Synthetic Amphiphile Vesicles

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1 Background

1.1 Liposomes and Model Membranes

At the beginning of the 20th Century the plasma membrane seemed to be a thin structure impregnated with lecithin and cholesterol which was likely to contain protein.[1] Quantitative data on thickness and composition scarcely entered into this description. There were no ideas on the structural role of lipids or molecular organization. 1925 yielded the classical result of Gorter and Grendel: 'Every chromocyte is surrounded by a layer of lipoids, of which the polar groups are directed to the inside and to the outside in much the same way as Bragg supposes the molecules to be oriented in a crystal of fatty acid, and as the molecules of a soap bubble are, according to Perrin'.[1] Further, Grendel in 1929 presented a value of 3.1 nm for such a lipoid layer based on calculations using the molecular lengths of lipid molecules and on his assumption that this membrane lipoid layer must be bimolecular, because it came in contact with water on both sides. A model of membrane structure, however, appeared only in 1935 in a paper by Davson and Danielli. The arrangement of the lipid molecules in the monolayers at the two membrane–water interfaces was derived from their work on lipid films at the air–water interface. Even today, with the maturation of the bilayer paradigm, a crucial question is whether or not the lipids in plasma membranes always take on the bimolecular leaflet form.

When lipids are mixed with water, organized lamellar structures called myelin figures are formed. 'Water enters the masses of phospholipids which do not usually take a spherical shape but often assume the most irregular and striking forms, reminding one irresistibly of the structure of cells' – a description given by Matthews in 1920. The liposomes of Bangham are a particular kind of myelin figure that forms closed systems and is composed of coherent concentric spheroidal bilayers.[1] They can be obtained simply by vortexing the phospholipids in aqueous solution. A great deal of research has been devoted to the physico-chemical properties of liposomes since their discovery in 1965.[1] From these early days up to the present, the development and diversification of the liposome 'membrane' model has been fascinating. They have been used as model membranes for anaesthetic action, 'test-beds' for membrane enzymes, carriers of encapsulated materials into cells, barriers to be invaded by viruses and bacteria, antigens to be attacked by anti-lipid antibodies, reconstituted membranes and presynaptic vesicles, binders for drugs, substrates for testing ionic channels, 'food' for phospholipases, and drug delivery systems.[1]

Ana Maria Carmona-Ribeiro graduated in Physics and in Biochemistry at the Universidade de São Paulo. There, in 1982, she obtained her Ph.D. in Biochemistry, developing the preparation of functional synthetic vesicles. She has lectured in the Biochemistry Department since 1987. In 1986 she visited the Department of Applied Mathematics at the Australian National University to measure directly interactions between amphiphile bilayers. In 1990 she visited the Department of Chemistry at the University of Reading to study interactions between synthetic bilayers and polystyrene microspheres. Her current research interests include the interaction between surfactants and polymers, membrane stability, adsorption, and fusion, and new uses for amphiphile vesicles.

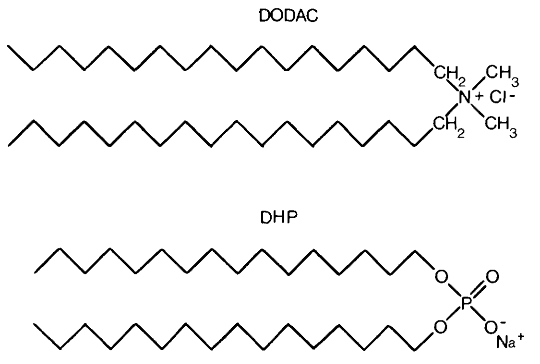

Figure 1 The chemical structure of some double-chain ionic amphiphiles which form bilayers in aqueous solution. Dioctadecyldimethylammonium chloride (DODAC) is a quaternary ammonium salt with a cationic polar head and chloride as counterion. Dihexadecylphosphate (DHP) is a derivative of phosphoric acid with an anionic phosphate polar head and protons or sodium ion as counterions. The acidic character of the DHP polar head originates a myriad of pH effects on DHP bilayer properties.[11]

Since 1976, other membrane mimetic systems composed of ionic synthetic amphiphiles with long alkyl double chains (Figure 1) have been proposed.[2–6] New developments and uses for these systems are, however, dependent on further characterizing the dispersions obtained in solution. In this respect, both the history and uses of the liposomes are very instructive. Methods employed for obtaining phospholipid dispersions can be successfully adapted to obtain vesicles of synthetic amphiphiles.[2–6] These highly-charged synthetic bilayers offer a challenge for colloid scientists. They are useful to test theoretical models for colloidal stability, electrical double layer, and amphiphile self-assembly. Futhermore, they accomplish the usual tasks that single-chain surfactants do, and at much lower concentrations.[7] Here about a decade of research on dioctadecyldimethylammonium chloride (DODAC) and dihexadecylphosphate (DHP) vesicles will be reviewed, focusing on the importance of good characterization of the dispersions in solution, on their analogies with and differences from phospholipid systems, and on their uses.

