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Incorporation of fullerene-C_{60} and C_{60} adducts in micellar and vesicular supramolecular assemblies; introductory flash photolysis and photoredox experiments in micelles [a]

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Abstract. The incorporation of fullerene-C_{60} and of C_{60} adducts into Triton X-100 (TX100) micelles and into liposomes made of phosphatidyl-ethanolamine from *Escherichia coli* has been studied. Incorporation curves and UV-Vis absorption spectra for the liposomes are presented. Flash photolysis experiments in TX100 micelles in the presence of the electron donors *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) and cytochrome *c* resulted in the observation of oxidized transient species.

Introduction

Compartmentalisation of components that constitute a photoinduced redox cycle, by using supramolecular assemblies such as micelles or liposomes (vesicles), is a very efficient way of combining the flexibility of choice of artificial redox mediators with systems of high biological interest. The study of micellar and vesicular supramolecular assemblies[1,2,3] which can be used for catalysis[4], proton motive force generation[5], solar energy conversion[6] and drug delivery[1], was initiated in the 1960s by *Bangham* and *Fendler*, and is still a topic of considerable interest[7].

The incentive for the present study was the eventual use (as visualised in Figure 1) of a fullerene-based material, incorporated in liposomes, as the photoactive unit to induce (with illumination) a proton-translocating redox cycle which uses mediators of biological origin (*e.g.* cytochrome *c* and ubiquinone), thus leading to the light-induced generation of a proton motive force.

In a liposomal environment only those diads that are incorporated in the lipid membrane with their donor side located towards the outside can interact with the membrane-impermeable cytochrome *c*. This can lead to the vectorial translocation of protons over the lipid membrane and hence the generation of a proton gradient: reduction and subsequent protonation of the secondary ubiquinone acceptor occurs on the inside of the liposomes, ubiquinol can diffuse through the membrane and (re)reduction of cytochrome *c* and proton release can only occur on the outside (*cf.* Ref. 5).

The use of fullerenes as photosensitizers for electron transfer and as electron mediators across a black lipid (bilayer) membrane (containing lipid, toluene, and decane), formed across a hole in a Teflon sheet has been reported earlier[8,9,10]. The system we studied, however, is a solution phase containing (virtually organic-solvent free) micellar or vesicular assemblies. The spectroscopic characterisation of intermediate (excited) states in the process is being pursued. The incorporation of fullerenes C_{60} and C_{70} into systems of the micellar or vesicular type has recently attained much attention. The incorporation of fullerenes into dioctadecyldimethylammonium bromide[11], dihexadecyl hydrogen phosphate[11], lecithin[11], Triton X-100[11,12,13], phosphatidylcholine[12] and poly(vinylpyrrolidone)[14] have been reported. Comparison of C_{60} and C_{60}-adduct incorporability and photophysical behaviour in such assemblies has not, however, attained any attention yet.

Here we report on these aspects and on electron transfer from water soluble electron donors such as cytochrome *c* to intramicellar fullerenes and fullerene adducts. Furthermore a preliminary comparison is made between two adducts (see Figure 2), one of which **1** is covalently

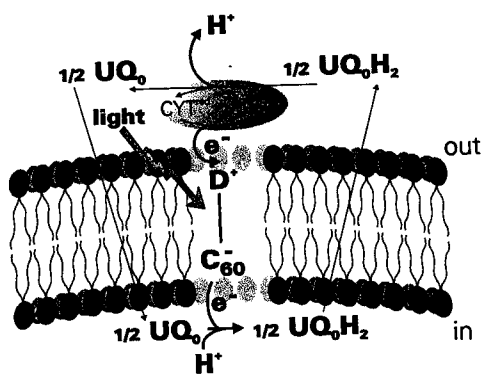


Figure 1. Visualisation of a redox system in which excitation of a donor-bridge-fullerene (acceptor) system leads to the formation of a charge-separated state which can accept an electron from cytochrome c and donate electrons to uniquinone. Translocation of the doubly reduced and protonated quinone and electron transfer to the oxidized cytochrome completes the redox cycle.

[a] Dedicated to Professor *Upendra Pandit* on the occasion of his retirement from the Department of Organic Chemistry of the University of Amsterdam.

functionalised with an electron-donating group; a second fullerene adduct **2** lacks this unit.

