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Evidence of entropically driven C_{60} fullerene aggregation in aqueous solution

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In the present work, we report the first experimental evidence of entropically driven C_{60} fullerene aggregation in aqueous solution, occurring with nearly zero enthalpy change. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4867902]

I. INTRODUCTION

The aggregation of C_{60} fullerenes has attracted much attention during the past decade due to a widely recognized influence of this process on physicochemical and biological properties of the fullerene-containing systems.[1–4] In particular, the link between antibacterial activity and the state and morphology of C_{60} fullerene aggregates in solution has recently been discovered,[5,6] suggesting the need for deeper understanding of physicochemical properties of pristine C_{60} fullerene aqueous colloid solution (C_{60}FAS) and invoking the general question regarding which form of C_{60} fullerene is bioactive, namely, a single molecule or its cluster?[5,7,8] It has been recently established that the aggregates of pristine C_{60} fullerenes provide an excellent template for formation of highly ordered inclusion complexes with other molecules, resulting in amplified physicochemical or biological properties.[9–11]

Although the kinetic and equilibrium aspects of pristine C_{60} fullerene aggregation have so far been characterized relatively well (see Refs. 12–14 and references therein), the knowledge of physical factors which regulate this process in water solution is still quite limited and is mainly based on the results of computer simulations. In particular, a very complex interplay between the van der Waals, electrostatic and hydrophobic interactions on C_{60} fullerene aggregation which presumably does not follow classical hydrophobic effect was shown.[15–17] However, the overall thermodynamic picture of C_{60} fullerene aggregation in aqueous solution still remains unclear.

In the present work, we provide the first experimental evidence suggesting that the aggregation of pristine C_{60} molecules in aqueous solution is a predominantly entropically driven process with negligible net enthalpic contribution.

II. MATERIALS AND METHODS

A. Preparation of pristine C_{60} fullerene aqueous solution

The pristine C_{60} fullerene aqueous colloid solutions (C_{60}FAS) used in the experiments were prepared as follows:[18,19] a saturated solution of C_{60} fullerene (99.5% purity) in toluene was mixed with the same amount of distilled water and the resulted two-phase system was subjected to the ultrasonic treatment until a completion of the toluene evaporation. Afterwards, the yellow-colored water phase was filtered for removing the un-dissolved C_{60} fullerenes. C_{60}FAS was prepared in 0.1 mg/ml (1.39×10^{-4} M) C_{60} fullerene concentration in distilled water.

Preliminary characterization of the obtained C_{60} fullerene products was carried out using UV/VIS spectroscopy in aqueous and toluene solutions. The UV/VIS absorption spectra were measured in the range of the wavelengths, $\lambda = 200$–700 nm, at room temperature. The results (not shown) agree with literature data well, indicating that C_{60} fullerene aqueous solution was formed (see Ref. 18 and references therein).

B. AFM characterization of C_{60} fullerene aqueous solution

The state of C_{60} fullerene in aqueous solution was monitored using atomic force microscopy (AFM; "Solver Pro M" system; NT-MDT, Russia). The sample was deposited onto cleaved mica substrate (V-1 Grade, SPI Supplies) by precipitation from an aqueous solution droplet. Sample visualization was conducted in semi-contact (tapping) mode using NSG10

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(NT-MDT) probes. AFM measurements were performed after complete evaporation of the solvent.

C. Dynamic light scattering study

The size distribution of C_{60} fullerene aggregates was measured by dynamic light scattering (DLS) at various temperatures in the range 15–60 °C on a DAWN® HELEOS™ 243-HHC (Wyatt Technology Corp.) DLS instrument equipped with a Ga-As laser (60 mW) operating at a wavelength of 658.0 nm. The measurements at each temperature point were performed in triplicate and mean value was calculated.

D. Isothermal titration calorimetry (ITC) study

All ITC experiments were performed in water at 25 °C using an AutoITC isothermal titration calorimeter (MicroCal Inc. GE Healthcare, Northampton, USA) with 1.4491 ml of sample and reference cells. The cell containing distilled water was used as reference. The data were processed with Origin 7 from MicroCal. An initial 2 μl was discarded from each data set in order to remove the effect of titrant diffusion across the syringe tip during the equilibration process. The experiment consisted of injecting 10.02 μl (29 injections, 2 μl for the first injection only) of distilled water or solution of C_{60} ($\sim$13.9 μM) into the reaction cell containing distilled water. All the solutions were degassed before titrations were performed. The titrant was injected in 4 min intervals to ensure that the titration peak returned to the baseline prior to the next injection. Each injection lasted 20 s. In order to achieve homogenous mixing in the cell, the stirrer speed was kept constant at 300 rpm.

