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Investigation of Encapsulation and Solvatochromism of Fullerenes in Binary Solvent Mixtures

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Fullerenes, when dissolved in certain binary solvent mixtures are found to exhibit strong solvatochromism and an unusual chemical inertness. From UV−vis optical absorption measurements, dynamic light scattering (DLS) size measurements and chemical tests on C_{60}/pyridine/water mixtures, we suggest that the origin of these unusual effects is the formation of monodispersive, spherical, and chemically inert C_{60} nanocapsules. Pyridine appears to act as a surfactant around fullerene molecules protecting them from chemical reagents. The hydrophobic surfactant interactions are thought to act in stabilizing the structures into chemically inert uniformly sized particles.

Introduction

Fullerenes and their derivatives have shown a very diverse range of properties, ranging from high-temperature superconductivity[1] to ferromagnetism.[2] More recently, the possibilities for their introduction into biological systems have been investigated and their use as possible HIV inhibitors has even made an impact in medical research.[3] Although C_{60} is insoluble in water, dissolution may be accomplished via derivitization,[3] by using water-soluble polymer[4] surfactant solutions containing micelles or liposomes[5,6] or by incorporation in water-soluble complexes such as γ-cyclodextrine (γ-CD).[7] In the latter case, at low γ-CD to C_{60} concentration ratios, solvatochromism is observed which is thought to arise from clustering of several C_{60} molecules surrounded by γ-CD. Solvatochromism is also observed in toluene−acetonitrile mixtures containing C_{70} in which, above a certain volume fraction of acetonitrile, a color change from reddish orange to pinkish purple[8] is observed. In this case also, the formation of fullerene clusters was suggested to be responsible for the observed changes in the optical absorbance. Subsequent light scattering studies confirmed the presence of clusters of various sizes ranging from 100 to 1000 nm, depending on the concentration of the fullerene.[9] The reversible aggregation of C_{60} is also observed at higher concentrations of C_{60} in neat benzonitrile. Evidence for the formation of C_{60} aggregates with a particle size distribution of about 100−500 nm is obtained from DLS and electron microscopy experiments.[10]

Here we report on a somewhat different type of solvatochromism which occurs when a strongly polar solvent (e.g., water) is added to a solution of fullerenes in pyridine or a similar solvent. The solvatochromism is usually quite dramatic, and the pyridine solution changes from a mauve color to clear lemon−yellow. We establish that the solvatochromism is associated with

the formation of chemically inert water-soluble C_{60}/pyridine "nanocapsules" in which the pyridine appears to act as a surfactant around the fullerene molecules protecting them from chemical reagents. We report on size measurements of such nanocapsules as a function of temperature, pH, and ionic strength using dynamic light scattering (DLS). We also report on chemical tests of the reactivity of such particles and discuss their structure.

Experimental Details

The organic solvents used in these experiments were generally spectroscopic grade (pyridine (Merck)) and were redistilled prior to use. C_{60} was purchased from Hoechst (Gold Grade Quality) and ^{13}C-enriched C_{60} from MER Corporation (10−15% ^{13}C, +99% C_{60}). For all experiments and measurements, a freshly prepared solution of C_{60} was used. H_2O with the resistivity of 10 MΩ was obtained from a deionized water system. Solutions with different values of pH were prepared by diluting an appropriate volume of standard solutions prepared from concentrated reagents (HCl, NaOH (0.1 mol/L, Titrial (Kemika)).

UV−vis spectra were recorded in a 10 mm path length cell on a HP 8453 UV−vis spectrophotometer at 23 ± 1 °C. Small amounts of water (5 μL) were added directly to pyridine (py) solutions of C_{60} in a quartz cell (volume 1.5 mL with concentration of C_{60} 0.05 mg/mL) by micropipet, and after the temperature of mixture had stabilized, the spectra were recorded.

