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Polymer-Encased Vesicles Derived from Dioctadecyldimethylammonium Methacrylate[1]

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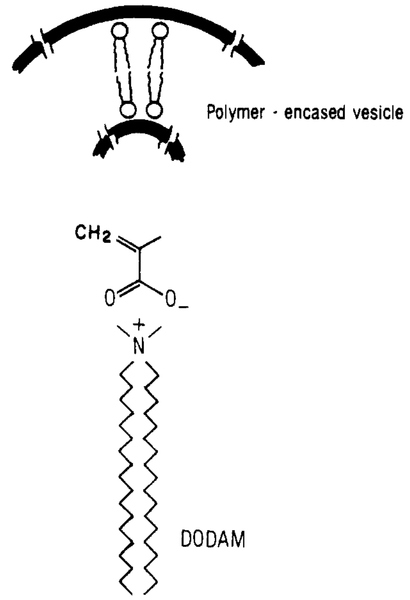
Abstract: Dispersal of dioctadecyldimethylammonium methacrylate (DODAM) in water via ultrasonic irradiation yielded small-diameter vesicles having a phase transition at ca. 42–46 °C. Photopolymerization (254 nm) at 30 and 60 °C resulted in the formation of polymer-encased vesicles which retained phase-transition behavior. Combination of dynamic light scattering, electron microscopy, and captured volume data provides strong evidence for vesicle shrinkage when polymerization is carried out at 60 °C; shrinkage occurring during photopolymerization at 30 °C is less certain. Poly(methacrylic acid), derived from 30°-polymerized vesicles (30°-polymerized means polymerized at 30 °C in this paper), was 75.4% syndiotactic, 22.3% heterotactic, and 2.3% isotactic and was significantly more soluble in DMF than poly(methacrylic acid) derived from 60°-polymerized vesicles; the latter polymer was predominantly syndiotactic. At 25 °C, nonpolymerized, 30°-polymerized, and 60°-polymerized vesicles showed similar permeability toward sucrose; at 60 °C, the 60°-polymerized vesicles were less permeable. Storage of the 60°-polymerized and the nonpolymerized DODAM vesicles for 2 months at room temperature revealed the former to be more stable. The monolayer properties and photopolymerization behavior of DODAM have been investigated at the air–water interface. Combination of surface pressure–area isotherms, surface viscosity measurements, and kinetic analysis of the photoreaction shows that the methacrylate and poly(methacrylate) ions are intimately associated with the dioctadecyldimethylammonium monolayer as tight ion pairs and that the photopolymerization is a true surface reaction, taking place at the gas–water interface, not in the bulk aqueous subphase.

Polymerized vesicles are of considerable current interest in the areas of biomembrane modeling, drug deliverly, and solar energy conversion.[3,4] They are similar to conventional (nonpolymerized) vesicles in terms of their structure and physical characteristics but are substantially more stable. In this paper, we describe the synthesis and characterization of a novel lipid assembly which represents a *hybrid* of polymerized and nonpolymerized vesicles. Specifically, we describe the preparation of "polymer-encased vesicles" derived from dioctadecyldimethylammonium methacrylate (DODAM). The uniqueness of these vesicles stems from the fact that the lipid bilayer is not covalently linked together but is, instead, ionically encased within two concentric poly(methacrylate) monolayers (Chart I).[5,6]

Chart I



Our choice of DODAM as the surfactant monomer was based on the following considerations. First, DODAM is very similar in structure to dioctadecyldimethylammonium chloride (DODAC); the only difference is the counterion employed. Extensive studies carried out with aqueous DODAC dispersions indicate that this surfactant forms well-behaved bilayer vesicles;[7,8] DODAM seemed likely to behave in a similar fashion. Second, polymerized-DODAM vesicles should react with hydrochloric acid, quantitatively, to yield poly(methacrylic acid) plus DODAC. Analysis of the polymer by ^{1}H NMR spectroscopy would provide direct information concerning the stereochemical course of the polymerization.[9] To the best of our knowledge, such information would represent the first clear stereochemical results obtained for any polymerization process carried out within a vesicle assembly and would be of intrinsic interest in and of itself. Third, based on the well-established antiviral activity, tumor growth inhibition and interferon induction associated with poly(acrylic acid), it seemed plausible that polymer-encased vesicles produced from DODAM

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(3) For recent reviews, see: Regen, S. L. *Ann. N. Y. Acad. Sci.* **1985**, *446*, 296. Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 305. Ringsdorf, H. *Adv. Polym. Sci.* **1985**, *64*, 1. Fendler, J. H.; Tundo, P. *Acc. Chem. Res.* **1984**, *17*, 3.

(4) See: *Polym. Prepr., Am. Chem. Soc., Div. Polym. Chem.* **1985**, *26*, 204.

(5) A preliminary account of this work has previously been published: Regen, S. L.; Shin, J. S.; Yamaguchi, K. *J. Am. Chem. Soc.* **1984**, *106*, 2446.

(6) Recently two other research groups have reported examples of polymer-encased vescicles: Aliev, K. V.; Ringsdorf, H.; Schlarb, B.; Leister, K.-H. *Makromol. Chem. Rapid. Commun.* **1984**, *5*, 345. Brady, J. E.; Evans, D. F.; Kachar, B.; Ninham, B. W. *J. Am. Chem. Soc.* **1984**, *106*, 4279. Two vesicle systems that have been stabilized by polymer coating have also appeared in the literature: Kunitake, T.; Yamado, S. *Polym. Bull.* **1978**, *1*, 35. Iwamoto, K.; Sunamoto, J. *J. Biochem.* (*Tokyo*) **1982**, *91*, 975.

