

# Surfactant Vesicles as Membrane Mimetic Agents: Characterization and Utilization

JANOS H. FENDLER

Department of Chemistry, Texas A&M University, College Station, Texas 77843

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Biological membranes organize molecules in their different compartments, provide suitable microenvironments, and allow for the controlled transport of solutes. Not surprisingly, membrane biology and biophysics are extremely active areas of current research.

Cell membranes are best considered in terms of the fluid-mosaic model, proposed by Singer and Nicolson.<sup>1</sup> According to this model the matrix of the membrane, a lipid bilayer composed of phospholipids and glycolipids, incorporates proteins, either on the surface or in the interior, and acts as a permeability barrier (Figure 1). Molecules are free to diffuse laterally in the plane of the membrane. Conversely, transverse diffusion (flip-flop) of large molecules is slow. This behavior can be rationalized in terms of the structure of lipids constituting the membrane. Lipids contain a hydrophobic moiety, generally an aliphatic double chain, phosphate, or carboxylate ester polar head groups and intermediate regions where hydrogen bonding can occur (Figure 1). Passive permeability is a function of the phase transition from ordered to liquidlike bilayers, and it depends on the lengths of the hydrocarbon chain, the position and number of double bonds, and the amount of cholesterol present.

Complexities of natural membranes have necessitated the use of models for investigations at the molecular level. These investigations fulfill two purposes. Firstly, better and more detailed understanding of biological membranes may be obtained. Secondly, mimicking membrane functions in relatively simple models may lead to novel chemistry of practical utility. Attention will be focused here on this latter aspect of membrane mimicking. In this respect, one need not attempt to reproduce Mother Nature slavishly. Simpler systems and approaches different from those utilized in vivo may well reproduce economically viable chemical processes presently mediated by membranes.

Synthetic surfactant vesicles, believed to be the simplest functional membrane models, are the subject of the present Account. These vesicles are defined as smectic mesophases of completely synthetic surfactant bilayers containing entrapped water. This definition includes spherical, ellipsoidal, single, lamellar, and multilamellar structures. Formation of vesicles composed of biological materials (e.g., phospholipids), referred to in the literature as liposomes or bangosomes, have been recognized for some time.<sup>2</sup> Vesicles or liposomes, once formed, do not disintegrate for weeks, even months. The slow leaving rates of monomers from vesicles are responsible for their kinetic stabilities.<sup>3,4</sup>

On prolonged standing, vesicles undergo fusion; however, larger and more polydisperse entities are formed.

Although formation of bilayer structures in simple surfactant dispersions has been inferred from their phase diagrams,<sup>5</sup> they were first recognized as potential membrane models by Gebicki and Hicks.<sup>6</sup> Shaking thin films of oleic and linoleic acids in aqueous buffers yielded closed bilayers, which they called ufasomes.<sup>6</sup> Ufasomes, unfortunately, are unstable outside the pH 6-8 range, do not concentrate on centrifugation, and retain substrates poorly.<sup>6</sup> More recently, formation of vesicles from a variety of single-chain amphiphiles of C8-18 chains has been reported.<sup>7</sup> These vesicles were found to be stable over a large pH range and behaved as ideal osmometers and entrapped solutes.<sup>7</sup> Vesicles consisting of only monoalkyl surfactants have been considered to be plausible prebiotic precursors of the cell membrane.<sup>8</sup>

This Account is devoted to work performed in the author's laboratories with dioctadecyldimethylammonium chloride (DODAC)<sup>9-13</sup> and dihexadecyl phosphate (DHP),<sup>13,14</sup> which are the best characterized and most investigated surfactant vesicles. Formation of cationic dialkyldimethylammonium halide,<sup>15-29</sup> an-

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Janos H. Fendler is Professor of Chemistry at Texas A&M University. His current interest is the development of chemistry based on membrane-mediated processes. For this work, he received the degree of D.Sc. from London University. In addition, he is fascinated by excited-state stereochemistry, enantiomeric recognition, and circularly polarized laser-initiated processes.

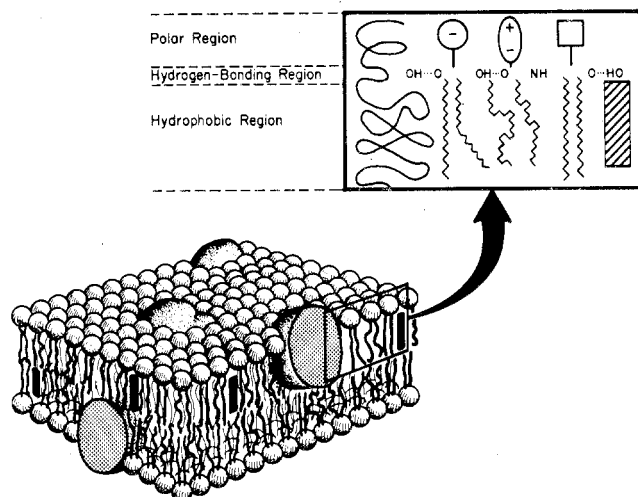


Figure 1. Fluid mosaic model of a cell membrane.<sup>1</sup>

Table I  
Weight-Averaged Molecular Weights,  $\bar{M}_w$ , and  
Hydrodynamic Radii,  $R_H$ , of Surfactant Vesicles<sup>a</sup>