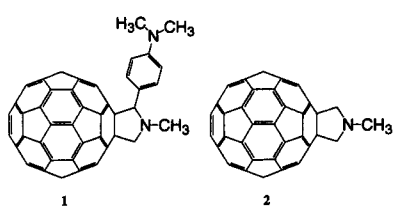
Experimental

Incorporation

Micelles. C_{60} and its adducts were incorporated into TX100 micelles by dissolving C_{60} in toluene, adding the required amount of TX100 and (after mixing) removing the solvent *in vacuo*. In this way a solution of C_{60} in pure TX100 was obtained, which after adding water/buffer and heating (50°C for 1 min) resulted in a clear micellar solution. A final TX100 concentration of 2% (w/v) was used ([TX100] ~ 32 mM).
A similar method has been reported[12,13].

Liposomes. Incorporation of C_{60} and the adducts into liposomes has been accomplished in the following way. The required amount of C_{60} was dissolved in benzene (solubility 1.7 mg/ml)[15] and a solution of phosphatidyl-ethanolamine (PE) in chloroform was added. The solvent was removed *in vacuo* to a concentration at which C_{60} crystallisation does not yet occur. Subsequently a small amount of acetone was added. This resulted in the formation of very small clusters of C_{60} (see also Ref. 16). (Note that if the $CHCl_3/C_6H_6$ mixture is further evaporated without acetone addition, C_{60} crystallisation occurred, resulting in a two-phase system consisting of a black C_{60} crystal phase and a colourless PE phase.)
From the mixture containing the small clusters of C_{60}, PE and the acetone/$CHCl_3/C_6H_6$ mixture, the solvent was further removed *in vacuo* and dried over $CaCl_2$ under reduced pressure. Next, the required amount of water or phosphate buffer was added, and the mixture was vigorously stirred with glass beads for *ca.* 15 min. A final concentration of 10 mg PE/ml water was used. Subsequent sonification (Branson sonifier 250, output 2, 30% duty cycle) for 45 min of aliquots of 3 ml was performed thermostated at 37°C (the lamellar gel to lamellar liquid crystalline phase transition temperature of PE from *E. coli* ranges between 20 and 40°C). Non-incorporated material was removed by 20-s centrifugation in an Eppendorf centrifuge at 14000 rpm (*i.e.* approximately 20000 *g*), whereby non-incorporated fullerene is pelleted. The supernatant consists of a homogeneous suspension of liposomes. These liposomes do not sediment at low-speed centrifugation. Samples were either used directly, or after storage at −20°C, followed by thawing and another 10 min of sonication.
The same method was used for the fullerene adducts, except for the solvent that induces fullerene aggregation: ethanol can be used for the adducts, but its addition is not essential since phase separation does not occur during evaporation of the $CHCl_3/C_6H_6$ solvent mixture (at low initial weight percentages).
The method described here is similar to that described in Ref. 12, except for the solvent-induced aggregation, which was found to be essential for reproducible incorporation.

Isopicnic centrifugation. Sucrose-density gradient centrifugation is an established method in biochemistry that separates different phases on the basis of density. As the density of non-incorporated fullerenes (density of $C_{60} = 1.65$; of the adducts ≈ 1.51 kg/dm^3)[17] does not change with particle size, this method is well-suited for the separation of liposome-incorporated fullerenes from non-incorporated material.
After tests with linear sucrose gradients the following method was found to be suitable. Centrifugation over a sucrose solution (with a density of 1.3 kg/dm^3) of various samples was performed with an ultracentrifuge (Centrikon T-1055, Kontron Instruments) at 35000 rpm in a swing-out rotor (type TST 41.14), for 19 h. After this treatment the non-incorporated fullerene material was present in the pellet, and the liposomal phase, containing the fullerenes, was on top of the sucrose solution. Extraction of the pellet with 1,2-dichlorobenzene and subsequent UV-VIS absorption allowed determination of the non-incorporated material. UV-VIS absorption of the yellow band on top of the sucrose solution allowed determination of the (relative) amount of incorporated fullerene.