(7) Kano, K.; Romero, A.; Djermouni, B. Ache, H. J.; Fendler, J. H. *J. Am. Chem. Soc.* **1979**, *101*, 4030.

(8) Carmona, A. M.; Chaimovich, H. *Biochim. Biophys. Acta* **1983**, *733*, 172.

(9) Klesper, E.; Johnsen, A.; Gronski, W. *J. Polymn. Sci., Polym. Lett. Ed.* **1970**, *8*, 369. For polymerization studies of methacrylic acid in organized media, see: Lando, J. B.; Semen, J. *J. Polymer Sci.* **1972**, *10*, 3003. And: Lando, J. B.; Morowetz, H. *J. Polymer Sci., Part C* **1964**, *4*, 789. We are grateful to a referee for bringing these references to our attention.

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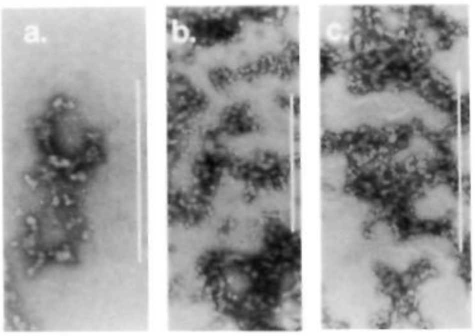

Figure 1. Transmission electron micrographs of (a) nonpolymerized DODAM vesicles, (b) 30°-polymerized DODAM vesicles, and (c) 60°-polymerized DODAM vesicles. Bar represents 1 μm.

could have immediate practical value as unique polymeric drugs, acting either alone or synergistically with an encapsulated agent.[10] Fourth, DODAM and related analogues should be readily obtainable by simple ion-exchange procedures using commercially available and relatively inexpensive starting material.

Results

Dioctadecyldimethylammonium Methacrylate. An anion ion-exchange resin (Rexyn 201, hydroxide form) was quantitatively converted into the methacrylate form by treatment with a methanolic solution of methacrylic acid (2-fold excess). Subsequent passage of dioctadecyldimethylammonium bromide (0.12 equiv based on pendant methacrylate) through the resin afforded a 99% yield of DODAM. Specific procedures described in the Experimental Section are unexceptional in nature and provide DODAM, conveniently, and in gram quantities.

Vesicle Formation. Vesicles derived from DODAM were prepared by coating the lipid onto the glass surface of a disposable thin-walled flat-bottomed ampule (chloroform evaporation) and then dispersing the lipid into distilled and deionized water via vortex mixing and irradiating the dispersion with ultrasound (bath-type sonicator) at 50 °C under an argon atmosphere to constant turbidity. Dynamic light scattering measurements carried out by using a Nicomp 200 instrument, equipped with a helium–neon laser (632.8 nm, scattering angle of 90°) and a computing autocorrelator, indicated particles having a mean diameter of 357 Å; reproducibility was ±5%. Sonication of samples in standard round-bottomed flasks, *which had not previously been subjected to ultrasound*, afforded similar dispersions. Reuse of such flasks for vesicle formation, however, led to substantial contamination by small glass particles. Passage of the dispersion through a Sephadex G-50 column afforded a 100% recovery in the void volume (nitrogen analysis). Examination by transmission electron microscopy (uranyl acetate), negative staining indicated spheres having an average diameter of 303 ± 41 Å (Figure 1). Temperature-dependent turbidity measurements (absorbance at 400 nm) revealed a phase transition, characteristic of an organized bilayer assembly (Figure 2A);[7,8,11] the transition temperature region was ca. 42–46 °C.

Vesicle Polymerization. Sonicated dispersions of DODAM were placed in quartz tubes, purged with a stream of argon, and polymerized by exposure to direct UV irradiation (254 nm). Polymerization reactions were carried out at temperatures above and below the phase-transition temperature, i.e., 60 and 30 °C, respectively. Freeze-drying of the dispersions, followed by ^{1}H NMR analysis of the residue (dissolved in $CDCl_3$), indicated the complete loss of the vinyl protons.

Examination of the 30°- and 60°-polymerized vesicles (30°- and 60°-polymerized means polymerized at 30 and 60 °C, respectively, in this paper) by light scattering (Nicomp 200) indicated mean diameters of 293 and 200 Å, respectively; transmission electron micrographs indicate average diameters of 368 ± 59 and 241 ± 49 Å, respectively (Figure 1). Gel filtration of the 30°-polymerized dispersion through a Sephadex G-50 column resulted in a 94% recovery in the void volume (nitrogen analysis); similar filtration of the 60°-polymerized dispersion afforded a 93% recovery. Raising the temperature of the 30°-polymerized dispersion from 26 to 70 °C resulted in a relatively small decrease in turbidity (Figure 2B). When the sample was cooled, however, a substantial increase in turbidity was noted. Subsequent heating and cooling cycles produced turibidity profiles which were similar to those observed for nonpolymerized DODAM vesicles, reflecting a restoration of phase-transition behavior. Dynamic laser light scattering of the 30°-polymerized dispersion (and also a nonpolymerized DODAM dispersion) after one heating–cooling cycle indicated no significant alteration in the mean diameter. The temperature-dependent turbidity behavior of the 60°-polymerized dispersion was similar to that of the 30°-polymerized sample, except that the first heating curve showed evidence of a phase transition (Figure 2C). Within experimental error, the phase-transition temperatures for the nonpolymerized, 30°-polymerized, and 60°-polymerized vesicles were identical; all were in the range of 42–48 °C.