1.2 Molecular Geometry, Bilayers, and Vesicles

Amphiphilic molecules as surfactants and lipids associate in aqueous solution and at interfaces to form a variety of structures.[8] The hydrocarbon chains are hydrophobic and the polar heads are hydrophilic so that the hydrophobic interaction induces the molecules to associate exposing the polar heads to the aqueous phase (Figure 2).

At thermodynamic equilibrium, the geometry of an amphiphile molecule in the aggregate is given by: the optimal area a, *i.e.* the surface area occupied per headgroup when the total interfacial energy per molecule in the aggregate is a minimum; the volume of the hydrocarbon chain or chains v; and the maximum effective length that the chains can assume l. From

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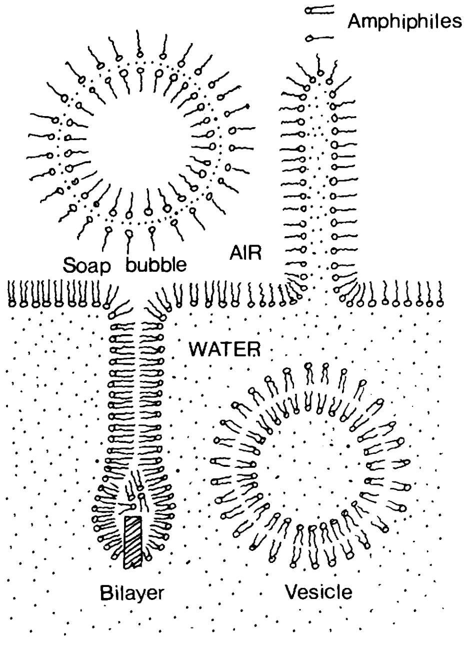

Figure 2 Association of amphiphilic molecules at air–air, air–water, and water–water interfacial regions. The hydrocarbon chains interact preferentially with each other and with air, whereas the polar heads are attracted to water.

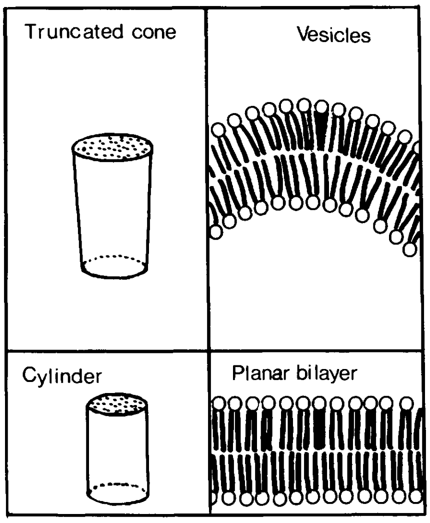

Figure 3 Molecular geometry of amphiphiles and lipids that are able to form planar bilayers and bilayer vesicles. A truncated conic molecular shape is required to form a curved bilayer and a cylindric one to form a planar bilayer. (Adapted from reference 8.)

the self-assembly model and the molecular geometry, it is possible to determine the nature, shape, and size of the aggregate into which the amphiphilic molecules can pack.[8]

Amphiphiles that form bilayers are those with small head-group areas or those with bulky hydrocarbon regions. The value of v/al must lie close to 1 and the critical packing shape has to be cylindrical (Figure 3). For a bilayer to curve, the amphiphiles in the outer monolayer must be able to pack into truncated cones and the value of v/al has to be smaller than 1 but larger than $\frac{1}{2}$. It should be noted that the self-assembly model does not predict tilting or interdigitation of the hydrocarbon chains. Furthermore, the model was constructed to predict the nature and shape of amphiphile aggregates in thermodynamic equilibrium, a condition often circumvented by nature and its metastable states.

Table 1 Calculation of the geometric parameter v/al for DODAC and DHP assuming that the area per monomer a is equal to the limiting area per monomer at 25 mN/m in DODAC and DHP monolayers. C is the NaCl concentration. (Adapted from references 9 and 10.)