surfactant vesicle	sonication		$10^{-6}\bar{M}_w$	$R_H, \text{ \AA}$	$R/R_0^c$
	time, min <sup>b</sup>				
DODAC	0.5		34.1	1000	5.1
DODAC	10.0		21.6	447	2.6
DODAC	50.0		13.4	407	2.8
DODAC	60.0		12.6	396	2.8
DHP	3.0		560.0	847	1.7
DHP	12.0		65.0	772	3.1
DHP	24.0		36.0	592	2.9
DHP	48.0		24.0	595	3.3
DHP	60.0		30.0	525	2.7

<sup>a</sup> Taken from ref 13; polydispersity indices are 0.026 and 0.22 for DODAC and DHP vesicles, respectively.

<sup>b</sup> Using a Branson B-12 sonifier. DODAC dispersions were sonicated at 55 °C at a setting of 3; DHP dispersions were sonicated at 65 °C at a setting of 7. <sup>c</sup> Measured hydrodynamic radius divided by the radius of a sphere calculated from the determined value of  $\bar{M}_w$ .

ionic dialkyl phosphate,<sup>30,31</sup> sulfonate,<sup>30</sup> carboxylate,<sup>30</sup> and zwitterionic<sup>20</sup> surfactant vesicles have also been reported by other investigators.

### Characterization of Surfactant Vesicles

Sonic dispersal of surfactants is the most general method for vesicle formation. Sonication has to be carried out above the phase-transition (vide infra) temperature. The type of sonicator, the applied power, and the time and temperature of sonication need to be specified. In the absence of sonication, shaking surfactant dispersions at high (well above phase transition) temperatures yields extremely large nonuniform par-

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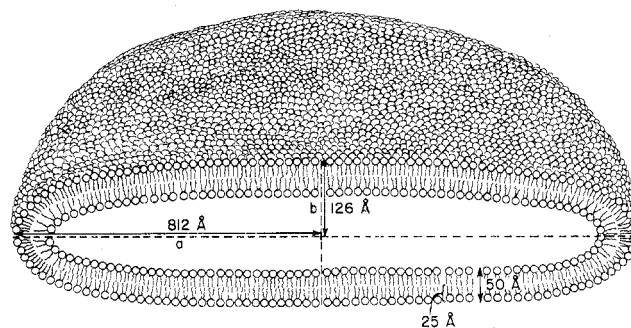


Figure 2. Schematic representation of a well-sonicated surfactant vesicle based on low-angle laser light scattering and photon correlation spectroscopy.<sup>13</sup>

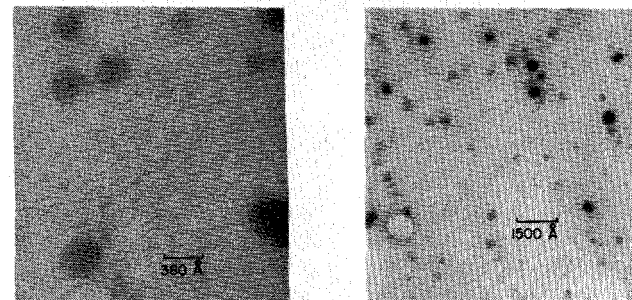


Figure 3. Electron micrographs of sonicated DODAC vesicles at 258 000 and 66 600 magnifications;<sup>11</sup> distribution of multicompartment vesicles are clearly seen.

Table II  
Morphological Comparisons of DODAC Vesicles and  
Phosphatidylcholine Liposomes

	DODAC vesicles <sup>a</sup>	phosphatidylcholine liposomes <sup>b</sup>
$10^{-6}\bar{M}_w$	32	2
number of molecules/vesicle	48 500	2450
radial parameters, Å	$a = 812; b = 126$	$r = 99$
bilayer thickness, Å	50	37
volume of bilayer, $10^{-6} \text{ \AA}^3$	36	3
outer surface area, $10^{-5} \text{ \AA}^2$	$10^{-5}$	1.2
inner surface, $10^{-4} \text{ \AA}^2$	57	4.8

<sup>a</sup> Assuming vesicles to be prolate ellipsoids. Data taken from ref 13. <sup>b</sup> Assuming liposomes to be spherical. Taken from ref 32.

ticles. Increasing the sonication time, at a given power setting, results in an exponential decrease in the viscosity and turbidity of the solution, down to a point beyond which further sonication has no appreciable effect. This point is considered to correspond to the appearance of single compartment bilayer vesicles. Centrifugation enhances the uniformity of the vesicles. Additionally, it removes titanium impurities released from the tip of the ultrasonic probe. Low angle laser light scattering and photon correlation spectroscopy substantiate this postulate.

