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On the Origin of C_{60} Fullerene Solubility in Aqueous Solution

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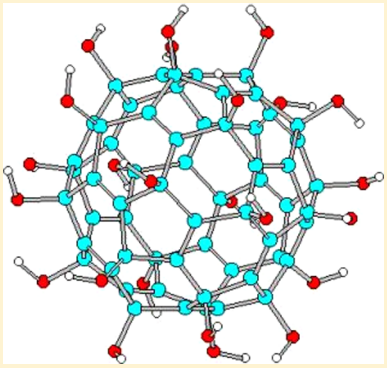
ABSTRACT: In this work, we report that the surface hydroxylation of C_{60} molecules is the most likely mechanism for pristine C_{60} fullerenes/C_{60} fullerene aggregate stabilization in water, being independent of the method of C_{60} fullerene aqueous solution preparation.



INTRODUCTION

Nanocarbon materials are used in a wide variety of biomedical applications, including biosensorics, targeted drug delivery, chemotherapy, cellular imaging, and diagnostics.[1] For example, the pristine C_{60} fullerenes as a unique class of carbon allotropes are able to penetrate the cell membrane,[2,3] to exhibit antioxidant properties,[4,5] and, being nontoxic (at least at low concentration),[6] to exert specific health effects (e.g., antibacterial,[7] antitumor,[8] and drug carrier[9]).

Biomedical applications require a dispersal of C_{60} fullerene in a solvent, with aqueous dispersions being preferred because of biocompatibility, safety, or environmental concerns. Although pristine C_{60} fullerenes have extremely low water solubility, they can form stable colloid solutions containing individual C_{60} fullerenes as well as C_{60} fullerene aggregates (clusters) in water when subjected to extended mixing, sonication, or solvent exchange[10−16] (to be further referred to as a C_{60} fullerene aqueous colloid solution (C_{60}FAS)). However, the origin of stabilization of such particles in water still remains poorly investigated.

At least two approaches have been suggested in order to explain the stability of fullerene particles in water solutions. One of them[17] postulates the formation of a water shell around C_{60} molecules stabilized simultaneously by the H-bonding network between the water molecules and charge transfer from water to the C_{60} fullerene. Another one[18] suggests that the sonication process induces the covalent attachment of water hydroxyls to C_{60} fullerene carbons, resulting in the formation of alcohol moieties that enable C_{60} fullerene dissolution. The problem here is that the properties of C_{60}FAS (including biological properties) as well as the mechanism of water solubility may depend on the method of preparation of C_{60} fullerene aqueous solution.[13,16,17] Indeed, these two approaches differ by the underlying method of C_{60}FAS preparation although the sonication step is presented in both methods. The question therefore arises as to whether the mechanism of solubility of C_{60} fullerene in water depends on the method of C_{60}FAS preparation. This question has been investigated in this article.

EXPERIMENTAL SECTION

Sample Preparation. For the preparation of C_{60}FAS, we used a saturated solution of pure C_{60} fullerene (purity >99.99%) in toluene with a C_{60} molecule concentration corresponding to maximum solubility near 2.9 mg/mL and the same amount of distilled water in an open beaker. The two phases that formed were treated in an ultrasonic bath. The procedure was continued until the toluene had completely evaporated and the water phase became yellow. Filtration of the aqueous solution allowed us to separate the product from undissolved C_{60} fullerenes. The pore size of the filter during the filtration of the aqueous solution was smaller than 2 μm (Typ Whatmann 602 h1/2).

The purity of the prepared C_{60}FAS samples was determined by HPLC and GC/MS techniques using standard protocols. Insoluble impurities in C_{60}FAS were determined by ultracentrifugation: it was found that their amount is less than 1 μg/mL. Toluene from the

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synthesis could not be detected in the water by GC/MS analysis. Moreover, the ^{1}H NMR spectrum (400 MHz) of C_{60}FAS did not reveal any residual proton signals.

The concentration of C_{60} fullerene in the prepared C_{60}FAS sample was determined as the concentration of total carbon in aqueous solution (Analytik Jena TOC analyzer multi N/C 3100). In our experiments, the C_{60}FAS samples with 0.15 and 0.026 (approximately 6 times less) mg/mL concentrations of C_{60} fullerene were used.

Sample AFM Characterization. The state of C_{60} fullerene in water was monitored using atomic force microscopy (AFM, Solver Pro M system, NT-MDT, Russia). In the AFM study, the samples were deposited by precipitation from an aqueous solution droplet onto a cleaved mica substrate (V-1 grade, SPI Supplies). The sample visualization in the AFM experiments was carried out in a semicontact (tapping) mode using NSG10 (NT-MDT) probes.