Amphiphile	C/M	pH	v/nm^3	l/nm	a/nm^2	v/al
DODAC	0	6.5	0.969	2.3	0.66	0.64
	0.0001				0.54	0.79
	0.0010				0.52	0.81
	0.0100				0.52	0.81
DHP	0	7.8	0.862	2.1	0.425	0.99
	0.0001	7.3			0.427	0.985
	0.0010	6.9			0.426	0.988
	0.0100	6.2			0.421	1

The geometry of DODAC and DHP molecules is given in Table 1. A complete test of the self-assembly model using these synthetic amphiphile vesicles would depend on determining the bilayer thickness and the areas per monomer in the bilayer vesicles for a range of experimental conditions. Assuming that the area per monomer a in DODAC and DHP vesicles is close to the limiting area per monomer at 25 mN/m of DODAC and DHP monolayers at an air–water interface,[9] v/al was calculated for a range of electrolyte concentrations.[10] As the pH decreases or the electrolyte concentration increases, the a value decreases and v/al becomes close to 1 indicating formation of planar bilayers.[7,10] The DHP molecule has an interesting geometry which depends on pH and ionic strength, and yields values of v/al close to 1. Using areas per DODAC molecule at 25 mN/m obtained for DODAC monolayers,[9] the calculated values of v/al for the DODAC system increase from 0.64 in water to 0.81 in 0.01 M NaCl (Table 1).[10] Since the pH and salt concentration determine the area per monomer, they also determine the vesicle size.[10,16]

2 Physical and Functional Properties

2.1 Preparation Methods

The physical and functional properties of synthetic amphiphile vesicles vary with the method of preparation. Three principal methods have been employed to prepare DODAC and DHP dispersions in aqueous solutions: (1) sonication in a bath;[3] (2) sonication with a tip;[3,4] (3) chloroform vaporization.[5,6] The first method yields multilammellar vesicles,[2] the second yields small unilamellar vesicles[3,4] and/or bilayer fragments,[12,13] and the third, large unilamellar vesicles.[5,6] With the exception of the large unilamellar vesicles obtained by chloroform vaporization, the dispersions obtained by the other two methods are not yet properly characterized. For a given medium composition, the three methods will yield different vesicle sizes. It is not possible for all of them to be in thermodynamic equilibrium.[12] At least two of these preparations must correspond to metastable states. Turbidity measurements as a function of time after vesicle preparation in water revealed that the dispersions obtained by sonication were not stable in contrast to the apparent stability over days of the dispersion prepared by chloroform vaporization.[6]

2.2 Captured Volume, Size, and Shape

The captured volume is one important parameter of vesicle preparations which is a function of the vesicle size only. It is defined as the volume enclosed by a given amount of amphiphile and expressed in litres entrapped per mole of total amphiphile (l mol^{-1}). It is usually measured using a water-soluble marker which labels the inner aqueous compartment without being adsorbed or included in the vesicle bilayer. Entrapped and free marker can be separated by dialysis[14] or by gel chromatography.[5,6] The captured volume for sonicated DODAC dispersions in water is close to zero (0.13 l mol^{-1})[5] and lower than that for equivalent phospholipid dispersions (0.5—0.8 l mol^{-1}).[15,16] The captured volume for large DODAC and DHP vesicles obtained by chloroform vaporization is 9.7[5] and 13.6 l mol^{-1},[6] respectively, and compares fairly well with that for large unilamellar phospholipid vesicles obtained by reversed phase vaporization, 11.7 l mol^{-1}.[17]

Vesicle sizes are determined by electron microscopy or photon correlation spectroscopy (PCS). After sonication (10 minutes for DODAC and 20 minutes for DHP), the DODAC and DHP dispersions are composed of non-spherical particles of around 45 and 59 nm mean hydrodynamic radius, respectively.[18] The DODAC and DHP dispersions obtained by chloroform vaporization in water are composed of spherical particles[6,19] of around 120 and 185 nm mean hydrodynamic radius, respectively.[16] The ratio between the hydrodynamic radius and the radius of a sphere with the molecular weight of a sonicated vesicle is close to 3 indicating a pronounced deviation from a spherical shape.[18] The turbidity of the dispersions obtained by chloroform vaporization is well described by the Joebst law for light-scattering by spherical particles[6,19,20] Thus, dispersions obtained by sonication produce small non-spherical particles whereas those obtained by chloroform vaporization yield large spherical ones. Vesicle size depends on electrolyte concentration and pH.[10,11] By increasing the NaCl concentration in the preparation medium from 0.1 to 0.5 mM, large DODAC vesicles almost doubled in size.[10] By increasing pH from 6.2 to 8, large DHP vesicles decreased in size from 325 to 240 nm diameter.[10]