Weight-average molecular weights,  $\bar{M}_w$ , and hydrodynamic radii,  $R_H$ , of DODAC and DHP vesicles decrease exponentially with increasing sonication time (Table I). Formation of DODAC vesicles is seen to require less sonic power than that needed for DHP vesicles. DHP vesicles are somewhat larger and less polydisperse than their cationic counterparts. Prolate ellipsoids describe best the structure of surfactant

vesicles (Figure 2). They are appreciably larger than liposomes (Table II). The considerably greater electrostatic head-group repulsions in the highly charged surfactant vesicles than those prevailing in liposomes are presumably responsible, in part, for the different packing arrangements.

Electron microscopy<sup>15-17,31</sup> substantiates the gross features of surfactant vesicles (Figure 3). Care is needed, however, in interpreting electron micrographs of surfactant vesicles, since both freeze-fracture and staining techniques may alter molecular arrangements.

Phase transitions of DODAC vesicles have been established to occur at 30 and 36 °C.<sup>11</sup> Below 30 °C DODAC vesicles are likely to consist of one-dimensional lamellae with the hydrocarbon chains fully extended and somewhat tilted.<sup>22</sup> Transformations from one- to two-dimensional structures occur at 30 °C, the pre-transition temperature. Between 30 and 36 °C, the fully extended surfactant molecules are distorted by periodic undulations. At the phase-transition temperature, 36 °C, the hydrocarbon chains "melt" by assuming mobile liquidlike arrangements and revert to one-dimensional lattices. The phase-transition temperature of DODAC vesicles is essentially identical with that observed for dipalmitoyl-DL- $\alpha$ -phosphatidylcholine liposomes (36.4 °C).<sup>32-34</sup>

Both cationic and anionic surfactant vesicles are osmotically active. In hyperosmolar solutions (electrolyte concentration greater on the outside than that within the vesicles) they shrink, while in hypoosmolar solutions they swell.<sup>11</sup> Osmotic shrinkage is the consequence of water effusion from the vesicles at an appreciably faster rate than solute infusion into them. The range of electrolyte concentrations, however, is considerably more limited than that used for liposomes. Electrolytes in excess of 0.1 M tend to precipitate surfactant vesicles. Osmotic activity implies, of course, that surfactant vesicles are closed and that water is contained in their interior. The extent and rates of osmotic shrinkage of DODAC vesicles are dependent on the temperature. Changes in these dependencies are indicative of the phase transition.<sup>11</sup>

Initial shrinkage rates of DODAC vesicles depend on the electrolytes used.<sup>11</sup> They increase with decreasing size of the alkali cation, implying some permeation of ions into DODAC vesicles.<sup>35</sup> This is a direct consequence of enhanced fluidities, caused by electrostatic repulsions of the positively charged head groups in DODAC vesicles. The observed increase in the initial shrinkage rates of phospholipid liposomes with increasing concentrations of added octadecylamine<sup>20</sup> is in accord with this interpretation. Enhanced fluidities of surfactant vesicles are also manifested in decreased viscosities in the bilayers (i.e., microviscosities) compared to liposomes. The microviscosity of DODAC vesicles is 144 cP while that of liposomes is 291 cP.<sup>9</sup> Interestingly, the use of benzophenone as a photochemical probe indicates multilamellar didodecyl phosphate vesicles to be considerably more rigid than

their single compartment analogues.<sup>28</sup>

Hydrogen ion concentrations in vesicle interiors and proton and hydroxide ion permeabilities across the bilayers have been determined by use of fluorescence probes.<sup>36,37</sup> Positively charged 2-aminopyridine hydrochloride and negatively charged trisodium 8-hydroxy-1,3,6-pyrenetrisulfonate, pyranine, were used for cationic DODAC and anionic DHP vesicles, respectively.<sup>37</sup> Electrostatic repulsions between the similarly charged probes and vesicle surfaces cause the probes to locate in the middle of the aqueous interiors. Free probes have been separated from those entrapped by the surfactant vesicles by gel filtration. In the absence of cholesterol, protons and hydroxide ions instantaneously permeate the vesicles. However, in cholesterol-containing DODAC vesicles, proton and hydroxide ion permeabilities become measurably slow and a pH gradient across the bilayer can be maintained for some time. Cholesterol also affects other properties of surfactant vesicles. In the presence of cholesterol, DODAC vesicles become smaller and less permeable and have altered phase transitions.<sup>9,11</sup>

Positively charged vesicles concentrate hydroxide ions, while negatively charged ones attract protons on their surfaces. This is seen by changes in the dissociation constants of vesicle-bound dyes. For example, the apparent  $pK_a$  of pyranine on the surface of DODAC vesicles is 6.15, compared with 7.23 in water. These  $pK_a$  values correspond to a surface potential<sup>38</sup> of 63.5 mV for DODAC vesicles.<sup>37</sup>

An important property of surfactant vesicles is that they entrap and retain large molecules in their interiors. For example, the entrapment of zwitterionic amino acids in DODAC vesicles is meager. The anionic form of 8-azaguanine, on the other hand, is incorporated extensively in cationic surfactant vesicles.<sup>8</sup> Once again, electrostatic binding is responsible for this behavior. Leakage of surfactant-entrapped substrates is minimal. Typically only 5–10% of the entrapped substrate is released in 20 h.<sup>9</sup> It should be borne in mind that entrapped molecules may alter the size and shape of vesicles.

