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Articles

Study of the Aggregation Properties of a Novel Amphiphilic C_{60} Fullerene Derivative

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An amphiphilic C_{60}-derivative, **AFE**, characterized by the presence of the chiral fragment of L-acetyl carnitine in its hydrophilic appendage has been synthesized. In binary (THF/H_2O) and ternary (THF/MeOH/H_2O) solutions, **AFE** exhibits a strong tendency to self-aggregation, provided that the Hildebrand polarity index, δ, of the solvent is higher than about 15. A stable aqueous solution of aggregated **AFE** was obtained. Partition experiments between *n*-octanol and water show that **AFE** cannot be spontaneously transferred from water into the organic solvent (and vice versa), although it is effectively "salted-out" by common electrolytes. Light scattering and reversed-phase liquid chromatography experiments carried out on the aqueous solution of **AFE** suggest for the aggregates an average diameter of 120 nm.

Introduction

Fullerenes and functionalized fullerenes appear to be particularly promising in the biological field,[1,2] as, for example, functionalized fullerenes can be used[3,4] in photodynamic therapy or for the inhibition of HIV enzymes. Most fullerene derivatives also have an unusually low toxicity compared with typical drugs.[5] The predominant hydrophobic character of the spherical carbon allotrope, however, hampers solubilization in polar media such as water, thus slowing the realization of the biological applications. Therefore, the design of water-soluble fullerene derivatives and functionalization of C_{60} with hydrophilic addends has become a central topic in synthetic fullerene chemistry.

Attachment of (only) a single hydrophilic addend promotes water solubility,[6] but strong adsorption forces between the fullerene cores usually lead to the formation of clusters.[7] Synthesis of amphiphilic fullerene monoadducts can be designed to obtain water-soluble supramolecular aggregates characterized by a hydrophobic core of fullerene spheres and a peripheral hydrophilic surface. Although several relatively water-soluble C_{60} derivatives have been recently synthesized,[6,8−11] the knowledge of the factors determining autoassembly is far from satisfactory. The aim of the present work is to find the experimental conditions under which the formation of aggregates occurs, in binary and ternary mixtures of water and organic cosolvents, for the following new fulleropyrrolidine derivatives, **AFE** and **FPO**.

Some peculiar properties of the aggregates have been investigated by UV−vis spectroscopy, and the dimensions of the clusters formed by **AFE** in aqueous solution have been determined by chromatography and static light scattering experiments.

Experimental Section

Syntheses. *5-(Fulleropyrrolidin-1-yl)-3-oxapentan-1-ol (**FPO**).* (See Scheme 1.) To a solution of 2-(2′-aminoethoxy)ethanol (2.94 g, 28.0 mmol) and TEA (5.5 mL, 40 mmol) in 100 mL of dry CH_2Cl_2 at 0 °C, benzylbromoacetate (5.7 mL, 36 mmol) was added over a period of 30 min, and then the solution was brought to room temperature and stirred overnight. The mixture was