Sample SANS Characterization. Small-angle neutron scattering (SANS) measurements were carried out at the YuMO small-angle diffractometer at the IBR-2 pulsed reactor (JINR, Dubna, Russia) in the time-of-flight regime. On the YuMO small-angle spectrometer, the two-detector setup with ring wire detectors was used.[19] The neutron wavelength range was 0.05−0.8 nm. The measured scattering curves were corrected for background scattering from buffer solutions. For an absolute calibration of the scattering intensity during the measurements, a vanadium standard was used. Treatment of the raw data was performed by the SAS program with a smoothing mode enabled.[20]

Sample FTIR Characterization. Fourier transform infrared (FTIR) spectroscopy was used to study the structural organization of pristine C_{60} fullerenes in water (0.15 mg/mL). The FTIR spectrum was recorded using a Perkin-Elmer BX-II spectrophotometer with a spectral resolution of 1 cm^{-1} in the range of 4.000−400 cm^{-1}. A C_{60}FAS sample was examined directly as a thin film placed between two NaBr plates by the liquid membrane method. The sample (for comparison) consisting of 20% C_{60} fullerene powder and 80% KBr was compressed (150 kgF) into a tablet with a size of 15 × 15 × 0.1 mm^3.

RESULTS AND DISCUSSION

In this work, we aimed to ascertain whether the mechanism of aqueous solubility of C_{60} fullerene particles may depend on the method of C_{60}FAS preparation. Two representative methods of stable C_{60}FAS preparation have been selected, which have so far been the most well characterized in terms of the morphological properties of the resultant fullerene solution and the mechanism of fullerene aqueous solubility.

Method 1[18,21] is based on prolonged mixing of the C_{60} fullerene powder and water with ultrasonic processing of the mixture and belongs to the group of methods that use extended mixing in water. It has been reported[18] that the aqueous solubility of C_{60} fullerene prepared by this method is a consequence of the formation of C−OH groups on the surface of C_{60} molecules, which facilitates the dissolution.

Method 2[12,13,21] is based on the technology of transferring C_{60} fullerenes from toluene (or other organic solvents) to an aqueous phase with the help of ultrasonic treatment and belongs to the group of solvent-exchange methods of C_{60}FAS preparation. It has been reported[17] that the aqueous solubility of C_{60} fullerene prepared by this method is a consequence of the formation of a stable water shell around fullerene molecules resulting from the H-bonding network and charge transfer from water to fullerene.

We used method 2 to determine whether the formation of C−OH groups may also occur during C_{60}FAS preparation. The first necessary step in such an analysis is the structural characterization of the prepared C_{60} fullerene solution.

Characterization of C_{60}FAS. To characterize the composition of the prepared C_{60}FAS, AFM and SANS measurements were performed.

The AFM picture in Figure 1 corresponds to various concentrations of C_{60} fullerene in water, viz. 0.15 (Figure 1a)

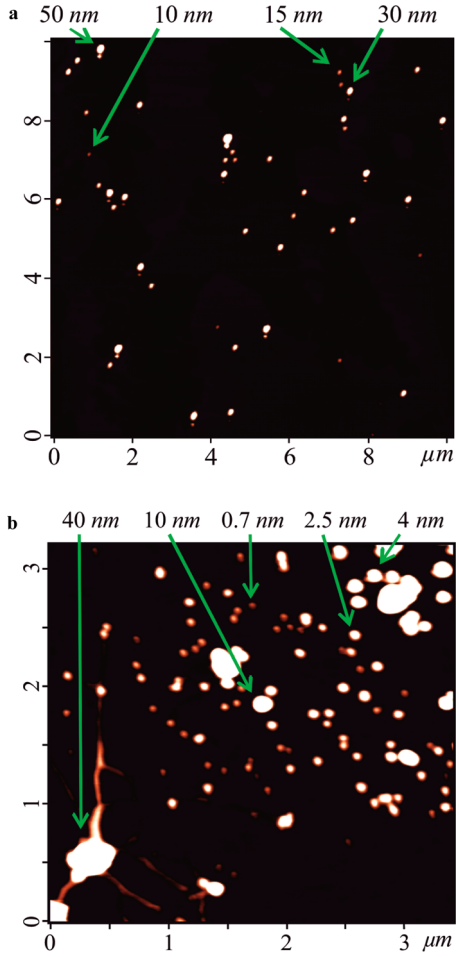

Figure 1. AFM images of C_{60} fullerenes on the mica surface, which were precipitated from the C_{60}FAS system at 0.15 (a) and 0.026 (b) mg/mL concentrations (3 months after preparation).

and 0.026 (Figure 1b) mg/mL. It demonstrates randomly arranged individual C_{60} molecules with a diameter ~0.7 nm and their bulk spherelike aggregates with a height of 2−50 nm.