Geometrical and compositional differences between the inner and outer monolayers are recognized properties of the cell membrane.<sup>32</sup> They influence ion permeabilities. Asymmetry and its consequences can most conveniently be investigated in surfactant vesicles. Differential charge densities and potentials between the inner and outer surface of DODAC vesicles have been created by judicious addition of sodium chloride.<sup>39</sup> DHP vesicles containing copper on their outer surfaces have also been prepared.<sup>37</sup> Functionalized asymmetrical surfactant vesicles will find interesting applications.

### Photochemical Solar Energy Conversion in Surfactant Vesicles

Since the excited state of a molecule is a better electron acceptor, as well as a better electron donor, than its ground state, light absorption can drive a redox reaction nonspontaneously:

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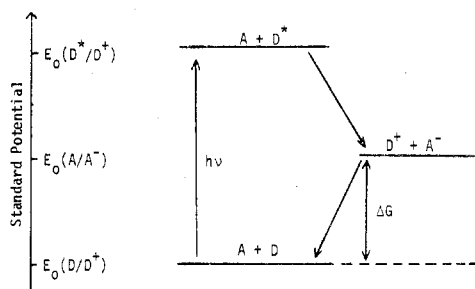
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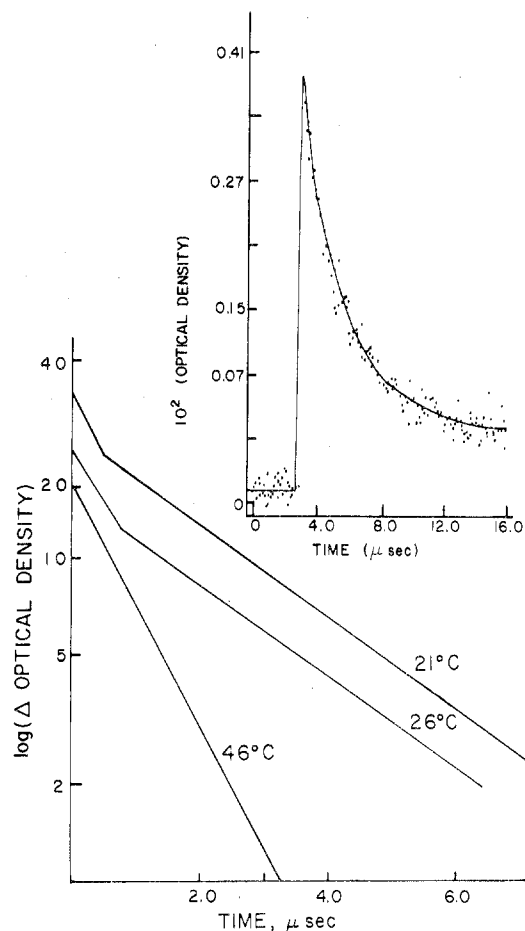
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Energy of an amount  $\Delta G$  can, therefore, be stored in  $D^+$  and  $A^-$ . Further, through a series of electron transfers to and from additional donors and acceptors, photoinduced catalytic water decomposition may be realized. Unfortunately, in solution, rapid combination of  $D^+ + A^-$  to  $D + A$  precludes the possibility of this type of photochemical energy storage and conversion. Separation in space of the photoproducts provides the most efficient method for obviating the back reaction. Indeed, this is accomplished by the photosynthetic membrane in photosynthesis.<sup>40</sup> Sensitized photoconversions have been carried out in micelles,<sup>41</sup> monolayers,<sup>42</sup> and liposomes.<sup>43</sup> These systems allowed some degree of organization and facilitated charge separation to some extent. Surfactant vesicles provide an alternative method for the selective organization of donor-acceptor couples. They are able to accommodate substantially more guest molecules per aggregate than can micelles. The amount of light absorbed by guest molecules is considerably greater than that absorbed by monolayers. Surfactant vesicles, therefore, possess all the desirable characteristics of liposomes, but they are chemically stable and easy to prepare and functionalize. Energy and electron transfer, as well as charge separation, have been demonstrated in surfactant vesicles.<sup>14,44-48</sup>

Organizational ability of DODAC vesicles is readily demonstrated by intermolecular energy transfer. The lysopyrene to pyranine transfer serves as an example.<sup>44</sup> The donor, lysopyrene, was anchored in the hydrophobic bilayer of the vesicles by its long hydrocarbon chain, which terminates in a choline group. The acceptor, pyranine, was electrostatically attached to the



