

On the Production of an Aqueous Colloidal Solution of Fullerenes

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The formation of a stable finely dispersed aqueous colloidal solution of fullerenes C₆₀ and C₇₀ with particle sizes $\leq 0.22 \mu\text{m}$ is reported and hypotheses concerning the nature of the aqueous solutions are discussed.

One of the challenges in fullerene chemistry is the production of solutions in polar solvents. A number of recent approaches, based on the assumption of the high hydrophobicity of purely carbon molecules, embrace a traditional set of methods for solubilization of hydrophobic compounds in water: synthesis of more hydrophilic derivatives, formation of host-guest inclusion complexes, *e.g.* with γ -cyclodextrin,^{1,2} generation of sols stabilized by dedicated additives, production of liposomes, micelles, vesicles *etc.* However, the idea of hydrophobicity was closely connected with the early anticipation of chemical inertness of fullerenes, which was found not to be so upon the discovery of a variety of chemical reactions.³ The high electron affinity of fullerenes facilitates not only covalent, but also charge-transfer and donor-acceptor interactions with nucleophilic compounds, which may also extend to water molecules. Parallel with electronic factors, some geometrical parameters may favour the inclusion of fullerenes into a network of water molecules. Based on these considerations, in the present work an attempt to prepare aqueous solutions of fullerenes in the absence of any additives was made, and this resulted in the generation of stable colloidal solutions with fullerene aggregate sizes $\leq 0.22 \mu\text{m}$.

The fullerenes studied were synthesized by the standard Krätschmer method,⁴ followed by toluene extraction in a Soxhlet apparatus. The solid fullerenes were then washed with diethyl ether and purified by recrystallization from toluene. The ratio C₆₀:C₇₀ in this sample was 10:7. Mass spectrometric measurements of all samples were performed using a time-of-flight MSBC mass spectrometer ('Selmi', Sumy, Ukraine).

A solution of fullerenes C₆₀ and C₇₀ in toluene (total concentration 0.2 mg cm⁻³) and deionized water were subjected to supersound under ambient conditions for several hours until the evaporation of toluene was complete.

The obtained solution was filtered through microfilters ('Millipore', USA, pore size 0.22 μm). The resulting transparent brightly coloured solution was brownish-orange and slightly opalescent in reflected light. The latter fact indicated the presence of light-scattering particles so suggesting a colloidal state with particles $\leq 0.2 \mu\text{m}$ in diameter. Some of fullerene was lost in the course of the production and filtration of the colloidal dispersion, and the final concentration of fullerenes may be roughly estimated as 5 $\mu\text{g cm}^{-3}$.

The evaluation of the characteristics of the obtained solution from the point of view of colloidal chemistry⁵ indicated high stability with no essential changes being evident on storing in the absence of light at low temperature for three months. The (nearly neutral) colloidal dispersion was also stable in the pH range 1-10 and was unaffected by boiling. No substantial reverse extraction of the fullerenes by toluene from the colloidal dispersion was observed.

The mass spectrum of the water-solubilized fullerenes, obtained by the ²⁵²Cf PID method (Fig. 1), showed prominent peaks corresponding to C₆₀ and C₇₀ in the same relative intensity as for the toluene solution. There was no indication of modified fullerenes being formed.

Comparison of the aqueous colloidal fullerene solution with aqueous γ -cyclodextrin-fullerene solutions (prepared as in ref. 2) showed similar UV-VIS band positions. Substantial broadening of the bands for both the aqueous colloidal fullerene

solution and the γ -cyclodextrin-fullerene solutions arises due to the presence of light-scattering particles, this being more pronounced for the latter. It should be noted, however, that aggregation of fullerenes *per se* takes place even in true solutions in nonpolar solvents, which was suggested to be a reason for anomalies in the temperature dependencies of their solubility.⁶ γ -Cyclodextrin itself, being in concentrations close to saturation, can form light-scattering aggregates with dimensions of the order of 0.2 μm ,⁷ which may contribute to the band broadening effect.

The ²⁵²Cf PID mass spectrum of the fullerene- γ -cyclodextrin solution is similar to that in Fig. 1 but also shows signals characteristic of γ -cyclodextrin (γ -CD) *viz.*, γ -CD·Na⁺, γ -CD·K⁺ and 2(γ -CD)·Na⁺. No peaks corresponding to the expected inclusion complexes were detected. Obviously the excitation energy transferred to the sample during ²⁵²Cf PID is sufficient for destruction of host-guest complexes bound by weak van der Waals forces; more stable hydrogen-bonded associates, *e.g.* the dimer of γ -CD itself, survive during desorption and ionization.

The possibility of the re-solution of the dried (under vacuum) solutes was checked. While the aqueous solution of the precipitate of γ -CD-fullerene obtained from toluene solution gave no meaningful signals, the mass spectrum corresponding to the water re-solute (Fig. 2) showed weak fullerenes signals. This indicated that 'washing' of fullerenes with water from the solid precipitate was assisted by γ -CD. The precipitate from solutions in the absence of γ -CD failed to redissolve in water.