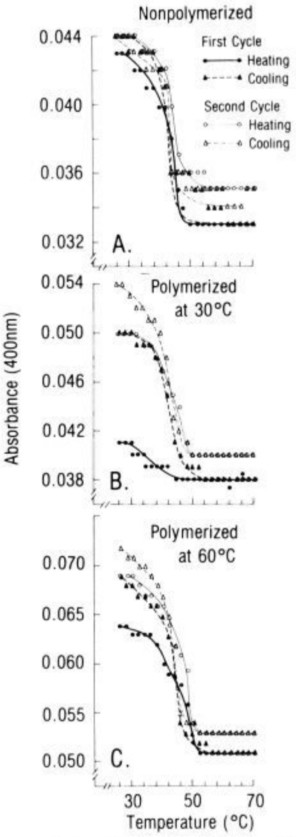

Figure 2. Plot of apparent absorbance (turbidity) at 400 nm as a function of temperature for (A) nonpolymerized DODAM vesicles, (B) 30°-polymerized DODAM vesicles, and (C) 60°-polymerized DODAM vesicles. First cycle: (●) heating; (▲) cooling. Second cycle: (○) heating; (△) cooling.

In our hands, all efforts to polymerize vesicles of DODAM using standard radical initiators such as azobis(isobutyronitrile) (AIBN), 4,4′-azobis(4-cyanovaleric acid) (ACVA), and hydrogen peroxide under thermal conditions proved unsuccessful. Similarly, attempted thermal polymerization of ethanolic solutions of DODAM using AIBN as the initiator or DODAM in the neat state (bulk polymerization) afforded only unreacted starting monomer.

Isolation and Tacticity of Poly(methacrylic acid). Treatment of a freeze-dried, 30°-polymerized vesicle dispersion of DODAM with hydrochloric acid in absolute ethanol afforded an 80% isolated yield of poly(methacrylic acid) (see Experimental Section). Similar isolation of poly(methacrylic acid) from the 60°-

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[10] Ottenbrite, R. M.; Regelson, W. In *Encyclopedia of Polymer Science and Technology*; Wiley-Interscience: New York, 1977; Suppl. 2, p 118.

[11] Yi, P. N.; McDonald, R. C. *Chem. Phys. Lipids* **1973**, *11*, 114. Tsong, T. Y.; Kanehisa, M. I. *Biochemistry* **1977**, *16*, 2674.

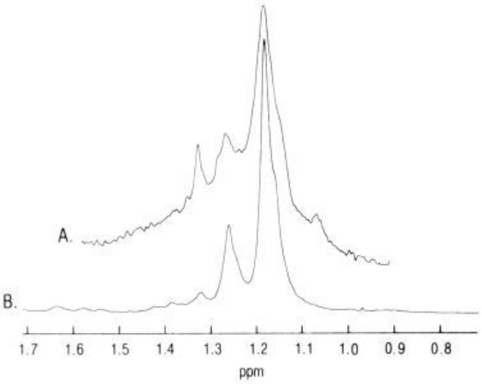

Figure 3. ^{1}H NMR spectrum of (250 MHz, DMF-d_7, 100 °C) of poly(methacrylic acid) derived from (A) 60°-polymerized DODAM vesicles and (B) 30°-polymerized DODAM vesicles. Methyl absorptions reflecting syndiotactic, heterotactic, and isotactic placement appear at δ 1.18, 1.27, and 1.32, respectively.

Table I. Entrapment and Permeability Toward [^{14}C]Sucrose

vesicle[a]	captured vol, L/mol	retention, % 1 h	4 h	12 h
NP DODAM	1.32	88 (38)	69 (20)	61 (8)
30 °P DODAM	1.36	77 (58)	69 (24)	62 (14)
60 °P DODAM	0.76	82 (60)	74 (41)	66 (27)

[a] Dispersions were formed from 15 mg of DODAM in 2.25 mL of distilled and deionized water containing 15 μCi of [^{14}C]sucrose; NP = nonpolymerized, 30 °P = polymerized at 30 °C, 60 °P = polymerized at 60 °C. Dialysis temperature was 25 °C; numbers in parentheses refer to dialysis experiments carried out at 60 °C. All numbers reported are the average values of three independent experiments.

polymerized dispersion resulted in a 56% yield. Each sample produced an IR spectrum which was identical with that of authentic poly(methacrylic acid). Both polymers exhibited relatively poor solubility in DMF-d_7, indicating a high degree of polymerization. Qualitatively, the 60°-polymerized polymer was significantly less soluble than the 30°-polymerized sample. Analysis of soluble fractions by ^{1}H NMR (250 MHz) at 100 °C revealed that both polymers were predominantly syndiotactic (Figure 3).[13] The poly(methacrylic acid) formed at 30 °C is estimated to be 75.4% syndiotactic, 22.3% heterotactic, and 2.3% isotactic. The broadness of the methyl resonances for the polymer formed at 60° precludes a quantitative assessment of its tacticity.

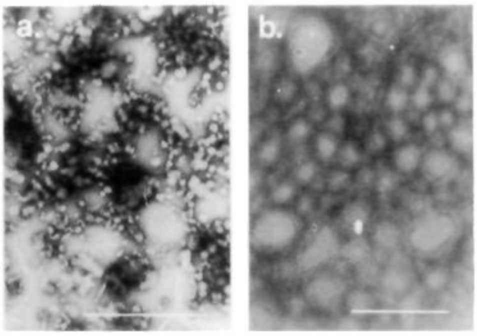

Figure 4. Transmission electron micrographs of (a) 60°-polymerized vesicles and (b) nonpolymerized DODAM vesicles, stored for 2 months at room temperature in the dark. Bar represents 1 μm.