2.3 Physical State and Bilayer Packing

At room temperature DODAC and DHP bilayers are in the rigid gel state.[5,11,21] As the temperature increases, so does the rotational freedom of the hydrocarbon chains. The transition from the ordered gel phase to the fluid liquid-crystalline state corresponds to an abrupt and large decrease in the packing density at a particular temperature, the phase transition temperature (T_c). The higher the packing density of the hydrocarbon chains in the gel state, the higher the T_c.[22] T_c values measured for DODAC vesicles were around 38 °C[5] whereas those measured for DHP vesicles varied between 57 and 72 °C, depending on pH.[11,21]. For the DHP system, vesicle size and bilayer packing (T_c) vary as a function of pH and electrolyte concentration. Both were at maximum at pH values corresponding to half-dissociation of the phosphate polar heads.[11] The largest DHP vesicles and the most tightly packed DHP bilayers were obtained at half-dissociation.[11]

The DHP system may also exist in the isotropic or hexagonal phase.[11,21] At high temperature, very low NaCl concentration, and pH at and around the pH of half-dissociation for the phosphate polar head, an abrupt and large increase in turbidity can be associated with vesicle aggregation and formation of the isotropic phase at the contact points in between vesicles.[11,21]

The determination of pressure–area isotherms using DODAC and DHP monolayers at the air–water interface provides an alternative method of studying the molecular packing in DODAC and DHP bilayer vesicles.[9] A high packing density in the vesicle bilayer usually corresponds to low limiting areas per monomer in the monolayer. At a surface pressure of 25 mN/m with water as sub-phase, the areas per monomer for DODAC and DHP are 0.66 and 0.41 nm^2, respectively.[9] This large difference correlates well with the large difference in T_c found for DODAC and DHP bilayers in water. A tight molecular packing in the gel state may also be associated with a low permeability towards several ionic and non-ionic substances. Permeabilities of large DHP vesicles are systematically smaller than those of large DODAC vesicles (6).

2.4 Inner Aqueous Compartment and Functional Properties

Closed bilayer systems are among the most investigated of membrane models. They usually perform such basic membrane functions as compartmentalization and passive transport of solutes and water. The captured volume of the sonicated synthetic amphiphile dispersions is close to zero (see Section 2.2). Thus, incorporation of water-soluble substrates and permeability studies have been restricted in the sonicated dispersions. In contrast, large DODAC or DHP vesicles obtained by chloroform vaporization entrap sizeable amounts of water-soluble substrates and exhibit ideal osmotic behaviour.[5,6] In Figure 4 the shrinkage of large DODAC vesicles due to an osmotic gradient of sucrose is seen as an increase in turbidity. The initial shrinking rate varies linearly with the applied osmotic gradient, as for an osmometer.[5,6] This ideal osmotic behaviour towards sucrose solutions suggests that large DODAC and DHP vesicles are non-permeant towards sucrose. Thus, radioactive sucrose could be used to determine the captured volume and cold sucrose could be used as a standard to determine relative permeabilities towards other solutes.[5,6] Most non-ionic and ionic solutes were non-permeant. Large DODAC and DHP vesicles at room temperature are impermeable even towards urea and permeable only to water, ethylene glycol, and ammonium acetate.[6] Synthetic amphiphile vesicles and phospholipid liposomes in the gel state behave similarly regarding passive transport.[6]

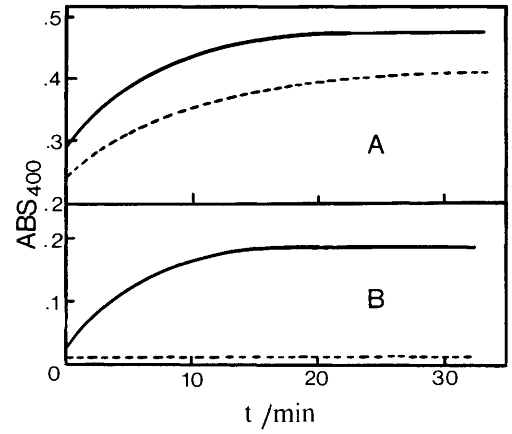

Figure 4 Time-dependent absorbance changes after addition of 0.05 M KCl (———) or 0.1 M sucrose (- - -) to large DODAC vesicles (A) or to DODAC dispersions obtained by sonication (B). The turbidity increase after sucrose addition is due to vesicle shrinkage (A) and is not observed for DODAC or DHP dispersions prepared by sonication (B). The absence of osmotic response for the sonicated dispersions possibly suggests the lack of an inner aqueous compartment or the impossibility of detecting changes in size using the turbidimetric method. The increase in the turbidity of the sonicated dispersion on KCl addition results either from vesicle fusion or from the coalescence of bilayer fragments.[19,24] (Adapted from reference 5.)

