tested for two types of HS in particular humic acids from coal and fulvic acids separated from Suwannee River. Several analytical methods are described in literature for determination of HS amount in aqueous solutions. They are size-exclusion chromatography [9-12] with

Table 1. Properties of HS used in the study.

HS index	Elemental composition			Ash, %	M_W, kDa	Content of carbon in the structural fragments, %			
	H/C	O/C	C/N			$\sum C_{C=O}$	$\sum C_{COO}$	$\sum C_{Ar}$	$\sum C_{Alk}$
CHA-Pow	0.87	0.50	53	7.8	9.4	5.7	19.0	62.7	12.5
FA,SR	1.38	0.33	50	0.6	7.6	5.0	17.0	57.0	22.0

Table 2. DLS results obtained for starting materials.

Material	Intensity-averaged diameter, nm	Polydispersity Index
Single-walled carbon nanotubes	182	0.23
Detonated nanodiamonds	100	0.02
Graphene	4774	0.30
Powhumus	369	0.28
FA,SR	246	0.47

either UV- or color coefficient analysis [13] and fractionation either by polyacrylamide gel electrophoresis [14] or by two-dimensional gel electrophoresis [15].

In previous research, we have shown that radioanalytical assay where tritium is used as a tracer has undoubted advantages comparison with UV-analysis [16]. If UV-analysis determines only aromatic part of HS radiotraser assay, using tritium (^{3}H)-labeled HS obtained by tritium thermal activation method allow following the adsorption of all fractions of HS. Tritium thermal activation method is a unique instrument that allowed obtaining radiolabeled organic materials of different classes and structural peculiarities. It was demonstrated in [17] that this method applied for HS radiolabeling results in ^{3}H-HS obtaining with equal tritium distribution in the HS components. Since tritium thermal activation technique is based on the bombardment of solid organic target with tritium atoms that are forms by thermal dissociation of tritium molecules on W-wire the method allows introduction of ^{3}H-label in any structural fragment of macromolecule, regardless of its nearest surrounding and HS origin. ^{3}H-HS obtained by tritium thermal activation method were successfully used for studying their adsorption and distribution in aqueous/organic liquid systems [18,19] and their interaction with bacteria and plants [19].

The goal of this research was to determine sorption capacity of carbon-based nanomaterials to humic substances. To this end, we compared sorption of brown coal humic acids to fulvic acids separated from Suwannee River. Detonated nanodiamonds, single-walled carbon nanotubes and graphene were used as a sorbent.

2. Experimental

2.1. Materials characteristics

Two types of HS were used brown coal humic acids (CHA-Pow) (commercially available preparation Powhumus (Humintech, Germany)) and fulvic acids separated from Suwannee River (FA,SR) (recommended as a standard by IHSS). HS were analyzed as it was previously described in Ref. [17]. The main properties of HS used in the study are summarized in Table 1.

Graphene and single-walled carbon nanotubes were synthesized in Moscow Institute of Elemeetoorganic Chemistry RAS. Detonated nanodiamonds were purchased from "Sinta" (Belarus). Specific surface of nanomaterials was characterized by dynamical method of specific surface determination by continuous flow method [20]. The technique is based on nitrogen adsorption by the sample at liquid nitrogen temperature from a gas stream of nitrogen-helium mixture and elution upon warming the sample. Carbon-black with specific surface 80 m^2/g was used as a standard. Measuring were carried out at gas stream rate 40 mL/min. The values obtained were 107, 260 and 350 m^2/g for graphene, SWNT and ND correspondingly.

Humic substances and carbon-based nanomaterials were subjected to dynamic light scattering analysis (DLS). Measurements were made, as it will be described below for modified materials (see Section 2.5). The intensity-averaged diameter and polydispersity indexes are summarized in Table 2.

