Original Paper

J. Jpn. Soc. Colour Mater. (SHIKIZAI), **74**(2), 63-67(2001)

Direct Observations of Assemblies of Novel Branched-Tail Fluorinated Anionic Surfactants in Water

Masanobu SAGISAKA*, Kyung Ok KWON**, Hideki SAKAI*,*** and Masahiko ABE*,***

Abstract

Molecular assembly formation of sodium 1, 2-bis(ω -perfluoroalkyldioxyethylene-carbonyl)-1-ethanesulfonate (nFS(EO)₄), novel double chain-type fluorinated anionic surfactants, in aqueous solutions was investigated by direct TEM observations and measurements of the entrapment efficiency of a water-soluble substance and the degree of counter ion dissociation on the assemblies.

The investigation revealed that all nFS(EO)₄ first form molecular assemblies of several tens nm sizes and then allow the assemblies to grow in size to several hundreds nm to several μ m as their concentration increases. The assemblies with sizes of several hundreds nm were identified as vesicles because they were found to form spontaneously and retain the water-soluble substance in their interior.

Key-words : Fluorinated Anionic Surfactant, Double Chain-type Surfactant, Oxyethylene, Vesicle, Transmission Electron Microscope

1. Introduction

Vesicles composed of fluorocarbon surfactant molecules are expected to be useful as drug carriers for drug delivery systems (DDS) and fields for organic chemical reactions¹⁾ since they have characteristics not shared by hydrocarbon surfactants such as high resistance to fusion²⁾ and low leakage of enveloped substances³⁾. They are also expected to be good as drug carriers capable of supplying oxygen because of their high affinity to oxygen⁴⁾. Fluorocarbon surfactant-based vesicles thus show potential as a functional material that can meet many of the diversified and complicated demands of modern society.

Yet, the use of fluorinated surfactants is not widespread and improvements are then necessary of their low solubility in water due to their strong hydrophobicity, also their high manufacturing cost needs to

be reduced in order to make them versatile. We have recently synthesized sodium 1, 2-bis(ω -perfluoroalkyldioxyethylenecarbonyl)-1-ethanesulfonates (nFS(EO)₄), fluorinated double chain-type anionic surfactants with a high water solubility that has never been observed for conventional long chain-type or double chain-type surfactants⁵⁾ and found that the surfactants synthesized form molecular assemblies of a discrete size distribution with several peaks. No detailed discussion has been made on the properties of the assemblies, including their shape, though a brief description has been given for their varying sizes such as micellar and much larger ones in our latest paper⁶⁾.

We will conduct in this study direct TEM observations and measurements of entrapment efficiency of water soluble substance and the degree of counter ion dissociation on molecular assemblies of nFS(EO)₄ in aqueous solution to examine the formation and shape of the assemblies in more detail.

2. Experimental

2.1 Materials

2.1.2 Surfactants

The surfactants used were sodium 1, 2-bis(ω -perfluoroalkyldioxyethylenecarbonyl)-1-ethanesulfonates with fluorocarbon chain lengths (n) of 4, 6,

Received August 25, 2000

* Faculty of Science and Technology, Science University of Tokyo, 2641, Yamazaki, Noda, Chiba 278-8510, Japan

** Korea Fire Equipment Inspection Corporation, 29-16, Shinheung-dong, 3ga, Chung-ku, Incheon City, Seoul, Korea

*** Institute of Colloid and Interface Science, Science University of Tokyo, 1-3, Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan

Fig. 1 Sodium 1, 2-bis (ω -perfluoroalkyldioxyethylenecarbonyl)-1-ethanesulfonate.

and 8, fluorinated double chain-type anionic surfactants, the chemical structure of which is shown in **Fig. 1**, and they are hereafter abbreviated to nFS(EO)₄. Their synthesis and purification were carried out according to the method in the earlier paper⁶⁾.

2. 1. 2 Water

Distilled water for injection (Ohtsuka Pharm. KK) was used as the water (pH 6.5).

2. 2 Methods

2. 2. 1 Electron microscopic observation by freeze-fracture method

Freeze-replica was prepared for each of nFS(EO)₄ solutions using a freeze-replica preparing apparatus (Hitachi Science System KK, Model FR-7000A) to observe the aggregation state in the solution. In the replica preparation, samples frozen in liquid propane were cut with a glass knife to remove the possible effects of cryohydrates, platinum was evaporated on the cut section at about 5 nm thickness at 45° to give shadows to the fine structures on the section, and then carbon was evaporated on the platinum-evaporated section at about 10 nm thickness at 90° to provide the cast. Metal replica films prepared using the cast were observed with a transmission electron microscope (JEOL KK, Model JEM-1200EX) at an acceleration voltage of 80 kV.

