Water-soluble [60]fullerene compositions with carbohydrates

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10.1070/MC2001v011n05ABEH001459

Water-soluble compositions of [60]fullerene with carbohydrates were mechanochemically synthesised and investigated by UV-VIS and solid-state high-resolution ^{13}C NMR spectroscopy.

For using fullerenes in biology and medicine, they should be converted into a water-soluble state by means of complexing agents. At present, several water-soluble fullerene-containing compounds have been obtained. These are [60]fullerene complexes with γ-cyclodextrin,[1,2] polyvinylpyrrolidone[3,4] water-soluble calix[*n*]-arenes,[5,6] poly(vinyl alcohol)[7] and tetraphenylporphine with polyvinylpyrrolidone.[8] Moreover, the preparation of stable colloid aqueous solutions of [60]fullerene with no stabilizing agents has also been described.[9,10]

However, no information is available on the products of fullerene interaction with low- and high molecular weight carbohydrates such as dextran, which is known in medicine as an effective blood plasma substitute. The probable reason is the absence of common solvents for fullerene and carbohydrates, making it possible to mix the components homogeneously and to investigate the products of their interaction.

The aim of this work was to obtain water-soluble [60]fullerene compositions with oligosaccharides and polysaccharides (dextran with a molecular weight of 40 kDa), to study the state of fullerene, and to find optimal conditions for the formation of a water-soluble product with carbohydrates characterised by different molecular weights.

To obtain fullerene compositions with carbohydrates, a modified mechanochemical method[7] was used. A carbohydrate was suspended in a fixed volume of [60]fullerene solution in CCl_4 at a concentration of 0.1 mg ml^{-1}. After evaporating the solvent in air, the mixture was ground in an agate mortar and then dried in a vacuum at 40 °C for 48 h to remove CCl_4 traces. The [60]fullerene content of the dry mixture with the carbohydrate ranged from 0.1 to 30%. A known volume of water was added to the finally dispersed powder; the mixture was stirred for 1 min and centrifuged; then, the solution was separated from the precipitate by decantation.

The water-soluble part of the composition was investigated by UV-VIS spectroscopy on a Specord M40 spectrophotometer.

High resolution solid-state ^{13}C NMR spectra were obtained at room temperature on a Bruker CPX-100 spectrometer using magic angle spinning (MAS), and cross-polarisation (CP MAS) techniques. The working frequency of ^{13}C NMR was 25.18 MHz, and the sample spin rate was 3–4 kHz. Tetramethylsilane (TMS) was used as reference.

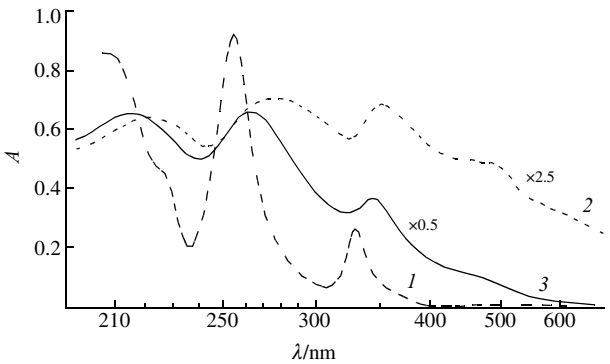

Figure 1 Absorption spectra of (*1*) a [60]fullerene solution in hexane (*2*) a [60]fullerene film on quartz and (3) an aqueous solution of sucrose–[60]fullerene composition (solid-phase [60]fullerene concentration is 2 wt%).

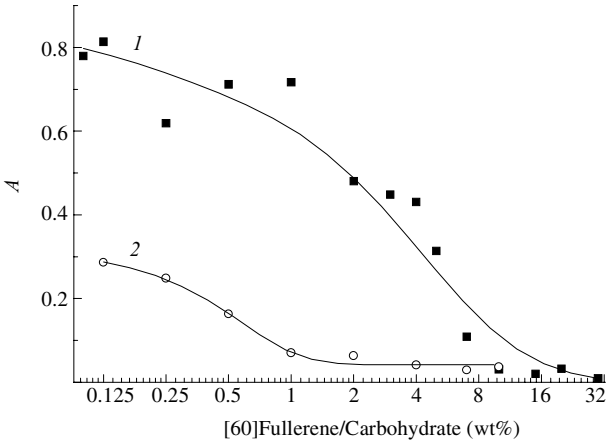

Figure 2 Absorbance (330 nm) of [60]fullerene in an aqueous solution as a function of [60]fullerene concentration in solid-phase compositions with (*1*) sucrose and (*2*) dextran (40 kDa).