2.2. Tritium labeling of humic substances

Tritium label was introduced in HS by means of the thermal activation method. The labeling technique and purification procedures were previously described in Ref. [17]. Briefly, 1 mL of 0.025 mg/mL of HS in the solution in 0.04 % sodium hydroxide was distributed on the walls of glass reaction vessel and lyophilized. The reaction vessel with prepared HS-target was connected to the special device for working with gaseous tritium and evacuated followed by the addition of tritium gas. HS-target was bombard with tritium atoms for 10 s. Tritium atoms were obtained by thermal dissociation of tritium molecules on W-wire at 1800 K. After 10 s reaction, HS-target was dissolved in 0.4 % sodium hydroxide. For purification and analysis of ^{3}H-HS 30 days dialysis through membrane 2 kDa cut-off against phosphate buffer (pH = 6.8) and size exclusion chromatography controlled by determination both UV-absorbance and radioactivity of HS fractions were applied. Purification procedure provides absence of exchangeable tritium in HS. Specific radioactivities of labeled products were 3.0 and 4.5 Ci/g for CHA-Pow and FA,SR correspondingly.

2.3. Sorption of humic substances by carbon-based nanomaterials

Sorption experiments were carried out at room temperature in polyethylene eppendorf tubes. Tree series of experiments were conducted distinguished by the sorbent. 3-7 mg sample of carbon nanomaterial was placed in the eppendorf tubes followed by the addition of 0.5 mL of ^{3}H-HS solution in phosphate buffer. The initial concentrations of HS were from 0.2 to 200 mg/L and the bulk radioactivity of HS solutions was *ca* 3 mCi/L. In each series to one sample of carbon nano-material 0.5 mL of buffer was added and this system was used as a control.

Ultrasonication of the dispersion was carried out for 90 minutes using a bath sonicator (GRAD, Model 28-35, Russia) with rated power of 50 W. The sonication efficiency and, hence, the quality of the dispersion varied with the volume of the solution sonicated. For best dispersions, the final sonication volume was kept at 0.5 mL. The solutions thus obtained were subjected to centrifugation at 12000 g (Allegra 64R centrifuge) for 60 min. The upper 75 % of the supernatant after ultra-centrifugation was filtered by 13 mm syringe filter with 0.2 μm PVDF membrane (Acrodisc LC, Life Sciences). 50 μL of filtered solution was stirred in 7 mL of scintillation cocktail OptiPhase HiSafe 3 (PerkinElmer). Counting rate was measured by liquid scintillation spectrometer RackBeta 1215 (Finland).

Equilibrium concentration (c_{eq}) and adsorption (Γ) were calculated as

$$c_{eq} = \frac{I}{\varepsilon \times V_1 \times a_{sp}} \tag{1}$$

$$\Gamma = \frac{(c - c_{eq}) \times V}{m \times S} \tag{2}$$

I is counting rate, ε is registration efficiency of tritium β-radiation (*ca* 50%), V_1 = 50 μL, a_{sp} is specific radioactivity of HS, c is initial concentration of HS solution, V = 0.5 mL, m is mass of nanomaterial and S is its specific surface.

2.4. Desorption experiments

Reversibility of HS adsorption on carbon-based nanomaterials was conducted in two steps. After sorption experiment, 1 mL of phosphate buffer was added to solid stuff followed by 30 minutes shaking. Then systems were centrifuged and 0.5 mL of supernatant was filtered followed by radioactivity measuring and concentration determination as it was described above. The procedure was repeated 10 times and after it a bit of solid stuff (*ca*. 2 μL of concentrate suspension) was picked for DLS (see Section 2.5). Remain solids was subjected another round of washing by 3 M sodium hydroxide.

2.5. Determination of size of particles

The solid stuff after sorption experiments was washed with 10 mL of buffer. A bit of solid stuff was picked out for DLS analysis [21]. Measurements were made on a Malvern Zetasizer Nano S (Malvern Instruments Ltd., UK) with a detection angle of 173°. All measurements in this study were taken at a temperature of 25 °C. At least three repeat measurements on each sample were taken to check for result repeatability. The intensity size distributions were obtained from analysis of the correlation functions using the Multiple Narrow Modes algorithm in the instrument software.