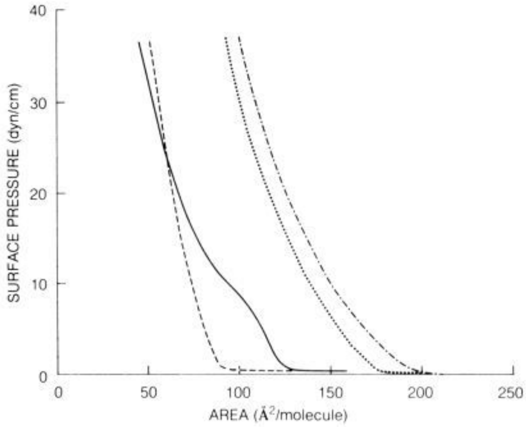

Figure 5. Surface pressure–area isotherms for nonpolymerized DODAM (—), UV-treated DODAM (- - -), dioctadecyldimethylammonium acetate (– –), and dioctadecyldimethylammonium bromide (···).

Table II. Surfactant Monolayer Properties at the Air–Water Interface

surfactant[a]	state	surface pressure, dyn/cm	A_0, Å^2/molecule	i	π_0, dyn/cm
DODAM	liquid-expanded	0.0–6.3	54	4.9	−17
P-DODAM	liquid-expanded	0.0–24.0	44	6.8	−22
DODAA	liquid-expanded	0.0–14.0	69	6.3	−16
DODAB	liquid-expanded	0.0–9.0	72	4.3	−15

[a] P-DODAM = dioctadecyldimethylammonium methacrylate after being exposed to UV light for 12 min. DODAA = dioctodecyldimethylammonium acetate. DODAB = dioctadecyldimethylammonium bromide.

Captured Volume and Permeability. In order to estimate the internal aqueous volume (captured volume) of DODAM vesicles in the polymerized and nonpolymerized state, [^{14}C]sucrose was entrapped within the aqueous interior by using standard procedures (see Experimental Section).[14] Table I summarizes apparent capture volumes and permeability toward sucrose.[15] Nonpolymerized vesicles had a capture volume which was similar to that of the 30°-polymerized sample; the 60°-polymerized dispersion exhibited a significantly lower volume. Dialysis experiments, carried out below the phase-transition temperature of the membranes (i.e., at 25 °C), indicate that within experimental error, all vesicles showed similar permeability toward sucrose. When the permeability was measured above the phase transition (i.e., at 60 °C), the 60°-polymerized vesicle was less permeable, relative to the nonpolymerized and 30°-polymerized samples.

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(12) Direct analysis of the aqueous vesicle dispersion by thin-layer chromatography (TLC), before and after UV irradiation, indicated a single spot having an R_f = 0.70 (silica gel, 3:1 $CHCl_3/CH_3OH$), which was identical with that of the starting monomer. Since our preliminary report of this work,[5] we have discovered that TLC cannot be used as a reliable criterion for vesicle polymerization, *in this specific system*. Overloading the TLC plates with polymerized or nonpolymerized surfactant, or insufficient drying of the plate prior to chromatography (i.e., less than 15 min under a stream of nitrogen), produces tailing and leaves significant quantities of the surfactant at the origin. Polymer formation is, however, firmly established by the NMR results together with the isolation of poly(methacrylic acid). It should be noted, however, that other vesicle systems that we have examined, where the polymerizable moiety is *covalently* incorporated into the lipid chain, have given highly reproducible TLC results.

(13) In our preliminary communication, we reported the tacticity of poly(methacrylic acid), isolated from a polymerized DODAM vesicle dispersion, to be 72% isotactic, 15% syndiotactic, and 13% heterotactic.[5] Polymerization conditions that were used were similar to those reported in this work, except that an ambient temperature inside of the Rayonet reactor was used; i.e., the temperature of the dispesion was ca. 22 °C, initially, and reached ca. 50 °C at the end of the polymerization. All efforts to repeat this "ambient" temperature experiment have yielded poly(methacrylic acid), having a ^{1}H NMR spectrum similar to that shown in Figure 2; i.e., the polymer is predominantly syndiotactic. We have no satisfactory explanation to account for this discrepancy, other than to suggest that authentic isotactic poly(methacrylic acid) samples, used to confirm peak assignments, may have been mistakenly recorded as vesicle-derived sample.

(14) Regen, S. L.; Singh, A.; Oehme, G.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 791.

(15) These numbers should be considered as approximate. Leakage of entraped sucrose, after gel filtration and before the start of dialysis, will result in low apparent captured volumes.

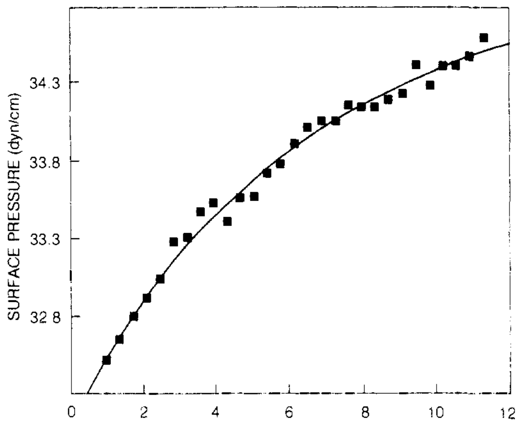

Figure 6. Surface pressure of DODAM as a function of UV-irradiation time; solid line represents the calculated curve based on (4).

Vesicle Shelf Life. Electron microscopic examination of nonpolymerized DODAM vesicles, stored in the dark at room temperature for 2 months, revealed the presence of very large oval-shaped structures (Figure 4). In contrast, the 60°-polymerized DODAM vesicles retained their spherical shape after 2 months but appeared somewhat larger; i.e., their average diameters increased to ca. 430 Å.

