Vesicles Formation of Double-Chain Cationic Surfactants with Different Chain-Lengths in Deep Sea Water

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Abstract: Vesicle forming ability of double-chain cationic surfactants {dialkyldimethylammonium bromides $\{(C_nH_{2n+1})_2(CH_3)_2N^+Br^-$; DDAB ($n=12$), DTDAB ($n=14$), DHDAB ($n=16$), DODAB ($n=18$)} in deep sea water was examined, and the effect of their chain length on the membrane properties and dispersability of vesicles formed was investigated.

Any of the surfactants failed to form vesicles in 100% of deep sea water while they formed vesicles in a mixture of deep sea water (5%) / purified water (95%) and in deep sea water treated with reverse osmosis membrane (RO water). The particle size of vesicles was found to increase with the chain length of the cationic surfactants (except for DHDAB). For DTDAB with 14 carbon atoms in the alkyl chain, vesicles in RO water were more stable than in purified water, being stable for six months after preparation. The experimental findings demonstrated that the stability of the vesicles depended on the preparation temperature and the gel-liquid crystal phase transition temperature of the surfactants (in each system).

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Key words: vesicle, deep sea water, alkyl chain length, stability

1 Introduction

Among those surfactants such as phospholipids and double-chain surfactants which possess both hydrophilic and hydrophobic groups in a molecule, there are some that can form liposomes and vesicles holding water in the inner phase of the closed bilayer structures, when they are forced to be dispersed by an external force like ultrasonication (1-4). Vesicles are expected to be used as a biomembrane model (5), a drug carrier in drug delivery systems (DDS) (6), a sensor base such as

stimulus-responsive membrane (7-9), a new organic reaction field (vesicle-catalytic reaction) (10, 11) and a remover of organic substances in super-filtration (12), since the inner aqueous phase of vesicles can encapsulate water-soluble materials and concurrently the bilayer membrane can take up oil-soluble materials. Then, effective usages of these vesicles and liposomes need not only encapsulation of water-soluble materials but also uptake of oil-soluble materials into the bilayer membrane, that is, concurrent solubilization of materials of different polarities in the different media (1-4).

It is in the sea where life emerged for the first time on the earth 3.8 billion years ago. Thus, the source of all living creatures is the "Sea", and its water always flows

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Table 1 Comparison between Component of Deep Sea Water and Surface sea Water.

head		unit	surface sea water (the depth of water 0 m)	deep sea water (the depth of water 200 m)
general conditions	temperature	°C	21.0 (16.5 ~ 29.0)	13.1 (10.8 ~ 15.2)
	pH		8.19	7.89
nutrtive salts	NO ₃ -N	μ gat/l	1.49	26.00
	PO ₃ -P		0.34	1.65
	SiO ₂ -Si		13.6	64.20
main elements	Na	%	0.97	1.00
	Mg	%	0.13	0.13
	Ca	mg/l	421	426
	K	mg/l	406	419
trance metals	Ni	mg/l	0.33	0.38
	Zn	mg/l	0.66	0.71

slowly and ceaselessly. "Deep Sea Water" in the deep flow has ripened for long years and contains a lot of useful minerals and nutritious salts similar to those in the living body. **Table 1** shows the ingredients contained in deep sea water (13, 14). Clearly, "Deep Sea Water" contains a lot of nutritious salts and minerals, in contrast to surface sea water (13-15). Because of this, deep sea water has been used in many fields including the food and medical fields and the range of its application is going to extend even to the cosmetic field in recent years (13, 14). In particular, if deep sea water can be applicable in the cosmetic field, new living body-friendly cosmetics would be expected as it is efficient in permeation through and moisturization of skin (14, 16-19).

In this work, we studied preparation of vesicles in deep sea water to find a new possibility for the water that has seldom been used so far in the cosmetic field. Preparation of vesicles comprising various cationic surfactants was conducted in particular in 100%, 50% and 5% of deep sea water as well as in RO water (deep sea water treated with reverse osmosis membrane). Besides, the effects of alkyl-chain length in surfactants were studied on the membrane properties and the stability of the vesicles.