3. Results and discussions

Tritium labeled humic substances were applied as tracers in sorption experiments. Figure 2 shows adsorption isotherms of HS on tree types of carbon-based nanomaterials. Equilibrium concentrations were four times lower than the initial one. One can see that for all tested nanomaterials under the same equilibrium concentration sorption of CHA-Pow was higher than FA,SR. The same result was previously obtained for adsorption at aqueous/organic liquid interfaces [18,19].

Adsorption isotherms were described by Langmuir equation with relative error 10% (solid lines in Figure 2).

$$\Gamma = \Gamma_{max} \frac{A \times c_{eq}}{1 + A \times c_{eq}} \tag{3}$$
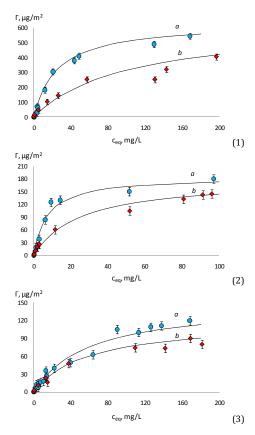
Here A is a coefficient that is equal to ratio between constants of rates of adsorption and desorption, Γ_{max} is maximum adsorption.

Reversibility of adsorption was also studied in pure buffer and in 3 M NaOH. It was found that desorption of both HS in buffer is *ca* 30 % for ND and *ca* 10 % for graphene and SWNT. Addition of 3 M NaOH increased desorption up to 55±5 %.

The fact that adsorption of natural organic matter is described by Langmuir equation was previously also described in literature [22]. In this research, we have calculated adsorption parameters for tested systems. Values of Γ_{max} and A are summarized in Table 3.

As one can see from Table 3 that values of maximum adsorption calculated by Langmuir equation of CHA-Pow and FA,SR were practically coincided, but values of A in cases of graphene and SWNT indicate higher adsorption ability of CHA-

Pow in comparison with FA,SR. In case of ND calculated value of Γ_{max} of CHA-Pow was higher than one calculated for FA,SR.

Figure 2. Adsorption of (a) CHA-Pow and (b) FA,SR on (1)-graphene; (2)-single-walled carbon nanotubes; (3)-detonated nanodiamonds. Solid lines obtained by calculations according Langmuir equation with parameters presented in Table 3.

Table 3. Values of maximum adsorption and coefficients A for HS adsorption on carbon-based nanomaterials calculated according to Langmuir equation.

Carbon-based nanomaterial	Humic substances			
	CHA-Pow		FA,SR	
	A, L/mg	Γ_{max}, mg/m²	A, L/mg	Γ_{max}, mg/m²
Graphene	0.040	0.637	0.010	0.630
Single-walled carbon nanotubes	0.134	0.175	0.040	0.180
Detonated nanodiamonds	0.017	0.150	0.017	0.120

Maximum adsorption of HS on graphene was 3.5 times higher than for SWNT despite of chemical resembling of these materials. SWNT is the result of curvature of the planar graphite thus, it share many of graphene's attributes. It has to be noted that structural peculiarities of SWNT make it surface partly unavailable for large HS molecules, while graphene sheets might be completely within the reach. Indeed, if we recalculate and compare maximum values of adsorption in mg of HS per g of carbon-based nanomaterial it results in graphene/SWNT 1.4 for FA,SR and 1.5 for CHA-Pow.

The results obtained allowed to conclude that sorption processes of HS on carbon surfaces are controlled by of hydrophobic interactions. For the data explanation, it is better to use HS model proposed by D. S. Orlov [23]. This model describes HS as substances, which consists in hydrophobic core and hydrophilic peripheral part. With the help of liquid scintillation spectrometry of tritium and ³H-HS with equal distribution of label among HS components hydrophobicity and surface activity scales were obtained and described in Ref. [19]. According to these scales CHA-Pow is more hydrophobic and

Table 4. DLS results obtained for carbon-based nanomaterials modified by Powhumus.