**Figure 4.** Decay of photoejected hydrated electron at different temperatures. The inset shows a typical digitalized electron decay at 26 °C.<sup>14</sup>

outer charged surface of DODAC vesicles. Depending on the concentration of pyranine, energy-transfer efficiencies up to 43% have been observed. In the absence of surfactant vesicles energy transfer is negligible. The dramatically enhanced energy transfer efficiency is a reflection of effective donor and acceptor localization in the relatively small volume of DODAC vesicles. Under the experimental conditions ( $8.0 \times 10^{-4}$  M stoichiometric DODAC,  $1.0 \times 10^{-8}$  M vesicle)<sup>44</sup> all the available outer surface of the vesicles are expected to be covered at a stoichiometric pyranine concentration of  $3.6 \times 10^{-5}$  M. This expectation was confirmed by determining energy-transfer efficiencies prior and subsequent to resonication.<sup>44</sup> Resonication opens up and reseals the vesicles and hence it results in a distribution of pyranine between the inner and outer surface of DODAC vesicles. At low stoichiometric pyranine concentration, the consequence of redistribution is a statistical decrease of the average interchromophoric distance between the donors and acceptors, which results in increased energy-transfer efficiencies. At  $2.5 \times 10^{-5}$  M stoichiometric pyranine concentration, resonication does not increase energy transfer efficiencies, suggesting that the outer surface had been effectively covered by the acceptor. Average interchromophoric distances between donor and acceptor molecules, calculated from the Förster equation,<sup>41</sup> varied between 45 and 60 Å. Considering the approximations involved, these values are in accord with those derived from the low-angle laser light-scattering experiment (shown in Figure 2).

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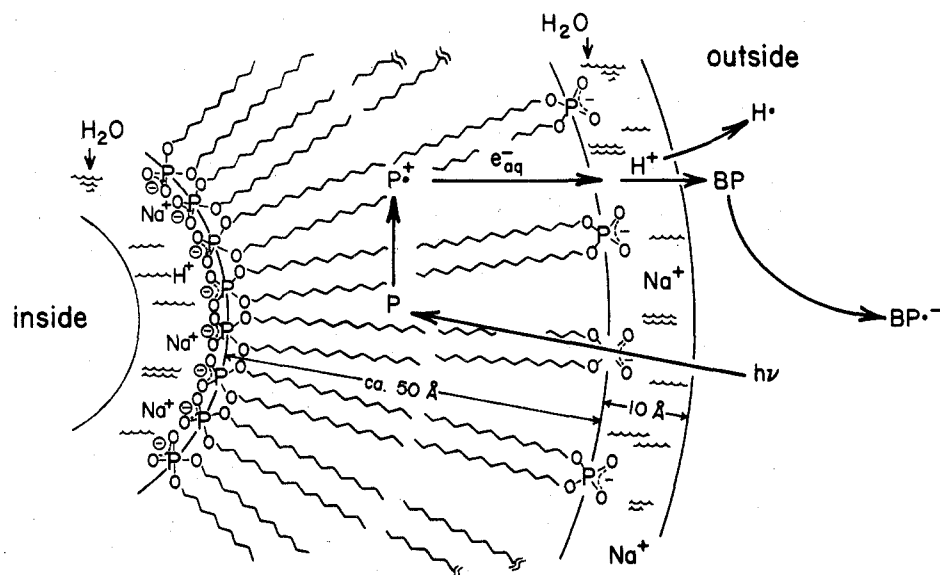
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**Figure 5.** Schematic representation of laser-induced photoionization of DHP-entrapped pyrene and subsequent electron transfer. Pyrene molecules are shown to be localized in the center of the bilayer. In reality, this probe is free to distribute itself dynamically and some probe may be localized somewhat close to the head group of the vesicle.<sup>14</sup>

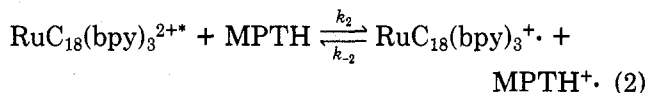
Efficient photoionization of DHP vesicle entrapped pyrene has also been demonstrated.<sup>14,49</sup> Excitation by a 347-nm laser pulse resulted in the development of transient absorption spectra corresponding to a pyrene triplet, a pyrene cation radical, and the hydrated electron. The photoejected hydrated electron decays by two consecutive first-order steps at or near ambient temperatures (Figure 4). Increasing the temperature increased the contribution of the slow electron decay until the process becomes monoexponential (Figure 4). These results have been rationalized in terms of altered reaction sites of the electron as a function of temperature-induced morphological changes of the DHP vesicles. Above the phase transition temperature surfactant vesicles become fluid. All the electrons readily exit, therefore, and decay in the aqueous phase monoexponentially. This decay corresponds to the longer lived component in Figure 4. At temperatures below the phase transition, DHP vesicles are more rigid and their head groups are closer together. Under these circumstances some of the electrons are scavenged by protons concentrated at the surface of the negatively charged DHP vesicles. The reaction of the hydrated electron gives hydrogen atoms and ultimately hydrogen. Electrons can be transferred to other acceptors, such as benzophenone.<sup>14</sup> The anion radical is repelled from the surface of the negatively charged vesicle and can undergo subsequent electron transfers. Figure 5 shows the proposed scheme for pyrene photoionization in DHP vesicles.