Monolayer Properties and Polymerization Behavior at the Gas-Water Interface. Monolayers of DODAM, dioctadecyldimethylammonium bromide, and dioctadecyldimethylammonium acetate were spread from a 9:1 (v/v) hexane–ethanol solution containing approximately 0.2 mg of lipid/mL. Isotherms were recorded at 22 °C by using a computerized MGW Lauda film balance and are shown in Figure 5. Analysis of these curves was made by using the *semiempirical* two-dimensional gas equation (1) and the Langmuir "duplex layer" equation (2) which describe the gaseous and liquid expanded monolayer states, respectively.

$$\pi(A - nA_0) = nRT \qquad (1)$$

$$(\pi - \pi_0)(A - nA_0) = inRT \qquad (2)$$

Here, π is the experimentally measured surface pressure, π_0 is the position of the horizontal asymptote of the hyperbola which characterizes the liquid-expanded region, A is the measured surface area for a given surface pressure, n is the number of moles of surfactant at the air–water interface, A_0 is the molar collisional area of the surfactant, $R = 8.3143 \times 10^7$ ergs mol^{-1} K^{-1}, and i is an empirical constant which is thought to reflect segmental motion. A nonlinear least-squares fit of the data for the surface pressure regions specified in Table II was excellent; computed values for i, A_0, and π_0 are presented in the table. For DODAM, an apparent phase transition to a liquid condensed state was observed at ca. 7 dyn/cm. Within experimental error, the collisional areas of the bromide and acetate salts were identical (ca. 70 Å^2). In contrast, the methacrylate salt occupied a significantly smaller area (54 Å^2).

Exposure of DODAM monolayers (having an initial surface pressure of 32.5 dyn/cm and confined to a fixed surface area) to UV irradiation (254 nm) resulted in a slight steady increase in pressure (Figure 6). Interestingly, the rate of this increase conforms well to the kinetics expected for a standard three-dimensional photochemical polymerization process, where the rate is governed by (3).[16] Here, R_p is the rate of polymerization, k_p

$$R_p = k_p\left[\frac{\phi\epsilon I_0[A]b}{k_t}\right]^{1/2}[M] \qquad (3)$$

is the propagation constant, k_t is the chain termination constant, [M] is monomer concentration in the monolayer, I_0 is the incident light intensity, ϕ is the quantum yield for initiation, [A] is the concentration in the monolayer of the species undergoing photoexcitation, ϵ is the extinction coefficient of A, and b is thickness of reaction system being irradiated. When the monomer itself serves as the initiator (i.e., when $[A] \propto [M]$), the polymerization rate is expected to have a 3/2-order dependence on the monomer concentration.[16] If we assume (1) that the initial monomer concentration in the monolayer equals $C(P_l - P_0)$ and the concentration of reacted monomer equals $C(P - P_0)$ (where C is a proportionality constant, P_l represents the limiting pressure at 100% conversion, P_0 is the initial pressure before UV irradiation, and P is the experimentally measured surface pressure) and (2) that the rate of polymerization obeys eq 3, then the observed pressure should vary with the time of irradiation, following eq 4 (integrated form of eq 3). As can be seen in Figure 6, a nonlinear

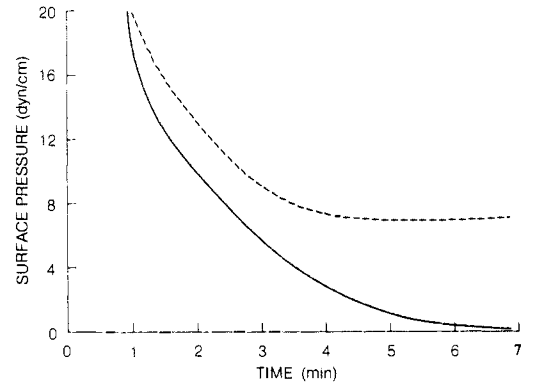

Figure 7. Surface pressure of nonpolymerized DODAM (—) and UV-treated DODAM monolayers (---), as a function of time of leakage, through a slit which was 0.05 mm wide and 3.9 cm long.

$$P = P_l - \frac{1}{\left[(P_l - P_0)^{1/2} + \frac{KC^{1/2}}{2}t\right]^2} \qquad (4)$$

$$\text{where} \quad K = k_p\left[\frac{\phi\epsilon I_0 b}{k_t}\right]^{1/2} \qquad t = \text{time of irradiation}$$

least-squares analysis of the entire experimental curve, using eq 4, gives an excellent fit, with a value of P_l of 35.6 dyn/cm. Furthermore, the data were inconsistent with a first-order or a second-order rate law.

Preliminary experiments aimed at determining the relative surface viscosities for polymerized and nonpolymerized DODAM monolayers at the argon–water interface have been carried out. Each monolayer was compressed to ca. 20 dyn/cm, using a movable barrier that was positioned directly behind a second barrier containing a slit which was 0.05 cm wide and 3.9 cm long. Subsequent and rapid movement of only the former resulted in surfactant flow through the slit, whose rate could be monitored by measuring the decrease in surface pressure. As can be seen in Figure 7, the nonpolymerized DODAM monolayer fell to 0 dyn/cm after ca. 5 min. In sharp contrast, the polymerized monolayer fell to 7 dyn/cm after 2.5 min but then showed a dramatic reduction in flow. From these preliminary results, it is clear that ca. 70% of the UV-treated DODAM monolayer has a surface viscosity which is at least 2 orders of magnitude greater than that of monomeric DODAM.