Material	Intensity-averaged diameter, nm	Polydispersity Index	Diffusion coefficient, $\mu m^2/s$
Single-walled carbon nanotubes	183	0.90	2.44
Detonated nanodiamonds	238	0.24	2.07
Graphene	1860	0.64	0.27

Table 5. DLS results obtained for carbon-based nanomaterials modified by FA,SR.

Material	Intensity-averaged diameter, nm	Polydispersity Index	Diffusion coefficient, $\mu m^2/s$
Single-walled carbon nanotubes	192	0.33	2.56
Detonated nanodiamonds	228	0.87	3.03
Graphene	359	0.37	1.37

possess higher surface active properties than FA,SR. High hydrophobicity of HS also explains the fact that adsorption of both CHA-Pow and FA,SR on graphene and on SWNT is higher than on ND. Values of Γ_{max} calculated for CHA-Pow on graphene and for FA,SR on SWNT were comparable with adsorption corresponding HS at aqueous/arene interfaces in plateau region of the isotherm [16,18,19]. We compared the results obtained for SWNT and graphene with arenes because of similarities of hybridization state of carbon atoms in these molecules.

Composites of HS with carbon-based nanomaterials and initial compounds were analyzed by DLS. The results obtained are summarized in Table 4 and 5 for carbon-based nano-materials modified by CHA-Pow and FA,SR correspondingly. Tables contain the intensity-averaged diameter in nanometers, the polydispersity index values and the calculated diffusion coefficients. The polydispersity index values are very sensitive to the presence of aggregates or dust. All samples were twice filtered before measuring. Thus, high value of polydispercity of samples related to properties of modified material i.e. possibility of aggregation.

Despite of differences in the adsorption values of CHA-Pow and FA,SR on the surface of each carbon-based material modified particles were practically of the same size for both humic substances. As one can see from Table 2, 4 and 5 that graphene modified by both CHA-Pow and FA,SR forms smaller particles then grephene itself, while modification of nanotubes and nanodimonds results in formation either larger or particles of the same size with starting materials. However, in each case the formation of suspensions of modified materials described by the diffusion coefficient presented in Table 4 and 5 occurred under easy shaking of the system, while starting material required sonication during several hours to achieved the same result. It was also observed that modified grephene and SWNT become rather hydrophilic and they form suspension in aqueous solution, which are very stable to sedimentation.

Furthermore, modification of graphene with HS gives rise 6-fold decrease of the average diameter of graphene particles. This result might be related with the formation of interlayer of adsorbed HS between graphene sheets that prohibit agglomeration as it was shown for nanotubes and peptides [24]. For detailed explanation of the results obtained further experiments involving scanning electron microscopy will be conducted.

4. Conclusions

In this work, we have demonstrated sorption capacity of carbon-based nanomaterials to such complex objects like humic substances. Usage of tritium labeled humic substances allowed for conducting the experiment in wide concentration range. The results obtained allowed to conclude that sorption processes of HS on solid surfaces are controlled by of hydrophobic interactions. Sorption capacity of single-walled carbon nanotubes and graphene is higher than of nanodiamond. Carbon-based nanomaterials modified by humic materials can find applications in soil sciences i.e. as proportioning HS agent in agro-industrial and so on.