Detailed studies have been carried out on photosensitized electron transfer and charge separation in DODAC. *N*-Methylphenothiazine, MPTH, was used as the electron donor and a surfactant derivative of tris(2,2'-bipyridine)ruthenium perchlorate,  $\text{RuC}_{18}(\text{bpy})_3^{2+}$ , acted

as the photoactive electron acceptor.<sup>45</sup>  $\text{RuC}_{18}(\text{bpy})_3^{2+}$  molecules were anchored onto the surface, while MPTH molecules were distributed among the hydrophobic bilayers of the vesicles. The metal-to-ligand charge-transfer excited ruthenium complex,  $\text{RuC}_{18}(\text{bpy})_3^{2+*}$ , may decay by luminescence and radiationless transition:



or it can capture an electron from MPTH:

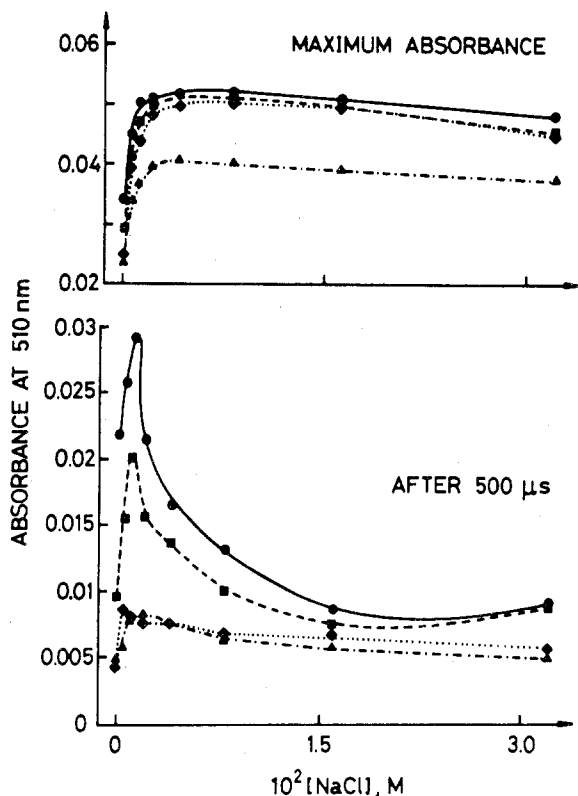


Three different pathways have been recognized for the reaction of  $\text{MPTH}^+$  with  $\text{RuC}_{18}(\text{bpy})_3^{+*}$  (reaction governed by  $k_{-2}$ ). Firstly, there is a rapid geminate combination at the very site of the generation of the cation radicals (reaction -2'). Secondly, some of the  $\text{MPTH}^+$  escapes into the vesicle-entrapped water pools and, due to spatial confinement, combination occurs at the inner surface of DODAC vesicles (reaction -2''). Finally, a part of  $\text{MPTH}^+$  escapes into the bulk solution where it survives for extended periods (reaction -2'''). Charge separation is promoted by electrostatic repulsions between the positively charged outer surface of DODAC vesicles and  $\text{MPTH}^+$ . Kinetics of these processes have been elucidated by laser flash photolysis.<sup>45</sup>

Addition of NaCl, even in low concentrations, has a profound effect on reactions 2, -2', -2'', and -2'''. In the range of concentrations used (Figure 6), NaCl does not penetrate the DODAC vesicles.<sup>11</sup> Added chloride ions decrease the fractional positive charges on the outer surface of DODAC vesicles. This has three important consequences. Firstly, the number of sites where the local electrostatic field prevented the exit of  $\text{MPTH}^+$  is reduced. Secondly, a dissymmetry is created between the inner and outer surface potential of the vesicles and this will increase the fraction of  $\text{MPTH}^+$  exiting into the bulk solution. Thirdly, the reduced net charge on the aggregates increases the rate of reaction 2''.

Addition of NaCl (up to  $2.0 \times 10^{-3}$  M) drastically increases the prompt amount of  $\text{MPTH}^+$  formed (see

(49) A reexamination of the system leads us to question the proposed monophotonic electron ejection. Particularly, fluorescence intensities at 400 nm, measured at the end of laser excitation of DHP vesicle entrapped pyrene, showed curvilinear dependencies on laser intensities. Additional and careful work is required (cf.: Thomas, J. K.; Picciolo, P. L. *J. Am. Chem. Soc.* 1979, 101, 2502). Regardless of whether electron photoionization is mono- or biphotonic, DHP vesicles have provided alternative pathways for electron reactions as well as hindered undesirable charge recombinations.