3 (Meta)stability

3.1 Stability of the Bilayer Structure and Colloidal Stability

A system is thermodynamically stable when its free energy is at a minimum. The invariancy of a given structural property after an annealing procedure identifies a structure that is apparently time-independent, *i.e.* stationary.[13] Stationary bilayer structures were obtained for DHP only for dispersions prepared by chloroform vaporization at pH values above 6. The DHP dispersions prepared by sonication were not stationary at any pH value.[13]

Two other simple methods of determining the stability (stationary character) of synthetic amphiphile dispersions are turbidity determination as a function of time after vesicle preparation[5,6] and determination of stability curves,[23,24] *i.e.* initial flocculation rates (v_0) as a function of monovalent salt concentration (Figure 5). Sonicated DODAC or DHP vesicles prepared in water are not stable in contrast to the large vesicles prepared by chloroform vaporization. In the presence of NaCl, the same trend was observed (Table 2). Critical coagulation concent-

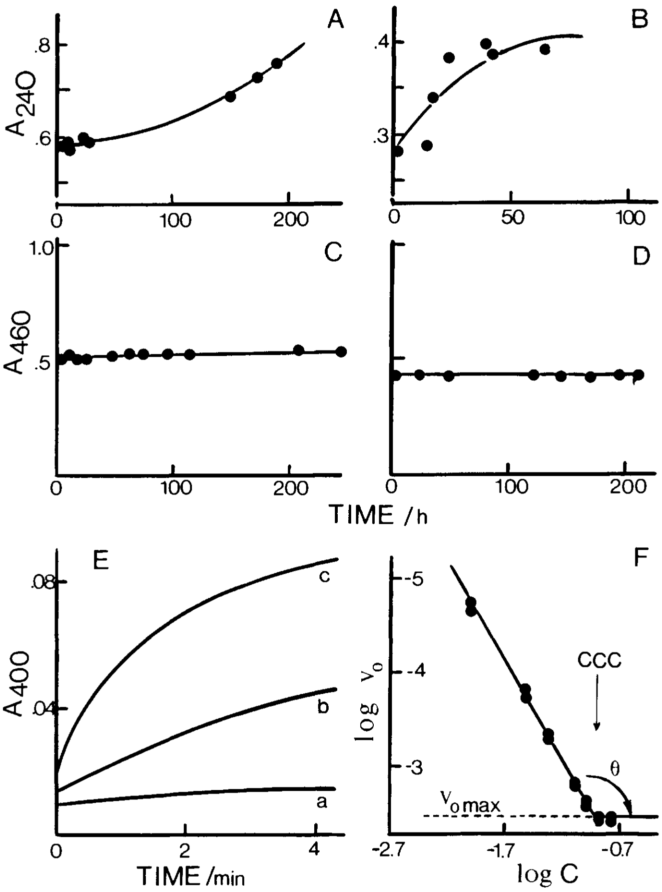

Figure 5 The stability of synthetic amphiphile vesicles in water (A, B, C, D) or in NaCl solutions (E, F). The turbidity of DODAC (A) or DHP (B) dispersions in water varies as a function of time after dispersion whereas DODAC (C) and DHP (D) dispersions obtained by chloroform vaporization are stationary. By adding salt solutions to vesicle samples under conditions of iso-osmolarity, the turbidity increases as a function of time indicating a low colloidal stability of the vesicles in the presence of salt (E). Quantification of stability is possible by measuring the initial flocculation rate (v_0) from the turbidity *versus* time curve at a given NaCl concentration C. As C increases, v_0 increases and the stability ($1/v_0$) decreases (from curve a to c, in E). Thus, stability curves like that in (F) were obtained for synthetic amphiphile dispersions in reference 24. The profile of log v_0 against log C is as predicted by the DLVO theory for the stability of colloidal dispersions (F). Important parameters defining the colloidal stability are indicated in the stability curve in (F): the critical coagulation concentration (*CCC*); the slope of the flocculation curve (tg θ); the maximal initial flocculation rate ($v_{0\max}$). (Adapted from references 6 and 24.)

ration (*CCC*) values were lower for the sonicated vesicles indicating a lowered stability in the presence of salt.[24] However, Stern potential values estimated from the slope of the stability curves were higher for the sonicated vesicles. Thus, the less stable dispersion has the highest electrostatic repulsion between vesicles.[24] There must be an extra attraction between the sonicated vesicles, that does not occur for the large vesicles, which overwhelms the electrostatic repulsion and yields the lower stability measured. In fact, assuming only an electrostatic repulsion and a van der Waals attraction between vesicles, the calculated theoretical stability value for the sonicated DHP dispersion is much higher than the stability value obtained experimentally.[25] An extra-attractive interaction force is needed to explain the low experimental stability of the sonicated DHP vesicles. This extra attraction can be due to a hydrophobic attraction between the edges of the bilayer fragments produced during sonication,[13] or to a return to the lamellar state due to salt addition,[10] or to both.

