Solubilization of [60]Fullerene in Water by Aquatic Humic Substances

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The solubilization of [60]fullerene (C_{60}) in water in the presence of aquatic humic substances (HSs) was investigated by means of a batch method. The apparent water solubility of C_{60} in the presence of HSs was 8–540 times higher than that in the blank solution. The size fractionation of C_{60} in aqueous solutions of HSs showed that the solubilization was due to the dispersion of C_{60} aggregates as a result of the adsorption of HSs. These results demonstrate that HSs can play a fundamental role in the solubilization of C_{60} in natural waters.

Environmental risk assessment for potentially hazardous nanomaterials is required in early stage of nanotechnology to avoid subsequent environmental issues that may arise.[1] In particular, [60]fullerene (C_{60}) is a typical example of one such nanomaterial needing risk assessment because of its toxicity to organisms.[2] However, our understanding of the environmental impact of C_{60} is currently far from complete, because the transport and fate of C_{60} in the environment is unknown. Although the solubility of C_{60} in natural waters is a major factor in the overall environmental transport and fate of C_{60}, available information on these issues is incomplete.

In general, C_{60} is a hydrophobic carbon allotrope that is insoluble in water. However, previous studies have shown that C_{60} can be solubilized in water by artificial surfactants such as Triton X-100,[3] poly(vinylpyrrolidone)[4] and fluoroalkyl end-capped oligomers.[5] On the other hand, natural surfactants are widely distributed in aquatic systems. Humic substances (HSs), which typically comprise more than 50% of the total dissolved organic carbon in natural waters,[6] typify such a natural surfactant and play an important role in the solubilization of apolar chemicals in natural waters. In fact, HSs reduce the surface tension and can form micelle-like aggregates above the critical micelle concentration.[7] In addition, the apparent water solubility of apolar chemicals such as DDT[8] and PAHs[9] is significantly enhanced in the presence of HSs. In addition to their surface activity, the hydrophobic backbone in the structure of HSs is characterized by cross-linked aromatic networks,[10] in which the aromatic compounds can preferentially associate via π–π interactions.[11] Thus, these findings suggest that HSs could contribute to the solubilization of C_{60} in natural waters.

In this work, to clarify the contribution of HSs to the solubilization of C_{60} in natural waters, the apparent water solubility of C_{60} in the presence of aquatic fulvic acid (FA) or humic acid (HA) was investigated. In addition, the size fractionation of C_{60} in aqueous solutions of HSs was also carried out, to elucidate the mechanism of the solubilization.

Aquatic HSs, FA and HA, were extracted from the Sahan River in Ukraina. The HSs were purified according to the procedure of the International Humic Substances Society.[12] The elemental compositions of HSs were as follows: C, 41.10; H, 3.63; N, 1.10; O, 51.48; Ash, 2.69% for FA; C, 49.43; H, 3.86; N, 2.42; O, 43.46; Ash, 0.83% for HA. Test solutions of HSs were prepared as: $100\ \mathrm{mg\,L^{-1}}$, pH 6.0, and 0.1 M NaCl. The C_{60} (>99.0% purity) was purchased from Tokyo Kasei Kogyo, Co., Ltd. and was used without further purification.

Solubilization experiments were performed by means of a batch method. A 2-mg sample of C_{60} powder was placed in an Erlenmeyer flask, and an aqueous solution of HSs (48 mL) was then added into the C_{60} powder. After sealing the flask with a silicon rubber cap, the mixture was stirred at 200 rpm and 25.0 ± 0.2 °C in dark. A 3-mL aliquot of the test solution was removed periodically and was filtered through a glass fiber filter (pore size, 0.4 µm). A 2-mL aliquot of the filtrate was mixed with a saturated aqueous solution of NaCl (0.5 mL), and the C_{60} in the solution was then extracted with toluene (1.0 mL). To determine the concentration of C_{60}, a 20 or 60 µL aliquot of the toluene phase was injected into an HPLC system. The detection wavelength was set at 335 nm. An Inertsil PH-3 column (4.6 mm i.d. × 150 mm) was used as the solid phase. Pure toluene was used as the mobile phase, and flow rate was set at $1.0\ \mathrm{mL\,min^{-1}}$.

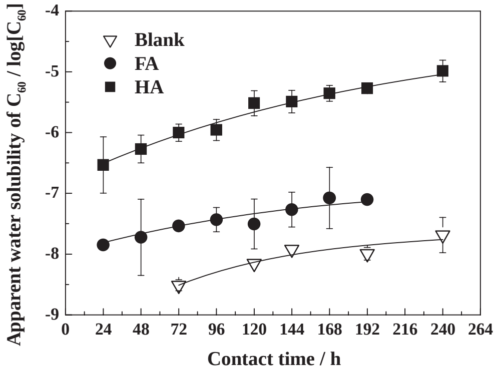

Figure 1. Relationships between contact time and apparent water solubility of C_{60} as a log [C_{60}] in the presence of HSs. Concentration of HSs $100\ \mathrm{mg\,L^{-1}}$, pH 6.0, ionic strength 0.1, and blank 0.1 M NaCl aq.