2 Experimental

2.1 Materials

2.1.1 Surfactants

Dialkyldimethylammonium bromides $\{(C_nH_{2n+1})_2(CH_3)_2N^+Br^-$; DDAB ($n=12$), DTDAB ($n=14$), DHDAB ($n=16$), DODAB ($n=18$) $\}$ as double-chain cationic sur-

factants were purchased from Tokyo kasei Co. and used without further purification.

2.1.2 Water

Waters for the preparation vesicles were used; Deep Sea Water taken at the coast of Muroto point, Kochi Prefecture, RO water (deep sea water treated with reverse osmosis membrane) and purified water (Ohtsuka Pharm. Co.). Purified water was used for comparison with deep sea water.

2.2 Methods

2.2.1 Preparation of vesicle dispersions

Preparation of surfactant vesicles was done using the ultrasonic irradiation method according to the previous paper (1). As in the previous paper (1, 2), an aqueous solution of surfactant (10mM) was irradiated in an ultrasonic irradiator (Branson Cleaning Equipment, Type B-220; output 125W) at a temperature higher than T_c (T_c : DDAB = 10°C, DTDAB = 25 ~ 27°C, DHDAB = 28 ~ 30°C, DODAB = 42 ~ 45°C) for 120 min or more to yield a coarse vesicle dispersion. Subsequently the dispersion was treated with a polycarbonate membrane filter (pore size 100 nm and 500 nm, Nuclepore Inc.) using an extruder (Lipex Biomembranes Inc.), resulting in a homogenous fine vesicle dispersion. The most suitable preparation temperature of vesicles was about 20°C higher than T_c as in the previous papers (1, 2) and the preparation temperatures were 35, 50, 55 and 65°C for DDAB, DTDAB, DHDAB and DODAB, respectively.

2.2.2 Preparation of vesicles containing fluorescent probe and measurement of fluorescence polarization

Preparation of vesicles containing 8-anilino-naphthalene-1-sulfonate (ANS) was conducted as in the previous paper (1). An aliquot of an ethanolic ANS solution was taken in a test tube with screw cap and the solvent was removed under reduced pressure to leave a thin film of ANS on the test tube wall. An aqueous dispersion containing vesicles prepared with the double-chain cationic surfactant (10 mM) was added to this test tube to make ANS concentration 0.5 mM and then the resultant dispersion was left standing for 24 h. In this way, a fluorescent probe-loaded vesicle dispersion was obtained.

A fluorophotometer (Shimadzu Model RF-3000) was used to measure the intensities of vertical and parallel polarizations to the exciting light, and the degree of fluorescence polarization, P , was calculated as in the previous paper (3).

2·2·3 Measurement of zeta-potential

The surfactant vesicle surface is positively charged due to the cationic hydrophilic group. The zeta-potential of vesicles was measured at 30°C using a NICOMP 380ZLS (Particle Sizing System Co.).

2·2·4 Particle size of vesicles

The particle size of vesicles was determined by a dynamic light scattering method. The distribution and time course of vesicle sizes were measured at 30°C using a NICOMP 380ZLS (Particle Sizing System Co.). The light source was a diode pump solid state laser (DPSS laser) with a wavelength of 535 nm and the scattering angle was 90°.

2·2·5 Observation of vesicle by TEM

Observation of vesicle bilayer membranes was carried out with freeze-replica TEM micrographs. Briefly,

a freeze replica of vesicles was made with a freeze-replica preparation equipment (FR-7000A, Hitachi Co. freeze-replica apparatus), and the replica thus obtained was observed using a TEM microscope (JEM-1200EX, JEOL Co.) with 80 kV acceleration voltage.

2·2·6 Measurement of differential scanning calorimetry (DSC)

The phase transition temperature of vesicle bilayer membranes was measured with a differential scanning calorimeter (DSC8420B, Rigaku Denki Co.). A vesicle dispersion was placed in a sampling vessel made of stainless steel (resistance to pressure at 50 atm, 3 mm × 5 mm), and then the vessel was sealed. The measurement conditions were 1 K min⁻¹ for the scanning rate, 30 ~ 60°C for the scanning range and 0.1 m cal s⁻¹ for the sensitivity.

