reproductibility of the experiment, it is in no way an absolute necessity and can be omitted. It is interesting to note the off-diagonal correlations of the amide resonances with the protons (A, Gly; B, Cys). The Glu-NH resonance is severely exchange broadened and hence is unobservable for all practical purpose. The JR spectrum of glutathione in the same conditions is shown (Figure 1A), presenting the characteristic phase inversion of this technique.

Applications of this general method can be developed for most 2-D experiments. As an example, this "phase" sequence can replace the preparation and evolution periods[11] of the 2-D NOE (followed by a selective pulse[8,9]) or the evolution and mixing periods of the multiple-quantum experiments.

We think that this new methodology should not be restricted to the specific cases discussed in this paper (non-deuterated solvents, under-solvent correlations) but should prove useful as an alternative approach for the residual protonated solvent suppression in most experiments.

(11) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246.

Spontaneous Vesicles

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Understanding the structure, stability, and dynamics of vesicles is important in elucidating biological self-assembly and in devising strategies for practical use of stable vesicles. The most widely studied vesicle systems are prepared using lecithin or double-chained surfactants.[1] Such systems require sonication to facilitate vesicle formation, are unstable, and invariably revert to the bilayers from which they emerged. Didodecyldimethylammonium hydroxide (DDAOH) is an exception to this rule. In previous reports[2-4] we have shown DDAOH vesicles form spontaneously, survive freeze–thaw cycles, and appear stable. Vesicles prepared from lecithin and DDAOH conceivably represent extremes in a spectrum of behavior regarding ease of vesicle formation and stability.

We have now found a wide range of didodecyldimethylammonium (DDA) salts form vesicles with surprising ease. In Figure 1 we show pictures of vesicles prepared from DDA carboxylates and polymerized acrylate obtained using video-enhanced differential interference contrast microscopy (VEDICM).[5-7] VEDICM, which employs a light microscope equipped with Nomarski optics,[8] a video camera, and frame processor, permits real time imaging of very fine structural details at the resolution limit of the light microscope (1500–2000 Å) and the detection of (diffraction enlarged) structures as small as 300 Å. The dynamics, growth, fusion, and flocculation of surfactant microstructures and other colloidal suspensions are readily visualized on a television screen. The frame processor permits real time image analysis and enhancement, freeze-frame, slow-motion, or time-lapse capability. Quantitative evaluation of data may be carried out with available image analysis technology (e.g., developed by NASA for satellite photograph analysis). The method is direct, rapid, independent of physical models (cf. light scattering etc.), and relatively free of artifacts, involves no sample alteration, and immediately reveals the presence of polydispersity.

As a guide to interpreting VEDICM images, we mention the following points. Images of particles smaller than the resolution limit of the microscope ($\lambda/2$, λ = wavelength of illuminating light) are diffraction enlarged relative to the true particle size. The highlighting of images produced by Nomarski optics may be altered by adjustment of movable beam combiner such that the *edges* of a *uniform* specimen can appear as dark on a light background or light on a dark background or so the entire image appears shadow cast.[8] Since the optical system is sensitive to differences in refractive index at edges, species such as vesicles will yield donut-like images.

Didodecyldimethylammonium salts were prepared by combining DDAOH (ion exchanged, REXYN 201 Fisher, from the bromide salt) with a stoichiometric amount of the appropriate acid.[2] Previously encountered ambiguities in the determination of DDAOH were avoided by employing a water–methanol mixture (10:90 v/v) as the titration medium. In this solvent system DDAOH behaves as a simple strong base, the titration products are soluble, pH electrode response is rapid and reproducible, and fouling of the reference electrode (saturated calomel) is absent. Hydrochloric acid served as titrant and was standardized with sodium borate decahydrate.

Two polymerized acrylate samples, one sonicated the other not sonicated prior to polymerization, were prepared with hydrogen peroxide as the initiator (100 μL of 30% H_2O_2 per 10 μL of sample; surfactant concentrations of 5.5, 6.4, and 55.0 mM).