III. RESULTS AND DISCUSSION

A. Analysis of DLS data

The AFM image (Figure 1) clearly demonstrates randomly arranged, individual C_{60} molecules and their bulk clusters with heights dispersed in the range 10–100 nm. The results obtained indicate polydispersity of the C_{60}FAS used in our study. Output from a typical DLS experiment (Figure 2) shows the distribution of light scattering particles according to their hydrodynamic diameters at a fixed solute concentration and room temperature. The main fraction of light scattering particles is distributed in the diameters range 30–230 nm and generally agrees with the AFM data. Corresponding DLS size distributions and cluster sizes of C_{60} fullerene aggregates were reported previously by different researchers.[12–14,20,21]

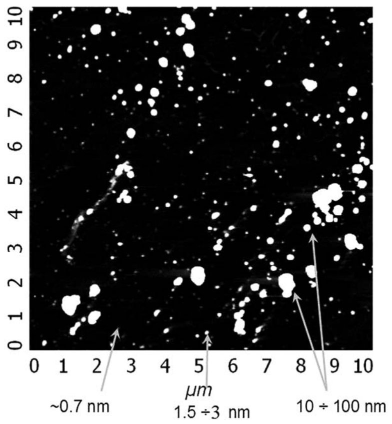

FIG. 1. The AFM picture of the distribution of C_{60} fullerenes in water at the concentration of 0.1 mg/ml.

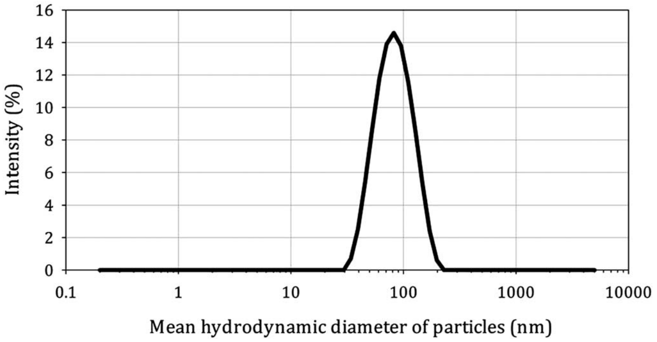

FIG. 2. Distribution of light scattering intensity over hydrodynamic diameters of dissolved C_{60} fullerene clusters.

The temperature dependence of mean hydrodynamic diameter, $d_z(T)$, for C_{60}FAS is shown in Figure 3. The most remarkable feature is the absence of any statistically meaningful trend of d_z with T in the temperature range studied. In order to verify the equilibrium state of C_{60}FAS and the absence of any irreversible changes in C_{60}FAS during the course of experiment, the temperature dependence was recorded in both heating and cooling modes, always resulting in similar diameters of particles, within the error limits. Such behavior may originate from two main reasons:

(i) no aggregation of C_{60} particles occurs in C_{60}FAS, thus leading to constant mean diameter $d_z(T) = \text{const}$. The constancy of $d_z(T)$ is well known for certain types of monodispersed micellar solutions (e.g., see Ref. 22). However, the C_{60}FAS is a highly dispersed system (see above), and the existence of C_{60} fullerene aggregation should be considered as a generally accepted fact (see the Introduction).

However, it should be noted that the constancy of $d_z(T)$ may also be explained in terms of existing of the polydispersed fullerene aggregates in kinetically arrested state under which the raise of temperature in the range studied does not induce dissociation of clusters. This hypothesis originates from the assumption that fullerene aggregation in solution may pass through two phases, viz., the rapid initial cluster formation phase and the slower phase leading to formation of larger clusters.[23] The latter phase may be associated with the kinetically arrested state. If so, DLS titration of concentrated C_{60}FAS should result in nearly unchanged (or random, if the titration procedure may invoke the breakage of clusters) value of mean hydrodynamic diameter. However, the experiment[14] has shown quite apparent and reproducible changes in d_z on concentration