Table 2 The stability of DODAC and DHP dispersions at pH 6.5 in the presence of NaCl. C_a is the amphiphile concentration; *CCC* is the critical coagulation concentration; $v_{0\max}$ is the maximal initial flocculation rate and Ψ_δ is the estimated Stern potential. These estimated Stern potentials are too low in comparison with the real surface potentials measured by electrophoresis[10,27] owing to the DLVO approximation used for the calculations but they reflect well the differences between the dispersions. (Adapted from references 10, 24, and 27.)

				Ψ_δ/mV	
Dispersion	C_a/mM	*CCC*/M	$10^3 v_{0\max}$	Calculated	Measured
Sonicated DHP	0.23	0.178	12.6	− 41.2	− 97.3
Injected DHP	0.18	0.229	3.8	− 19.3	− 70.7
Sonicated DODAC	0.21	0.112	1.5	26.8	85.0
Injected DODAC	0.24	0.129	0.1	8.5	84.8

3.3 Aggregation and Fusion

DODAC and DHP dispersions prepared by sonication with a tip undergo fusion upon NaCl addition.[19,24] For the large DHP vesicles, however, fusion has never been observed.[9,19,24] Thus, fusion in the sonicated dispersions could be associated with the formation of vesicles or coalescence of the bilayer fragments present in these dispersions.[12,13] Nevertheless, the contraction of DODAC monolayers at the air–water interface as the NaCl concentration increases in the sub-phase indicates a trend towards the formation of larger vesicles upon salt addition.[9] In contrast, the DHP monolayer shows the opposite trend and expands as the NaCl concentration increases in the sub-phase.[9] Furthermore, the high packing density of the DHP bilayer in the gel state does not favour fusion.[11,21] Adhesion, however, has often been described for the DHP system.[6,9,11,21] The adhesive force between DHP monolayers adsorbed on hydrophobic mica at 0.1—0.3 nm separation decreases as the NaCl concentration increases (pH 5.5).[9] A salt-induced shift of the half-dissociation of the phosphate polar heads to lower pH values might be increasing the charge density and the electrostatic repulsion[11,21] and/or salt might disrupt adhesion originating from the formation of interbilayer hydrogen bonds at half-dissociation. Both phenomena would be observed as a decrease in the adhesive force as the salt concentration increases.[9] At high temperature, very low NaCl concentration, and pH 6.5 (close to the phosphate half-dissociation), a reversible temperature-induced vesicle aggregation has been described.[11] The extent of aggregation markedly decreased as the NaCl concentration was increased. This was explained also by a salt-induced disruption of the interbilayer hydrogen bridges.[11] Some direct measurements of adhesion between DHP layers are given in Table 3.

Whereas the DODAC system seems to be stabilized solely by electrostatic repulsion,[26] fusing when this repulsion decays due to salt addition,[19,26] the DHP system has a tightly packed bilayer[11,21] which may adhere through hydrogen bonds at half-dissociation[11] or return to the lamellar state at higher salt concentration.[7]

3.4 The Surface Potential and Theoretical Stabilities

Whether the classical DLVO theory for the stability of spherical charged colloidal particles is sufficient to describe the stability of the DODAC vesicles is still an open question.[25] To further address this issue, theoretical stabilities must be calculated using the adapted DLVO model for vesicles and compared with the experimental ones.[24,25] However, Stern potentials are needed for calculating the electrostatic term of the interaction energy. We are presently measuring electrophoretic mobilities of large DODAC vesicles over an extensive range of NaCl concentration

Table 3 Direct measurements of adhesion forces between DHP layers adsorbed onto hydrophobic mica at room temperature as a function of NaCl concentration (*C*) and pH. The force (*F*) is normalized to the curvature radius on mica and expressed as mN/ m. (Adapted from reference 9.)

C/M	pH	F/mN m^{-1}
.0002	5.5	− 5.4
.0010	5.5	− 3.7
.0100	5.5	− 3.3
.0100	3.0	− 7.2
.0100	9.5	0

to check the suitability of the DLVO forces to describe the interaction between synthetic amphiphile vesicles.

