NMR and UV–VIS Investigation of Water-soluble Fullerene-60–γ-Cyclodextrin Complex

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Fullerene-60 can be made water-soluble by forming an inclusion complex with γ-cyclodextrin (γ-CD). The complex between C_{60} and γ-CD is selectively formed; neither β- or α-CD, nor C_{70} takes part in complex formation. The UV–VIS and ^{13}C NMR spectra of C_{60} in the complex are almost identical to those of C_{60} in organic solvents. The ^1H NMR spectrum of the host, γ-CD, in the complex has been identified. Only small differences to the spectrum of the free host are observed; this is interpreted as being due mainly to a conformational change to a more conical structure in the host. Both 2:1 and 1:1 complexes between γ-CD and C_{60} are believed to exist in water. On heating in water, the complexes are transformed into water-soluble aggregates containing several fullerenes and γ-CD molecules. On addition of excess γ-CD to this solution and further heating, the initial complexes, monomeric in C_{60}, are reformed.

The chemical properties of a compound depend on its level of aggregation which can be modified by a solvent or by host–guest complexation. Beautiful examples of the latter have been reported by Cram and others.[1] The new class of carbon modification, the fullerenes,[2] should be no exception to this general observation. Fullerene-60 can be used as an example. It is relatively soluble in hydrocarbon solvents but not so in water.[3] We have reported on a method to make C_{60} water-soluble, that is by formation of a host–guest complex with γ-cyclodextrin (γ-CD) in boiling water.[4] Another more recent example has been reported by Verhoeven and co-workers.[5] They used a calix[8]arene with sulfonic acid side chains to dissolve C_{60} in water.

Results and Discussion

When C_{60} or a mixture of C_{60} and C_{70} (obtained by extraction of the soot from the standard procedure of fullerene production) is boiled with a 0.08 mol dm^{-3} aqueous solution of γ-CD for at least 48 h, the UV–VIS spectrum of the magenta coloured solution, after cooling and precipitation of some complex and excess γ-CD, is almost identical to that of C_{60} in toluene or hexane (Fig. 1).[6] The region of the spectrum above 450 nm where weak absorptions occur is only slightly changed and the peak near 400 nm is shifted a few nm. The formation of the complex is relatively slow; this will be discussed later in relation to the stability of the complex. We have found no evidence for the complexation of C_{60} with α- or β-CD under similar reaction conditions. Although α- and β-CD generally give stronger complexes with small molecules than γ-CD, the size of the cavities in α- and β-CD are too small to accommodate a C_{60} molecule.[7] Furthermore, the selectivity between C_{60} and C_{70} in complex formation with γ-CD is also high. A mixture of C_{60} and C_{70} boiled with γ-CD in water (0.08 mol dm^{-3}) for 48 h gave essentially pure C_{60}–γ-CD complex. The C_{60}/C_{70}-selectivity estimated from analysis of the UV–VIS spectra is *ca.* 150–200. The high selectivity for γ-CD–C_{60} complex formation is evidence for the formation of a true host–guest complex with C_{60} located inside the γ-CD in the hydrophobic cavity. However, we have not yet been able to obtain crystals so an X-ray structure of the complex is not available; only indirect pieces of evidence for its structure are reported, namely UV–VIS and NMR spectroscopies and photophysical studies.

The aqueous solution of the C_{60}–γ-CD complex contains an excess of γ-CD. The concentration of the complex is at best 10^{-4} mol dm^{-3}, *cf.* the concentration of γ-CD of 0.08 mol dm^{-3}. NMR spectra of this solution show the presence only of free γ-CD. However, if the solution is cooled and the precipitate is collected, a violet residue is obtained. This residue still contains excess of γ-CD but can be dissolved into water by careful warming. The resulting violet solution shows the same UV–VIS spectrum as monomeric C_{60}. Heating to 60–70 °C, even for a short time, produces a yellowish solution as mentioned in our previous papers.[4,8] This decomposition of the complex will be discussed later. The ^1H NMR spectrum (D_2O) of the violet solution shows the presence of two different γ-CD species; one has the same shifts as free γ-CD, the other has slightly shifted proton signals (see Fig. 2). The relative proportions of these species vary with washing and heating procedures but the amount of free γ-CD always exceeds that of CD bound to C_{60}.