3 Results and Discussion

3·1 Preparation of Vesicle Dispersion Using Deep Sea Water

First, we investigated the effect of the properties of water on vesicle formation. **Figure 1** shows pictures of vesicles (from left to right) prepared with purified water, 100% deep sea water, 50% deep sea water, 5% deep sea water, and RO water, respectively. RO water was the reverse osmotic filtrate of deep sea water (14). The pictures in the figure revealed that preparation of vesicles was impossible with 100% deep sea water, because crystalline aggregates were formed on the upper part of the solution.

Since the ion strength of deep sea water is relatively high due to the mineral ingredients such as nitrate,

Fig. 1 Photograph of Purified Water, Deep Sea Water, Mixture of Deep Sea Water with Purified, RO Water Including DDAB Molecules.

Table 2 Particle Size of DDAB Vesicles Prepared by the Ultrasonication Method.

solvent		particle size of vesicles aggregate /nm (after 1 day)
H ₂ O	Deep Sea Water / %	
100	0	20
95	5	250
50	50	300
0	95	—
RO water		180

Fig. 2-1 Freeze-replica TEM Micrograph of DDAB-RO Water Solution. **Fig. 2-2** Micrograph of DDAB-RO Water Solution.

phosphate salts and those of magnesium and calcium cations, it is likely that the ion dissociation of surfactant was restricted and crystalline aggregates were formed in 100% deep sea water. On the contrary, when the mixing ratio of deep sea water with purified water was reduced to 50% and 5%, the vesicle dispersion became bluish semitransparent, suggesting fine vesicle formation. A similar bluish color was also observed for DDAB vesicle dispersion prepared in purified water (1, 21). The same tendency was seen for vesicle dispersions prepared with RO water. In short, since deep sea water contains a lot of nutritious salts and minerals, preparation of vesicles is possible only when it is mixed with purified water or RO water is used.

We then measured the effect of deep sea water concentration on the particle size of vesicles of surfactants having different alkyl tail lengths. **Table 2** shows that the particle size of vesicles increases with increase in deep sea water concentration. The particle size of aggregates formed in 100% deep sea water could not be measured due to their precipitation.

Although vesicles were formed in 50% deep sea water solution, the vesicles were less stable and crystalline aggregate formation was seen after 4 or 5 days. On the other side, the particle sizes of vesicles in 5% deep sea water and RO water were 250 nm and 180 nm, respectively, and they were highly stable “deep sea water vesicles” in both cases.

We then directly observed the vesicles formed in RO water, which is frequently used in many industries, by TEM using the freeze replica technique (**Fig. 2-1**) and by optical microscopy (**Fig. 2-2**) about one month after preparation, on the assumption that deep sea water vesicles are made practicable. In **Fig. 2-1** are seen roughly spherical particles and the particle size was slightly larger (400 ~ 500 nm) than that immediately after preparation. On the other hand, a doughnut like structure characteristic of vesicles was observed in the optical micrograph (**Fig. 2-2**), confirming the formation of vesicles holding water in the inner phase because the vesicles could hold water-soluble drugs (data are not given here).

3·2 Stability of Vesicles in Deep Sea Water

Table 3 shows the particle size of vesicles in RO water prepared using 4 kinds of double-chain cationic surfactants with different chain lengths. Vesicles formation was confirmed beforehand for all surfactants. With

Fig. 3 Photograph of RO Water Vesicles.
(after 1 day)

the exception of DHDAB, the vesicle size in RO water was larger than that in purified water as is shown in the table. This would be due to the increased aggregation number of surfactant molecules brought about by the increase in the ionic strength of the medium to suppress the degree of counterion dissociation. On the other hand, the vesicle size increased with increasing alkyl chain length of the double-chain cationic surfactant. The particle size of DHDAB vesicles had a broad distribution (20 ~ 2000 nm) and showed a polydispersity.

