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Spontaneous Vesicles Formed from Hydroxide Surfactants: Evidence from Electron Microscopy

Abstract. *Dialkyldimethylammonium hydroxide surfactants are highly soluble in water and form spontaneous stable vesicles. These vesicles can be grown to size with added acid, and appear to provide an ideal membrane mimetic system for the study of fusion and ion transport. These phenomena are a consequence of strong hydration forces that are not necessarily limited to the hydroxide ions. The forces can be used to design a variety of model systems whose behavior differs from that of systems in which double-chained surfactants form insoluble liquid crystalline phases in water and unstable vesicle suspensions on prolonged sonication.*

We report here on vesicle suspensions that form spontaneously, are stable and fairly monodisperse, and can be grown to a prescribed size by titration with acid. These are prepared from the double-chained ionic surfactant didodecyldimethylammonium hydroxide $[(C_{12}H_{25})_2(CH_3)_2NOH]$. The existence of such spontaneous vesicles is of interest for the following reasons. (i) Vesicles prepared by the usual sonication or solvent extraction methods are unstable and eventually revert to the (lowest free energy) liquid crystalline state from which they emerged (*1*, *2*). This led to the belief that all vesicles, unlike micelles or liquid crystals, are a peculiar nonequilibrium state of matter to which the usual theories of self-assembly based on statistical mechanics cannot be applied. According to theory (*3*), it should be possible to produce some stable vesicle systems by suitable design of surfactant (to control bilayer curvature) and choice of suspending medium (to control interaggregate forces). Our system appears to fulfill these two criteria. (ii) Vesicles have been suggested as model systems (*1*) for photocatalysis and ion transport mechanisms in membranes. The inability of investigators to show that vesicles can be stable has inhibited progress in these areas. (iii) The particular hydroxide vesicles described here provide dramatic evidence for the existence of strong hydration forces characteristic of the hydroxide ion. These are qualitatively different from forces associated with other anions. This observation, along with the demonstration of hydration forces (*4*) for cations (*5*), extends the boundaries of classical colloid science to hitherto inaccessible regions including biological macromolecules and surfaces (*6*).

Didodecyldimethylammonium hydroxide is readily prepared by gently mixing the insoluble bromide surfactant with hydroxide ion-exchange resin (Rexyn 201, Fisher Scientific) at 25°C, filtering off the supernatant, and repeating the exchange process a second time (*2*). Typically, 3 g of the bromide surfactant are added to 50 ml of resin (previously rinsed with several batches of water and drained of excess water) in a 250-ml stoppered Erlenmeyer flask. After the surfactant has been thoroughly mixed with the damp resin, 50 ml of water are added and the slurry is gently mixed with a magnetic stirring bar. The resin is separated from the surfactant solution by passing through filter paper and the resin is rinsed with about 20 ml of water. After the second exchange, a small portion of the solution is checked for completeness of exchange by acidifying with nitric acid (pH ~ 4) and adding silver nitrate. The exchange process yields about 1.5 g of surfactant (0.05*M*).

For the exchange and filtering operations, we used a polyethylene glove bag filled with CO_2-free air. Other than gentle mixing and gravity filtration, the solutions are not subjected to any stirring or agitation. Measurements with pH paper give values ranging from 11 to 13, depending on surfactant concentration. Our attempts to use pH electrodes were unsuccessful because of precipitation of the surfactant at the liquid junction of the reference electrode. Most of our studies with added salt have involved partial neutralization with hydrobromic acid. Preliminary measurements of vesicles neutralized with hydrochloric or hydrofluoric acid indicate that there may be significant differences in vesicle size and stability depending on counterion (*7*). Unlike the halides which form liquid crystals (and vesicles only on prolonged sonication), the hydroxide surfactant is highly soluble (> 1.0*M*) in water. The supernatant can be dried by lyophilization to a fine white powder which dissolves instantly on addition of water. Freeze-thaw cycles give vesicles that spontaneously reconstitute to the structures illustrated, indicating thermodynamic stability.

We focus here on the direct evidence for vesicles as revealed by cold-stage electron microscopy. Frozen specimens of the dispersions are prepared by trapping a thin layer of liquid, ideally less than half a micrometer thick, between two polyimide film-covered electron microscope grids. These specimens are then plunged into liquid nitrogen and stored in the cryogen until their transfer into the microscope (*8*). The frozen specimen is transferred into the microscope, a JEOL JEM 100CX, without excessive heating or frost deposition with a special cold-stage transfer module (*9*) and is kept in the microscope at 95 K. This technique applied to vesicular and liquid crystalline dispersions is free from the artifacts introduced by staining and drying techniques (*10*). Data from previous work (*11*) on the contrast mechanism responsible for images of vesicular dispersions enable one to distinguish easily among vesicles, liquid crystals, and globular precipitates found in frozen specimens. However, with this technique we cannot distinguish between unilamellar and multilamellar vesicles.

Figure 1a shows structures observed in a frozen suspension of the hydroxide surfactant ($1.4 \times 10^{-2}M$). These vesicles are fairly monodisperse and have an average diameter of 300 ± 80 Å (± standard deviation) as determined from measurements on 537 vesicles. Structural features of the ice matrix, such as grain boundaries and defects in the crystalline ice, can also be seen. Partial neutralization (25 percent) with hydrobromic acid results in vesicle suspensions typified by Fig. 1b. The average size here is 460 Å, and the vesicles seem to be less monodisperse. When the vesicles are 50 percent neutralized with HBr, pronounced Tyndall scattering is observed, and some of the vesicles (as characterized by electron microscopy) grow considerably and some become liquid crystals (Fig. 1c).