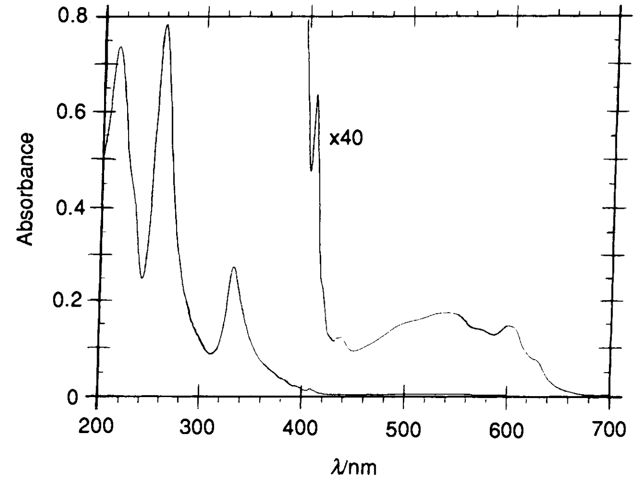

Fig. 1 UV–VIS spectrum of water-soluble C_{60}–γ-CD complex

The T_1 relaxation times for the various proton signals were measured and the values shown in Table 1 were obtained. The T_1 values for the protons in the complex are greater than those of 'free' CD. The mass of γ-CD in D_2O (1.32 kd) and the viscosity of water (D_2O) are such that the rotational correlation time, τ_c, is close to that of the T_1 minimum. Thus, 2:1 or 1:1 γ-CD–C_{60} complexes (3.36 and 2.04 kd, respectively) should have τ_c values which give greater T_1 values than that of free γ-CD. Cross-peak determination by DQ COSY experiments on the sets of signals from the free and bound γ-CD showed that