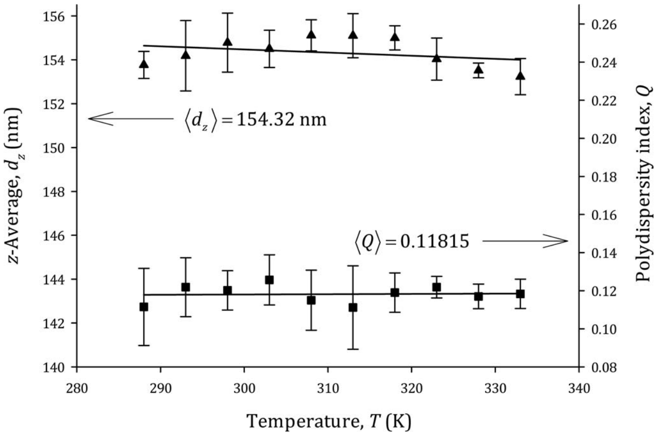

FIG. 3. Temperature dependence of z-average and polydispersity index.

consistent with aggregation. In addition, the estimations of the magnitude of C_{60}FAS equilibrium aggregation constant (see Ref. 14 for more discussion) available in literature fall in the range of values typical for the system under thermodynamic equilibrium.

In summary, it may be concluded that most likely the observed constancy of $d_z(T)$ in Figure 3 is not the manifestation of the kinetically arrested state of fullerene aggregates in solution;

(ii) the aggregation of C_{60} particles occurs in solution with equilibrium constant K,

$$K = \exp\left(\frac{T\Delta S - \Delta H}{RT}\right), \tag{1}$$

where ΔH and ΔS are the changes in enthalpy and entropy. In this case the equilibrium distribution of aggregated particles and the intensity-averaged quantity, d_z, are dependent on K. Hence, if d_z is independent on T, so the K must be independent too. Let us further apply this assumption to Eq. (1).

The derivative of Eq. (1) by temperature under constant pressure gives the condition of constancy of K in the form:

$$\frac{1}{T}\frac{\partial(\Delta H)}{\partial T} - \frac{\Delta H}{T^2} - \frac{\partial(\Delta S)}{\partial T} = 0. \tag{2}$$

Introducing the standard definition of heat capacity change under constant pressure, i.e., $\Delta C_P = \frac{\partial(\Delta H)}{\partial T} = T\frac{\partial(\Delta S)}{\partial T}$, one gets the trivial solution of Eq. (2) as $\Delta H = 0$. It should be noted that this result is dependent on the assumption that the d_z quantity and the dynamic equilibrium in solution are interconnected via the equilibrium constant. An alternative and more direct measure of aggregation in solution is the polydispersity index, Q. It is given as the normalized variance of decay rates for intensity autocorrelation function,[14] and is typically available in software package supporting the DLS measurements. It is seen from Figure 3 that the Q quantity is independent on T, confirming that the aggregation state of C_{60}FAS remains essentially unchanged on temperature variation.

The DLS data obtained above suggest close to zero enthalpy change of fullerene aggregation. This result may be directly verified by measuring thermal effects of fullerene aggregation in ITC study.

B. Analysis of ITC data

In order to check thermal effects of possible C_{60}FAS aggregation we applied ITC. Thermograms representing titration of water with water (control) and water with C_{60}FAS are shown in Figure 4. The obtained data show no essential differences between magnitudes of recorded peaks. Determined thermal effects of titration of water with water and water with C_{60}FAS also do not exhibit significant differences (Figure 5). All determined values of experimental points as well as heat difference obtained by subtraction heat effects mentioned above do not differ and oscillate near zero. The performed ITC analysis indicates no enthalpy changes ($\Delta H \approx 0$) on aggregation of C_{60} particles.