The experimental SANS curves for the C_{60}FAS system at two C_{60} fullerene concentrations (0.15 and 0.026 mg/mL) are presented in Figure 2a. The absence of specific peculiarities on the curves suggests that the particles are polydisperse in size. It should be noted that a 0.026 mg/mL concentration of C_{60} fullerene is the minimum value at which the SANS signal can be detected. (The signal is close to the background level.) One can see similar SANS curves in the case of a 0.15 mg/mL concentration of C_{60} fullerene after 3 and 6 months of preparation, which points out similar aggregate structures in both solutions. The experimental SANS curves were processed by indirect Fourier transform according to ref 20. The satisfactory fit was obtained only for a 0.15 mg/mL concentration of C_{60} fullerene in the C_{60}FAS system. The pair distance distribution, $P(r)$, inside C_{60} fullerene aggregates, obtained using spherically symmetric basis functions, is shown in Figure 2b. The resulting values of the radius of gyration, R_g = 18.4 ± 0.8 nm (after 6 months of preparation) and R_g = 15.7 ± 0.2 nm (after 3 months of preparation), correspond to the radius for homogeneous spheres equal to 20 nm and are in

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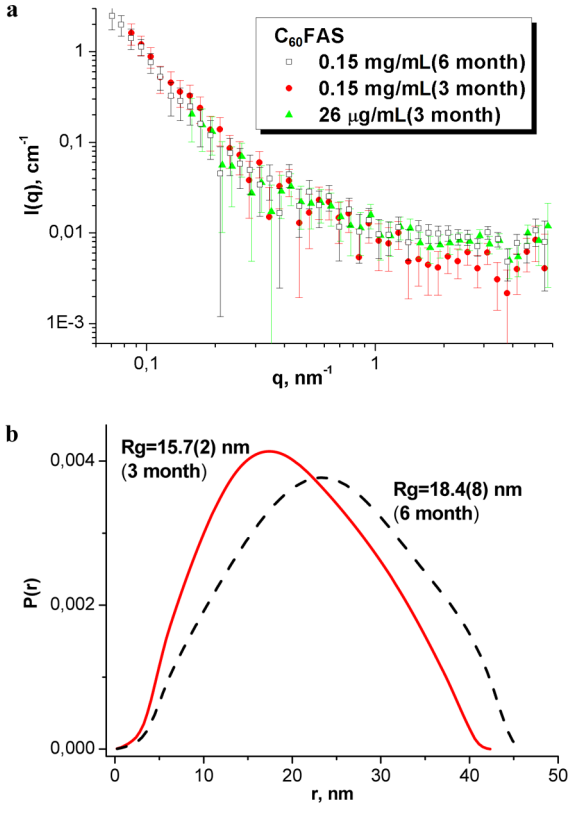

Figure 2. SANS curves (a) for C_{60}FAS systems at 0.15 (squares, 3 months after preparation; and circles, 3 months after preparation) and 0.026 (triangles, 3 months after preparation) mg/mL concentrations of C_{60} fullerene; the obtained pair distance distribution, $P(r)$, (b) for C_{60} fullerene clusters in solution at a 0.15 mg/mL concentration (solid line, 3 months after preparation; and dashed line, 6 months after preparation).

agreement with the aggregate sizes, as seen via AFM (Figure 1a). The measured sizes are slightly smaller than those reported previously[22] using essentially the same method of C_{60}FAS preparation by toluene extraction, which may be associated with different concentrations and/or minor differences in the protocols of preparation employed, as noted before.[23] At the same time, the main morphological and spectroscopic properties of our C_{60}FAS[11,23] are in good agreement with data reported by other authors.[16,21,24]

In summary, the SANS and AFM data indicate that polydisperse C_{60} fullerene aggregates (0.7−90 nm in size) present in the C_{60}FAS system and the prepared fullerene aqueous solution remain stable for quite a long time. These features appear to be similar for different methods of C_{60}FAS preparation.