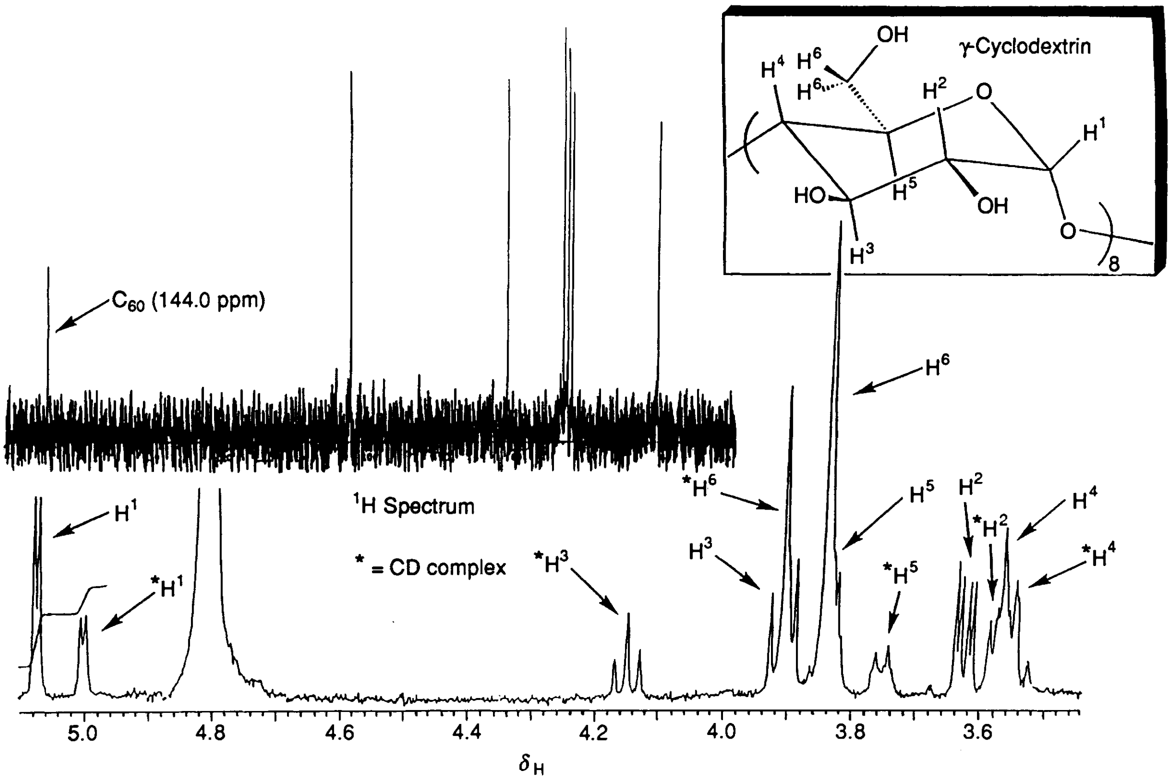

Fig. 2 NMR spectra of free γ-CD and bound to C_{60} in a complex in D_2O

Table 1 T_1 relaxation times for the protons in free γ-CD and bound to C_{60} in a complex in D_2O at 25 °C

	1-H	2-H	3-H	4-H	5-H	6-H
Free γ-CD	0.96 ± 0.01	1.07 ± 0.1	1.61 ± 0.03	1.07 ± 0.04	0.67 ± 0.01	0.67 ± 0.01
Complex	1.33 ± 0.04	1.3 ± 0.1[a]	1.96 ± 0.08	1.2 ± 0.1[a]	1.22 ± 0.04	1.0 ± 0.1[a]

[a] Could not be determined accurately due to spectral overlap.

Table 2 ^1H NMR shifts of α-, β- and γ-cyclodextrin in D_2O

	1-H	2-H	3-H	4-H	5-H	6-H
Free α-CD	5.05	3.63	3.98	3.58	3.86	3.90
Free β-CD	5.07	3.65	3.96	3.58	3.88	3.88
Free γ-CD	5.10	3.65	3.93	3.59	3.87	3.87
γ-CD complex	5.03	3.58	4.17	3.55	3.78	3.92

both sets have the same number of signals and the same coupling patterns. This was also verified by selective decoupling experiments of the relevant signals. We have observed that the stability of the solution and the solubility of the complex both increase with the concentration of free γ-CD. All data are consistent with the assumption that the new γ-CD signals come from the C_{60} complex.

NMR studies of CD complexes generally show rapid exchange of guest molecules between the hosts,[9] resulting in averaged shifts and no distinct species on the NMR time-scale. Our unusual observation of a slow rate for the exchange of C_{60} between γ-CD molecules is consistent with our finding that the complex is formed only slowly on prolonged boiling of C_{60} in water containing excess γ-CD.

This observation provides a unique chance to directly observe the influence of C_{60} on the ^1H NMR chemical shifts of nearby hydrogens. The induced ^1H NMR shifts in γ-CD upon complexation with C_{60} are small. In the C_{60} complex, the signals for the protons 3- and 6-H are shifted slightly downfield, whereas small upfield shifts are noted for 1-, 2-, 4- and 5-H (see Table 2 and Fig. 2). The guest should be free to rotate because the D_8-symmetry of γ-CD cannot be matched by C_{60}. There is no reason to believe that any particular orientation of the C_{60} guest should be preferred. Instead, the H-3 protons scan the π-electron cloud of C_{60} as the latter rotates, thereby feeling the diamagnetic and paramagnetic ring current effects. According to the theoretical treatment of these effects,[10] they can be significant locally but small taken together over the entire C_{60} molecule. The observed induced ^1H NMR shifts for all but one of the protons are indeed very small, and more probably due to small conformational changes in γ-CD upon complexation than ring current effects from C_{60}. For comparison, the chemical shifts of the protons in α-, β- and γ-CD are shown in Table 2. Of the three CDs, the α-macrocycle has the most conical shape.[7] There are several possible reasons for the largest shift difference upon complexation being observed for the 3-Hs. One is the effect of exchanging the internal water molecules for C_{60}; another is the possibility that the paramagnetic ring current effect of the five-membered rings and the diamagnetic one of the six-membered rings of C_{60} do not completely average out.