DLS experiments were performed with a He−Ne laser (λ = 632.8 nm), and the intensity correlation function of scattered light was obtained with an ALV 5000 correlator. Pyridine solutions of C_{60} and distilled water are filtered through suitable micrometer sized filters to remove dust particles and microcrystalites. For all the measurements described in this paper, the same C_{60} concentration of 0.5 mg/mL pyridine was used. For the DLS measurements, 50 μL of the pyridine solution of C_{60} was added by micropipet to 5 mL of water and the spectra recorded after 1 min. For chemical reactions hydrazine mono-

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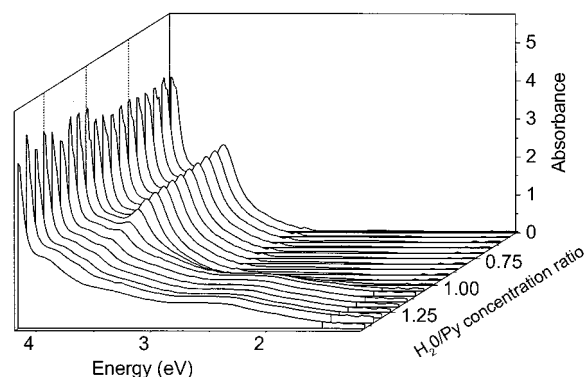**Figure 1.** UV−vis absorption spectra for C_{60}/py/water at different molar concentration ratios $C = C_{water}/C_{py}$. The C_{60}/py concentration was 0.5 mg/mL.
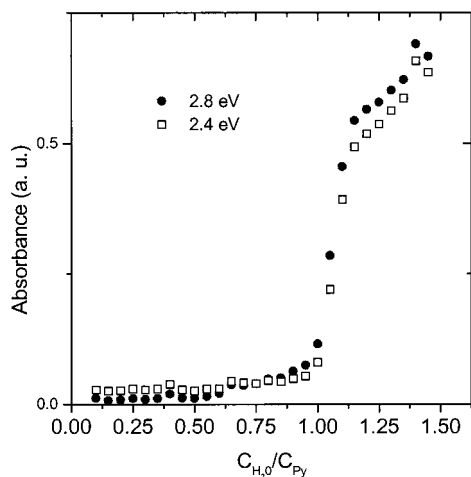

Figure 2. Absorbance shows the critical-like behavior with increasing concentration indicated by the abrupt increase in absorbance at 2.4 and 2.8 eV for $C_{water}/C_{py} > C_{critical}$.

hydrate was used (98% hydrazine monohydrate, Aldrich). The ^{13}C NMR spectra were recorded in standard NMR tubes (Aldrich) on a Varian VXR-300 spectrophotometer. Pyridine-d^5 (Aldrich (99% atom % D) and D_2O (100% atom D) were used as deuterated solutions for NMR spectroscopy.

Optical Characterization

UV−vis Spectroscopy. In Figure 1, we show UV−vis spectra for C_{60}/py/water mixtures at different molar concentration ratios of added water to pyridine $C = C_{water}/C_{py}$. Below a critical concentration, C_c, the absorption spectrum is indistinguishable from a fresh C_{60} solution in pure pyridine. However, as the water concentration increases above C_c, the spectrum changes rather abruptly. Rather than a featureless power law increase in the apparent absorbance at shorter wavelengths which might be caused by Rayleigh light scattering, the absorption spectra show new bands at $E_2 = 2.78$ eV ($\Delta_{Efwhm} = 0.5 \pm 0.1$ eV) and $E_3 = 3.66$ eV ($\Delta_{Efwhm} = 0.42 \pm 0.05$ eV) and a weaker narrow band at $E_1 = 2.02$ eV ($\Delta_{Efwhm} = 0.2 \pm 0.05$ eV).

Figure 2 shows the absorbance measured at 2.4 and 2.8 eV as a function of C, showing abrupt, critical-like change in absorbance associated with the phase change. It's important to note here that the process described above is entirely reversible; if primary solvent (pyridine) is added to dilute the mixture below C_c, a mauve solution results again whose spectrum is indistinguishable from the original solution.

Dynamic Light Scattering Measurements. DLS measurements show that the solvatochromism described above is
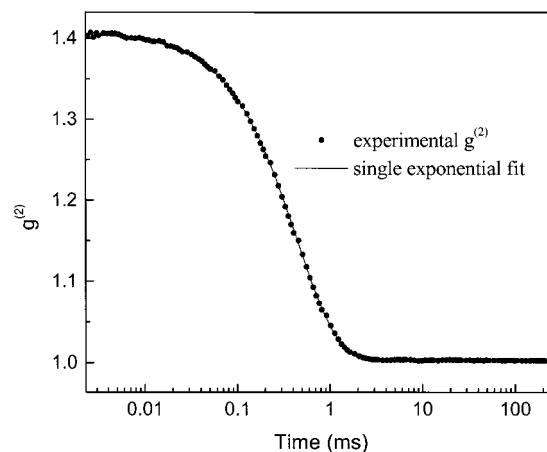

