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Structural Features of Highly Stable Reproducible C_{60} Fullerene Aqueous Colloid Solution Probed by Various Techniques

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The method of preparation of highly stable reproducible C_{60} fullerene aqueous colloid solution is described. The structural organization of C_{60} fullerenes in aqueous solution was studied and analyzed in detail using various techniques such as chemical analysis, UV/VIS spectroscopy, atomic force and scanning tunneling microscopy, dynamic light scattering, and zeta potential methods.

Keywords: atomic force microscopy, dynamic light scattering, C_{60} fullerene aqueous colloid solution, chemical analysis, scanning tunneling microscopy, UV/VIS spectroscopy, zeta potential

Introduction

C_{60} fullerenes have been intensively investigated in the last decades mainly because of the vast range of their potential applications in biomedicine (1, 2). Due to its nanometer size, the pristine C_{60} fullerenes are able to interact with biomolecules (3, 4) and penetrate through the cell membrane (5, 6). They exhibit antioxidant properties (7) and, being non-toxic (at low concentration at least) (8–13), exert specific health effects (e.g., suppress the growth of malignant tumors (14, 15)). Although these molecules have extremely low water solubility (16), they form stable colloid solutions containing individual C_{60} fullerenes as well as C_{60} fullerene aggregates (clusters) in water (17, 18), when subjected to extended mixing, sonication, or solvent exchange (19, 20). To understand behavior of C_{60} fullerene in the biological medium (at the levels of cell, tissue and organ), it is necessary to know exactly its concentration in water (dose effect), distribution in size and shape (size effect). Because the biomedical effects of the C_{60} fullerene nanoparticles directly depend on these properties (14, 21, 22), their knowledge will enable understanding of "which form of C_{60} fullerene is bioactive, namely a single molecule or its cluster?"

The aim of this paper is to describe in detail the technology of relatively cheap production of highly stable C_{60} fullerene aqueous colloid solution (C_{60}FAS), comprising the method of preparation and detailed analysis (characterization) of the morphological properties of the prepared solution. It should be noted that the method for obtaining aqueous colloid solution of C_{60} fullerene was first proposed by Andrievsky et al. (23), however, the procedure outlined in the cited paper was not reproducible. Water soluble C_{60} fullerene prepared from toluene is known in literature as nano-C_{60} (9, 10).

Experimental

Method of Preparation of C_{60} Fullerene Aqueous Colloid Solution

Carbon soot was generated by evaporating spectral carbon rods (Fa. Schunk) in a d.c. arc at 24 V in a He atmosphere (0.2 bar). The soot was extracted for 6 h in boiling toluene. Undissolved soot particles were eliminated by filtration. The filtrate was then gently warmed under flowing nitrogen to evaporate the solvent. Preparative separation of C_{60} and C_{70} fullerenes was performed through flash chromatography on silica gel/activated carbon with toluene as eluent. The C_{60} fullerene fraction had a purity of >98%. For further

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separation and analysis the fullerene mixture was dissolved in toluene and fractionated with a preparative high-performance liquid chromatography (Jasco PU-2086) coupled to a multi-wavelength UV/VIS detector (Jasco UV-2077) and an autosampler. A preparative Cosmosil Buckyprep Packed Column with toluene mobile phase was used. The flow rate was set to 20 mL/min. The resultant C_{60} fullerene fraction had a purity of >99.5%. Further purification was done by sublimation of the C_{60} fullerene in a high vacuum (purity 99.99% by HPLC analysis).

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^{-1}, and the same amount of distilled water in an open beaker. The two phases formed were treated in ultrasonic bath. The procedure was continued until the toluene had completely evaporated and the water phase became yellow colored. Filtration of the aqueous solution allowed to separate the product from undissolved C_{60} fullerene. The pore size of the filter during the filtration of the aqueous solution was smaller then 2 μm (Typ Whatmann 602 h1/2).

Different concentrations of C_{60} fullerene in water (from 0.1 to 0.01 mg mL^{-1}) are obtained by this method. The concentration of C_{60} fullerene in the prepared C_{60}FAS sample was determined as the concentration of total organic carbon in aqueous solution and equals to 0.1 mg mL^{-1} ($1.39 \cdot 10^{-4}$ M) (Analytik Jena TOC Analyser multi N/C 3100). The obtained C_{60}FAS is stable for about 12 months at $T = 277$ K.