The ^{13}C NMR spectrum of a water solution of C_{60}–γ-CD reveals the presence of free γ-CD and of C_{60} giving one sharp peak at δ 144.0, close to that reported for C_{60} in organic solvents.[8] The relaxation time (T_1) of ^{13}C in C_{60} for C_{60}–γ-CD in water is much shorter than that of C_{60} in benzene.[11] The spectrum shown in Fig. 2 was recorded with 5 s between two successive (π/4) pulses. Increasing the delay time to 10 s had

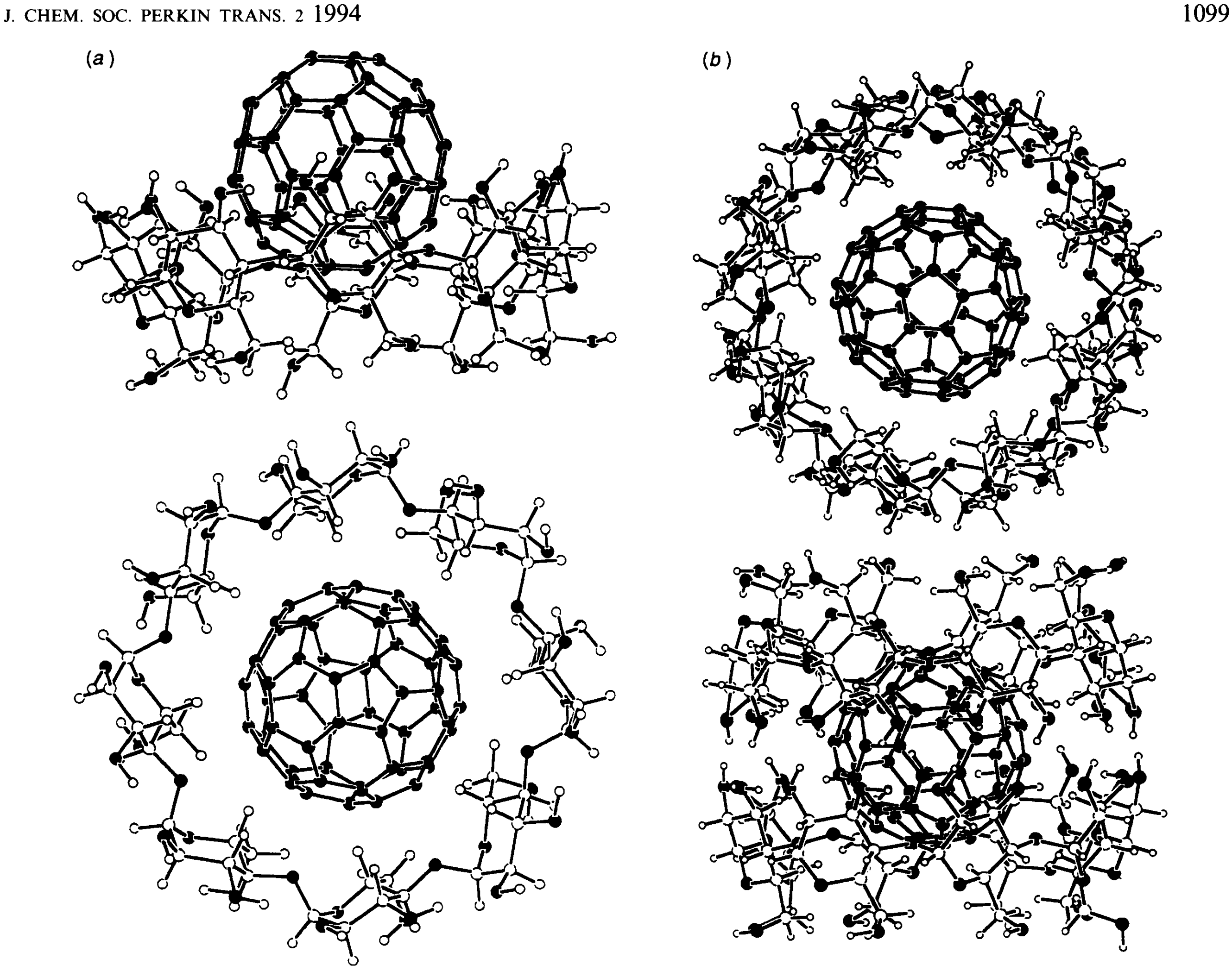

Fig. 3 Structures of the 1:1 and 2:1 complexes between γ-CD and C_{60} from Biograph modelling experiments: (*a*) two views of the 1:1 γ-CD and C_{60} complex and (*b*) two views of the corresponding 2:1 complex