Origin of C_{60} Fullerene Solubility in Water. To clarify the possible mechanism of C_{60} fullerene hydrophilic character after exposure to water, the FTIR measurements were performed.

The FTIR spectrum of C_{60}FAS (0.15 mg/mL) is shown in Figure 3 together with the reference spectrum of pristine C_{60} fullerene powder. The spectra display four intense peaks at 1423, 1180, 576, and 526 cm^{-1} attributed to C−C vibrational modes of the C_{60} molecule.[25] For the C_{60}FAS system, however, additional peaks are observed: the bands at 3372 and 1666 cm^{-1} correspond to O−H stretching and bending, respectively. But the most interesting feature of the FTIR spectrum of

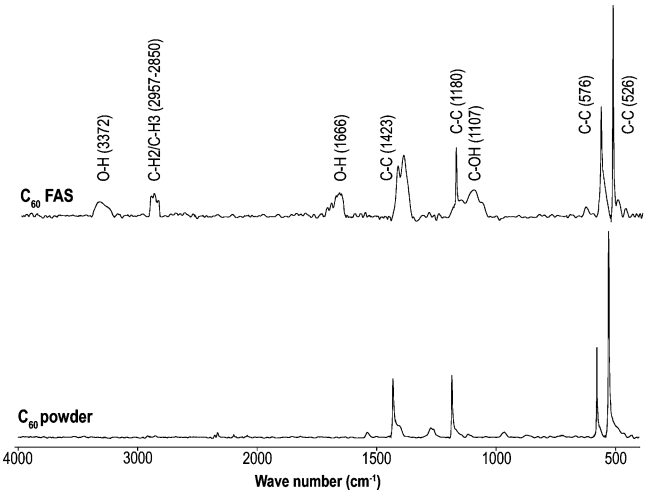

Figure 3. Comparison in the FTIR spectra of the C_{60}FAS system (0.15 mg/mL) and the original pristine C_{60} fullerene powder.

C_{60}FAS is the broad, intense band near 1107 cm^{-1} attributed to C−OH stretching.[26] The FTIR spectrum of C_{60}FAS is also characterized by the C−H stretching bands at 2957−2850 cm^{-1}, which are obviously absent in pristine C_{60} fullerene powder. Given the composition of the C_{60}FAS system, the observed C−O vibrations strongly suggest that the C_{60} fullerene cage is hydroxylated and hydroxyls forming alcohol functional groups exist in the structure of C_{60}FAS.

The hydroxylation of the fullerene cage found in the present work for C_{60}FAS prepared by method 2 is in agreement with the same experimental evidence reported in ref 18 by FTIR for C_{60}FAS prepared by method 1. It strongly suggests that the primary mechanism of C_{60} fullerene solubilization in water is the attachment of the OH groups to C_{60} fullerene carbons, which explains why the lone C_{60} molecules and their clusters exist at equilibrium in solution for quite a long time (see above), a feature that is independent of the method of C_{60}FAS preparation. It also explains the irreversible character of the adsorption/desorption isotherms reported earlier,[27] the minimal extraction of C_{60} fullerene from water−colloid solutions by toluene,[28] and the ability of C_{60} fullerenes from C_{60}FAS to hold water molecules even in vacuum.[17] The covalent attachment of the OH groups does not exclude the possibility of electron transfer from water molecules to C_{60} fullerene, enabling us to explain the negative charge on fullerene particles.[17,18,28] However, the previous model of stabilization of hydrated C_{60} fullerene by water molecules joined together by an H-bonding network[17,23] needs to be corrected in view of the available results of molecular dynamics simulations of hydrated C_{60} fullerenes[29,30] reporting the weakening and breakage of the H bonds between water molecules in the immediate vicinity of the C_{60} fullerene surface as a result of the overbalancing effect of C_{60} fullerene−water with respect to the water−water interaction.

■ CONCLUSIONS

It may be concluded that the surface hydroxylation of C_{60} molecules is the most likely mechanism for pristine C_{60} fullerenes/C_{60} fullerene aggregates stabilization in water, being independent of the method of C_{60}FAS preparation. The general importance of hydroxyls in the state of C_{60} fullerenes in water has been noted by numerous workers in

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the past[17,21,31] for different methods of fullerene aqueous solution preparation, although the exact role played by hydroxyls in the stabilization of C_{60}FAS has so far been unclear. The covalent attachment, revealed in the present work, gives deeper insight into this problem. However, further studies are required in order to clarify the mechanism by which this attachment occurs.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) *Medicinal Chemistry and Pharmacological Potential of Fullerenes and Carbon Nanotubes*; Cataldo, F., Da Ros, T., Eds.; Carbon Materials: Chemistry and Physics; Springer: Dordrecht, The Netherlands, 2008; Vol. *1*.