UV/VIS Measurements

UV/VIS absorption spectrum of C_{60} fullerene in water was recorded using a double-beam spectrophotometer SQ-4802 (UNICO, USA). The solution was poured into a polymethylacrylate cuvette (Spain) having an optical path length of 1 cm, which enabled to perform the measurements in the range of 200–700 nm. The temperature was maintained constant at $T = 298$ K.

AFM and STM Measurements

The state of C_{60} fullerene was monitored using atomic force microscopy (AFM; "Solver Pro M" system; NT-MDT, Russia) and scanning tunneling microscopy (STM; NT-MDT, Russia) technique. Under AFM and STM study the samples were deposited by precipitation from an aqueous solution droplet onto a cleaved mica substrate (V-1 Grade, SPI Supplies) or Au (111) surface (SPI Supplies), respectively. Measurements were performed after complete evaporation of the solvent. The sample visualization in the AFM experiments was carried out in a semicontact (tapping) mode using NSG10 (NT-MDT) probes. Typical values of the tunneling current and voltage in the STM experiments were chosen within the ranges of 0.01–0.1 nA and 0.1–0.8 V, respectively.

DLS Measurements

Measurement of the size distribution for C_{60} fullerenes in aqueous solution was performed by dynamic light scattering (DLS) at $T = 298$ K on a Zetasizer Nano-ZS90 (Malvern, Worcestershire, UK). DLS instrument equipped with a He-Ne laser (max 5 mW) operating at the wavelength of 633 nm, was used.

Zeta Potential Measurements

Zeta potential measurement for C_{60}FAS was carried out on a Zetasizer Nano-ZS90 (Malvern, Worcestershire, UK) at $T =$ 298 K. The results were evaluated using the Smoluchowski approximation, which is known to be rigorously valid only for spherical-like particles.

Results and Discussion

Chemical Analysis of C_{60} Fullerene Aqueous Colloid Solution

The purity of prepared C_{60}FAS sample (i.e., the presence/absence of any residual impurities, for example carbon black, toluene phase) is an important factor, which influences its toxicity (8, 13). The purity of prepared C_{60}FAS sample was determined by HPLC and GC/MS using standard programs. Insoluble impurities were determined by ultra-centrifugation.

Insoluble impurities in the prepared solution were found to be less than 1 μg mL^{-1}. Toluene from the synthesis could not be detected in the water by GC/MS analysis. ^{1}H NMR spectrum (400 MHz) of C_{60}FAS recorded in heavy water did not reveal any residual proton signals.

Absorption Spectrum of C_{60} Fullerene Aqueous Colloid Solution

To confirm the presence of C_{60} fullerene in water, the UV/VIS absorption spectrum was recorded (0.1 mg mL^{-1}) in the range of the wavelengths $\lambda = 200$–700 nm (Figure 1). Three

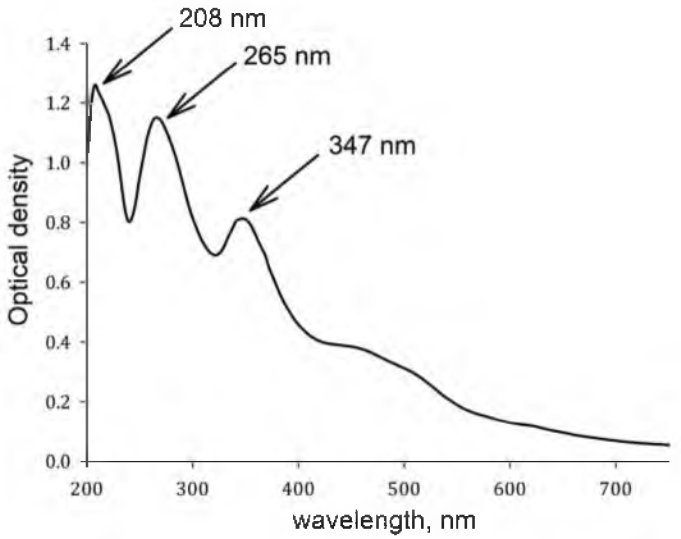

Fig. 1. UV/VIS absorption spectrum of C_{60}FAS (0.1 mg mL^{-1}).

intense broad UV absorption bands with maxima at 208, 265, and 347 nm dominate which is in good agreement with literature data (24).

AFM and STM Characterization of C_{60} Fullerene Aqueous Colloid Solution

In order to additionally characterize the composition of the prepared C_{60}FAS, the state of C_{60} fullerene was monitored using atomic force microscopy and scanning tunneling microscopy technique.