**Figure 6.** Absorbances due to  $\text{MPTH}^+$   $1 \mu\text{s}$  (maximum absorbance) and  $500 \mu\text{s}$  after laser pulse as function of added NaCl in  $2.55 \times 10^{-3} \text{ M}$  DODAC intercalated  $8.9 \times 10^{-5} \text{ M}$   $\text{RuC}_{18}(\text{bpy})_3^{2+}$  containing  $1.49 \times 10^{-4} \text{ M}$  ( $\blacktriangle$ );  $2.98 \times 10^{-4} \text{ M}$  ( $\blacklozenge$ );  $5.95 \times 10^{-4} \text{ M}$  ( $\blacksquare$ ), and  $1.2 \times 10^{-3} \text{ M}$   $\text{MPTH}^+$  ( $\bullet$ ).<sup>39</sup>

upper portion of Figure 6). Apparently, when the NaCl concentration reaches that of the DODAC, most of the positive charges on the outer surface of the vesicles are neutralized. Further addition of electrolytes can no longer influence the local electrostatic field. Increased fractions of the long-lived component of  $\text{MPTH}^+$  with increasing NaCl concentrations are manifestations of the dissymmetry in the electrostatic field between the inner and the outer surface of the vesicles. While no further increase in the prompt amounts of  $\text{MPTH}^+$  formed can be obtained on addition of NaCl beyond  $2.0 \times 10^{-3} \text{ M}$  (see the upper portion of Figure 6), the reduction of the net surface charge on the aggregates causes a drastic loss of the long-lived species (see the lower portion of Figure 6) via acceleration of reaction  $-2''$ .

Photosensitized electron transfer has also been demonstrated in anionic DHP vesicles.<sup>46</sup> Both duroquinone and diphenylamine were intercalated in the hydrophobic regions of the surfactant vesicles. Electron transfer from diphenylamine to duroquinone triplet resulted in the formation of the corresponding anion and cation radicals. DHP surfactant vesicles appreciably stabilized these species.<sup>46</sup>

Chlorophyll *a* has been entrapped in surfactant vesicles. Excitation in cationic DODAC vesicles by a 694-nm pulsed ruby laser resulted in the development of long-lived triplets and cation radicals of chlorophyll *a*.<sup>48</sup> Similarly to  $\text{MPTH}^+$ , chlorophyll *a* cation radicals escaped the potential field of the surfactant vesicles where they survived for an appreciable amount of time. Attempts are under way to arrange chlorophyll molecules in surfactant vesicles so that they may provide

working models for the in vivo light-harvesting antenna.

### Reactivity Control in Surfactant Vesicles

Rates and mechanisms of chemical reactions are expected to be modified in the environments of surfactant vesicles. Conceptually, inter- and intravesicle reactions can be visualized. In principle, both reactants can be entrapped in the aqueous inner compartments, intercalated in the bilayers, or attached to the outer or inner surface of the vesicles. Alternatively, one of the reactants can be distributed in the bulk aqueous phase or be localized in the vesicle-entrapped water pools, while the other is associated with the vesicles. Manipulation of the sizes, charges, and hydrophobicities of surfactant vesicles and reagents allows a variety of organizations. Reactivities in the environments of surfactant vesicles are, therefore, more diverse and at the same time more complex than those in micelles.<sup>3</sup> Relatively few kinetic investigations have been carried out to date.

In a pioneering work, Kunitake and Sakamoto have investigated the kinetics of hydrolysis of *p*-nitrophenyl acetate and palmitate in didodecyltrimethylammonium surfactant vesicles.<sup>19</sup> The cholesteryl ester of imidazole carboxylic acid was used as catalyst. Two types of experiments were performed. In the intervesicle reaction, the substrate and the catalyst were placed in different vesicles. Intravesicle reactions have been investigated by localizing both substrate and catalyst in the same vesicle. The reaction was initiated by adding the base (pH 9.5) to the vesicles. The intervesicle hydrolysis of *p*-nitrophenyl palmitate was slower than that of *p*-nitrophenyl acetate. Conversely, the long-chain ester underwent faster intravesicle hydrolysis than the short-chain one. This latter phenomenon is related to the anchoring of the substrate. Arrhenius plots of intravesicle hydrolysis of *p*-nitrophenyl palmitate showed breaks which correspond to the phase transition of the surfactant vesicles used.<sup>24</sup> More significantly, intravesicle hydrolysis of *p*-nitrophenyl palmitate was found to be some 200-fold faster than the corresponding intervesicle reaction. This point illustrates beautifully the advantage of localizing substrates and catalysts in close proximity. Their rigid alignments among the bilayers of surfactant vesicles provide opportunities for maximal interactions.