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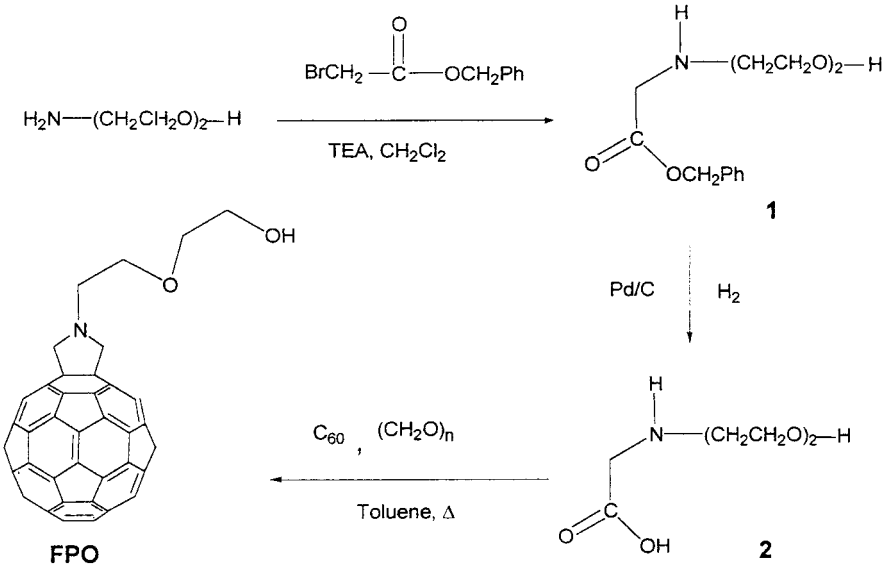
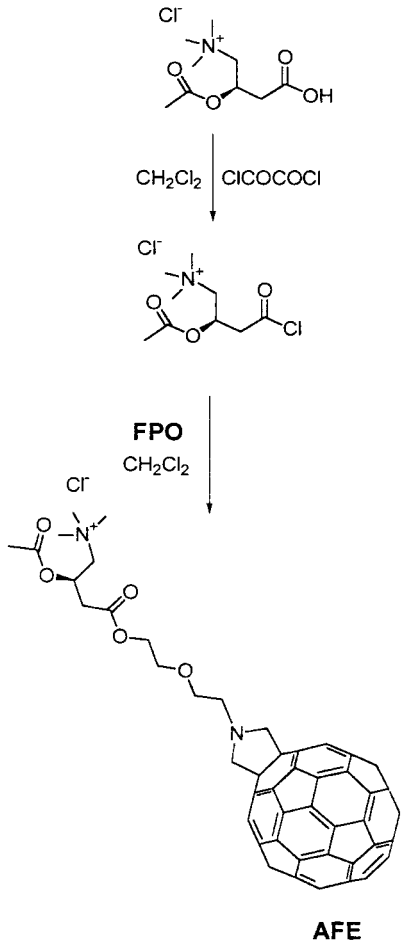
Scheme 1[a]



[a] **FPO**: 5-(**F**ullero**P**yrrolidin-1-yl)-3-**O**xapentan-1-ol.

Scheme 2[a]



[a] **AFE**: (*R*)-2-**A**cetyl-carnitine (5-(**F**ulleropyrrolidin-1-yl)-3-oxapentan-1-yl) **E**ster; IUPAC name, {(*R*)-2-acetoxy-3-[2-(2-fulleropyrrolidin-1-yl-ethoxy)-ethoxycarbonyl]-propyl}-trimethylammonium chloride.

concentrated under reduced pressure, and the product was purified by flash chromatography (SiO_2, ethyl acetate/ethanol/30% aq NH_3 85:10:5) affording 3.75 g (53%) of *N*-[(ethyleneoxy)-2-hydroxyethyl]glycinebenzylester (**1**) as a clear oil. Pd/C catalyst (50 mg) was added to a solution of **1** (0.5 g, 2.0 mmol in 50 mL of ethanol). Hydrogen was bubbled for 1 h, and then the catalyst was filtered over a pad of Celite and washed with methanol. The solution was concentrated under reduced pressure, and the recovered solid was dried in vacuo affording *N*-[(ethyleneoxy)-2-hydroxyethyl]glycine (**2**) in nearly quantitative yield (320 mg).

A solution of C_{60} (440 mg, 0.61 mmol), **2** (102 mg, 0.62 mmol), and paraformaldehyde (56.6 mg, 1.90 mmol) in 300 mL of toluene was heated to reflux temperature for 2 h. The mixture was loaded on top of a SiO_2 column, and the product was purified by flash chromatography using toluene to remove unreacted C_{60} and then toluene/ethyl acetate 7:3. The product was dissolved in the minimum amount of toluene and precipitated by dropwise addition of acetonitrile; 204 mg (39%) of **FPO** was isolated as a brownish powder.

*5-(Fulleropyrrolidin-1-yl)-3-oxapentan-1-ol L-Acetylcarnitine Ester (**AFE**).* (See Scheme 2.) To a suspension of *O*-acetyl-L-carnitine hydrochloride (48 mg, 0.2 mmol) in dry CH_2Cl_2 (0.5 mL), oxalyl chloride (0.026 mL, 0.3 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was dissolved in dry CH_2Cl_2 (3 × 3 mL) and concentrated under reduced pressure to give the corresponding acyl chloride as a white solid, which was used for the next step without further purification. To a solution of the above acyl chloride in dry CH_2Cl_2 (1 mL), a solution of **FPO** (172 mg, 0.2 mmol) was added over a period of 10 min at 0 °C. The mixture was stirred for 10 min at room temperature, sonicated for 3 min, stirred for a further 5 min, and concentrated under reduced pressure at room temperature to give 211 mg (98%) of **AFE** as a brown solid.

