Electron microscopy study of amino acid derivatives of [60]fullerene in non-aqueous solution

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It has been shown that in non-aqueous solution amino acid derivatives of [60]fullerene form associates.

Recently, by application of Scanning Electron Microscopy (SEM) and Scanning Tunneling Electron Microscopy (STEM) we showed that water soluble amino acid and peptide derivatives of [60]fullerene form oval shape associates in aqueous solutions, their size depended on the nature of the amino acid (peptide) and the concentration of the solution.[1,2] It has been found that these associates represent anisometric particles varying in diameter from 0.5 to 10 μm. Upon strong dilution, some of the amino acid (peptide) derivatives of [60]fullerene studied give true solutions.

A number of amino acid derivatives of [60]fullerene such as *p*-aminobenzoic acid, ω-aminocaproic acid and proline ($HC_{60}NHXCOOH$) ones are water-insoluble but are soluble in organic solvents (pyridine, DMSO, DMF). Interestingly, none of these solvents dissolve [60]fullerene. From this, the conclusion might be drawn that amino acid (peptide) derivatives are present in organic solutions in the form of associates.

To ascertain this, we again used the SEM and STEM methods. The study was carried out on a Hitachi S-2500 Scanning Electron Microscope operating in a secondary electron mode (resolution 3.5 nm) and on a PTM-1 scanning tunnelling microscope (made in St. Petersburg, Russia) working in profilometer mode with a vertical resolution of 10 nm. The latter instrument provides the possibility of obtaining a 3D-image with which to study the surface profile by tracing the surface of the object at any chosen region.[3] The simultaneous application of both instruments allows one to obtain information on the 3D-structure of associates.

Preparation of the samples for SEM and STEM was carried out by two methods: (i) by evaporating the solution under air and (ii) by a conventional freeze-drying procedure usually employed in the electron microscopy of biological objects and polymeric systems.[4,5] The application of a cryogenic technique allows one to avoid the aggregation of particles that can be caused by the action of surface tension during the air drying procedure. Besides, a low temperature fixation of the sample structure excludes the effect arising from concentration changes when the solution is dried in air. However, the cryogenic method cannot prevent the occurrence of artefacts. Thus, it would be appropiate to use both preparation methods.

This study revealed that all [60]fullerene derivatives studied, *i.e.* ones of *p*-aminobenzoic acid **1**, ω-caproic acid **2** and proline **3** formed associates when dissolved in pyridine and DMSO. The structure of the associates is of two different types, either oval or spherical with sharp borders and definite sizes or representing aggregates consisting of plates or other groups positioned in disorder and differing in size and shape even for the same object. The size of the latter particles varies mostly from 0.3 μm for **2** and **3** to 4 μm for **1**, larger associates of up to 15 μm occasionally occur as seen in Figure 1 for compound **1** dried in air from pyridine solution. However, the effect of increasing concentration may have occurred in this case since the evaporation took rather a long time.

Figure 2 shows the electron microphotograph of the compound **1** associate obtained from DMSO solution. The

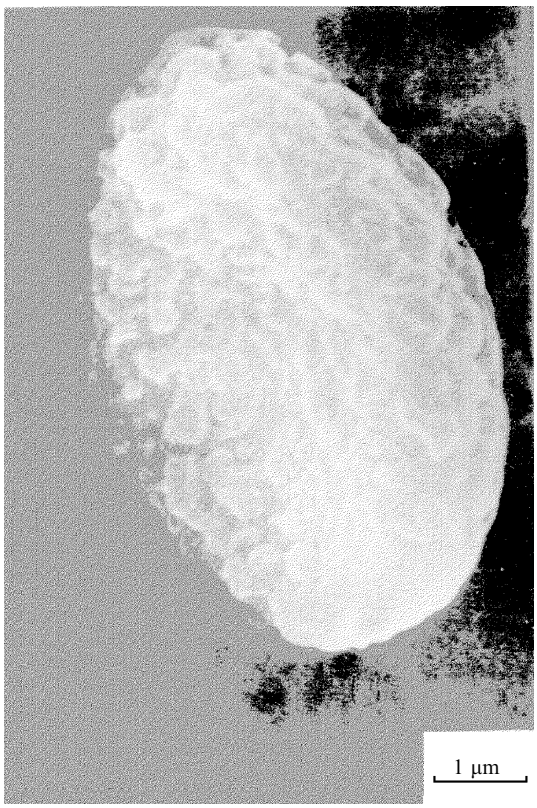

Figure 1 Electron micrograph of [60]fullerene *p*-aminobenzoic acid in pyridine (dried in the air).

Figure 2 Electron micrograph of [60]fullerene *p*-aminobenzoic acid in DMSO (dried in the air).