1 2

Figure 2. The fullerene adducts used in this study.

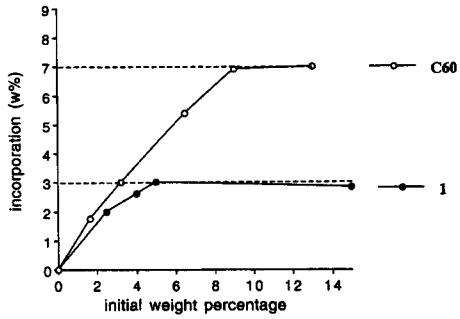


Figure 3. Representation of the incorporation curves of C_{60} and adduct **1** *in PE. The* x *axis denotes the amount of fullerene with which the incorporation procedure was started, and the* y *axis denotes the amount that is incorporated into liposomes after sonication and sucrose centrifugation (see experimental section).*

Spectroscopy and materials

UV-Vis absorption spectra were recorded on a CARY 3 spectrophotometer. Transient absorption spectra were obtained with a gated Optical Multichannel Analyzer of EG&G instruments as described earlier[18]. As excitation and white probe sources, a Nd-YAG laser (355 or 532 nm, ns pulses) and a 450-W high-pressure Xe arc (white light of 300-880 nm) coupled to a Müller Elektronik MSP05 pulser were used, respectively. The excitation beam was at right angles to the probing beam. The probing path length was 1 cm. The volume through which the analytical light passes was 10×2×2 mm. Samples were adjusted to an absorption of *ca.* 1 (1 cm) at the excitation wavelength. Laser power was between 10 and 50 mJ per pulse (0.2 cm^2). Samples were de-aerated by bubbling with argon for 20 min. Fullerene concentrations ranged from 10^{-4} M (for 355 nm excitation) to 10^{-3} M (for 532 nm excitation). C_{60} was obtained from MER corporation, the fullerene adducts **1** and **2** were synthesized as reported earlier[17]. Triton X-100 (TX100) was obtained from Merck. Liposomes were made from acetone washed L-α-phosphatidyl-ethanolamine (PE) type IX from *E. coli* (Sigma). Cytochrome *c*, from horse heart Type V (Sigma) was reduced with dithionite and purified using a Sephadex column; ubiquinone (UQ_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone) was obtained from Sigma. Stock solutions of 2.5 mM (cytochrome *c* in phosphate buffer pH 7) and 100 mM (UQ_0 in ethanol) were stored at −70°C.

Results and discussion

Incorporability

Micelles. In contrast to the reported[11] method of stirring solid C_{60} in an aqueous solution of TX100, the toluene method allows introduction of an exact (predetermined) amount of C_{60} into the micelles, as long as the system is not saturated with C_{60}.
As reported by others[12,13] we find that the state of aggregation in micelles changes at low C_{60} concentrations. However, incorporation of truly monomeric C_{60}, which would be expected to give a purple solution and the characteristic 400 to 630 nm absorption with a very minor absorption between 420 to 450 nm, could not be accomplished.

Liposomes. Quantifying the incorporation of C_{60} and C_{60} adducts into liposomes is less straightforward than incorporation in micelles. This is because during sonication at high initial weight percentages a phase separation tends to occur and also at lower concentrations the incorporation efficiencies are less than 100%. Thus a method is needed to determine the amount actually incorporated. The methods reported for establishing incorporation of fullerenes into vesicles[11,12] differ and appear not to be unequivocal. The sucrose-density centrifugation method

described in the experimental section, however, turns out to provide a useful method for establishing incorporation unequivocally. Via this method the incorporation efficiencies were estimated to be approximately 90% for C_{60} and 66% for the adducts from the data presented in Figure 3 (*i.e.* when starting the procedure with an initial weight percentage of 4% fullerene adduct, this results in the incorporation of only $4 \times 0.66 = 2.65\%$). The maximum incorporability of C_{60} and our adducts **1** and **2** in PE liposomes were determined in this way. Figure 3 shows that maximum incorporability of C_{60} is about twice as high, 7% (w), as that of the adducts, 3% (w). Incorporability is expressed as weight percentage, based on the amount of lipid used. This implies that C_{60} can be incorporated into liposomes to a rather high degree. If an average liposome contains 10^5 lipid molecules, then one liposome can contain up to 6800 C_{60} molecules (*i.e.* a molecular lipid/C_{60} ratio of 15). For the adducts, these numbers are 2300 molecules per liposome and a molecular ratio of 43. For reaction centres and light-harvesting systems positioned in a liposome these numbers vary from 100 to 500 systems per liposome[19]. As the diameter of C_{60} is only 1 nm, and the reaction and harvesting centres are *ca.* 10 nm in diameter[19], these numbers imply in a quite reasonable structural resemblance.