little effect on the size of the ^{13}C signal in C_{60}. The ^{13}C relaxation time in C_{60} when surrounded by γ-CD containing several protons close to the guest in the complex should be shorter than that of free C_{60} in [$^2H_6$]benzene. However, there might be other reasons for this observation. The ^{13}C NMR spectrum of the complex did not show distinct signals from γ-CD bound to C_{60}.

Molecular modelling (BIOGRAPH, BioDesign, Inc.) of two possible structures of the complex have been reported.[4] The cavity in γ-CD is just a little too small to accommodate C_{60} within its hydrophobic cavity, as shown in Fig. 3(*a*). Thus, in a 1:1 complex, the C_{60} molecule will expose a relatively large area towards water. In a 2:1 complex [see Fig. 3(*b*)], the guest is perfectly enclosed by the two host molecules, which are close enough to allow for hydrogen bonding between the secondary hydroxy groups. In this case C_{60} will, however, not completely fill the hydrophobic cavities of the two hosts and some water molecules will remain inside the γ-CD cavities.

The observed high selectivity of C_{60}- over C_{70}-complex formation is difficult to rationalize when only considering the 2:1 complexes, even if the entropy term is very important. The C_{60} molecule should be completely free to rotate within the complex whereas in the hypothetical C_{70} complex, the guest can only rotate around the long axis, parallel to the symmetry axis of the γ-CDs. It is more tempting to speculate that the selectivity is a kinetic effect determined by the slow formation of the 1:1 complex, which is a very reasonable precursor to the 2:1 complex. The entropy argument is still valid, but in the 1:1 complex, the exposure of the C_{70} molecule to water is larger and energetically more unfavourable than the exposure of C_{60} to water. From a series of photophysical measurements on the lifetime of the triplet state of C_{60} complexed with excess γ-CD in water, we found that the rate of oxygen quenching of the triplet corresponds to *ca.* half of the rate in toluene.[12] More importantly, the rate of annihilation of triplets of the complex in water is only one quarter of the rate in toluene. These observations are consistent with the presence of a stable 2:1 complex in which the excited C_{60} molecule is well protected from collision.[13]

The structure of the complex could not be unambiguously solved even after quantitative NMR analysis of the amount of free and complexed γ-CD and UV determination of the C_{60} concentration. Consistent ratios between bound γ-CD and C_{60} were not obtained; the ratio increases with the amount of free γ-CD and decreases with the concentration of free and complexed γ-CD. In concentrated solutions a 2:1 complex dominates, in dilute solutions a 1:1 complex might be more favourable. The solubility of the C_{60}–CD complex is low at room temperature; to get a significant amount of the complex into solution, the solvent has to be heated for a short time. However, such heating can then induce decomposition of both the 1:1 and 2:1 complexes. A major problem then, is that equilibrium conditions cannot be obtained when determining the ratio of the two components of the complex.

The change in solubility as well as the variation in composition of the C_{60}–γ-CD complex with the concentration of free γ-CD must be due to an equilibrium between the 1 : 1 and 2 : 1 complexes according to eqns. (1) and (2). At high

$$\gamma\text{-CD} + C_{60}\text{-}\gamma\text{-CD} \rightleftharpoons \gamma\text{-CD-}C_{60}\text{-}\gamma\text{-CD} \quad (1)$$

$$K = [\text{CD-}C_{60}\text{-CD}]/[C_{60}\text{-CD}][\text{CD}] \quad (2)$$

concentrations of free γ-CD as under the initial preparation of the complexes, the 2 : 1 complex dominates whereas the 1 : 1 complex is formed when the concentration of free γ-CD drops. Thus, it is not surprising that we have been unable to produce 'pure' 2 : 1 or 1 : 1 γ-CD–C_{60} complexes with the methods used so far, the question of the composition of the complex has different answers depending on the concentration of γ-CD in solution.