In addition, vesicle dispersions of DDAB, DTDAB and DODAB were stable without separating any crystalline precipitates even after one year, whereas the particle size of DHDAB vesicles grew gradually and crystalline aggregates precipitated 3 days after preparation, thereby making it difficult to obtain stable vesicle dispersions in DHDAB system. We then performed observations by TEM and optical microscopy on stable DODAB vesicles and unstable DHDAB vesicles, respectively, in order to study the factors affecting stability of vesicles. **Figure 4 (A)** shows the uniformity in the particle size of DODAB vesicles (500 nm). While,

the particle size of DHDAB vesicles was heterogeneous (20 nm ~ 2 μ m) aggregates were also observed in **Fig. 4 (B)**. In other words, the system that give homogeneous vesicles was suggested to have a good dispersability and stability even in RO water as in purified water.

We further studied changes with time of the vesicle size in RO water (**Fig. 5**) and the zeta-potential (**Fig. 6**) in order to understand vesicle stability. **Figures 5 and 6** show that all of DDAB, DTDAB and DODAB kept their vesicle size and zeta-potential at almost a constant level. Also, though not shown in the figure, no precipitates such as crystals were observed in these vesicle dispersions. In contrast, the particle size and zeta-potential of DHDAB vesicles increased with the elapse of time, and they could not be measured due to crystalline precipitates suspending in the dispersion.

These findings would demonstrate a high difficulty of preparing stable vesicles in the DHDAB system even with RO water. In fact, DHDAB forms a polydispersed vesicle system where the particles collide with each other to aggregate with ease.

3.3 Effect of the Phase Transition Temperature of Bilayer Membranes of Deep Sea Water Vesicles

In order to examine the state of the hydrophilic moieties of the bilayer surface, vesicles containing ANS, a fluorescence probe, were prepared and the degree of ANS fluorescence polarization was measured (**Fig. 7**). The degree of fluorescence polarization increased with increase in the alkyl chain length of the cationic surfactant for vesicles prepared with RO water and purified water (except for DHDAB). In general, the degree of ANS fluorescence polarization is known to increase with increase in the packing density of the hydrophilic groups of surfactant molecules in the vesicle surface (1, 19). Rupert *et al.* (1, 20) mentioned that in order for the packing density of the surface hydrophilic groups (cationic groups in our case) to become higher, the electrostatic repulsion between the hydrophilic groups involved should be reduced by the adsorption of the oppositely charged species (anions) to the surface hydrophilic groups and the hydrophilic groups should also be dehydrated.

Meanwhile, the fluorescence polarization of ANS in the bilayer membranes of DHDAB vesicles prepared with purified water was small as compared to that of

Table 3 Particle Size of Vesicles Aggregates Prepared by the Ultrasonication Method.

surfactant	particle size of vesicles/nm (after 1 day)	
	H ₂ O	solvent Deep Sea Water (RO water)
DDAB (n=12)	20	180
DTDAB (n=14)	165	300
DHDAB (n=16)	170	20 ~ 2000
DODAB (n=18)	200	400 ~ 500

Fig. 4 Freeze-replica TEM Micrographs and Optical Micrographs for DODAB, DHDAB-RO Water Solution.

those prepared with RO water. Although the reason for this is not clear at the moment, it would be related to the low stability of DHDAB vesicles as shown in the previous paper (1).

We reported in the previous paper (21) that changes in the fluorescence polarization value observed become smaller as the vesicle size is reduced because the hydration structure is more rigid for smaller vesicles. It is

then necessary for larger vesicles to have a lower electrostatic repulsion between the surfactant hydrophilic groups through suppressing their ionization, because larger vesicles show a bilayer membrane structure having a relatively small curvature. As a result, the fluorescence polarization value for larger vesicles is smaller than that for smaller vesicles. Contrary to this expectation, the fluorescence polarization value of vesicles pre-

Fig. 5 The Apparent Particle Size of RO Water Vesicles as a Function of Time.

Fig. 6 The Apparent Zeta-potential of RO Water Vesicles as a Function of Time.

Fig. 7 Relationship between the Fluorescence Polarization Degree of ANS in Vesicles and the Carbon Number of Surfactants.

pared with RO water was larger than that of those prepared with purified water (smaller vesicles). Consequently, the degree of ANS fluorescence polarization depends on the packing density of the hydrophilic groups in vesicle bilayers, and the vesicles prepared with RO water have a stronger bilayer membrane structure and are more stable.