If we express the maximum incorporability reported by *Yamakoshi* et al.[14] as a weight percentage of the amount of polyvinylpyrrolidone, this leads to 8% for C_{60} (and 4% for C_{70}, *i.e.* similar values to those reported here).

Interestingly, small amounts of the adducts (but not of C_{60}) can be incorporated into liposomes by prolonged stirring with glass beads (without sonication). After 3 days an incorporation of *ca.* 1% (w) can be accomplished. This implies that it is likely that a certain directionality of incorporation is obtained via a low energy path, as found for certain proteins[20]. This directionality of incorporation of the donor-acceptor system in liposomes is a prerequisite for an efficient proton motive force generation (see introduction).

Spectroscopy

The UV-Vis absorption spectra of C_{60} in PE liposomes are shown in Figure 4. The high similarity to spectra of C_{60} in micelles reported by others[11–14] suggests that the systems contain a similar fullerene phase, consisting of aggregates, as indicated by a new absorption and characteristic band around 450 nm.

Interestingly, however, flash photolysis of a solution of C_{60} in TX100 micelles (see also Ref. 21) results in a transient (see Figure 5) that is similar to the transient obtained in organic solvents like benzene or hexane, where no ground-state aggregation occurs. A slight difference of the triplet-triplet absorption in micelles compared to that

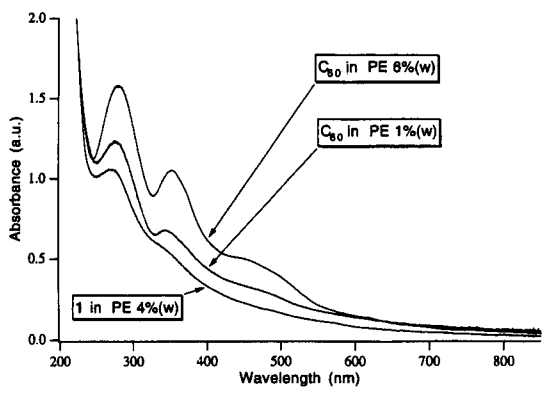


Figure 4. UV-Vis absorption of C_{60} in PE liposomes at 6% (w) and 1% (w), and of adduct **1** *in liposomes (4% (w)).*

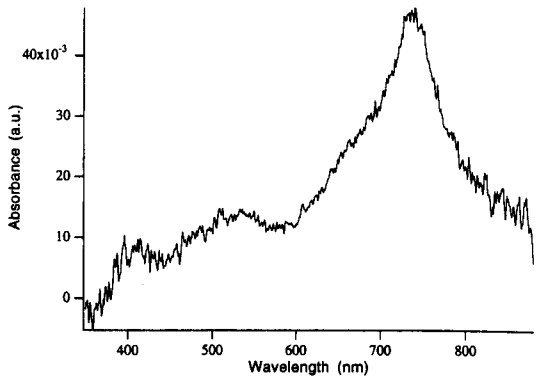


Figure 5. Representative transient absorption spectrum of C_{60} in TX100 micelles.

obtained in organic solvents near the UV region (at 380 nm) is, however, observed. The characteristic 750 nm absorption is clearly present. The species generated is obviously the triplet state which, in contrast to the aggregational state implied from the UV-Vis absorption, can be considered to be a molecular triplet. The lifetime of this triplet is 48 μs. A lower limit of the triplet yield is 10% (obtained by signal intensity comparison with C_{60} in toluene). Exact numbers are hard to determine as there is no reference compound for the determination of triplet yields in micelles and the triplet yield may depend on the ratio of fullerene/TX100 (*i.e.* the degree of aggregation). Triplet-triplet annihilation and exciton formation in clusters is expected to be responsible for the reduction of the triplet yield. Nevertheless it is evident that the triplet yield in micellar solution is strongly reduced with respect to that in organic solvents (approximately 100%)[17].