The results from electron microscopy are substantiated by dynamic light scattering, viscosity, trapping experiments (*7*), and video-enhanced contrast polarization microscopy (VCPM) (*12, 13*), which will be described in detail elsewhere (*14*). Briefly, growth in aggregate size with addition of acid or salt is confirmed by viscosity and light scattering measurements. Leakage of hydroxide from initially neutralized supsensions (a trapping experiment) was followed by titration with acid and the use of phenolphthalein as an indicator. The vesicles thus provide a model biological system for fusion and transport studies, with different counterions and *p*H interior and exterior. Suspensions with compositions like those in Fig. 1, a and b, remain in apparently stable configurations for months.

Analysis by dynamic light scattering (*7*) suggests that the hydroxide solutions contain a mixture of two populations, anomalous micelles (which cannot normally form with double-chained surfactants) and the vesicles. The relative proportions of these two species and the size of the vesicular component are determined by surfactant concentration, added sodium hydroxide, and temperature. (Micelles cannot be seen by the cold-stage electron microscopy technique.) Fluorescence excimer studies (*15*) show that there are about 50 surfactant molecules per micelle. With added hydroxide, or acid, the micelles disappear completely.

VCPM studies permit direct visualization of particles larger than 1000 Å. The degradation of vesicles to liquid crystals upon addition of acid (Fig. 1c) has been followed and recorded on video tape. Other observations with VCPM confirm that vesicle size increases with decreasing surfactant concentration and depends on temperature and salt concentration. Neutralized suspensions of vesicles at high surfactant concentration ($10^{-2}M$) seen under VCPM grow through a tubular regime (on partial neutralization) into pancake shapes reminiscent of red blood cells (long axes of the order of a micrometer, and diameter several thousand angstroms). They turn into long, thin microtubule-like structures with excess acid, and are quite unlike liquid crystalline systems as seen by this technique. Dioctadecyldimethylammonium hydroxide surfactants have the same behavior, forming more concentrated vesicle suspensions at the same surfactant concentrations.

An explanation of these phenomena can be deduced from a study of the corresponding single-chained surfactants $[R(CH_3)_3NOH]$ (*15, 16*). The critical micelle concentrations and degree of "ion binding" for the hydroxides are very different from the corresponding bromides or chlorides and reflect the extreme hydrophilicity of this ion. An analysis (*17*) of the data suggests that unlike the halide ions located close to the surface of the aggregate, the hydroxide ion is located 4 to 5 Å from the surface. The presence of hydroxide appears to alter local water structure in a manner that effectively lowers the "local dielectric constant" seen by the surfactant head groups. Addition of NaOH or increasing the surfactant (and therefore OH^-) concentrations enhances this effect, causing increased head group repulsion and a corresponding inhibition of aggregate size increases.

We have also observed similar behavior with insoluble anionic surfactants. A change of counterion for Na^+ to H^+ again induces different curvature. Besides curvature effects induced by changes in head group, transitions from liquid crystalline structures to spontaneous vesicles can also be induced by changes in solvent that affect aggregation forces (for example, mixtures of water with 6*M* urea or ethylene glycol).

These observations suggest that there are a remarkable number of ways to control curvature and thus dictate aggregate shape and stability. In particular, by balancing head group and hydrocarbon repulsion and controlling interaggregate forces it becomes possible to impose stability and size on vesicle suspensions.

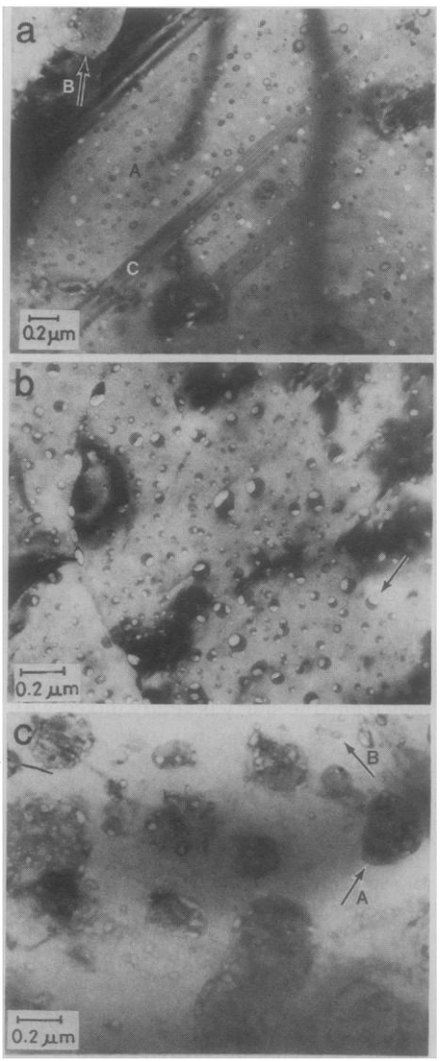

Fig. 1. (a) Frozen aqueous dispersion of 0.014*M* $(C_{12}H_{25})_2(CH_3)_2NOH$. Note vesicles (A), grain boundary (B), and stacking faults in the ice matrix (C). (b) Frozen samples of an aqueous dispersion of 0.014*M* $(C_{12}H_{25})_2(CH_3)_2NOH$ that was partially neutralized (25 percent) with HBr. Note the large vesicles (arrow). (c) Frozen sample of an aqueous dispersion of 0.014*M* $(C_{12}H_{25})_2(CH_3)_2NOH$ that was partially neutralized (50 percent with HBr). Note liquid crystalline particles (A) and vesicles (B).

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