The didodecyldimethylammonium salt samples were prepared for VEDICM analysis by depositing a small drop of solution on a microscope slide and placing a cover slip on top. This method of preparation subjects the surfactant solution to a small uncontrolled amount of shear as the fluid flows between the two glass surfaces. In a second series of experiments shear was minimized by permitting water to diffuse into a concentrated surfactant solution contained between the slide and coverslip, and the emergence of vesicles was followed as the solution was diluted. The VEDICM equipment was described in detail previously.[4]

Didodecyldimethylammonium fluoride, formate, acetate, propionate, butyrate, glycinate, tartarate (as dianion), and oxalate (as $C_2O_4^{2-}$) give clear isotropic solutions up to at least 0.1 M which have low to moderate viscosities (<500 cp at 0.13 M).

In concentrated solutions (~0.13 M) no evidence of vesicle formation is detected by VEDICM. Dilution of these solutions by a factor of 100–10 000 yields vesicles, see Figure 1. This behavior parallels that observed for DDAOH.[2,3] In concentrated DDAOH solutions, small micelles with aggregation numbers of ~40 are obtained by the double-exponential fluorescence decay method;[9] dilution yields vesicles. The existence of DDAOH vesicles has been established by VEDICM, electron microscopy, and light-scattering measurements.[2-4] The transformation of DDAOH and DDA carboxylate micelles to vesicles appears surprisingly rapid (~60 s). Samples of DDA formate subjected to freeze–thaw cycles yield similar to those shown in Figure 1c. This provides a crude indication the DDA carboxylate vesicles

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(1) See, e.g.: Fendler, J. H. "Membrane Mimetic Chemistry"; Wiley: New York, 1983; Chapter 6.
(2) Talmon, Y.; Evans, D. F.; Ninham, B. W. *Science (Washington, D.C.)* **1983**, *221*, 1047.
(3) Ninham, B. W.; Evans, D. F.; Wei, G. J. *J. Phys. Chem.* **1983**, *87*, 5020.
(4) Kachar, B.; Evans, D. F.; Ninham, B. W. *J. Colloid Interface Sci.*, in press.
(5) Inoué, S. *J. Cell Biol.* **1983**, *89*, 346.
(6) Allen, R. D.; Allen, N. S.; Travis, J. L. *Cell Muscle Motil.* **1981**, *1*, 275.
(7) Allen, R. D.; Travis, J. L.; Allen, N. S.; Yelmaz, H. *Cell Muscle Motil.* **1981**, *1*, 291.
(8) Spencer, M. "Fundamentals of Light Microscopy"; Cambridge University Press: London, 1982; Chapter 8.
(9) Hashimoto, S.; Thomas, J. K.; Evans, D. F.; Mukerjee, S.; Ninham, B. W. *J. Colloid Interface Sci.* **1983**, *95*, 594.