4 Uses

4.1 Storage of Solar Energy and Catalysis of Chemical Reactions

Charged synthetic amphiphile vesicles concentrate oppositely charged substrates at their bilayer/water interface. Consequently they may be used to separate charges generated by photoionization of certain molecules[28,29] or they may catalyse certain chemical reactions by concentrating one of the reactants at the bilayer/water interface.[28,30] In this respect, synthetic amphiphile vesicles are as useful as ionic micelles and can be considered as a model system for the storage of light energy allowing compartmentalization of electron donors and acceptors relative to water.[29] In addition, the selective incorporation of a charged substrate at the inner or outer vesicle surface and the permeation control of the other reactant can be used to modulate the reaction rate.[30]

4.2 Bilayer Adsorption

DODAC and DHP bilayers from DODAC and DHP dispersions prepared by sonication or by chloroform vaporization deposit onto oppositely charged polystyrene microspheres with high adsorption constants.[7] At maximum adsorption, the areas per amphiphile molecule adsorbed, the electrokinetic properties, and the sizes measured for the covered particles are consistent with bilayer deposition.[7] The deposition process is controlled not only by the interaction forces between the vesicle and the microsphere but also by the restrictions of packing inside the bilayer. Vesicles tending to return to the lamellar state, *i.e.* to a state with larger curvature radius, have a high affinity for larger microspheres depositing as multibilayers, and a low affinity for smaller microspheres depositing as single bilayers. Interbilayer hydrogen bonds were found to enhance the multibilayer deposition.

4.3 Flocculation and Stabilization of Dispersions

DODAC and DHP vesicles are effective flocculants or stabilizers for oppositely charged dispersions in solution (Figure 6). In contrast to single chain surfactants, they are effective at very low concentration (10—200 μM).[7] The division between their action as flocculants or stabilizers is defined by the equality between the total surface area for each dispersion. When there is more vesicle than particle surface area available then the vesicles act as stabilizers and when there is more particle than vesicle surface they act as flocculants. Another advantage over surfactants with short hydrocarbon chains is the irreversible character of the bilayer adsorption, as shown by the high adsorption constants obtained.[7]

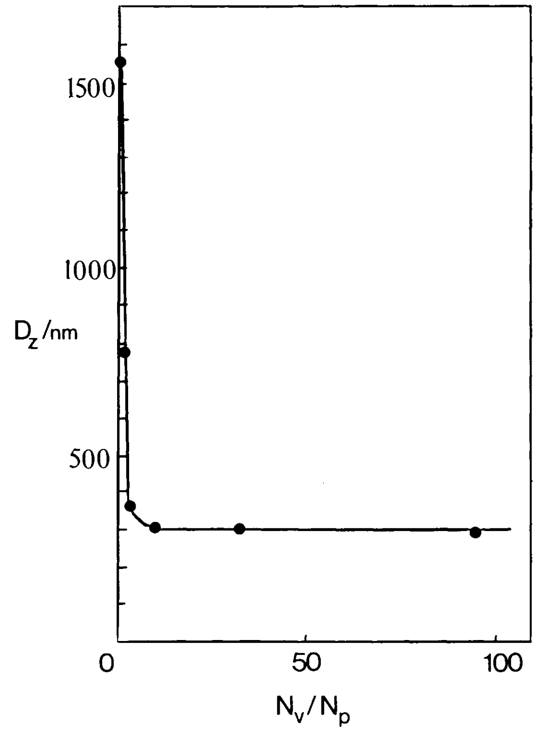

Figure 6 Synthetic amphiphile vesicles acting as flocculants or as stabilizers of oppositely charged polystyrene microspheres. Large DODAC vesicles with a mean z-average (D_z) of 256 nm were mixed with sulfate polystyrene lattices (285 nm) 4 h before the D_z determination. One week after mixing, flocculation was visible for samples in which the ratio between number densities for vesicles and polystyrene microspheres (N_v/N_p) was smaller than 1 and absent when $N_v/N_p > 1$. (Adapted from reference 7.)

4.4 Production of Model Colloids

Large DODAC or DHP vesicles are perfectly rigid spherical shells composed of a bilayer enclosing an aqueous compartment. They are highly charged and have a much smoother interface than other colloids. Because of these properties, they behave as model colloids and have been used to test theoretical models.[24,25] However their polydispersity is still a disadvantage compared with highly monodisperse systems such as polystyrene microspheres.[7] On the other hand, the possibly hairy, rough, or conducting surface of these microspheres often presents a problem. Thus, the interaction between synthetic amphiphile vesicles and oppositely charged polystyrene microspheres leads to the production of an ideal model colloid: *homodisperse* and *smooth* bilayer covered polystyrene microspheres (Table 4). From this system, significant new developments in colloid science can be expected.