Figure 1 shows the relationships between the contact time and the apparent water solubility of C_{60} as a logarithmic concentration (M). In all systems, the apparent water solubility of C_{60} increased with increasing the contact time, while no equilibration point was observed at the contact time examined. After contact for 192 h, the apparent water solubility of C_{60} in the blank solution (i.e. 0.1 M NaCl aqueous) was determined to be 1.0×10^{-8} M. In the presence of HSs, the solubility was determined to be 7.9×10^{-8} M for FA or 5.4×10^{-6} M for HA. These results show that the apparent water solubility of C_{60} in the presence of HSs was 8–540 times higher than that in the blank solution. Thus, C_{60} is clearly solubilized in water by

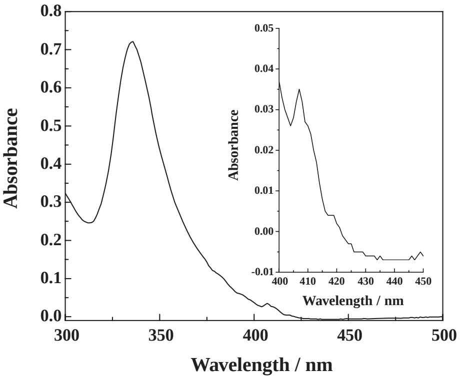

Figure 2. UV–vis spectrum of C_{60}, which was extracted into the toluene phase from an aqueous solution of HA after being in contact for 192 h.

HSs. Figure 2 shows the UV–vis spectrum of C_{60}, which was extracted into the toluene phase from an aqueous solution of HA. The spectrum showed the characteristic bands of C_{60} dissolved in toluene at 335 and 407 nm wavelengths, while no C_{60} epoxide band at a wavelength of 424 nm[13] was observed (insert in Figure 2). This indicates that no oxidation of C_{60} occurred during the time it was in contact with HSs. Thus, the solubilization of C_{60} can be attributed as being due to an interaction with HSs.

To understand the mechanism by which C_{60} is solubilized, C_{60} in an aqueous solution of HSs was fractionated using three types of inorganic filters with different pore sizes (i.e., 20, 100, 200 nm). The results of the size fractionation of C_{60} after being in contact with HSs for 192 h are summarized in Table 1. C_{60} was detected in aqueous solutions of HSs in all size fractions. Approximately 85% of the total solubilized C_{60}, defined as the fraction below 400 nm, was enriched in the size fraction from 200 to 400 nm, while the remaining 15% was distributed below 200 nm. In the blank solution, however, C_{60} was detected only in the size fraction from 200 to 400 nm. Previous studies have shown that the size of aquatic HSs is below 10 nm.[14] The HS concentration examined in this study is much lower than the aggregation point of HSs (690–12500 mg L^{-1}).[7] Thus, these findings indicate that the solubilization of C_{60} by HSs is due to the dispersion of C_{60} aggregates as a result of adsorption to HSs.

At each size fraction, the apparent solubility of C_{60} in the presence of HA was 47–280 times larger than that in the presence of FA (Table 1). On the other hand, no decrease in the absorbance of HSs at a wavelength of 400 nm was observed, even after passing the solution through a filter with a pore size of 20 nm. This shows that the adsorption of FA or HA to C_{60} aggregates was in a saturated state. Therefore, these results indicate that HA is more effective in dispersing C_{60} aggregates than FA. In general, HA has a low charge density[15] and a relatively large hydrophobic backbone consisting of cross-linked aromatic networks,[10] compared to FA. Such structural characteristics of HA result in its higher surface activity and affinity for aromatic compounds than that of FA. Although the relationship between the structural characteristics of HSs and the degree of dispersion of C_{60} aggregates is unclear at this time, both the surface activity and hydrophobic structural features of HSs should be a factor determining the degree of dispersion of C_{60} aggregates. Further work will be necessary to clarify the issue.

Table 1. Size distribution of C_{60} in the aqueous solution of HSs after being in contact for 192 h

HSs	Abundance of C_{60}/%				Apparent water solubility[c] /μM
	F1[a]	F2[a]	F3[a]	F4[a]	
Blank[b]	100	N.D.[d]	N.D.	N.D.	0.010
FA	85.8	7.9	1.9	4.4	0.079
HA	83.6	5.3	7.8	3.6	5.41

[a]Fractions are represented as F1: 200–400 nm, F2: 100–200 nm, F3: 20–100 nm, F4: <20 nm. [b]Blank: 0.1 M NaCl aq. [c]Apparent solubility is defined as the fraction below 400 nm. [d]N.D., "Not detected".