A dramatic  $7.0 \times 10^4$ -fold enhancement has been observed in the thiolysis of *p*-nitrophenyl acetate by *N*-methylmercaptan in dialkyldimethylammonium chloride vesicles.<sup>29</sup> This effect was attributed to specific substrate localization in the surfactant vesicles and to changes in the apparent dissociation constant of the thiol.<sup>29</sup>

Up to now in our own laboratories, attention has been focused on reactions in which one reagent is in the bulk solution while the other is associated with the vesicles. Scavenging of the sodium 2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxylate spin probe,  $\text{R}^{\cdot}\text{Na}^+$ , by sodium ascorbate has been examined, in the presence of DODAC vesicles, by EPR techniques.<sup>12</sup> Second-order rate constants for this reaction increase sigmoidally with increasing DODAC concentration. The kinetic data have been treated quantitatively by assuming partitioning of the reactants between the outer surface of DODAC vesicles and bulk water and by considering different reactivities at these two locations. Application of the kinetic treatment, derived for second-order re-



actions occurring in the presence of aqueous micelles,<sup>50</sup> gave good plots<sup>12</sup> which allowed the calculation of substrate-vesicle binding constants and the rate constant for  $R^-Na^+$  scavenging at the surface of vesicles. The binding constant for the association of  $R^-Na^+$  with DODAC vesicles,  $328 M^{-1}$ , agrees well with that obtained independently from the line-shape analysis of the EPR spectra ( $375 M^{-1}$ ), thereby justifying the assumptions involved in the kinetic treatment.<sup>12</sup> The rate constant for scavenging at the surface of vesicles is some sevenfold smaller than that in bulk water if true concentrations of reactants are used. This rate retardation is not unexpected, since two negatively charged reactants ( $R^-Na^+$  and ascorbate ion) are less likely to encounter when bound to a surface at close proximity than when they are free to move about. A similar situation has been encountered in reversed micelles.<sup>51</sup>

Interestingly, ascorbate ions did not promptly destroy all  $R^-Na^+$  in the presence of DODAC vesicles.<sup>12</sup> Dependent upon the conditions there remained up to 7% residual  $R^-Na^+$ . It is proposed that this residual  $R^-Na^+$  is located at the inner surface of DODAC vesicles, after having "flipped" inside.

The rate constant for the base-catalyzed hydrolysis of Ellman's reagents (5,5'-dithiobis[2-nitrobenzoic acid]) is up to 500-fold greater in the environment of DODAC vesicles than in water.<sup>37</sup> The negatively charged reagent is bound to the outer and inner surface of the surfactant vesicles where there is a high local hydroxide ion concentration. Treating the kinetic data according to the Berezin-Cordes-Romsted equations<sup>50</sup> leads to a substrate-surfactant vesicle binding constant of  $2.5 \times 10^3 M^{-1}$ . This value agrees well with that determined independently by spectrophotometry.<sup>37</sup> Using the "true" hydroxide ion concentration at the surface of DODAC vesicles results in a second-order rate constant which is identical with that in bulk water. The role of surfactant vesicles is simply to concentrate the reactants in their environments. Kinetic treatments derived for micellar catalyses<sup>37</sup> are apparently applicable for this type of reactions involving synthetic surfactant vesicles.

### Surfactant Vesicles as Drug Carriers

Liposomes are being increasingly utilized as drug carriers.<sup>52</sup> The advantages of the method are reduced dosages, and hence decreased allergic, toxic, and immunological side effects. Subsequent to its introduction, the liposome encapsulated drug is transported, essentially intact, to the different organs of the body

and its distribution depends on a number of factors. Most liposomes are taken up by the liver, the spleen, and, to a lesser extent, the lungs and bone marrow. Having reached the cell, the drug-carrying liposome enters it either by fusion or by endocytosis. Once inside the cell, the carrier is broken down and the drug is free to act. Considerable effort is being directed toward target-directing drug-carrying liposomes.<sup>53</sup> The relative difficulty of entrapment and chemical instability of phospholipid liposomes warrant mechanistic investigations on simpler models.

In a preliminary investigation, substantial entrapments of 8-azaguanine, methotrexate, and 6-mercaptopurine have been reported in DODAC vesicles.<sup>10</sup> The extents of drug entrapments in DODAC vesicles exceeded those in liposomes, but leakage was similar in the synthetic and biological vesicles. These studies have laid the foundation for detailed investigations of drug entrapment, release, and target directing in functionalized surfactant vesicles.

### Conclusion

Completely synthetic surfactant vesicles were first recognized as membrane mimetic agents in 1977. Within a remarkably short time, considerable progress has been made in their characterization and utilization. Much has been inferred from the available information on liposomes. Indeed, to a first approximation, surfactant vesicles can be expected to behave just like liposomes. Such an analogy cannot be carried too far, however. Importantly, surfactant vesicles, unlike liposomes, can be highly charged. Dialkylammonium halide and dialkyl carboxylate or sulfonate vesicles can now be critically compared to phospholipid liposomes. What is so special about the phosphate head groups in membranes? Are they unique or are they evolutionary freaks? How do mobilities, hydrations, and geometries of the different vesicles compare and contrast? Deducing answers to these and related questions will allow the better exploitation of surfactant vesicles to mimic membrane-mediated processes. Initial results on the utilization of surfactant vesicles in photochemical solar energy conversion and storage, in reactivity control and drug delivery, detailed in the present Account, are encouraging. Structural simplicities and chemical stabilities of vesicle-forming surfactants render them amenable to functionalization. Functionalized surfactant vesicles hold the key to new types of highly relevant and, at the same time, fascinating chemistry.

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