UV-Vis absorption of fullerene adducts **1** and **2** in TX100 and in liposomes (see Figure 4) do not show the sharp features observed in organic solvents, or any extra absorption band (expected around 450 nm as for C_{60}) that might be attributed to aggregation. Flash photolysis of a solution of adduct **2** in TX100 micelles results in a transient (see Figure 6) that has again great similarity to the transient obtained in organic solvents like benzene or hexane[17]. The species generated is obviously a molecular triplet state. Triplet lifetime is 50 μs. The lower limit of the triplet yield is approximately 10%.

While in non-polar organic solvents the adduct **1** produces a triplet transient spectrum identical to that of **2**[17], the transient spectra of **1** and **2** were found to be significantly different in micellar solution, both in spectral shape (see Figure 6) and in lifetime (50 μs for **2** and 10 μs for **1**). In a difference spectrum (see Figure 6) it appears that for the transient formed by excitation of **1** broad extra ab-

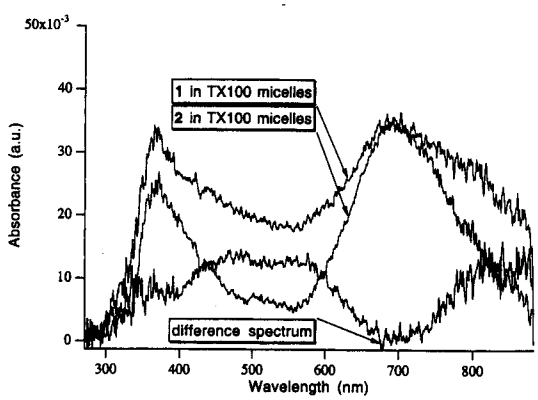


Figure 6. Transient absorption spectra of fullerene adducts **1** *and* **2** *in TX100 micelles. The difference spectrum between the two spectra (scaled at 700 nm) is also shown.*

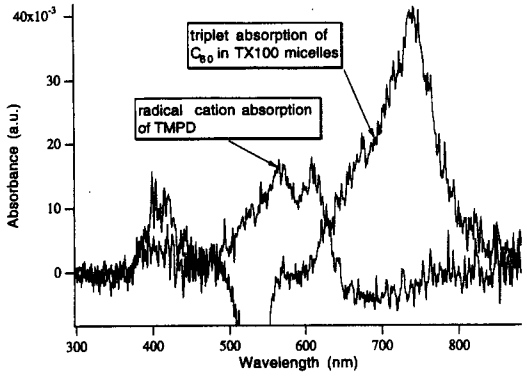


Figure 7. Transient absorption spectra of C_{60} in TX100 micelles in the presence of 0.2M TMPD at pH 7. The first transient is taken in the laser pulse (532 nm) and clearly shows the triplet state of C_{60}. The second transient is taken after 100 μs and is characteristic of the radical cation of TMPD.

sorption bands are present, particularly around 480 and 880 nm. We tentatively attribute this to partial (reversible) transformation of the fullerene triplet in **1** into a charge transfer state[22] in which an electron has been transferred from the aniline group to the fullerene unit. From studies in homogeneous solution[17] it is known that this process becomes energetically accessible for **1** in more polar environments but under such conditions the lifetime of the charge separated state is very short. Apparently, the micellar environment not only energetically allows for charge transfer to take place, but also stabilizes the resulting dipolar state kinetically, making it available to participate in redox photochemistry. That the micellar environment allows intramolecular charge transfer to take place in **1** is also supported by the observation that the fluorescence of **1** in TX100 micelles is quenched for 80% as compared to that of **2**.

Redox photochemistry

As has amply been demonstrated in organic solvents[23], a photoexcited fullerene can act as an electron-accepting unit. Figure 7 demonstrates that excitation of C_{60} in TX100 in the presence of TMPD (0.2 M TMPD + 0.15M ascorbate (functions as electron pool), pH 7) leads to a short-lived (ns time scale) triplet state, producing a long-lived radical cation of TMPD (lifetime of 1.6 ms). The radical anion has not yet been observed (it is expected to show strong absorption at 1100 nm).