0002-7863/84/1506-4279$01.50/0

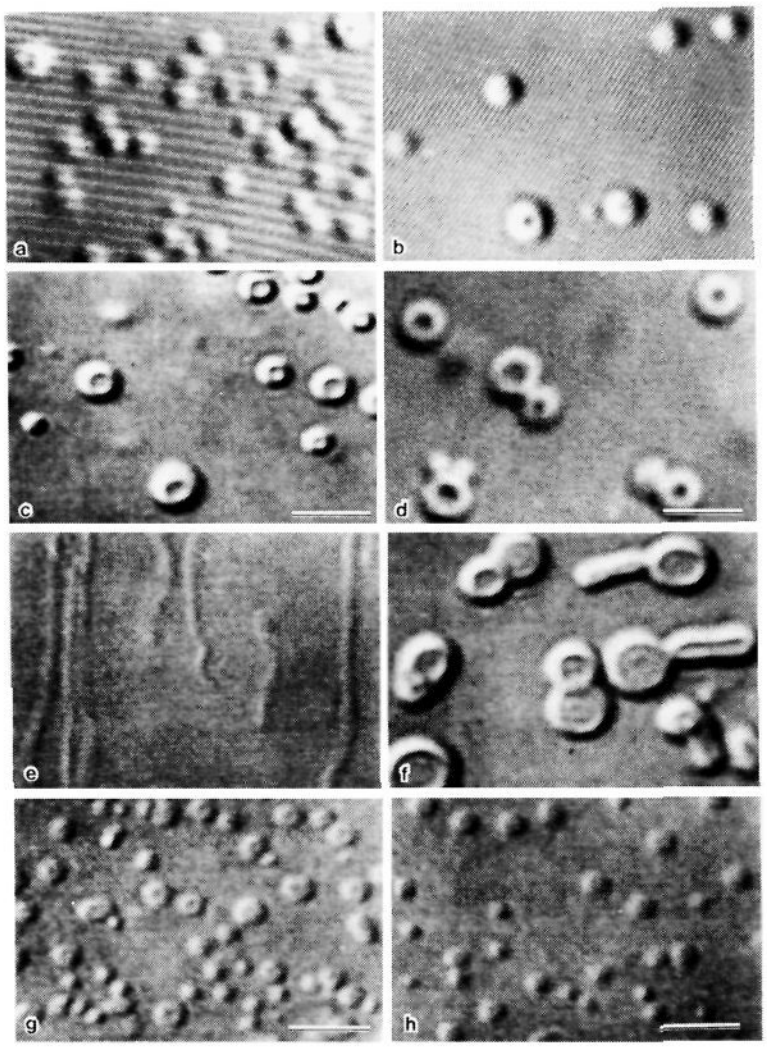

Figure 1. Photographs taken directly from the video screen of spontaneously formed DDA carboxylate vesicles as detected by VEDICM: The vesicles shown by larger than those obtianed from DDAOH although structural transformations remain rapid. (a) DDA acetate 0.13 mM, (b) DDA acetate 0.13 mM, (c) DDA formate 0.13 mM, (d) DDA propionate 0.40 mM, (e) DDA formate 0.13 mM under shear, (f) DDA acetate 0.80 mM at rest after shear, (g) DDA acrylate 5.45 mM sonicated prior to addition of H_2O_2, (h) DDA acrylate 5.45 mM not sonicated prior to addition of H_2O_2. Bar length shown is 2.5 μm and applies to all photographs.

are stable and not merely kinetically favored species. The rapidity of structural transformations in these systems also supports this view.

Other anions examined include trifluoroacetate, trichloroacetate, bromoacetate, benzoate, octanoate, perchlorate, oxalate (as $HC_2O_4^-$), and perfluorobutyrate. These yield birefringent or turbid solutions at 0.1 M and upon dilution; vesicles are not detected. We note in passing the vesicle-forming anions are fairly weak acids (pK_a = 3.9–9.8) while those yielding birefringent or turbid solutions are strong acids (pK_a < 2.7; benzoate (pK_a = 4.2) and octanoate (pK_a = 4.9) are exceptions).

The photographs shown in Figure 1 illustrate a number of features of vesicles formed from the DDA carboxylates. We emphasize these still photographs cannot convey the system dynamics that are directly accessible from the television screen. Figures 1a–d establish that vesicles are formed from DDA carboxylates; whether these relatively small structures are single- or multi-walled cannot be established by VEDICM alone. However, the larger structures shown in Figure 1f certainly appear to be multilamellar vesicles. The carboxylate vesicles are considerably larger than those found with hydroxide as the counterion. Figure 1e illustrates some of the effects of fluid flow and shear on these vesicle systems. The tubules shown in Figure 1e result when the sample is subjected to shear—for example, by moving the coverslip relative to the microscope slide. The tubules slither about and gradually (over ~30 min) revert to the vesicles shown in Figure 1c). Understanding the dynamic response of these vesicles to shear and fluid flow will certainly be important to many potential applications such as controlled drug delivery or tertiary oil recovery.