References

[1].   Kleinhempel, D. *Albercht-Thaer-Arch.* **1970**, *14*, 3-11.
[2].   Frimmel, F. H. *Agronomie* **2000**, *20*, 451–463.
[3].   Tan, W. F.; Koopal, L. K.; Weng, L. P.; Riemsdijk, W. H. V.; Norde W. *Geochim. Cosmochim. Acta* **2008**, *72*, 2090–2099.
[4].   Hyung, H.; Kim, J. H. *Environ. Sci. Technol.* **2008**, *42*, 4416–4421.
[5].   Su, F.; Lu, Ch. J. *Environ. Sci. Health A* **2007**, *42*, 1543–1552.
[6].   Lu, J.; Li, X.; Yan, Shi, B.; Wang, D.; Tang, H. *Colloid. Surface A* **2009**, *347*, 90–96.
[7].   Mauter, M. S.; Elimelech, M. *Environ. Sci. Technol.* **2008**, *42*, 5843–5859.
[8].   Venkata, K.; Upadhyayula K.; Deng, S.; Mitchell, M. C.; Geoffrey, B. S. *Sci. Total Environ.* **2009**, *408*, 1–13.
[9].   Kudryavtsev, A. V.; Perminova, I. V.; Petrosyan V. S. *Anal. Chim. Acta* **2000**, *407*, 193–202.
[10]. Perminova, I. V.; Frimmel, F. H.; Kudryavtsev, A. V.; Kulikova, N. A.; Abbt – Braun, G.; Hesse, S.; Petrosyn, V. S. *Environ. Sci. Technol.* **2003**, *37*, 2477-2485.
[11]. De Nobili, M.; Chen, Y. *Soil Sci.* **1999**, *164*, 825-833.
[12]. Balcke, G. U.; Kulikova, N. A.; Hesse, S.; Kopinke, F. D.; Perminova, I. V.; Frimmel, F. H. *Soil Sci. Soc. Am. J.* **2002**, *66*, 1805–1812.
[13]. Kantor, Ch.; Shimmel, P. Biophysical chemistry. Moscow 1984 (in Russian).
[14]. Trubetskoj, O. A.; Trubetskaya, O. E.; Afanas'eva G. V.; Reznikova, O. I.; Saiz-Jimenez, C. *J. Chromatogr. A* **1997**, *767*, 285-292.
[15]. Aoyama, M.; Karim, S.; Okuyama Y. 15th IHSS Meeting 2010. Puerto de la Cruz, Tenerife, Canary Islands, 27 June - 2 July 2010. P. 27-30.
[16]. Chernysheva, M. G.; Badun, G. A.; Perminova, I. V.; Korobkov, V. I.; Tyasto, Z. A.; Belyaeva, E. Yu.; Kudryavtsev, A. V.; Tsvetkova, E. A.; Kulikva, N. A. Proc. 14th IHSS Meeting 2008, P. 509-512.
[17]. Badun, G. A.; Chernysheva, M. G.; Tyasto, Z. A.; Kulikova, N. A.; Kudryavtsev, A. V.; Preminova, I. V. *Radiochim. Acta* **2010**, *98*, 161-166.
[18]. Badun, G. A.; Chernysheva, M. G.; Konstantinov, A. I.; Kulikova, N. A.; Perminova, I. V. Proc. 15 IHSS Meeting. Puerto de la Cruz, Tenerife, Canary Islands, 27 June - 2 July 2010. P. 132-134.
[19]. Kulikova, N. A.; Perminova, I. V.; Badun, G. A.; Chernysheva, M. G.; Koroleva, O. V.; Tsvetkova, E. A. *Appl. Environ. Microbiol.* **2010**, *76*, 6223–6230.
[20]. Nelsen, F. M.; Eggersten, F. T. *Anal. Chem* **1958**, *30*, 1387-1390.
[21]. Kaszuba, M.; McKnight, D.; Connah, M. T.; McNeil-Watson, F. K.; Nobbmann, U. *J. Nanopart. Res.* **2008**, *10*, 823-829.
[22]. Su, F.; Lu, Ch. J. *Environ. Sci. Health A*, **2007**, *42*, 1543–1552.
[23]. Orlov, D. S.; Grishina, L. A. Handbook of Humus Chemistry; Moscow State University Publisher: Moscow, 1981. (In Russian).
[24]. Dieckmann, G. R.; Dalton, A. B.; Johnson, P. A.; Razal, J.; Chen, J.; Giordano, G. M.; Muňoz, E.; Musselman, I. H.; Baughman, R. H.; Draper, R. K. *J. Am. Chem. Soc.* **2003**, *125*, 1770-1777.