Flash photolysis of a solution of **1** in TX100 micelles with cytochrome *c* (reduced form, concentration of 0.1 mM) results in a transient that not only contains the fullerene triplet but also a bleaching signal at 530 and 550 nm (see Figure 8), that shows striking similarity to the difference spectrum of the reduced and oxidized form of cytochrome *c* (see Figure 8, upper spectrum). Clearly, excitation of adduct **1** in micelles can lead to the oxidation of cytochrome *c* with a lifetime of *ca.* 10 μs. Addition of the oxidized form of ubiquinone (4 mM) to this mixture leads to a reduction of the lifetime of the cytochrome *c* bleach signal to less than 0.5 μs, implying that the electron taken from the cytochrome by fullerene-adduct excitation is, via the quinone, returned to the cytochrome. These preliminary results strongly suggests that a photoexcited fullerene is capable of initiating a redox cycle that has cytochrome *c* and ubiquinone as redox mediators. Redox midpoint potentials[17,24] of the compounds involved are given in Table I.

Excitation of cytochrome *c* and ubiquinone (separate compounds or a mixture) under similar circumstances does not lead to transients.

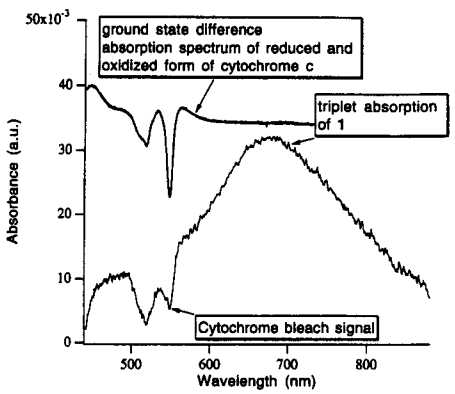


*Figure 8. Representative transient obtained on excitation (excitation wavelength is 532 nm) of adduct **1** in TX100 micelles in the presence of the reduced form of cytochrome c, together with the UV-Vis absorption difference spectrum of the reduced and oxidized form of cytochrome c (this spectrum is vertically shifted and has an absorption of zero 700 nm).*

Table I Redox data of the compounds used in this study.

	V (SCE) [a]	V (NHE) [b]	n [c]	Ref.
E ($C_{60} \rightarrow C_{60}^-$	−0.40	(−0.16) [d]	1	17
E ($\mathbf{1} \rightarrow \mathbf{1}^-$)	−0.57	(−0.33) [d]	1	17
E (UQ → UQH_2)	(−0.14) [d]	+0.10	2	24
E [cytc(ox) → cytc(red)]	(−0.02) [d]	+0.22	1	24
E (TMPD → $TMPD^+$)	+0.16	(+0.40) [d]	1	17
E ($\mathbf{1} \rightarrow \mathbf{1}^+$)	+0.17	(+0.95) [d]	1	17

[a] In CH_3CN or CH_3CN/toluene mixtures.
[b] In H_2O at pH 7, 25°C.
[c] Number of electrons involved.
[d] Calculated via E (SCE) = E (NHE) − 0.24.

Conclusions

The incorporation of fullerene-C_{60} and the adducts **1** and **2** into micelles and liposomes has been accomplished. The incorporability of the adducts in liposomes is lower than that of C_{60}. It is tentatively concluded that a reduced aggregational tendency of the adducts[25] **1** and **2**, compared to C_{60}, reduces their incorporability in liposomes (*i.e.* C_{60} has more aggregational tendency and therefore can be incorporated up to higher levels).

The excited state created in micelles for C_{60} and adduct **2** is spectroscopically similar to that created in (nonpolar) organic solvents, *i.e.* a molecular triplet state. In addition, for adduct **1** a state with charge transfer character appears to be populated. This implies that micellar bound C_{60} and adduct **2** can be used as singlet oxygen sensitizers, as in organic solvents, but that their efficiency will be less (lower estimate: 10%). Micelle-incorporated fullerene-C_{60} and C_{60} adducts are thus expected to be phototoxic and might perhaps find uses in phototherapy. We have also shown that C_{60} and the adducts **1** and **2** are able to enter into photo-redox chemistry with biologically relevant redox partners in micellar solution. Further experiments will be carried out to establish whether this can also be accomplished in liposomal systems, and to what extent the possibility of intramolecular charge transfer in **1** modifies its behaviour in this respect compared to **2** and native C_{60}.