(2) Foley, S.; Crowley, C.; Smaihi, M.; Bonfils, C.; Erlanger, B. F.; Seta, P.; Larroque, C. Cellular localization of a water-soluble fullerene derivative. *Biochem. Biophys. Res. Commun.* **2002**, *294*, 116−119.

(3) Prylutska, S.; Bilyy, R.; Overchuk, M.; Bychko, A.; Andreichenko, K.; Stoika, R.; Rybalchenko, V.; Prylutskyy, Yu.; Tsierkezos, N. G.; Ritter, U. Water-soluble pristine fullerenes C_{60} increase the specific conductivity and capacity of lipid model membrane and form the channels in cellular plasma membrane. *J. Biomed. Nanotechnol.* **2012**, *8*, 522−527.

(4) Gharbi, N.; Pressac, M.; Hadchouel, M.; Szwarc, H.; Wilson, S. R.; Moussa, F. [60]Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. *Nano Lett.* **2005**, *5*, 2578−2585.

(5) Prylutska, S. V.; Grynyuk, I. I.; Matyshevska, O. P.; Prylutskyy, Yu.I.; Ritter, U.; Scharff, P. Anti-oxidant properties of C_{60} fullerenes in vitro. *Fullerenes, Nanotubes and Carbon Nanostruct.* **2008**, *16*, 698−705.

(6) Johnston, H. J.; Hutchison, G. R.; Christensen, F. M.; Aschberger, K.; Stone, V. The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. *Toxicol. Sci.* **2010**, *114*, 162−182.

(7) Lyon, D. Y.; Brunet, L.; Hinkal, G. W.; Wiesner, M. R.; Alvarez, P. J. J. Antibacterial activity of fullerene water suspensions (nC60) is not due to ROS-mediated damage. *Nano Lett.* **2008**, *8*, 1539−1543.

(8) Prylutska, S. V.; Burlaka, A. P.; Prylutskyy, Yu.I.; Ritter, U.; Scharff, P. Pristine C(60) fullerenes inhibit the rate of tumor growth and metastasis. *Exp. Oncol.* **2011**, *33*, 162−164.

(9) Anilkumar, P.; Lu, F.; Cao, L.; Luo, P. G.; Liu, J.-H.; Sahu, S.; Tackett, N. K., II; Wang, Y.; Sun, Y.-P. Fullerenes for applications in biology and medicine. *Curr. Med. Chem.* **2011**, *18*, 2045−2059.

(10) Brant, J.; Lecoanet, H.; Wiesner, M. R. Aggregation and deposition characteristics of fullerene nanoparticles in aqueous systems. *J. Nanoparticle Res.* **2005**, *7*, 545−553.

(11) Prylutskyy, Yu.I.; Buchelnikov, A. S.; Voronin, D. P.; Kostjukov, V. V.; Ritter, U.; Parkinson, J. A.; Evstigneev, M. P. C_{60} fullerene aggregation in aqueous solution. *Phys. Chem. Chem. Phys.* **2013**, *15*, 9351−9360.

(12) Andrievsky, G. V.; Kosevich, M. V.; Vovk, O. H.; Shelkovsky, V. S.; Vashenko, L. A. On the production of an aqueous colloidal solution of fullerenes. *J. Chem. Soc., Chem. Commun.* **1995**, *12*, 1281−1283.

(13) Duncan, L. K.; Jinschek, J. R.; Vikesland, P. J. C_{60} colloid formation in aqueous systems: effects of preparation method on size, structure, and surface charge. *Environ. Sci. Technol.* **2008**, *42*, 173−178.

(14) Torres, V. M.; Posa, M.; Srdjenovic, B.; Simplicio, A. L. Solubilization of fullerene C_{60} in micellar solutions of different solubilizers. *Colloids Surf., B* **2011**, *82*, 46−53.

(15) Maples, R. D.; Hilburn, M. E.; Murdianti, B. S.; Koralege, R. S. H.; Williams, J.; Kuriyavar, S. I.; Ausman, K. D. Optimized solvent-exchange synthesis method for C_{60} colloidal dispersions. *J. Colloid Interface Sci.* **2012**, *370*, 27−31.