1, **2**, **FPO**, and **AFE** were characterized by ^1H and ^{13}C NMR, TLC, IR, MS, and microanalysis [Supporting Information is available].

Aggregates of AFE. Aqueous solutions of aggregated **AFE** can be obtained from a mother solution of about 1×10^{-4} M **AFE** in THF. In a typical experiment, a diluted solution (2.5 mL of mother solution in 50 mL of water) is evaporated in a rotative evaporator at 60 °C and 350 mmHg for 15 min, until about 10% of its volume is evaporated off. After resetting the volume to about 50 mL with water, the solution is sonicated for 1 min. The procedure is repeated four times, and the solvent is evaporated under reduced pressure (70 mmHg), to quantitatively remove traces of THF. The final solution is diluted to 50 mL with water in a volumetric flask and centrifuged (3000 runs/min) for 30 min.

Results and Discussion

AFE was the target molecule of the synthetic project of a fullerene derivative characterized by a presumably unbiased combination of hydrophilic and hydrophobic

Table 1. Solubility of AFE, S_{AFE}, in Solvents of Different Polarities

	solvent	$S_{AFE}/1 \times 10^{-6}$ (mol/dm^3)	ϵ^a (M^{-1} cm^{-1})	δ^b (Kcal$^{1/2}$ dm$^{-3/2}$)
1	water			23.4
2	acetonitrile	1.70		12.1
3	methanol	2.50		14.5
4	ethanol	9.60		12.7
5	2-propanol	11.0		12.0
6	butan-1-ol	240	69 573	10.9
7	dichloromethane	980	98 659	9.9
8	tetrahydrofuran	980	117 358	9.1
9	1,4-dioxane	450	99 890	10.1
10	*n*-hexane	4.20		7.3
11	*n*-octanol	170	80 362	9.0
12	chloroform	380	90 000	9.3

[a] ϵ = extinction coefficient at 254 nm. [b] δ = Hildebrand's polarity index.

regions, prone to spontaneous autoassociation in aqueous media. The connection of L-acetyl carnitine (in the form of hydrochloride) to fulleropyrrolidine by means of a simple oxapentanol spacer turned out to satisfy the above requirements. In particular, acetyl carnitine was chosen as the hydrophilic addend due to its well-known biological activity[12−16] in the transport of fatty acid through mitochondrial membrane surfaces. Molecular mechanics calculations gave a packing parameter of **AFE** close to unity, theoretically compatible with the formation of bilayers and vesicles in solution.[17]

The UV−vis spectra of **AFE** in organic solvents of different polarity show absorption maxima at 254, 320, and 430 nm. These results are in agreement with the reported[18,19] spectroscopic characteristics of monofunctionalized fullerenes present in solution in the form of monomers. The corresponding solubility values, S_{AFE}, in the investigated solvents are collected in Table 1. **AFE** is effectively solubilized by solvents of intermediate polarity, and it is only sparingly soluble in solvents of low or high polarity and appears to be virtually insoluble in water (but see later). In particular, S_{AFE} reaches its maximum value in THF and dichloromethane at a Hildebrand δ value[20] of about 9.5. The aggregation tendency of this amphiphilic molecule has been verified by adding to a solution of **AFE** in THF (where, as mentioned above, this fullerene derivative is present in the monomeric form) increasing amounts of water. The UV−vis spectrum of 1.04×10^{-4} M **AFE** in THF/H_2O mixtures in the range 0−90% (v/v) H_2O is regularly modified according to the change in polarity of the medium (see Figure 1). A progressive loss of resolution of the spectrum can be observed, due to a decrease in absorbance at lower wavelengths and an increase at higher wavelengths in water-rich solution. At the same time, the absorption maximum at 254 nm in THF is shifted to 260 nm in the 10% THF−90% H_2O (v/v) mixture. This spectroscopic behavior can be ascribed to the formation of colloidal clusters[6,7,18,21] arising from the autoassociation of C_{60}