Acknowledgement

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References and notes

1 *J.H. Fendler*, Acc Chem. Res. **13**, 7 (1980).
2 *J.H. Fendler*, J. Phys. Chem. **84**, 1485 (1980).
3 *J.-H. Fuhrhop, J. Mathieu*, Angew. Chem. **96**, 124 (1984).
4 *J.H. Fendler, E.J. Fendler*, Catalysis in micellar and macromolecular systems (1975).
5 *D. Molenaar, W. Crielaard, K.J. Hellingwerf*, Biochemistry **27**, 2014 (1988).
6 *J.H. Fendler*, J. Phys. Chem. **89**, 2730 (1985).
7 *P.J. Clapp, B. Armitage, P. Roosa, D.F. O'Brien*, J. Am. Chem. Soc. **116**, 9166 (1994).
8 *K.C. Hwang, D. Mauzerall*, J. Am. Chem. Soc. **114**, 9705 (1992).
9 *K.C. Hwang, D. Mauzerall*, Nature **361**, 138 (1993).
10 *R.V. Bensasson, J.-L. Garaud, S. Leach, G. Miquel, P. Seta*, Chem. Phys. Lett. **210**, 141 (1993).
11 *H. Hungerbühler, D.M. Guldi, K.-D. Asmus*, J. Am. Chem. Soc. **115**, 3386 (1993).
12 *R.V. Bensasson, E. Bienvenue, M. Dellinger, S. Leach, P. Seta*, J. Phys. Chem. **98**, 3492 (1994).
13 *A. Beeby, J. Eastoe, R.K. Heenan*, J. Chem. Soc., Chem. Commun. 173 (1994).
14 *Y.N. Yamakoshi, T. Yagami, K. Fukuhara, S. Sueyoshi, N. Miyata*, J. Chem. Soc., Chem. Commun. 517 (1994).
15 *R.S. Ruoff, D.S. Tse, R. Malhotra, D.C. Lorents*, J. Phys. Chem. **97**, 3379 (1993).
16 *W.A. Scrivens, J.M. Tour, K.E. Creek, L. Pirisi*, J. Am. Chem. Soc. **116**, 4517 (1994).
17 *R.M. Williams, J.M. Zwier, J.W. Verhoeven*, J. Am. Chem. Soc. **117**, 4093 (1995).
18 *I.H.M. van Stokkum, T. Scherer, A.M. Brouwer, J.W. Verhoeven*, J. Phys. Chem. **98**, 852 (1994).
19 *W. Crielaard, K.J. Hellingwerf, W.N. Konings*, Biochim. Biophys. Acta **973**, 205 (1989).
20 *G.D. Eytan, M.J. Matheson, E. Racker*, J. Biol. Chem. **251**, 6831 (1976).
21 *D.M. Guldi, R.E. Huie, P. Neta, H. Hungerbühler, K.-D. Asmus*, Chem. Phys. Lett. **223**, 511 (1994).
22 Similar traces, with increased absorption around 880 nm and an absorption between 400 and 500 nm are obtained by excitation of C_{60} in benzonitrile containing 0.01M dimethylaniline, which leads to the solvated ion pair with a lifetime of *ca.* 2 μs. In this case the recombination of the ionic species is obviously slowed down by ion-pair dissociation. It thus appears likely that the kinetic stabilisation of the charge transfer state observed in **1** in micellar solution is made possible by the interaction with nearby ground-state molecules.
23 See Ref. 17 and references cited therein.
24 *L. Stryer*, Biochemistry 310 (1975).
25 *R.A.J. Janssen, M.P.T. Christiaans, K. Pakbaz, D. Moses, J.C. Hummelen, N.S. Sariciftci*, J. Chem. Phys. **102**, 2628 (1995).