2. 2. 2 Measurement of entrapment efficiency

Measurements of entrapment efficiency of D-glucose (Nippon Rika Co., Ltd, reagent grade) in molecular assemblies were made by the method described in a previous paper⁷⁾ using nFS(EO)₄ solutions in 0.28 mol·dm⁻³ D-glucose solution.

2. 2. 3 Optical microscopic observation

Optical microscopic observations of molecular assemblies were performed at 30°C with an inversion-type optical microscope (Olympus KK, Model IMT-2) under the Normalski differential interference.

2. 2. 4 Measurement of counter ion dissociation

Counter ion concentration of molecular assemblies was determined at 30°C with an ion concentration meter (TOA Electronics Ltd., Model IM-40S) combined with a sodium ion electrode (TOA Electronics Ltd., Model NA-115B). The degree of counter ion dissociation was evaluated by the method of Botre et

al.^{8,9)} using the determined counter ion concentrations. A saturated calomel electrode (TOA Electronics Ltd., Model HS-305DS) was used as the reference electrode.

3. Results and Discussion

As reported in the previous paper⁶⁾, the aggregated state of nFS(EO)₄ in aqueous solution changes with increasing concentration in such a way that small molecular assemblies, A_s, formed at low concentrations grow to vesicles of several hundreds nm sizes and those of several μm sizes, large molecular assemblies, A_M, and A_L, respectively, and all of these assemblies are simultaneously present in a wide concentration range. We then directly observed these molecular assemblies of different sizes, A_s, A_M, and A_L, with the transmission electron microscope using the freeze-fracture method to examine the assemblies in detail. Typical electron micrographs are given in **Fig. 2** showing molecular assemblies in an nFS(EO)₄ solution containing the three kinds of assemblies. Figure 2(A) is a micrograph of the smallest particles observed in the present study, which are molecular assemblies with sizes almost identical to those of A_s determined by the dynamic light scattering (DLS) method⁶⁾. Thus, the particles are A_s and spherical or disk-like molecular assemblies with sizes ranging from several to several tens nm as the micrograph indicates. These molecular assemblies can be regarded as micelles since they were found to have no water phase in their interior as mentioned later. Figures 2(B) and 2(C) are respectively micrographs of the middle-sized and largest particles observed and they were identified as A_M and A_L, respectively, since their sizes coincided with those obtained by DLS measurements for A_M and A_L⁶⁾. A_M and A_L are spherical molecular assemblies of sizes ranging from several hundreds nm to several μm and they are regarded as vesicles because as described later they have an inner water phase and 8FS(EO)₂, a fluorinated double chain-type surfactant having fewer added moles of ethylene oxide by 2 than nFS(EO)₄, forms vesicles of several hundreds nm sizes⁷⁾.

Glucose entrapment efficiency by molecular assemblies was then measured to confirm the presence of vesicles in aqueous solutions of nFS(EO)₄ (**Fig. 3**). The entrapment efficiency started to rise at a certain surfactant concentration below which it was practically zero for all surfactants. Consequently, the surfactant concentration at which the entrapment effi-

Fig. 2 Electron micrographs of freeze-fracture replica prepared from 6FS(EO)₄ solution at $4.3 \times 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$. Bar length is 100 nm.

Fig. 3 Relationship between entrapment efficiency E_T of nFS(EO)₄ assemblies and concentration of nFS(EO)₄.

Table 1 CAC and CVC of nFS(EO)₄ at 30°C.

nFS(EO) ₄	n=4	n=6	n=8
CAC/mol·dm ⁻³	2.3×10^{-5}	3.1×10^{-6}	2.4×10^{-5}
CVC/mol·dm ⁻³	1.2×10^{-4}	1.3×10^{-5}	2.3×10^{-5}

ciency starts to rise was designated as the vesicle formation concentration (Critical Vesicle Concentration: CVC). Since this concentration coincided with the concentration at which A_M and A_L appear, these large molecular assemblies with a water phase in the interior were identified as vesicles. Table 1 shows the values of CVC obtained in this work and those of CAC (Critical Assembly Concentration) reported in the earlier work⁶⁾. The table indicates no dependence on fluorocarbon chain length of the CAC and CVC values for nFS(EO)₄. In addition, nFS(EO)₄ are suggested to have a chemical structure suitable for forming vesicles in a wide concentration range because they were found to form vesicles of several tens μm

Fig. 4 A differential interference optical micrograph for 6FS(EO)₄ solution at $1.0 \times 10^{-2} \text{ mol} \cdot \text{dm}^{-3}$.

sizes even at concentrations 1000 times higher than CVC as shown in Fig. 4.