Figure 3. Measured correlation function $g^{(2)}$ for C_{60}/H_2O/py and a superimposed single-exponential fit indicating a monodispersive solution. The C_{60}/py concentration was 0.5 mg/mL.
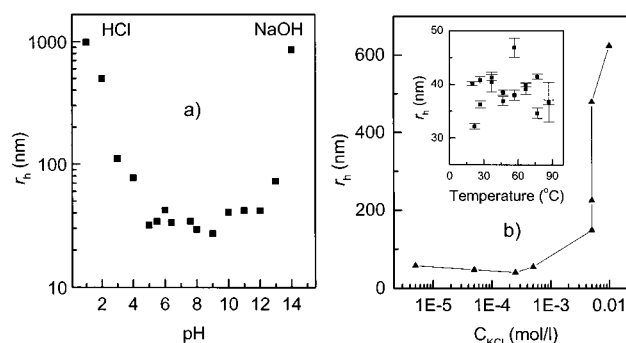

Figure 4. (a) Particle radius r_h as a function of pH. The C_{60}/py concentration used was 0.3 mg/mL. (b) r_h as a function of KCl ionic concentration. The increase of r_h above 10^{-3} mol/L is attributed to the aggregation of the nanoparticles. The insert shows the radius r_h as a function of temperature.

associated with the formation of small nanoparticles in the solution. The hydrodynamic radius r_h of the particles was determined from the homodyne intensity correlation function $g^{(2)} = 1 + A e^{-2Dq^2t}$ using the Einstein relation for the diffusion coefficient $D = k_B T/6\pi r_h \eta$.[11] Here q is the scattering vector, η is the viscosity of the solvent, T is the temperature, k_B is Boltzman's constant, and A is a fitting parameter.

The correlation function for the C_{60}/py/water mixture is shown in Figure 3. We obtain a hydrodynamic radius $r_h = 30$ nm, which is significantly larger than the van der Waals radius of the buckyballs (∼7 Å). An unusual feature of the solution is that the particles in solution are completely *monodispersive*, shown by the excellent single-exponential fit to the autocorrelation function, $g^{(2)}$ ($\sigma < 5 \times 10^{-3}$) (Figure 3). Furthermore, the particles are very close to spherical (less than 1% difference in aspect ratio) as deduced from the depolarization intensity ratio of the scattered light.

Micelles in solutions are known to change their properties and even disassemble if ions are introduced into the solution in sufficiently high concentration. With this in mind, we have performed particle size measurements in C_{60}/py/water solutions (a) with different pH and (b) with KCl dissolved in various concentrations. The results of DLS measurements on solutions with different pH are shown in Figure 4a. The particle radius appears to be more or less constant, $r_h \approx 30$ nm, in the range $4 <$ pH < 9. However, it increases rapidly for pH < 4 and pH > 9. Beyond these limits, the particles are not stable in solution, and after a few minutes precipitation is observed. Performing DLS as a function of temperature (using deionized water) we

find that r_h is T-independent up to 90 °C (insert to Figure 4a), above which they disassemble. The effect of introducing ions into the solution is shown in Figure 4b. By dissolving KCl in different concentrations in the deionized water prior to mixing with the C_{60}/py solution, we find that the nanocapsules with r_h < 50 nm are stable up to a KCl concentration of approximately 10^{-3} mol/L. Above this concentration, r_h rapidly increases and C_{60} is precipitated from solution.

Chemical Tests

To try to determine the structure of the particles involved, we can perform chemical reactivity tests on C_{60}/py/water solution. Neutral nucleophiles such as amines are known to add to fullerene double bonds at room temperature.[12,13] For example, hydrazine or other primary aliphatic amines reacts immediately on contact with fullerenes and all the fullerene is rapidly consumed, provided sufficient reagent is present. Surprisingly, with hydrazine added to the C_{60}/py/water solution, no changes are observed even after many hours, which is confirmed by the fact that there are no observed changes in the UV−vis spectra. To further confirm that no reactions have taken place, we evaporated the solvents and dried the C_{60}/py/water/hydrazine mixture. The result was found to be a pure fullerene residue, which was confirmed with electronic ionization mass spectrometry (EI-MS), liquid-phase chromatography, and by redissolving in toluene as well as by UV−vis spectroscopy. (For comparison, we found that a control solution of C_{60} in pyridine immediately reacted with hydrazine, as expected.) From these chemical tests we conclude that the C_{60} molecules do not form simple aggregates in the C_{60}/py/water mixture, but they are *encapsulated* by pyridine molecules in such a way that they are completely chemically protected from aggressive reagents in the solution. This test also shows that the C_{60} molecules are not present on the surface of the nanoparticles. Clearly, if the C_{60} molecules were on the surface, they would be exposed to the reagent and a reaction would take place which would be immediately evident.