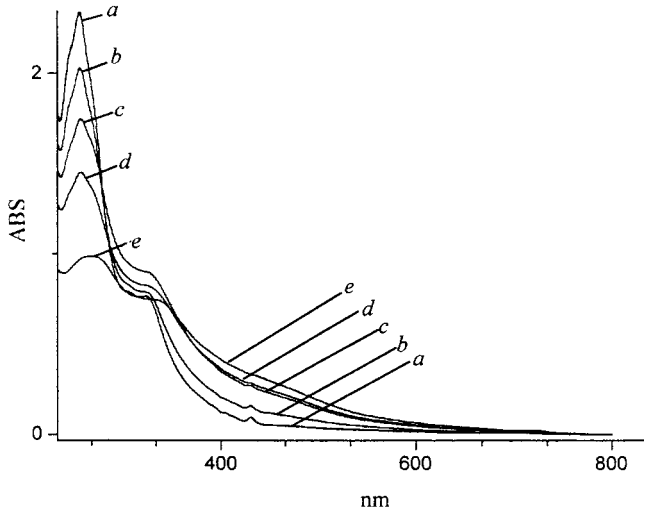


Figure 1. UV−vis spectra of **AFE** in THF−H_2O mixtures in the range 100−10% THF (v/v): a, 100% THF; b, 60% THF; c, 50% THF; d, 40% THF; e, 10% THF ([**AFE**] = 5×10^{-5} M).

spheres. The existence of supramolecular aggregates in water-rich solutions is confirmed by the fact that if a cationic (5×10^{-2} M CTAB) or an anionic (5×10^{-2} M SDS) surfactant is added to an initial 4×10^{-6} M solution of **AFE** in THF, the "normal" UV−vis spectrum of monomeric **AFE** is recorded after progressive additions of water.[19] On the other hand, the formation of clusters is probably best evidenced by considering the difference between the absorbance at 254 nm and that at 410 nm, normalized within the 0−100% range,[22] $(\Delta Abs254-430)_{norm}$, as a function of solvent composition. A plot of $(\Delta Abs254-430)_{norm}$ against the composition of the THF/H_2O or the THF/MeOH/H_2O mixtures (Figure 2) shows clearly that the aggregation of **AFE** is not a linear process. Instead, in the THF/H_2O mixture aggregation begins only at about 40% (v/v) H_2O and is essentially completed at about 75% (v/v) H_2O (Figure 2, curve a). The 60% THF−40% H_2O mixture has a Hildebrand δ value of 15, and a polarity value of this order appears to be necessary to start up the aggregation process of **AFE** in aqueous organic solvents. Indeed, by repetition of the above spectroscopic experiments in ternary mixtures of THF−(70% MeOH−30% H_2O) (v/v), the beginning of the aggregation process of **AFE** occurs at about 20% THF (Figure 2, curve c), that is, at a δ value of 15.6. The above-described cluster formation as a function of the medium polarity appears to be a reversible process. In fact, by addition of increasing amounts of THF to a THF/H_2O solution in which the molecules of **AFE** are extensively associated, the original UV−vis spectrum of the monomeric form is eventually recovered (see Figure 2, curve b).

It has been known for some time[7,9] that aggregation enhances the solubility in water of hydrophilic fullerene derivatives. To test if this is the case for **AFE** too, we have applied the protocol described in the Experimental Section, to promote solubility in water and carefully remove any residual amount of the organic cosolvent which might affect the solubility of aggregated **AFE** in the final aqueous solution. The maximum formal concentration of **AFE** in

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(22) The difference in absorbance was calculated from the following equation: $(\Delta ABS254-430)_{norm} = (\Delta ABS_{THFint} - \Delta ABS_{THFmin})/(\Delta ABS_{THFpure} - \Delta ABS_{THFmin}) \times 100$, where the subscripts THFmin, THFpure, and THFint refer to differences in absorbance of **AFE** in the investigated solvent mixtures at minimum, maximum (100%), and intermediate percentage of THF, respectively.