Many methods are known at present for preparing vesicles including those of mixing solutions of more than two kinds of surfactants (for example, cationic and anionic surfactant solutions)¹⁰⁻¹³⁾ and exerting external forces such as ultrasonic irradiation on solutions of single surfactants capable of forming plane lamellar structures typical of lipids^{14,15)}. Moreover, examples of spontaneous vesicle formation are reported in certain single surfactant solutions¹⁶⁻¹⁸⁾. Vesicles were also found to form spontaneously in single surfactant solutions of nFS(EO)₄ in the absence of external force in the present work.

It is generally accepted that the shape of molecular assemblies depends mainly on the hydrophobe-hydrophile balance and steric structure of surfactant molecules. Estimation of the degree of counter ion dissociation was then made for molecular assemblies of nFS(EO)₄ through measurements of sodium ion activity as a function of surfactant concentration to examine

Table 2 Degree of counter ion dissociation of nFS(EO)₄ assemblies at 30°C.

Phase	Degree of counter ion dissociation/%		
	n=4	n=6	n=8
Micelle	1.9	9.7	—
Vesicle	1.9	2.5	2.1

the steric structure of fluorinated surfactant molecules involved in vesicle formation (**Table 2**). The degree of counter ion dissociation for 6FS(EO)₄ decreased to 9.7% at the concentrations above CAC (micellar state) and reduced further down to 2.5% at the concentrations above CVC (vesicular state) as shown in the table. Similarly, 4FS(EO)₄ and 8FS(EO)₄ exhibited an extremely low degree of counter ion dissociation at the concentrations above CVC (vesicular state). According to the critical packing parameter theory^{19–22}, molecular assemblies of surfactant take an aggregated state with a low curvature in aqueous solution when the area occupied by its hydrophilic group on the surface of the assemblies becomes small compared with the size of its hydrophobic group. For nFS(EO)₄ in the present study, the dissociation of counter ions at the molecular terminal is suppressed since the ionic strength of the solution increases with increasing surfactant concentration and this causes dehydration of oxyethylene chains²². As a consequence, surfactant molecules take a cylinder-like structure suited for vesicle formation (**Fig. 5**) because of their double fluorocarbon chains and

the decreased occupied area of their hydrophilic groups. Here, the molecular model shown in **Fig. 5** is the one for R-form and the steric structure of S-form is the mirror image of that for R-form. Since nFS(EO)₄ have two highly hydrophobic fluorocarbon groups and strongly hydrophilic groups (an SO₃[−] and four oxyethylene groups) at the opposite ends of the molecule and the ether bond of their oxyethylene group allows the rigid fluorocarbon chains to orient reasonably in molecular assemblies, their structure is highly effective in enhancing the stability of bilayer structures and the order of molecular orientations²³. The fluorinated surfactants thus form vesicles spontaneously because they have two fluorocarbon chains, a good hydrophobe-hydrophile balance, and ether bonds in their molecule. Coexistence of vesicles with different curvatures for nFS(EO)₄ at concentrations above CVC would result from the asymmetric carbon atom (that connects the two chains) in their molecule. Actually, both R- and S-form optical isomers of nFS(EO)₄ are present in their solutions since the asymmetric carbon atom is generated through the reaction of a maleic acid diester derivative, an intermediary compound, with sodium hydrogen sulfite in the synthesis of nFS(EO)₄. This would be the cause for the coexistence of vesicles with different curvatures in nFS(EO)₄ solutions and the phenomenon will be investigated in detail.

4. Conclusions

The aggregation behavior in aqueous solutions of

Fig. 5 Steric structure models of nFS(EO)₄ molecules (R-form).

- Lone pair ○ Hydrogen ● Carbon
- Oxygen ⊗ Fluorine ⊙ Sulfur

sodium 1, 2-bis(ω -perfluoroalkyldioxyethylenecarbonyl)-1-ethanesulfonates, nFS(EO)₄, was surface-chemically examined with emphasis on their vesicle formation and the following facts were revealed.

All of nFS(EO)₄ were found to form micelles with sizes of several tens nm at concentrations above CAC and vesicles with sizes of several hundreds nm or larger at concentrations above CVC in their aqueous solutions. This would result from the fact that all of nFS(EO)₄ have two fluorocarbon chains and ether bonds in their molecule, a structure favorable for vesicle formation.