The reversibility of the encapsulation, which was already indicated by the measurements of UV−vis spectra discussed in the previous section, can be effectively confirmed with the chemical tests described above. By gradually adding pyridine to the C_{60}/py/water/hydrazine mixture, a reaction of C_{60} with hydrazine is observed as soon as the water/py concentration ratio C falls below C_c. The reaction is nearly instantaneous at room temperature, giving a yellow product containing a mixture of compounds. The UV−vis visible spectrum of the product mixture is quite distinct and easily identifiable, showing bands only below 325 nm.

Similar chemical reactivity tests procedures were carried out also with C_{70} and give qualitatively the same results as with C_{60}. To emphasize the difference in behavior between aggregates and nanocapsules, it is important to mention here that in contrast to inertness of the nanocapsules outlined above, a reaction of C_{70} aggregates in acetonitrile/toluene mixture and also C_{60} aggregates in benzonitrile with hydrazine is immediately apparent.

Discussion

A key question to understanding the described phenomena is the microscopic structure of the nanocapsules. The two obvious possibilities are drawn schematically in Figure 5. The first possibility is that simple polidispersive aggregation of C_{60} is responsible for the unusual solvatochromism (see Figure 5a),
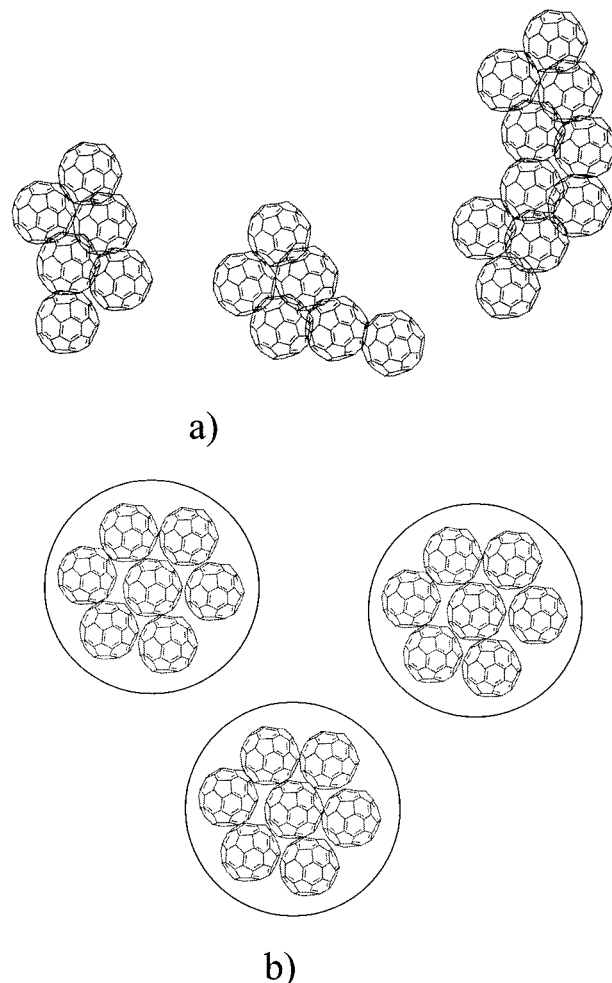

Figure 5. Schematic picture of (a) C_{60} aggregates and (b) encapsulated aggregates with multimolecular cores as discussed in the text.

similarly as was suggested for the C_{70} in toluene/acetonitrile mixtures[8] or C_{60} in benzonitrile.[10]

The second possibility (Figure 5b) is that during mixing pyridine solution of C_{60} with water monodispersive and spherical aggregates of C_{60} are formed. These aggregates, indicated by the chemical reactivity tests, are encapsulated by an outer layer of pyridine in such a way that fullerene double bounds are well protected against chemicals. In the following discussion, we will first compare the properties of C_{60} aggregates in pyridine solution with those of the C_{60}/py/water solution and subsequently argue in favor of the formation of nanoparticles composed of C_{60} cores with pyridine encapsulation.