The solubility of C_{60} with γ-CD in water varies with the temperature and the concentration of free γ-CD. We believe that the 2 : 1 complex is the more soluble one and its solubility is the limiting factor at high concentrations of free γ-CD. The 1 : 1 complex is considerably less soluble and stable which makes the preparation of 'pure' 2 : 1 or 1 : 1 γ-CD–C_{60} complexes even more difficult. During the initial preparation of the C_{60}–γ-CD complexes, the concentration of free γ-CD is 0.08 mol dm^{-3} and the maximum concentration of the complexes are *ca.* 10^{-4} mol dm^{-3} in C_{60}. Assuming that these are near-equilibrium conditions and that the only complex present is the 2 : 1 one, a hypothetical equilibrium constant for the 2 : 1 complex at 100 °C can be calculated according to eqn. (3), where $[C_{60}]_s$ is set to

$$K_{2:1} = [\text{CD-}C_{60}\text{-CD}]/[C_{60}]_s[\text{CD}]^2 = 1/64 \quad (3)$$

unity and the reaction is considered to be two-phase, between solid C_{60} and γ-CD in water solution. By comparison, the corresponding hypothetical equilibrium constant for the 1 : 1 complex under the same conditions is given by eqn. (4).

$$K_{1:1} = [C_{60}\text{-CD}]/[C_{60}]_s[\text{CD}] = 1/800 \quad (4)$$

As already mentioned, the stability of our solutions is limited and colour changes appear on heating. The stability is also very much affected by the concentration of free γ-CD. Notably, the observed colour change to yellow is accompanied by the disappearance of the ^{1}H NMR signals assigned to the γ-CD–C_{60} complex. It is most likely that the decomposition is initiated *via* the 1 : 1 complex. The decomposition starts by the reversal of the complex-forming reaction (1). The 1 : 1 complex may then form aggregates or further decompose [eqn. (5)]. This

$$C_{60}\text{-CD} \longrightarrow C_{60} + \text{CD} \quad (5)$$

decomposition requires elevated temperatures to be reasonably fast, as does the initial complex-forming reaction. The fate of the monomeric C_{60}–CD or 'free' C_{60} in water is discussed later.

The hypothesis presented in this paper states that two different C_{60}–γ-CD complexes exist in solution under certain conditions. However, we do observe only one set of ^{1}H NMR signals for the complexed CD. This may be due to (*a*), exactly the same chemical shifts for CD in both the 2 : 1 and 1 : 1 complexes (*b*), a rapid exchange of CD between the 1 : 1 and 2 : 1 complexes but not with the free CD or (*c*), only the 2 : 1 complex giving the observed ^{1}H NMR spectrum and the 1 : 1 complex easily aggregating so that its NMR signals broaden too much to be observed. We believe hypothesis (*c*) to be most probable. As already mentioned, the induced shifts in complexed CD are believed to be due to a change of conformation of the host: the dihedral angles around the bridging oxygen–carbon bonds between the sugar units in γ-CD change slightly and the shape of the host becomes more like a cone.

The rate of CD-exchange between the two complexes must be relatively slow on the NMR timescale. However, some broadening of the signals of CD in the complexes always occurs. Furthermore, the ^{13}C NMR signals from the complexes have not yet been observed, possibly due to broadening caused by this slow exchange process, whereas the signal from C_{60} is quite strong (see Fig. 2). Further work to confirm this hypothesis of slow exchange is necessary.

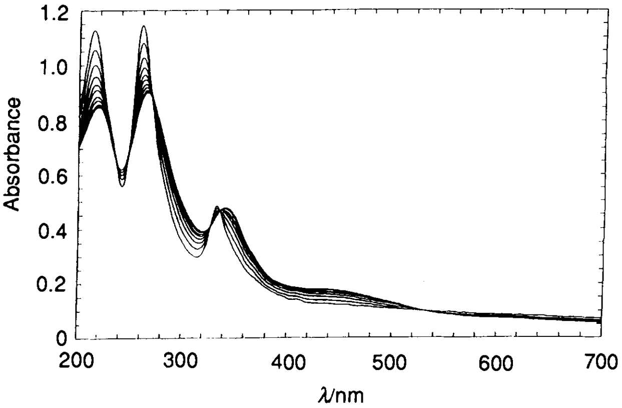

Fig. 4 UV–VIS spectrum of a solution of C_{60}–γ-CD complex heated to 70 °C for 5–60 min