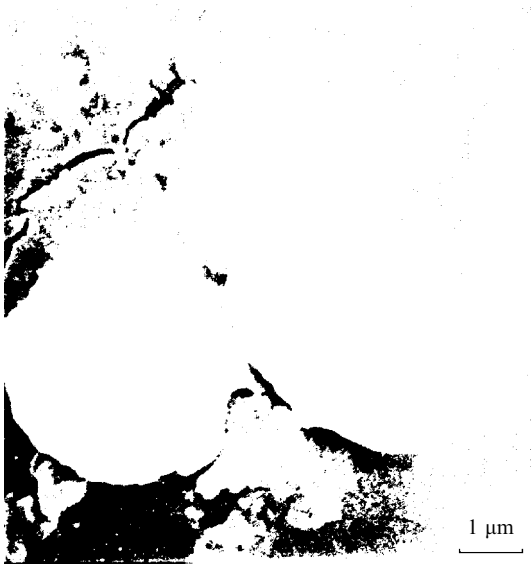

Figure 3 Electron micrograph of [60]fullerene *p*-aminobenzoic acid in pyridine (freeze-drying).

analogous photograph of **1** obtained from pyridine-solution is presented in Figure 3, with the oval shape associates and plate aggregates being seen distinctly. Interestingly, in contrast to water-soluble [60]fullerene derivatives, in organic solvents associates do not form aggregates constituted from chains of 2–3 associates even in those areas where they are located closely.[6] The lamellae structure aggregates observed in all the samples studied (Figure 4) concealed small size associates which occurred especially often for solutions of **1** and **2** and possessed a spherical form with a diameter of 0.3–0.7 mm and less (Figure 5).

For the large associates, the inner structure can sometimes be disclosed. Thus, Figure 1 demonstrates that the associates consist of 0.5–1 mm lamellae, showing these plates to be closely packed. This notion of the structure is supported by the data obtained by means of STEM (Figure 6).

The STEM data showed that upon applying the drying-in-air procedure to the compound **1** solution, the associates observed had a diameter to thickness ratio of 1 : 2 or 1 : 3 whereas the different size associates of amino acid derivatives of [60]fullerene in aqueous solutions are characteristic of 1 : 2 or 1 : 10 diameter to thickness ratios.

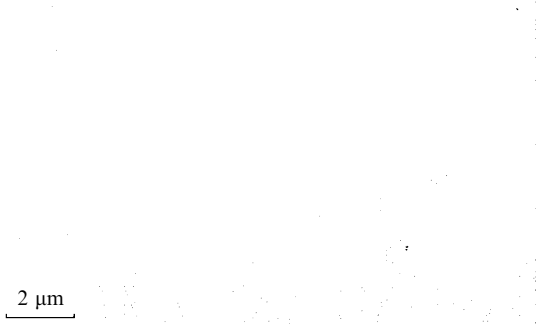

Figure 4 Electron micrograph of [60]fullerene *p*-aminobenzoic acid in pyridine (cryogenic method).

Figure 5 Electron micrograph of [60]fullerene proline in pyridine (cryogenic method).

This indicates that organic solutions of compound **1** dried in air brings about a flattening which is less than in the case of aqueous solutions of amino acid derivatives of [60]fullerene.

The SEM and STEM results provide grounds to consider the compound **1**–**3** associates in DMSO solution as ones consisting of closely packed lamellae while water soluble amino acid derivatives form more friable aggregates.

The present and previous studies allow the following conclusions to be drawn:

(1) Amino acid and peptide derivatives of [60]fullerene form associates both in aqueous and organic solutions.

(2) The structure of associates in aqueous solution differs from that in organic solution: associates are thicker and their shape is more spherical in organic solvents as compared with water systems where they predominantly exist as oval shape particles.

(3) The structure of associates in organic solvates depends on the nature of the starting amino acid and the solvent.

This investigation was financially supported by the Russian Foundation for Basic Research (grant no. 95-03-08417), Russian Foundation of the Scientific-technical program 'Fullerene and Atomic Clusters' and the International Scientific Technical Centre program no. 079.

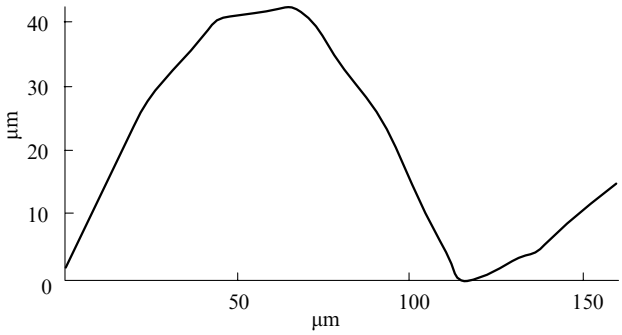

Figure 6 Profilogramme of associates of [60]fullerene *p*-aminobenzoic acid in pyridine.

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Received: Moscow, 1st March 1996
Cambridge, 15th April 1996; Com. 6/01592A