Comparison of Aggregated C_{60} and Nanocapsules in C_{60}/ py/Water Mixtures. It is known that C_{60} dissolved in pure pyridine exhibits weak solvatochromism upon standing[14] and the color of the solution eventually changes from mauve to reddish. Oxygen plays an important role in this process, and much slower aggregation is observed if the solution is prepared in an argon atmosphere and kept in the dark. The UV−vis spectra of such a solution (Figure 6a) show a very distinct Reyleigh scattering contribution, for which the apparent absorbance decreases as the fourth power of the wavelength. Subtracting the spectra of aggregated solution from the spectrum of a fresh solution recorded immediately after preparation, we obtain a virtually perfect $1/\lambda^4$ Rayleigh scattering law (Figure 6b). This difference in apparent absorbance is sufficient to give rise to the change in color from mauve to wine-red and is a
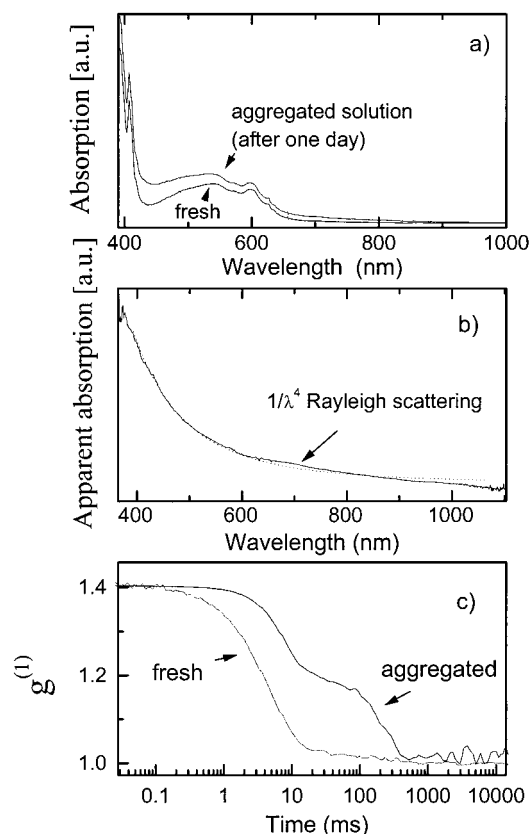
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Figure 6. (a) UV−vis spectra of C_{60} in pure pyridine showing changes due to aggregation. (b) The difference spectra of (a) above can be attributed to $1/\lambda^4$ scattering from the aggregates. (c) DLS of fresh C_{60} in pyridine and after a few hours showing clearly the effect of the aggregation. The C_{60}/py concentration was 0.5 mg/mL.

Figure 7. A comparison of ^{13}C-enriched NMR of C_{60} capsules with ^{13}C NMR spectra of C_{60} shows a 4-fold increase in line width (from 0.07 to 0.29 ppm) for the ^{13}C line. The C_{60}/py concentration was 0.5 mg/mL.

consequence only of light scattering from C_{60} aggregates similar to that seen in the solution of C_{60} in benzonitrile.[10]

It is quite different from the much larger changes in the optical spectra exhibited by the nanocapsules shown in Figure 1 where the changes in the spectra cannot be attributed to Rayleigh scattering.

Clear differences between aggregates and the present system are also evident in particle size measurements. DLS measurements show that C_{60} in pyridine forms a *polydispersive* solution with a large range of particle sizes present.[9] Their size and polydispersivity increase with time as the solution is left standing, eventually leading to precipitation of the larger particles. In contrast, the particles in C_{60}/py/water solution are completely *monodispersive* and show only a single particle size which remains in solution and does not change with time over long periods.

A very convincing piece of evidence that the nanoparticles are different from simple aggregates comes from performing the same hydrazine chemical test on aggregated C_{60} in pyridine solution which we performed on the nanocapsules. Whereas the C_{60}/py/water/hydrazine solution is stable, aggregated C_{60} in pyridine solution immediately displays a reaction with hydrazine, and all the fullerene is consumed immediately. Clearly, the C_{60} is not protected from the hydrazine in this case as in the case of the nanocapsules formed in C_{60}/py/water mixtures.