As already mentioned, the 2 : 1 complex between C_{60} and γ-CD is not stable and the violet solution containing monomeric C_{60} rapidly turns yellow on heating at 60–70 °C. At first, this may seem surprising since the complex is formed after boiling for 48 h. The formation of the yellow solution is accompanied by the disappearance of the ^{1}H NMR signals for the CD complex and an increase in those of free CD. The UV–VIS spectrum also changes, as shown in Fig. 4. The peaks become broad and a new weak band appears just below 500 nm, which causes the solution to appear yellow instead of violet. On prolonged heating, the UV–VIS spectrum shows only broad peaks with the peak originally at 331 shifted to 345 nm. The ε value of this peak stays very much the same whereas the ε values for the two intense bands at 215 and 260 nm decrease. The final spectrum is very similar to that of the calixarene complex reported by Verhoeven and co-workers,[5] as well as to those reported for a thin film or an L-B-film or other aggregates of C_{60}. In the calixarene complex, the yellow band is rationalized as a charge-transfer band to the central C_{60} molecule from the surrounding aromatic rings. In our case, we suggest that the new yellow band comes from closely interacting C_{60} molecules. A theoretical study of weak interactions in dimers and oligomers of C_{60} is under way.

Our yellow solutions are usually very stable and no C_{60} is precipitated from them. If the yellow solution is heated with excess γ-CD, the monomeric C_{60} complex is reformed. Thus, the formation of the yellow species is reversible and we do not consider it to be a chemical reaction but rather the formation of an aggregate or polymer of C_{60} and γ-CD. Aggregates consisting of C_{60} clusters surrounded by γ-CD molecules is one simple structural possibility, a two-dimensional C_{60} plate with γ-CDs bound along the two sides is a less probable alternative. Another possibility is of a linear polymer of C_{60} and γ-CD; such a structure has been suggested for the 1 : 1 complex of 1,2-dicarbadodecaborane(12) with α-cyclodextrin.[14] CD is also known to crystallize in stacks in the presence of large guest molecules.[15] The cavity in γ-CD has almost the same diameter as length and thus could function as a proper building block in a linear polymer. It is interesting to note that in the water-soluble complex between C_{60} and calixarene, there seems to be no severe hindrance towards aggregation and thus neither to C_{60}–C_{60} interactions.

Further work, including light scattering studies of the yellow solutions, is under way and will eventually reveal the structure of the water-soluble C_{60} aggregates containing γ-CD.

Experimental

NMR spectra were recorded on a Varian Unity 500 operating at 11.74 T with D_2O solvent. For the quantitative determination of free and complexed γ-CD, an internal standard {sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate} was added to the water solution. ^1H Spin–lattice relaxation times were measured by the inversion recovery method using a 180° composite pulse. In all cases, standard deviations were measured to $< 4\%$. No exact determinations of T_1 for the protons 2-, 4- and 6-H could be carried out due to spectral overlap. All shifts were measured relative to that of sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]-propionate.

Pure C_{60} powder (2–3 mg) was added to a solution of γ-CD (1.55 g, 0.08 mol dm^{-3}) in water (15 cm^3) and the mixture was refluxed for 4–5 days. The resulting magenta coloured solution was filtered and allowed to cool to room temperature. A precipitate was slowly formed and collected by centrifugation.

One portion of the precipitate was heated with D_2O (1 cm^3) in a test tube for 10–20 s in boiling water. The remaining precipitate was separated from the magenta coloured solution by centrifugation. The ^1H NMR spectrum of the solution always showed the presence of excess γ-CD.

Another portion of the precipitate was treated with water (2–3 cm^3), the mixture heated to 60–70 °C and its UV–VIS spectrum recorded at intervals. The solution turned yellow (see Fig. 4) at different rates depending on the temperature and amount of excess of γ-CD. On addition of a large excess of γ-CD to the resulting yellow solution followed by further heating, the UV–VIS spectrum of the solution became identical with that of monomeric C_{60}.

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