Regen et al.[10] recently reported stabilized polymer-encased vesicles prepared from dioctadecyldimethylammonium methacrylate. We show similar polymerized vesicles prepared from didodecyldimethylammonium acrylate sonicated (Figure 1g) and unsonicated (Figure 1h) prior to polymerization. The behavior of DDA acrylate monomer is intermediate to that of DDA bromide and DDA carboxylates in that solutions are turbid at high concentration (55 mM) with eventual formation of a surfactant precipitate (in contrast DDA bromide remains finely dispersed), while dilution causes immediate clearing of the solution.

We believe the preliminary results reported here are significant on several counts. Although the relationship between surfactant structure and vesicle stability has received considerable attention, specific counterion effects have remained unexplored. These counterion effects clearly constitute an important means for controlling surfactant microstructure stability and focus attention on the delicate nature of forces that dictate the transitions between bilayers, vesicles, microtubules, and micelles seen in self-assembly systems. The ease of vesicle formation by DDAOH illustrated an important point regarding surfactant self-assembly. However, DDAOH is not easily amenable to careful study; the DDA carboxylates are. The results above, as well as our previous studies on DDAOH, clearly illustrate that subtle chemical or physical differences can lead to pronounced morphological effects when dealing with large-scale aggregates. This has obvious implications regarding our understanding of biological self-assembly as well as on the practical design and utilization of surfactant microstructures.

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Registry No. DDA fluoride, 90790-94-6; DDA formate, 90790-95-7; DDA acetate, 16613-01-7; DDA propionate, 90790-96-8; DDA butyrate, 90790-97-9; DDA glycinate, 90790-98-0; DDA acrylate, 90790-99-1; DDA tartarate (dianion), 90791-00-7; DDA oxalate (dianion), 90791-01-8; DDA trifluoroacetate, 90791-02-9; DDA trichloroacetate, 90791-03-0; DDA bromoacetate, 90791-04-1; DDA benzoate, 90791-05-2; DDA octanoate, 71156-48-4; DDA oxalate (monoanion), 90791-06-3; DDA perfluorobutyrate, 90791-07-4.

(10) Regen, S. L.; Shen, J.-S.; Yamaguchi, K. *J. Am. Chem. Soc.* **1984**, *106*, 2446.

Spin-State Relaxation Dynamics in Iron(III) Complexes: Photochemical Perturbation of the $^2T \rightleftharpoons {}^6A$ Spin Equilibrium by Pulsed-Laser Irradiation in the Ligand-to-Metal Charge-Transfer Absorption Band

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Considerable attention has been devoted recently to investigation of the photophysics and spin-state relaxation dynamics of iron(II) and iron(III) complexes in solution.[1-3] In the case of iron(II) there is substantial evidence[1,2a] that intersystem crossing to lig-

(1) (a) Bergkamp, M. A.; Brunschwig, B. S.; Gütlich, P.; Netzel, T. L.; Sutin, N. *Chem. Phys. Lett.* **1981**, *81*, 147. (b) Creutz, C.; Chou, M.; Netzel, T. L.; Okumura, M.; Sutin, N. *J. Am. Chem. Soc.* **1980**, *102*, 1309. (c) Street, A. J.; Goodall, D. M.; Greenhow, R. C. *Chem. Phys. Lett.* **1978**, *56*, 326. (d) Kirk, A. D.; Hoggard, P. E.; Porter, G. B.; Rockley, M. G.; Windsor, M. W. *Ibid.* **1976**, *37*, 199.

(2) (a) McGarvey, J. J.; Lawthers, I. *J. Chem. Soc., Chem. Commun.* **1982**, 906. (b) Binstead, R. A.; Beattie, J. K.; Dewey, T. G.; Turner, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 6442. (c) Beattie, J. K.; Binstead, R. A.; West, R. J. *Ibid.* **1978**, *100*, 3044. (d) Dose, E. V.; Hoselton, M. A.; Sutin, N.; Tweedle, M. F.; Wilson, L. J. *Ibid.* **1978**, *100* 1141.

(3) Berkgamp, M. A.; Gütlich, P.; Netzel, T. L.; Sutin, N. *J. Phys. Chem.* **1983**, *87*, 3877.

0002-7863/84/1506-4280$01.50/0