Other Solvents. Apart from the combination of pyridine and water, for which most of the quantitative measurements were presented so far, we have also performed qualitative experiments with other secondary solvents including tetrahydrofuran, acetone, ether, hexane, methanol, ethanol, acetonitrile, and ethylene
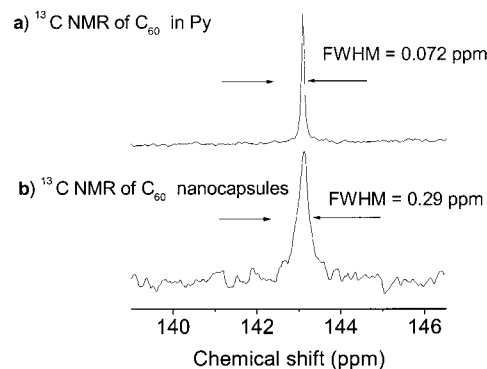
glycol. The dielectric constant of the second solvent appears to be very important for the effect to occur, and water ($\epsilon = 78$), methanol (33), acetonitrile (38), or ethylene glycol (38), all of which have high dielectric constants, $\epsilon > 30$, have all been found to display similar solvatochromism. In contrast, ethanol, ether, hexane, and acetone ($\epsilon < 30$) do not. The importance of the dielectric constant is particularly evident from the comparison of C_{60}/pyridine/ethanol ($\epsilon_{et} = 23$) and C_{60}/pyridine/methanol ($\epsilon_{met} = 33$) mixtures; the ethanol mixtures display no solvatochromism while the methanol mixtures do. Furthermore, after 1 h the C_{60} completely precipitates from the py/ethanol solution, while the methanol mixture remains transparent with C_{60} remaining in solution.

We have also attempted to use a different *primary* solvent and find, for example that replacing pyridine with chloropyridine, similar solvatochromism is observed. We deduce that the effect is rather general, although it may differ in the details for the different solvent mixtures.

Discussion of the Structure of the Nanocapsules. Some important information about the structure of particles involved can be obtained from UV−vis spectra. The features in UV−vis spectra of mixture C_{60}/py/water and crystalline thin film[15] are very similar. The absorption at 330 nm which is characteristic for C_{60} and effected by its environment is shifted in both cases for 8 cm^{-1} to 338 cm^{-1}. Additionally, in UV−vis spectra of the C_{60}/py/water mixture, a new strong absorption at 450 cm^{-1} appears. Similar absorption has been reported for various thin films[16] of C_{60} and attributed to aggregates to C_{60}. This prediction also supports the results of NMR spectroscopy. The ^{13}C spectra in C_{60}/py/water shows a 4-fold increase in line width in comparison with C_{60} dissolved in pyridine (Figure 7). The line broadening in the mixture C_{60}/py/water suggests a more solidlike environment for C_{60} on aggregation. The DLS measurements show that these aggregates are spherical and monodispersive with a size of about 30 nm.

It is quite clear from experiments that it is water which induces the stability of the pyridine shell around the C_{60} acting as a *surfactant*. Most probably, the hydrophobic surfactant interactions hold these entities together.

The stable shell of pyridine which is formed when enough water is added not only maintains the stability and solubility of capsules in polar solvent but also protects the double bounds of C_{60} against hydrazine. The driving force for self-assembly is probably provided by the homophobic interaction between the C_{60} and the secondary polar solvent.

Conclusion

UV−vis, DLS measurements and chemical tests show that solvatochromism of pyridine C_{60} solution mixtures with high dielectric constant polar solvents is a consequence of the formation monodispersive, spherical capsules of C_{60}, in which C_{60} is well protected against chemical reagents with a shell of pyridine around the C_{60} surface. Similar encapsulation was found to occur also with C_{70}, some simple fullerene derivatives, and surprisingly, also with carbon nanotubes and appears to be quite general. With increases of ion concentration in solution, the capsules are not stable and the fullerene completely precipitates out, an effect which could be very useful for simple isolation of fullerenes or their derivatives from other chemicals in solution. Preliminary experiments on such separation technique have been found to be very promising. However, the problem of the detailed microscopic structure, particularly regarding the relative orientation of the pyridine around the fullerene and the surrounding water, still remains to be solved. Once this is known, the mechanism for the formation of the fullerene nanocapsules can also be elucidated.

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