Although we reported in the previous paper (21) that DTDAB vesicles prepared with purified water had a poor stability and floating substances like liquid crystals were observed several days after preparation, DTDAB vesicles prepared with deep sea water were stable even six months after preparation. We then determined the phase transition temperature of DTDAB vesi-

cles prepared with RO water by DSC to be 32°C. Bilayer of DTDAB vesicles prepared with purified water were in an unstable state at 30°C because the phase transition temperature of the vesicles was 27°C, which is close to the observation temperature (30°C) in this work. On the other hand, the vesicles prepared with RO water showed a better stability because the phase transition temperature rose to 32°C due to the deep sea water ingredients, and this gave a good stability to the bilayer membrane structures at the lower observation temperature. The reason for the observed poor dispersibility of DHDAB vesicles in both purified water and RO water would be that the phase transition temperatures of the vesicles in both systems were almost the same as the

observation temperature.

4 Conclusion

The experimental results mentioned so far clearly demonstrated that the preparation of surfactant vesicles using "Deep Sea Water" is possible. Furthermore, the stability of surfactant vesicles was found to depend on the mixing ratio of deep sea water with purified water and the alkyl chain length of the surfactant. Since the stability of vesicles prepared with RO water was very high and their use is expected to expand. Then, if we can prepare liposomes with deep sea water can encapsulated in the inner phase and oil-soluble materials solubilized in the bilayers, we expect these liposomes to be used cosmetics and medicines for delivering the contents deep inside skin.

References

1. Kondo, Y., Yoshino, N., Kaise, C., Sakai, H. & Abe, M. (1997) *J. Jpn Oil Chem. Soc. (J. Oleo Sci.)*, **46**, 323-329.
2. Nojima, S., Sunamoto, J. & Inoue, K. (1988) *Liposome*, Nankoudou publications.
3. Shobha, J., Srinivas, V. & Balasubramanin, D. (1989) *J. Phys. Chem.*, **17**, 93.
4. Christian, S. D., Smith, G. A. & Tucker, E. E. (1985) *Langmuir*, **1**, 564-567.
5. Singer, S. J. & Nicolson, G. L. (1972) *Science*, **175**, 720-731.
6. Nakajima, T. & Toyoda, T. (1994) *Gekkan kaiyou*, **26**, 134-135.
7. Tabufe, I. & Mthuo, T. (1979) *Kagaku-zoukan 82 Asunoenerugi-to-kagaku - jinnkou-hikarigousei*, Kagakudou-jin.
8. Toyoshima, Y. (1978) *Maku*, **3**, 163-164.
9. Okahata, Y. & Seki, T. (1984) *Chem. lett.*, 1251.
10. Okahata, Y., Tanamachi, S. & Kunitake, T. (1981) *NIPPON KAGAKU KASHI*, **8**, 442-448.
11. Kunitake, T. & Higashi, N. (1985) *Macromol. Chem., Suppl.*, **14**, 81.
12. Abe, M. & Kondo, Y. (1999) *CHEMTECH*, **29**, 33-42.
13. Kaise, C. (1999) *Material Technology*, **17**, 334-341.
14. Ostro, M. J. & Cullis, P. R. (1989) *Am. J. Hosp. Pharm.*, **46**, 1576.
15. Kubota, S. (1996) *Atarashii-mizu-no-kisochishiki*, Ohmushya press, 55-56.
16. Sakurai, H. (1996) *Kinzoku-ha-naze-jinntainihituyouka*, Koudansha press.
17. Nomura, T. (1985) *The state and subject of ocean method of treatments*, **73**.
18. Atopic dermatitis Institute, Report on Effectiveness of Deep Sea Water on Atopic dermatitis, 1996. (in Japan)
19. Inoue, T., Muraoka, Y., Fukushima, K. & Shimozaawa, R. (1988) *Chem. Soc.*, **46**, 107.
20. Rupert, L. A. M., Hoekstra, D. & Engberts, J. B. F. N. (1986) *J. Am. Chem. Soc.*, **108**, 3920.
21. Kaise, C., Sakai, H., Kondo, Y., Yoshino, N. & Abe, M. (1997) *J. Jpn. Soc. Colour Mater. (SHIKIZAI)*, **70**, 242-250.