Discussion

Dispersal of DODAM in water via ultrasonic irradiation produces vesicles in high yield, as evidenced by gel filtration, transmission electron microscopy (TEM), and dynamic light scattering. The average diameter for nonpolymerized DODAM vesicles, indicated by TEM, is similar to the 300 Å diameter vesicles which have previously been reported for sonicated dispersions of DODAC.[7] The high yield of recovery of DODAM in the void volume of the Sephadex column, together with the vesicle diameters estimated by light scattering, establishes that soluble lipid monomer and/or small surfactant aggregates are

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[16] *Principles of Polymerization*, 2nd ed.; Odian, G., Ed.; Wiley-Interscience: New York, 1981; p 210.

present in negligible concentrations. Temperature-dependent turbidity profiles seen for nonpolymerized dispersions of DODAM are similar to those which have been observed for DODAC;[8] such profiles have been used as strong evidence for the existence of organized bilayer assemblies.[8,11] The transition temperature region is slightly higher than that reported for DODAC. This result presumably reflects a head-group effect on the cooperative disordering and packing of the hydrocarbon chains (see monolayer properties discussed below). The estimated capture volume for nonpolymerized DODAM vesicles is 1.3 L/mol and is within the range expected for small unilamellar vesicles.[15,17]

Photopolymerization of the vesicular assembly at 30 °C produces a decrease in its diameter, as seen by light scattering. Transmission electron micrographs establish the retention of small microspheres after polymerization but do not confirm vesicle shrinkage. Absolute values obtained by both electron microscopy and light scattering should be considered only as approximate. The similarity between the captured volume of the nonpolymerized and 30°-polymerized vesicles indicates that the vesicles are similar in size. From all these results, we conclude that if there is a change in the vesicle's diameter upon polymerization at 30°, it is relatively small. The high recovery of the polymerized lipid in the void volume of a Sephadex G-50 column provides further confirmation that the vesicle structure is retained after UV treatment. Temperature-dependent turbidity measurements of the 30°-polymerized sample indicate the formation of a metastable state; heating–cooling cycles apparently anneal the bilayer into a more stable assembly. The fact that the mean vesicle diameter remains essentially unchanged after the first heating–cooling cycle, as seen by dynamic light scattering, argues strongly against interference in the turbidity profiles arising from aggregation phenomena. The retention of a phase transition in the polymerized state indicates that the cooperative disordering of the hydrocarbon chains remains essentially intact. Photopolymerization of DODAM vesicles, carried out at 60 °C, results in a significant reduction in vesicle size; this conclusion is strongly supported by the combination of dynamic light scattering, TEM, and captured volume data. Qualitatively, the temperature-dependent turbidity profiles for these vesicles are similar to those of the 30°-polymerized sample, except that the "annealing process" is less pronounced during the first heating–cooling cycle. Presumably, partial membrane annealing takes place during the course of the polymerization.

Permeabilities of polymerized and nonpolymerized DODAM vesicles toward sucrose have been examined above and below the apparent phase-transition temperature of the bilayer. A clear interpretation of the data presented in Table I is hampered due to (a) the small differences in permeability observed and (b) the lack of understanding of the mechanism(s) by which solutes diffuse across lipid bilayers. One of the current theories of the transport of polar nonelectrolytes across vesicle membranes is that permeation occurs through water-filled channels or "pores", whose existence may be transient.[18] In the case of polymerized DODAM vesicles, very thin poly(methacrylate) monolayers which encase the bilayer are also likely to play an indirect role in membrane permeability. At 25 °C, there is no significant difference in the permeability among nonpolymerized, 30°-polymerized, and 60°-polymerized vesicles toward sucrose. Here, the bilayers are presumed to lie in a closely packed, ordered array (gel state). The similarity in vesicle permeability strongly suggests that diffusion across the *monomeric bilayer* is rate-limiting in each case and that the number of transient pores per unit time is similar. Above the phase-transition temperature (at 60 °C), the bilayers are presumed to change to a state in which the hydrocarbon chains are more loosely packed and display considerably more rotational freedom (fluid state). The lower permeability of the 60°-polymerized vesicle *at this temperature* indicates that a poly(methacrylate) "casing" can significantly decrease the transport rate of sucrose out of the vesicle. The polymer backbone, itself, does not appear to provide an additional barrier toward diffusion, since it does not modify the membrane's permeability at 25 °C. The relatively low permeability of the 60°-polymerized vesicle at 60 °C most likely reflects a reduction in the number of "transient pores" in the bilayer through ionic interaction with lipid domain.

Isolation of poly(methacrylic acid) from UV-treated vesicle dispersions at 30 and 60 °C provides unambiguous confirmation that polymerization has taken place in the vesicle state. The failure of DODAM vesicles to polymerize with standard free-radical initiators is surprising. It may be that the radical precursors tested prefer to selectively reside in the hydrocarbon bilayer, the aqueous vesicle interior, and/or the external aqueous phase, rather than the inner and outer vesicle surface. Also, if the initiator was strongly adsorbed to the vesicle surface, cage effects could reduce considerably its initiator efficiency. The need for DODAM to be in a highly organized state for successful polymerization is evident from its inability to be polymerized in concentrated *solution* and in the bulk phase. While we cannot provide absolute molecular weight data for the 30°- and 60°-polymerized samples at this time,[19] their low solubility in DMF and their ^{1}H NMR spectra indicate that both polymers are high polymers and that the 60°-polymerized sample is higher in molecular weight.

^{1}H NMR analysis of poly(methacrylic acid) formed at 30 and 60 °C in the vesicle state shows that both polymers are predominantly syndiotactic. Since we have not yet been able to polymerize DODAM in a nonordered state, we cannot judge the influence that ordering has on the stereochemistry of the photopolymerization. The similarity between the tacticity of vesicle-derived poly(methacrylic acid) and that produced via free-radical polymerization of methacrylic acid in dioxane at 60° (62% syndiotactic, 33% heterotactic and 5% isotactic,)[9] does, however, suggest that ordering of the monomeric counterion has only a small influence, if at all, on the stereochemistry of the polymer.