The AFM picture in Figure 2(a) corresponds to the concentration of C_{60} fullerene in water equal to 0.1 mg mL^{-1}. Figure 2(b), which corresponds to ten times less concentration of C_{60} fullerene in water (0.01 mg mL^{-1}), demonstrates randomly arranged individual C_{60} molecules with diameter ~0.7 nm and their bulk sphere-like aggregates with a height of 2–50 nm. Figure 3(a) shows the STM image of surface area covered by lone particles and their clusters. Analysis of the image in the cross-section (Figure 3(b)) indicates that the observed objects have the height of ~0.7 nm, which corresponds to the size of lone C_{60} fullerene. The obtained results are in good agreement with theoretical calculations (25) and literature data from electron microscopy (26, 27).

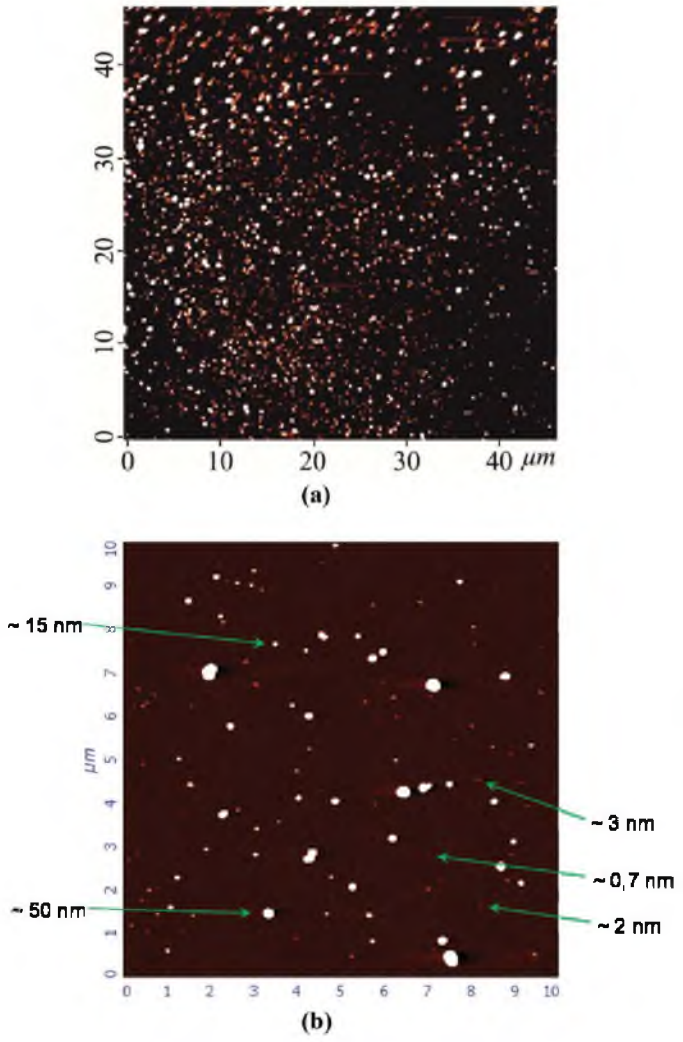

Fig. 2. AFM images of C_{60} fullerenes on mica surface, which were precipitated from C_{60}FAS with 0.1 (a) and 0.01 (b) mg mL^{-1} concentration.

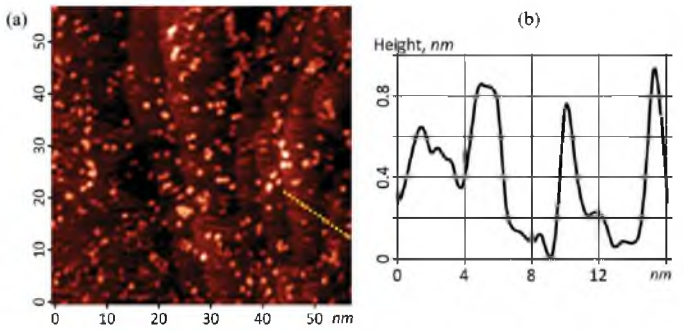

Fig. 3. STM image of C_{60} fullerenes on gold surface (a) and its profile along the marked line (b). C_{60} fullerenes were precipitated from C_{60}FAS with 0.01 mg mL^{-1} concentration.