The main aim of solubilization of fullerenes in water is to enable studies of their interactions in aqueous media, particularly with biomolecules, to elucidate their biological activity. Thus the colloidal aqueous fullerene solution was tested with phosphatidylinositol (PI), a component of biomembranes. In the ²⁵²Cf PID mass spectrum of a dried aqueous solution of the

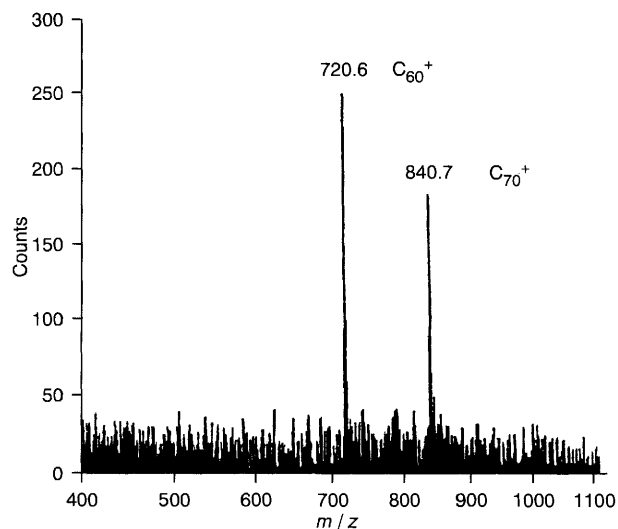


Fig. 1 ²⁵²Cf Plasma desorption mass spectrum of water-solubilized fullerenes, obtained from a droplet of solution, dried as a thin film on the gilt sample holder

fullerenes with PI, weak signals of fullerene-PI (1:1) clusters were observed, which indicates a stronger binding of the fullerene with PI relative to γ -CD. Interestingly, the pattern of mass spectrometric fragmentation of PI differed from that of pure PI, which indicates a change of conformational and electronic parameters of PI caused by complexation with fullerenes. These changes may be due to donor-acceptor interactions of fullerenes with the polar head of PI. A more detailed study of such complexes may form a basis for understanding the recently reported observations of incorporation of fullerenes into membranes.^{8,9}

The observed non-covalent interaction of fullerenes with PI indicates the reactivity of aqueous colloidal fullerene solutions and their incorporation into complexes with PI as single molecules.

While the formation of colloidal solutions of otherwise insoluble substances under supersonic treatment is no surprise, the high stability of the very finely dispersed colloidal fullerenes is of more significance. Sensitive mass spectrometric measurements show no detectable admixtures in the system (except traces of alkali metal ions Na and K), which may serve as colloid stabilizers. This stabilization presumably arises from fullerene-water interactions. Ultrasonic treatment, which pro-

duces high pressure during cavitation,¹⁰ may facilitate the inclusion of fullerenes into cavities in the water structure¹¹ and formation of clathrate-like networks of water molecules¹² around fullerenes and their aggregates, stabilized due to the low conformational mobility of fullerenes and geometrical matching between the structures, which may be formed by hydrogen bonding of water molecules in the clathrate and covalent bonds of the fullerene carbon atoms.¹³⁻¹⁵

In parallel with geometrical factors, the electronic properties of fullerenes may lead to the possibility of donor-acceptor and charge-transfer interactions^{3,16,17} which may promote weak intermolecular water-fullerene interactions. Such interactions have been invoked to explain peculiarities of fullerene behaviour in other solvents.^{16,17}

To summarize, this work has demonstrated the formation of extremely stable finely dispersed colloidal aqueous solutions of fullerenes and the possibility of utilization of such solutions in biochemical-biophysical studies.

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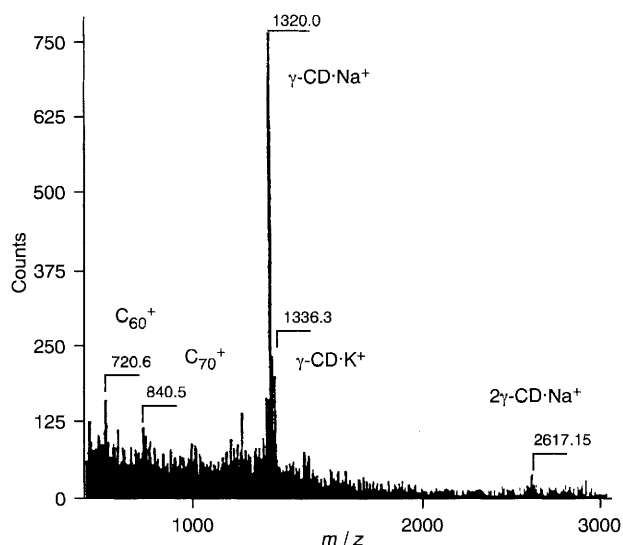


Fig. 2 ²⁵²Cf Plasma desorption mass spectrum of the water re-dissolved solid precipitate of γ -CD-fullerene complexes, obtained by sonication of a mixture of a toluene-fullerene solution and a water-ethanol solution of γ -CD under ambient conditions until evaporation of toluene was complete, followed by filtration through 0.3 μ m microfilters