The concentration and the molar absorption coefficient of water-soluble [60]fullerene were determined photometrically by isolating fullerene from aqueous solutions by TLC on silica gel plates prewashed with MeOH using $CHCl_3$ as an eluent. Under these conditions, carbohydrate complexes were decomposed and the fullerene remained entirely at the solvent front. Subsequently, the part of the silica gel layer containing fullerene was transported to a Schott filter, [60]fullerene was desorbed from silica gel wih CCl_4, the solution obtained was photometrically examined at λ = 328 nm. The concentration (C) and molar absorption coefficient (ε) of fullerene passed into water were determined. The RSD for C and ε was better than 15%.

Figure 1 shows the electronic spectra of a [60]fullerene solution in hexane, the spectra of its films on a quartz support and the spectra of an aqueous solution of a [60]fullerene composition with sucrose. The spectra of the composition and the film are similar and greatly differ from that of fullerene. The bands are wider, the bathochromic shifts of band maxima is observed, the ratio of their intensities change and new spectral features in the long-wave region appear. A similar bathochromic shift and band widening in the spectra of solutions of the products of [60]fullerene interaction with γ-cyclodextrin and liposomes was attributed previously[8] to the presence of [60]fullerene aggregates in solutions. Similar results were obtained for [60]fullerene–pyridine–water mixtures,[9] which were explained by the formation of monodisperse, spherical, and chemically inert [60]fullerene nanocapsules.

The absorbance of solutions depends on the fullerene content of the initial composition (Figure 2). The solutions obtained from a composition with a high fullerene content (10% or higher) and, correspondingly, with a lower carbohydrate content, are characterised by low absorbance. This is probably due to a low concentration of fullerene passed into solution (for sucrose, less than 1×10^{-5} mol dm^{-3}). The increasing carbohydrate fraction in the composition at a fixed quantity of [60]fullerene leads, first, to a drastic and then to a gradual increase in absorbance. When the sucrose content of the solid-phase composition is higher than 99.7 wt%, the fullerene concentration in water increases

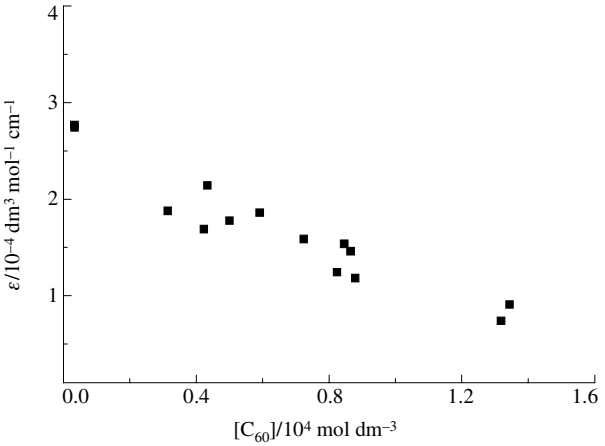

Figure 3 The concentration dependence of the molar absorption coefficient (ε) of water-soluble fullerene in aqueous solutions of a [60]fullerene–sucrose composition (λ = 440 nm).

up to (1–2)×10^{-4} mol dm^{-3}. The absorbance and hence concentration are lower for dextrans than for sucrose.

To better understand the properties of the products, the concentration dependence of the molar absorption coefficient of water-soluble fullerene was studied using the above technique (Figure 3). The molar absorption coefficient increases with decreasing the concentration of fullerene passed into water. This indicates that the ratio of components passed into solution from the compositions with different carbohydrate contents is not constant. A maximal concentration of fullerene in water at its concentration of 4% in the solid-phase composition was attained with the use of sucrose, and it was 5×10^{-4} mol dm^{-3}.