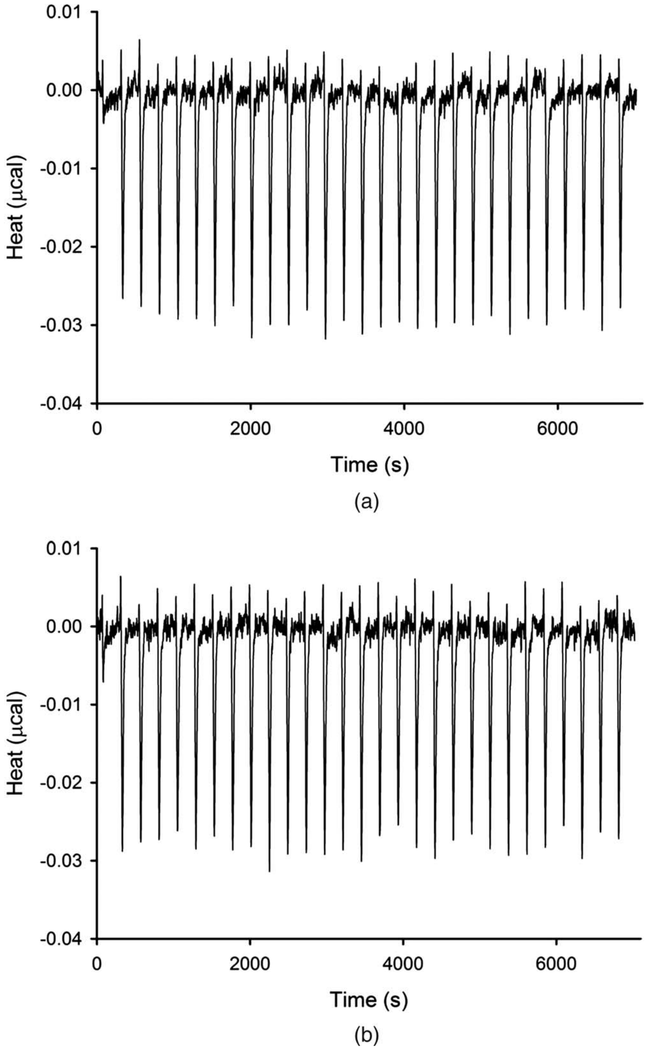

FIG. 4. Microcalorimetric titrations of water with water (a) and water with C_{60} solution (initial concentration ~13.9 μM) with water (b).

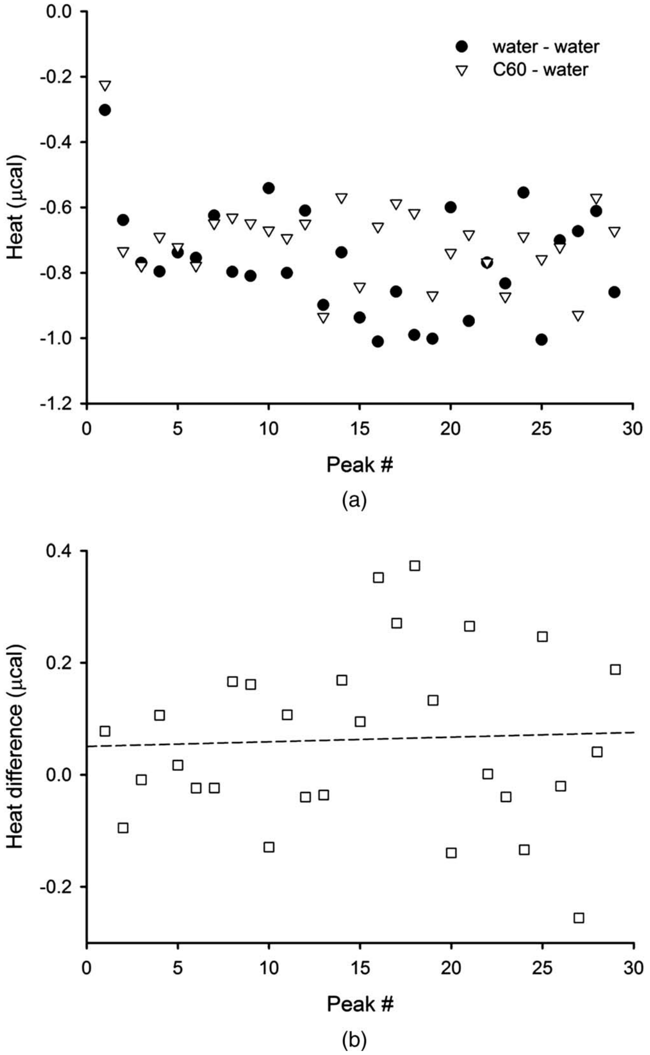

FIG. 5. Thermal effects of microcalorimetric titration of water with water, water with C_{60} solution (initial concentration ~13.9 μM) (a) and the heat differences between thermal effects mentioned above (b). Dashed line represents trend line.