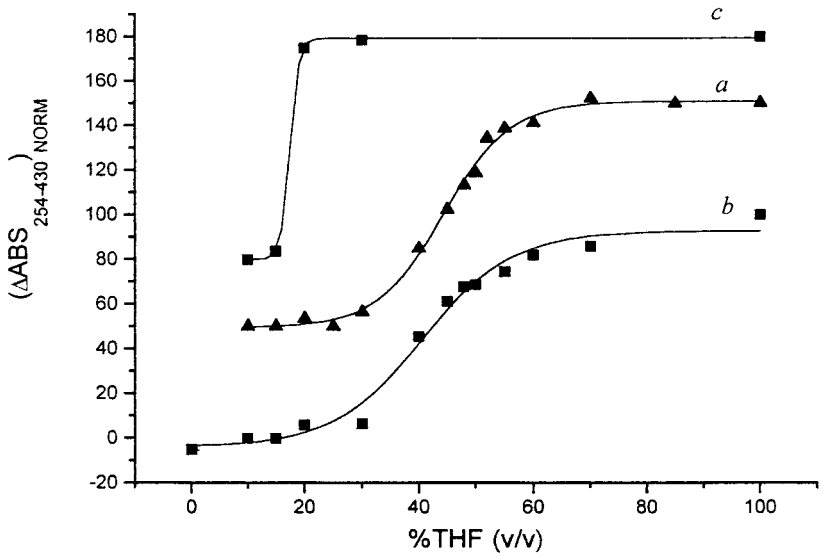

Figure 2. Aggregation (curves a and c) and dissociation (curve b) of **AFE** as a function of solvent composition in binary (THF−H_2O, curves a and b) and ternary ([THF−(70%MeOH−30%H_2O)], curve c) mixtures (v/v). Curves a and c have been translated along the ordinate axis, by 50 and 80 percentual units, respectively, for clarity.

aqueous solution attainable by this method is 1.0×10^{-5} M. The UV−vis spectrum of this solution displays a broad absorption maximum at 260 nm [$\epsilon = 7.9 (\pm 0.2) \times 10^4$ M^{-1} cm^{-1}] and a shoulder at 320 nm [$\epsilon = 4.3 (\pm 0.3) \times 10^4$ M^{-1} cm^{-1}], while the peak at 430 nm, typical of the monomeric form of **AFE**, is absent. This spectrum is similar to that recorded for aggregated **AFE** in the 10% THF−90% H_2O (v/v) solution (Figure 1, spectrum e). **AFE** in the aggregated form is quite stable in aqueous solution: at 25 °C, 80% of its initial absorbance at 260 nm persists after 20 days. Despite the above-mentioned reversibility of the aggregation process, as observed in THF/H_2O mixtures of variable composition (see Figure 2, curves a and b), the clusters of **AFE**, once formed, are insensitive to dilution. The remarkable stability of the colloidal aqueous clusters of **AFE** was also confirmed by some partition experiments between water and *n*-octanol at constant temperature. Absorbance readings (at $\lambda_{max} = 260$ nm in water and $\lambda_{max} = 254$ nm in *n*-octanol), taken at regular time intervals, show that a conventional partition equilibrium is not reached after 48 h, starting neither from a solution of **AFE** in *n*-octanol nor from a solution of aggregated **AFE** in water. These experiments suggest that the aqueous clusters are stable as such, probably as a consequence of the strong $\pi-\pi$ interaction among the C_{60} spheres within the core of the assembled structure as well as of the extensive hydration of the hydrophilic external surface of the aggregate. When some simple electrolytes (i.e., $KClO_4$, KCl, KNO_3) are added to a biphase system, made up of *n*-octanol and an aqueous solution of aggregated **AFE**, a strong "salting out" of the dissolved **AFE** can be observed. The salting out depends on the ionic strength of the aqueous solution rather than on the specific electrolyte.

Finally, we have performed a reversed-phase liquid chromatography (RPLC) analysis of aqueous solutions of **AFE** on a C_{18} column (pore diameter of the stationary phase, 100 Å). The aggregate particles, identified by means of their UV−vis spectrum, are eluted in a peak preceding that of the dead volume, indicating their exclusion from the pores of the stationary phase, and this fact is in agreement with dimensions larger than 10 nm. The direct determination of the dimensions of these aggregates was obtained by static light scattering performed on 5×10^{-6} M aqueous solutions of **AFE**. The average diameter of the aggregates was 121 ± 44 nm, spanning over an interval from 40 to 300 nm.

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Supporting Information Available: Used instrumentation and characterization of all synthesized products. This material is available free of charge via the Internet at http://pubs.acs.org.

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