Improved stability of DODAM vesicles upon polymerization is evident from electron microscopic examination of aged (2 months) samples. Nonpolymerized dispersions revealed the presence of only very large, oval structures, presumed to be derived from fusion events occurring with time,[20] whereas the 60°-polymerized DODAM vesicles largely retained their original structure.

Analysis of the force–area curves for DODAM, dioctadecyldimethylammonium bromide, and dioctadecyldimethylammonium acetate at the gas–water interface yields π_0 values characteristic of the liquid-expanded state. The significantly lower collisional area observed for DODAM relative to the other two salts is a likely result of the greater hydrophobicity of the methacrylate ion. Increased hydrophobicity of the counterion should lead to tighter ion pairing and reduced electrostatic repulsion between the positively charged ammonium ions at the gas–water interface; the net result is a more compact and less compressible monolayer.

Treatment of the DODAM monolayer with UV irradiation results in substantial polymerization as evidenced by (1) changes in the surface pressure (at constant surface area), (2) surface viscosity measurements, and (3) analysis of the surface pressure–area isotherms. The fact that the observed 3/2-order dependence of the rate on monomer concentration is identical with that found for standard photochemical polymerization reactions in bulk solution is intriguing. Whether this is just fortuitous or

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(18) Fendler, J. H. *Membrane Mimetic Chemistry*, Wiley-Interscience: New York, 1982; p 156.

(19) Efforts to obtain precise viscosity-average molecular weights of the poly(methacrylic acid) samples have been hampered due to their poor solubility in 0.002 M HCl. The similarity in tacticity of the 30°- and 60°-polymerized samples provides further evidence that the observed differences in solubility reflect true differences in molecular weight; i.e., the solubility differences are not derived from differences in stereoregularity of poly(methacrylic acid) [Lando, J. B.; Semen, J.; Koenig, J. L. *J. Macaromol. Sci., Phys. B* **1973**, *7* (*2*), 319. Finally, attempted esterification with diazomethane in THF afforded poly(methyl methacrylate) as evidenced by gel permeation chromatography. Due to incomplete solubilization in THF, however, meaningful molecular weight distributions could not be obtained.

(20) Rupert, L. A. M.; Hoekstra, D.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **1985**, *107*, 2628. Shimomura, M.; Kunitake, T. *Chem. Lett.* **1981**, *1001*.

whether it reflects a close relationship between chain initiation and chain termination events, however, remains to be established. Based on the calculated limiting pressure, P_1, of 35.6 dyn/cm, we estimate an extent of polymerization within the monolayer of ca. 70%, under the specific conditions used. The surface viscosity measurements also show that at least 70% of the UV-treated DODAM monolayer has a surface viscosity which is at least 2 orders of magnitude greater than that of monomeric DODAM. The similarity between the estimated extent of polymerization (70%) and percentage of surfactant that possesses a high surface viscosity provides added support that the DODAM monolayer has undergone extensive and high polymer formation upon UV irradiation. Final proof of polymerization comes from the observed surface pressure–area isotherms, before and after UV treatment. After the UV treatment, the monolayer is far less responsive to the external force exerted by the moving barrier; i.e., it is much less compressible. In addition, it should be noted that the apparent phase transition that was present before irradiation is now absent. Interestingly, a "cross-over point" exists for the polymerized and nonpolymerized isotherms at ca. 24 dyn/cm. Below this pressure, the nonpolymerized monolayer occupies greater area than the polymerized film; above this pressure, the reverse is true. The collisional area of DODAM, calculated from the liquid expanded region of the force–area curve, is ca. 54 Å^2/molecule. This value implies that the average distance between the nitrogen atoms of DODAM in a tightly packed monolayer is ca. 7.3 Å (assuming circular cross sections and hexagonal packing in the fully compressed monolayer). Space filling models (CPK) for syndiotactic poly(methacrylic acid) indicate that adjacent pendant carboxylate ions have a maximum average spacing of ca. 6 Å, when the polymer backbone is in an all-trans conformation; heterotactic and isotactic segments will further reduce this spacing. To a first approximation, therefore, pendant carboxylate groups of a poly(methacrylate) "casing" should lie in closer proximity toward one another than the carboxylate groups of DODAM in a tightly packed monolayer. Strong ionic interaction between the poly(methacrylate) casing and the dioctadecyldimethylammonium monolayer, coupled with a reduction in the average distance between the carboxylate groups upon polymerization, is undoubtedly responsible for the reduced compressibility of UV-treated DODAM. Above the cross-over point (ca. 24 dyn/cm, 58 Å^2/molecule), the polymer backbone restricts further compression of the monolayer, relative to nonpolymerized DODAM. Taken together, all these results provide compelling evidence that *methacrylate and poly(methacrylate) ions are intimately associated with the dioctadecyldimethylammonium monolayer as tight ion pairs and that the photopolymerization is a true surface reaction, taking place at the gas–water interface, not in the bulk aqueous subphase.*

By analogy, strong ionic interactions are likely to be present at the inner and outer surface of the polymer-encased DODAM vesicle. This strong interaction, together with a reduction of the average distance between the carboxylate groups, upon polymerization, should result in lateral compression of the bilayer and to overall shrinkage of the vesicle; the fewer the number of polymer chains per vesicle (i.e., the higher the degree of polymerization), the greater the shrinkage. In the case of the 60°-polymerized DODAM vesicle, dramatic shrinkage is clearly evident. The formation of a metastable state upon polymerization at 30°, and to a lesser extent at 60°, confirms that the polymer chains interact strongly with the lipid assembly and that the state of the bilayer has been altered. The reduced permeability of the 60°-polymerized vesicles found at 60° are consistent with an overall "tightening up" of the bilayer. Structural studies aimed at elucidating the metastable and annealed states of polymerized DODAM vesicles are now in progress.