C. Nature of forces governing the fullerene aggregation in aqueous solution

The principal result obtained above from analysis of DLS and ITC data, viz., $\Delta H \approx 0$, enables one to conclude that the observed in Figure 3 independence of the particles' diameters in C_{60}FAS on temperature is likely due the fact that C_{60} fullerene aggregation in solution is an *entropically driven process* with negligible contribution from enthalpy change. The instances of purely entropic aggregation processes are well-known in physical chemistry for micellar solutions (e.g., Ref. 24). We can also cite the only known example related to aggregation of modified C_{60} fullerenes,[25,26] where the dependence of hydrodynamic diameter was also reported to be constant, although no interpretation to this result was provided. The entropic origin of C_{60} fullerene aggregation can also be deduced from the recently reported results of decomposition of the experimentally measured Gibbs free energy change, ΔG, in terms of a sum of energy contributions from various physical factors.[14] The reported value, $\Delta G = -6.5$ kcal/mol (at $T = 298$ K), which is the sum of van der Waals, electrostatic, hydrophobic, and various entropic factors, is characterised by close to zero net effect of the principal enthalpic factors, i.e., $\Delta G_{\text{van der Waals}} + \Delta G_{\text{electrostatic}} = 0\ldots3$ kcal/mol, which is within the limits of the error of computations in Ref. 14. In contrast, among the set of entropic factors (hydrophobic, change in the number of translational, rotational, and vibrational degrees of freedom), the hydrophobic factor was shown to overbalance the change in the number of degrees of freedom. Hence, within the context of the present work, the hydrophobic factor may be treated as the main driving force to C_{60} fullerene aggregation, providing additional support to the hypothesis of entropically driven fullerene aggregation in aqueous solution.

The importance of hydrophobic factor in fullerene aggregation is not unusual and might be expected taking into account that the process occurs in aqueous solution. However, the close to zero enthalpy change could not be expected from the very beginning because the interaction of aromatic surfaces in solution is commonly accompanied by relatively high negative value of ΔH.[27] We may suggest the following rationale in order to explain this observation.

Experimental[18,21,30] and theoretical[16,17] evidences suggest that fullerene aggregation in water proceeds in nonclassical hydrophobic manner featuring exceptionally strong fullerene-water interaction, which presumably overbalances the fullerene-fullerene interactions. Typical manifestations of this situation are the irreversible character of the adsorption/desorption isotherms,[28] the minimal extraction of C_{60} fullerene from water-colloid solutions by toluene,[29] and the ability of C_{60} fullerenes from C_{60}FAS to hold water molecules even in vacuum.[30] This feature enables one to conclude that during formation of fullerene clusters and aggregates in solution the first hydration shell surrounding each fullerene molecule remains essentially unchanged resulting in a contact distance between the surfaces of the neighboring fullerene molecules larger than observed for typical aromatic-aromatic stacking (~0.34 nm). It follows that the contribution of short-range van der Waals forces into the stacking of C_{60} molecules should be much lower as compared to classical π-stacking of aromatic surfaces. Under such conditions the expected enthalpy change may be close to zero and the stabilization of aggregates mainly occurs due to hydrophobically favorable removal of water molecules from the weakly attached second- and higher-level hydration shells. This qualitative model, from our viewpoint, may explain the nature of the observed entropy-driven aggregation of fullerene particles in aqueous solution.

IV. CONCLUSIONS

In the presented research, basing on dynamic light scattering study of the temperature dependence of mean hydrodynamic diameter of C_{60} fullerene clusters in aqueous solution, and isothermal titration calorimetry study of C_{60} fullerene aqueous solution, we report entropically driven C_{60} aggregation proceeding with nearly zero enthalpy change. The origin of the observed effect is considered to be due to exceptionally strong fullerene-water interaction, overbalancing the fullerene-fullerene interactions, and emphasising the importance of hydrophobically favorable water rearrangement in the second- and higher-level hydration shells of fullerene particles. These results deepen our understanding of the nature of factors stabilizing fullerene clusters in aqueous solution.

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