In conclusion, C_{60} is significantly solubilized in water in the presence of aquatic HSs. This demonstrates that aquatic HSs can play a fundamental role in the solubilization of C_{60} in natural waters. Thus, our results will be useful for understanding the transport and fate of C_{60} in aqueous environments.

References

1 V. L. Colvin, *Nat. Biotechnol.* **2003**, *21*, 1166; R. F. Service, *Science* **2003**, *300*, 243; F. Proffitt, *Science* **2004**, *305*, 762; M. R. Wiesner, G. V. Lowry, P. Alvarez, D. Dionysiou, P. Biswas, *Environ. Sci. Technol.* **2006**, *40*, A4336.
2 E. Oberdörster, *Environ. Health Perspect.* **2004**, *112*, 1058; C. M. Sayes, J. D. Fortner, W. Guo, D. Lyon, A. M. Boyd, K. D. Ausman, Y. J. Tao, B. Sitharaman, L. J. Wilson, J. B. Hughes, J. L. West, V. L. Colvin, *Nano Lett.* **2004**, *4*, 1881; C. M. Sayes, A. M. Gobin, K. D. Ausman, J. Mendez, J. L. West, V. L. Colvin, *Biomaterials* **2005**, *26*, 7587; D. Y. Lyon, L. K. Adams, J. C. Falkner, P. J. J. Alvarez, *Environ. Sci. Technol.* **2006**, *40*, 4360.
3 R. V. Bensasson, E. Bienvenue, M. Delliger, S. Leach, P. Seta, *J. Phys. Chem.* **1994**, *98*, 3492.
4 Y. N. Yamakoshi, T. Yagami, K. Fukuhara, S. Sueyoshi, N. Miyata, *J. Chem. Soc., Chem. Commun.* **1994**, 517.
5 H. Sawada, J.-I. Iidzuka, T. Maekawa, R. Takahashi, T. Kawase, K. Oharu, H. Nakagawa, K. Ohira, *J. Colloid Interface Sci.* **2003**, *263*, 1.
6 E. M. Thurman, *Organic Geochemistry of Natural Waters*, Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, **1985**, pp. 103–110.
7 K. Hayase, H. Tsubota, *Geochim. Cosmochim. Acta* **1983**, *47*, 947; M. Terashima, M. Fukushima, S. Tanaka, *Colloids Surf., A* **2004**, *247*, 77; M. Terashima, M. Fukushima, S. Tanaka, *Chemosphere* **2004**, *57*, 439.
8 R. L. Wershaw, P. J. Burcar, M. C. Goldberg, *Environ. Sci. Technol.* **1969**, *3*, 271; C. T. Chiou, R. L. Malcolm, T. I. Brinton, D. E. Kile, *Environ. Sci. Technol.* **1986**, *20*, 502.
9 S. Tanaka, K. Oba, M. Fukushima, K. Nakayasu, K. Hasebe, *Anal. Chim. Acta* **1997**, *337*, 351.
10 F. J. Stevenson, *Humus Chemistry*, 2nd ed., John Wiley & Sons, Inc., New York, **1994**, pp. 285–302.
11 T. D. Gauthier, E. C. Shane, W. F. Guerin, W. R. Seitz, C. L. Grant, *Environ. Sci. Technol.* **1986**, *20*, 1162; T. D. Gauthier, W. R. Seitz, C. L. Grant, *Environ. Sci. Technol.* **1987**, *21*, 243.
12 G. R. Aiken, in *Humic Substances in Soil, Sediment and Water: Geochemistry and Isolation*, ed. by G. R. Aiken, D. M. McKnight, R. L. Wershaw, P. MacCarthy, Wiley-Interscience, New York, **1985**, pp. 363–385.
13 K. M. Creegan, J. L. Robbins, W. K. Robbins, J. M. Millar, R. D. Sherwood, P. J. Tindall, D. M. Cox, *J. Am. Chem. Soc.* **1992**, *114*, 1103.
14 R. Beckett, Z. Jue, J. C. Giddings, *Environ. Sci. Technol.* **1987**, *21*, 289; J. R. Lead, K. J. Wilkinson, K. Starchev, S. Canonica, J. Buffle, *Environ. Sci. Technol.* **2000**, *34*, 1365.
15 M. M. Yee, H. Kodama, T. Miyajima, N. Takisawa, *Humic Subst. Res.* **2005**, *2*, 27; M. M. Yee, T. Miyajima, N. Takisawa, *Colloids Surf., A* **2006**, *272*, 182.

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