(16) Mchedlov-Petrossyan, N. O. Fullerenes in liquid media: an unsettling intrusion into the solution chemistry. *Chem. Rev.* **2013**, *113*, 5149−5193.

(17) Andrievsky, G. V.; Klochkov, V. K.; Bordyuh, A. B.; Dovbeshko, G. I. Comparative analysis of two aqueous-colloidal solutions of C_{60} fullerene with help of FTIR reflectance and UV-vis spectroscopy. *Chem. Phys. Lett.* **2002**, *364*, 8−17.

(18) Labille, J.; Masion, A.; Ziarelli, F.; Rose, J.; Brant, J.; Villieras, F.; Pelletier, M.; Borschneck, D.; Wiesner, M. R.; Bottero, J.-Y. Hydration and dispersion of C_{60} in aqueous systems: the nature of water-fullerene interactions. *Langmuir* **2009**, *25*, 11232−11235.

(19) Kuklin, A. I.; Islamov, A.Kh.; Gordeliy, V. I. Two-detector system for small-angle neutron scattering instrument. *Neutron News* **2005**, *16*, 16−18.

(20) Soloviev, A. G.; Solovieva, T. M.; Stadnik, A. V.; Islamov, A. H.; Kuklin, A. I. The upgrade of package for preliminary treatment of small-angle scattering spectra. *JINR Commun.* **2003**, *10*, 2003−2086.

(21) Brant, J. A.; Labille, J.; Bottero, J. Y.; Wiesner, M. R. Characterizing the impact of preparation method on fullerene cluster structure and chemistry. *Langmuir* **2006**, *22*, 3878−3885.

(22) Avdeev, M. V.; Khokhryakov, A. A.; Tropin, T. V.; Andrievsky, G. V.; Klochkov, V. K.; Derevyanchenko, L. I.; Rosta, L.; Garamus, V. M.; Priezzhev, V. B.; Korobov, M. V.; Aksenov, V. L. Structural features of molecular-colloidal solutions of C_{60} fullerenes in water by small-angle neutron scattering. *Langmuir* **2004**, *20*, 4363−4368.

(23) Scharff, P.; Risch, K.; Carta-Abelmann, L.; Dmytruk, I. M.; Bilyi, M. M.; Golub, O. A.; Khavryuchenko, A. V.; Buzaneva, E. V.; Aksenov, V. L.; Avdeev, M. V.; Prylutskyy, Yu.I.; Durov, S. S. Structure of C_{60} fullerene in water: spectroscopic data. *Carbon* **2004**, *42*, 1203−1206.

(24) Chen, K. L.; Elimelech, M. Aggregation and deposition kinetics of fullerene (C_{60}) nanoparticles. *Langmuir* **2006**, *22*, 10994−11001.

(25) Kratschmer, W.; Lamb, L. D.; Fostiropoulos, K.; Huffman, D. R. C_{60}: a new form of carbon. *Nature* **1990**, *347*, 354−358.

(26) Lu, C. Y.; Yao, S. D.; Lin, W. Z.; Wang, W. F.; Lin, N. Y.; Tong, Y. P.; Rong, T. W. Studies on the fullerol of C_{60} in aqueous solution with laser photolysis and pulse radiolysis. *Radiat. Phys. Chem.* **1998**, *53*, 137−143.

(27) Labille, J.; Brant, J.; Villieras, F.; Pelletier, M.; Thill, A.; Masion, A.; Wiesner, M.; Rose, J.; Bottero, J.-Y. Affinity of C_{60} fullerenes with water. *Fullerenes, Nanotubes, Carbon Nanostruct.* **2006**, *14*, 307−314.

(28) Deguchi, S.; Alargova, R. G.; Tsujii, K. Stable dispersions of fullerenes, C_{60} and C70, in water. Preparation and characterization. *Langmuir* **2001**, *17*, 6013−6017.

(29) Li, L.; Bedrov, D.; Smith, G. D. A molecular-dynamics simulation study of solvent-induced repulsion between C_{60} fullerenes in water. *J. Chem. Phys.* **2005**, *123*, 204504−204508.

(30) Li, L.; Bedrov, D.; Smith, G. D. Water-induced interactions between carbon nanoparticles. *J. Phys. Chem. B.* **2006**, *110*, 10509−10513.

(31) Rudalevige, T.; Francis, A. H.; Zand, R. Spectroscopic studies of fullerene aggregates. *J. Phys. Chem. A* **1998**, *102*, 9797−9802.