References

- 1) T. Kunitake, N. Higashi: *Macromol. Chem., Suppl.*, **14**, 81 (1985).
- 2) T. Kunitake, Y. Okahata, S. Yasunami: *J. Am. Chem. Soc.*, **104**, 5547 (1982).
- 3) N. Azuma, T. Kunitake: *Membrane*, **10**, 138 (1985).
- 4) E. Kissa: "Fluorinated Surfactants: Synthesis• Properties• Applications", p. 254, Marcel Dekker, New York, (1994).
- 5) Y. Kondo, E. Yokochi, S. Mizumura, N. Yoshino: *J. Fluorine Chem.*, **91**, 147 (1998).
- 6) M. Sagisaka, A. Ito, Y. Kondo, N. Yoshino, K. Ok Kwon, H. Sakai, M. Abe: *Colloids Surf. A: Physicochemical and Engineering Aspects*, in press.
- 7) M. Abe, Y. Kondo, M. Sagisaka, H. Sakai, Y. Morita, C. Kaise, N. Yoshino: *J. Jpn. Soc. Color Mater.*, **73**, 8 (2000).
- 8) C. Botre, V. L. Crescen Zi, A. Mele: *J. Phys. Chem.*, **63**, 650 (1959).
- 9) K. Ogino, N. Tsubaki, M. Abe: *J. Colloid interface Sci.*, **98**, 78 (1984).
- 10) E. W. Kaler, A. K. Murthy, B. Rodriguez, J. A. N. Zasadzinski: *Science*, **245**, 1371 (1989).
- 11) E. W. Kaler, K. L. Herrington, A. K. Murthy, J. A. N. Zasadzinski: *J. Phys. Chem.*, **96**, 6698 (1992).
- 12) D. J. Iampietro, E. W. Kaler: *Langmuir*, **15**, 8590 (1999).
- 13) H. Sakai, A. Matsumura, S. Yokoyama, T. Saji, M. Abe: *J. Phys. Chem. B*, **103**, 10737 (1999).
- 14) A. D. Bangham, R. W. Horne: *J. Mol. Biol.*, **8**, 660 (1964).
- 15) S. M. Johnson, A. D. Bangham, M. W. Hill, E. D. Korn: *Biochim. Biophys. Acta*, **233**, 820 (1971).
- 16) B. W. Ninham, D. F. Evans, G. J. Wel: *J. Phys. Chem.*, **87**, 5020 (1983).
- 17) Y. Talmon, D. F. Evans, B. W. Ninham: *Science*, **221**, 1047 (1983).
- 18) A. Masuyama, Y. Nakatsuji: *Hyomen*, **32**, 8 (1994).
- 19) C. Tanford: "The Hydrophobic Effect", Chap. 9, Wiley-Interscience, New York, (1973).
- 20) J. N. Israelachvili, D. J. Mitchell, B. W. Ninham: *Biochim. Biophys. Acta*, **470**, 185 (1977); J. N. Israelachvili: "Intermolecular and Surface Forces", Academic Press, London (1985).
- 21) P. R. Cullis, B. De Kruijff: *Biochim. Biophys. Acta*, **559**, 399 (1979).
- 22) J. N. Israelachvili: *Chem. Scripta*, **25**, 7 (1985).
- 23) N. Kimizuka, T. Takasaki, T. Kunitake: *Chem. Lett.*, 1911 (1988).

水溶液中における新規二鎖型フッ素系アニオン界面活性剤分子集合体の観察

鷺坂将伸*・Kyung Ok Kwon**・酒井秀樹***・阿部正彦***

*東京理科大学理工学部 千葉県野田市山崎 2641 (〒278-8510)

**Korea Fire Equipment Inspection Corporation, 29-16, Shinheung-dong, 3ga, Chung-ku, Incheon City, Seoul, Korea

***東京理科大学界面科学研究所 東京都新宿区神楽坂 1-3 (〒162-8601)

要 旨

新規二鎖型フッ素系アニオン界面活性剤である Sodium 1, 2-bis(ω -perfluoroalkyldioxyethylenecarbonyl)-1-ethanesulfonate (nFS(EO)₄) の水溶液中における分子集合体の形成が、透過型電子顕微鏡による直接観察、水溶性物質の保持効率、分子集合体の対イオン解離度などの測定により検討された。

その結果、いずれの nFS(EO)₄ の場合も、その濃度増加にともない、まず数十 nm 以下の分子集合体が形成した後、数百 nm の分子集合体や数 μ m の大きな分子集合体が形成した。これらのうち、数百 nm 以上の大きな分子集合体はその内部に水溶性物質を保持できたことからベシクルであることがわかり、それはまた自発的に形成することもわかった。