The concentration dependence of absorption, the UV-VIS spectra of water-soluble fractions (broadening and shift of fullerene bands in the UV region and new spectral features in the long-wave range) show that fullerene passes into water as aggregates. They exhibit high stability and do not form smaller fragments when the solution is diluted. It is impossible to extract fullerene from aqueous solutions by organic solvents.

The solid-state ^{13}C MAS NMR spectrum of a composition

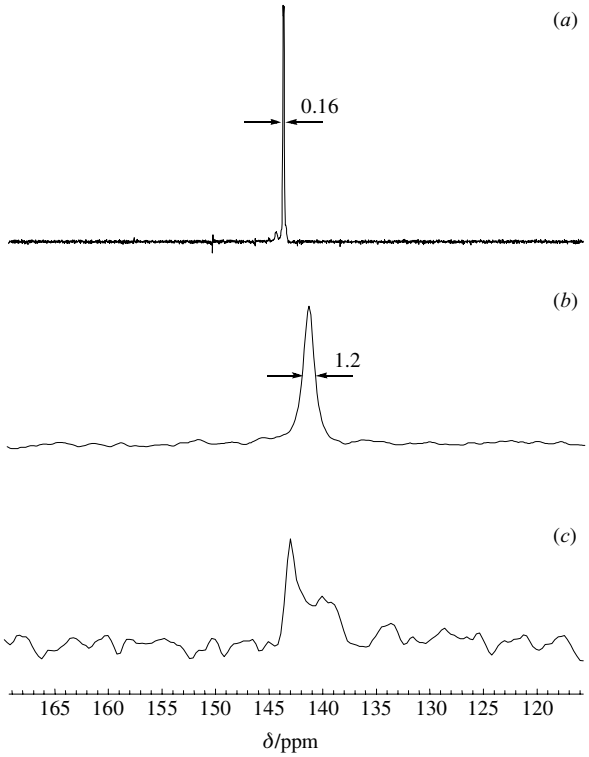

Figure 4 High-resolution solid-state ^{13}C NMR spectra of (*a*) [60]fullerene, (*b*) and (*c*) sucrose–[60]fullerene composition (20 wt%) in MAS (*a*), (*b*) and CP MAS (*c*) modes.

with dextran contains a signal characteristic of [60]fullerene (143.6 ppm). The half-width of the line increased to 1.2 ppm, whereas for pure fullerene it is 0.16 ppm. The spectrum of the same composition obtained by a CP MAS technique also contains a [60]fullerene signal, which indicates that proton-containing groups are located near the fullerene surface.

In the spectra of the composition with sucrose, the [60]fullerene line in the MAS spectrum is broadened (1.2 ppm) and shifted upfield (δ_{MAS} 141.3 ppm). Moreover, in the CP MAS spectrum, a new low-intensity peak appeared at the higher magnetic field ($\delta_{CP\,MAS}$ 139.2 ppm). The line splitting and upfield shift of the [60]fullerene signal in CP MAS can be interpreted by the interaction of fullerene with hydroxyl groups in the carbohydrate. This suggestion is consistent with published data,[2] according to which the interaction in the [60]fullerene complex with γ-cyclodextrine takes place *via* oxygen atoms including those in hydroxyl groups. This interaction is of the donor–acceptor character.[2] It was found[13,14] that when [60]fullerene is included in capsule-like cage molecules (palladium-linked bis-porphyrins and homooxacalix[3]arene dimeric capsules) the ^{13}C NMR spectra also exhibit an additional upfield line of encapsulated [60]fullerene. The NMR data confirm the fullerene interaction with the carbohydrate in the composite.

In conclusion, water-soluble compositions of [60]fullerene with sucrose and dextran (40 kDa) were prepared mechanochemically. Sucrose is a better solubilising agent for [60]fullerene than dextran.

Two types of interactions occurred in the test samples: fullerene–carbohydrate (complexation) and fullerene–fullerene (aggregation). The stability of an aqueous solution of fullerene with respect to [60]fullerene extraction by such solvents as toluene and chloroform suggests that fullerene passes into solution in the form of aggregates in a carbohydrate shell. Moreover, the surface carbohydrate layer forms a van der Waals complex with fullerene.

This work was supported by the Russian Research and Development Programme ‘Fullerenes and Atomic Clusters’ (grant no. 94053).

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Received: 3rd April 2001; Com. 01/1785