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5 References

1 'Liposome Letters', ed. A. D. Bangham, Academic Press, London, 1983.
2 T. Kunitake, Y. Okahata, K. Tamaki, F. Kumamaru, and M. Takayanagi, *Chem. Lett.*, 1977, 387.
3 R. A. Mortara, F. H. Quina, and H. Chaimovich, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 1080.
4 K. Kano, A. Romero, B. Djermouni, H. Ache, and J. H. Fendler, *J. Am. Chem. Soc.*, 1979, **101**, 4030.
5 A. M. Carmona-Ribeiro and H. Chaimovich, *Biochim. Biophys. Acta*, 1983, **733**, 172.
6 A. M. Carmona-Ribeiro, L. S. Yoshida, A. Sesso, and H. Chaimovich, *J. Colloid Interface Sci.*, 1984, **100**, 433.

Table 4 Homodisperse and smooth bilayer covered polystyrene microspheres are produced by mixing sonicated DODAC dispersions and sulfate polystyrene microspheres (150 nm mean z-average diameter). The ratio between the vesicle number density and the microsphere number density is 7 ($N_v/N_p = 7$). At $N_v/N_p = 7$, the limiting diameter (D_z) for the mixture upon increasing dilution before mixing is 160 nm, *i.e.* the size of the microsphere plus about 10 nm which suggests surfactant deposition of one-bilayer thickness. (Adapted from reference 7.)

C_a/mM	$N_p 10^{12}$cm^{-3}	mean D_z/nm	Polydispersity index
1	1.04	172.0	0.065
0.4	0.42	158.0	0.066
0.2	0.21	162.0	0.061
0.05	0.05	160.0	0.044
0	1.04	150.0	0.043

7 A. M. Carmona-Ribeiro and B. R. Midmore, *Langmuir*, 1992, **8**, 801.

8 J. N. Israelachvili, 'Intermolecular and Surface Forces', Academic Press, London, 1985.

9 P. M. Claesson, A. M. Carmona-Ribeiro, and K. Kurihara, *J. Phys. Chem.*, 1939, **93**, 917.

10 A. M. Carmona-Ribeiro and B. R. Midmore, *J. Phys. Chem.*, 1992, **96**, 3542.

11 A. M. Carmona-Ribeiro and S. Hix, *J. Phys. Chem.*, 1991, **95**, 1812.

12 R. B. Pansu, B. Arrio, J. Roncin, and J. Faure, *J. Phys. Chem.*, 1990, **94**, 796.

13 A. M. Carmona-Ribeiro, C. E. Castuma, A. Sesso, and S. Schreier, *J. Phys. Chem.*, 1991, **95**, 5362.

14 O. Augusto and A. M. Carmona-Ribeiro, *Biochemical Education* 1989, **17(4)**, 209.

15 D. W. Deamer and A. D. Bangham, *Biochim. Biophys. Acta*, 1976, **443**, 629.

16 D. Papahadjopoulos and W. J. Vail, *Ann. N.Y. Acad. Sci.*, 1978, **308**, 259.

17 F. Szoka and D. Papahadjopoulos, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4194.

18 H. Herrmann and J. H. Fendler, *Chem. Phys. Lett.*, 1979, **64**. 270.

19 A. M. Carmona-Ribeiro and H. Chaimovich, *Biophys. J.*, 1986, **50**, 621.

20 A. L. Koch, *Biochim. Biophys. Acta*, 1961, **51**, 429.

21 A. M. Carmona-Ribeiro, *J. Colloid Interface Sci.*, 1990, **139**, 343.

22 A. G. Lee, *Biochim. Biophys. Acta*, 1977, **472**, 237.

23 H. Reerink and J. Th. G. Overbeek, *Disc. Faraday Soc.*, 1954, **18**, 74.

24 A. M. Carmona-Ribeiro, L. S. Yoshida. and H. Chaimovich. *J. Phys. Chem.*, 1985, **89**, 2928.

25 A. M. Carmona-Ribeiro, *J. Phys. Chem.*, 1989, **93**, 2630.

26 R. M. Pashley, P. M. MacGuiggan, B. W. Ninham, J. Brady, and D. F. Evans, *J. Phys. Chem.*, 1986, **90**, 1637.

27 I. M. Cuccovia, E. Feitosa, H. Chaimovich, L. Sepulveda, and W. Reed, *J. Phys. Chem.*, 1990, **94**, 3722.

28 J. H. Fendler, *Acc. Chem. Res.*, 1980, **13**, 7.

29 P. Bratt, Y. S. Kang, L. Kevan, H. Nakamura, and T. Matsuo, *J. Phys. Chem.*, 1991, **95**, 6399.

30 I. M. Cuccovia, M. K. Kawamuro, M. A. K. Krutman. and H. Chaimovich, *J. Am. Chem. Soc.*, 1989, **111**, 365.