It is important to note that some individual C_{60} aggregates with a height of ~100 nm were also seen in the probe microscopy images. Numerous experimental investigations have demonstrated the polydisperse nature of C_{60}FAS, including either monomers or aggregates having diameters ranging from several to hundreds of nanometers (26, 28, 29), which fully agrees with our AFM/STM results presented above.

DLS Study of C_{60} Fullerene Aqueous Colloid Solution

A typical result of DLS experiment shown in Figure 4 gives the distribution of light scattering particles according to their hydrodynamic diameters at a fixed solute concentration (0.1 mg mL^{-1}). The main fraction of light scattering particles had diameters in the range of 100 nm. This result is in a good agreement with literature data (30) and the data of laser

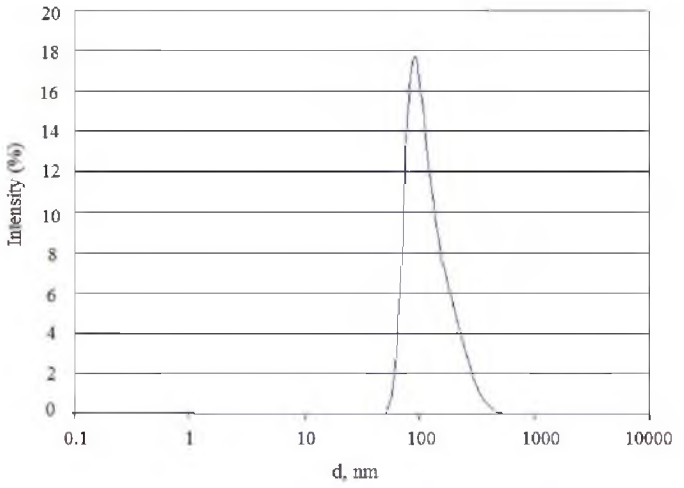

Fig. 4. Distribution of the scattered light intensity according to the diameters of light scattering C_{60} fullerene particles (0.1 mg mL^{-1}).

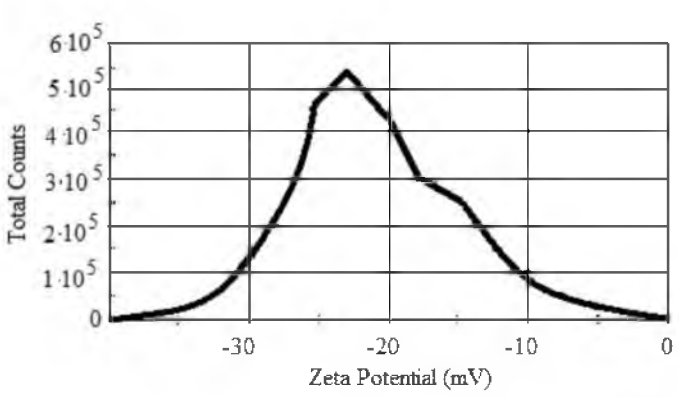

Fig. 5. Zeta potential of C_{60}FAS (0.1 mg mL^{-1}).

correlation spectroscopy of pristine C_{60} fullerene water colloid solution, which confirm that the average hydrodynamic radius of nanoparticles is 50 nm and no further agglomeration is observed (31).

Zeta Potential Study of C_{60} Fullerene Aqueous Colloid Solution

The magnitude of the zeta potential is related to the stability of colloid dispersions, because it determines the degree and nature of the interaction between the particles of the disperse system. The value of zeta potential for C_{60}FAS was equal to −23 mV (Figure 5). This result satisfactory agrees with the previously published data for colloid C_{60} fullerenes in water: −30 mV (24) and −38 mV (32). At the same time, Mchedlov-Petrossyan et al. reported much smaller zeta potential of −9 mV (33). A high negative charge of colloid clusters (or, more strictly, the electrostatic repulsion between the negatively charged clusters) seems to play significant role in the stabilization of C_{60}FAS (i.e., it disfavors the aggregation and makes the solution electrically stable).

Conclusions

The method of preparation of highly stable, purified, and reproducible C_{60} fullerene aqueous colloid solution is described. The morphological properties of the prepared C_{60}FAS have been investigated by means of chemical analysis, UV/VIS spectroscopy, atomic force and scanning tunneling microscopy, dynamic light scattering, and zeta potential methods. It was found that the properties of the prepared aqueous colloid solution well agree with literature data.

The suggested method of preparation is relatively cheap, reproducible and allows getting highly concentrated fullerene solutions ready for further use in various fields of nanobiotechnology.

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