Experimemental Section

General Methods. Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and used without further purification. Dioctadecyldimethylammonium bromide (Eastman Kodak Co.) was used directly; methacrylic acid (Aldrich Chemical Co.) was distilled under reduced pressure prior to use. Distilled water was purified by filtration through a "Milli-Q" reagent water purification system (Millipore Co.). [^{14}C]Sucrose (560 mCi/mmol, 20% ethanol solution) was obtained from ICN Laboratories. Dialysis experiments were carried out by using Spectropor No. 6 dry membrane tubing (cylindrical diameter of 14.6 mm; M_W cutoff 6000–8000) obtained from Spectrum Medical Industries. Chloroform used in depositing DODAM onto the walls of the glass flask was HPLC-grade (Fischer Scientific). Turbidity was determined by measuring the apparent absorbance at 400 nm. ^{1}H NMR, IR, and UV spectra were recorded on a Varian EM 360L or a Bruker WM 250-MHz, Beckman Acculab 7, and Bausch & Lomb Spectronic 2000 spectrometers, respectively. Chemical shifts for poly(methacrylic acid) in DMF-d_7 at 100 °C are reported relative to the aldehyde proton, occurring at 8.01 ppm. All other chemical shift data are reported relative to tetramethylsilane. Phase-transition measurements were carried out by using a Perkin-Elmer 320 spectrophotometer coupled with a digital temperature controller. Chromatographic separations were carried out by using precoated Merck 0.25-mm silica gel 60 TLC plates, 70–230-mesh silica gel, and Sephadex G-50-150 (Sigma). A commercial anion-exchange resin (Rexyn 201–hydroxide form, 1.3 mequiv/mL in wet state, Fischer Scientific) was continuously extracted (Soxhlet) with methanol for 12 h prior to use. Detection of components on the TLC plates was made when using iodine vapor or by charing after spraying with 10% sulfuric acid. Sonications were performed by using a Heat Systems Model W-375 R bath-type sonicator in a pulse mode. Vortex mixing was carried out by using a VWR Scientific mixer (Model K-550 G). Specific precedures used for electron microscopy and entrapment of [^{14}C]sucrose were similar to those previously described;[13] electron micrographs using negative staining (2% uranyl acetate) were recorded by using a Philips 400 STEM microscope. All ultraviolet-induced polymerization reactions were carried out with a Rayonet photochemical reactor (Southern N.E. Ultraviolet Co., Hamden, CT), equipped with 16 2537 Å Rayonet photochemical reactor lamps and a quartz vessel maintained at constant temperature via water circulation.

DODAM has an absorption maxima in ethanol at 239 nm, with substantial absorption at 254 nm. Freeze drying of vesicle dispersions was carried out by using a Virtis freeze dryer. Liquid scintillation was performed with a Nuclear Chicago Mark II instrument, Model 4643, using a liquid scintillation cocktail comprised of 70% 1,2,4-trimethylbenzene plus 30% surfactant ("Mini Blend", ICN Laboratories). Dynamic light scattering measurements were carried out by using a Nicomp 200 instrument, equipped with a helium–neon laser (632.8 nm, scattering angle of 90°) and a computing autocorrelator.

Nitrogen Analysis. Nitrogen analysis of vesicle dispersions was carried out by using a modification of Nessler's method.[21] Calibration curves were constructed in the range of 0–10 μg using ammonium chloride as the standard; standard methanolic solutions of dioctadecyldimethylammonium bromide (0.1 mg/mL) produced identical calibration curves.

Dioctadecyldimethylammonium Methacrylate. A 15 mm diameter glass column was packed with 30 mL (0.039 mol) of wet Rexyn 201 (hydroxide form) and converted into the methacrylate form by passage of 100 mL of methanol containing 6.7 g (0.078 mol) of methacrylic acid. After washing the resin with 300 mL of methanol, 3.01 g (4.77 mmol) of dioctadecyldimethylammonium bromide in 200 mL of methanol was then passed through the column. The eluent was concentrated under reduced pressure, and the residue was dissolved in 50 mL of benzene and freeze-dried to give 2.99 g (99%) of dioctadecyldimethylammonium methacrylate: mp 66–69 °C; IR (KBr) $\nu_{C=C}$ 1630 cm^{-1}; $\nu_{C=O}$ 1560 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6 H, CH_3), 1.25 (s, 64 H, CH_2), 1.95 (m, 3 H, $CH_3C{=}$), 3.19–3.63 (m, 10 H, $(CH_3)_2N(CH_2)_2$), 5.12 (m, 1 H, vinyl), 5.75 (m, 1 H, vinyl).

Vesicle Formation. Typically, 155.5 mg (0.244 mmol) of DODAM was dissolved in a minimum volume of chloroform and placed in a 100-mL round-bottomed flask (a new flask or one which had not been previously subjected to ultrasound). After the chloroform was evaporated by passing a stream of argon through the flask, the thin lipid film was dried under reduced pressure [25 °C, 12 h (0.05 mmHg)]. Distilled water (100 mL) was then added to the flask and the lipid dispersed via vortex mixing at room temperature. The flask was sealed by using a no-air stopper and the dispersion purged with argon. The dispersion was then sonicated at 50 °C to constant turbidity (ca. 15 min). For small-scale preparations, a disposable thin-glass, flat-bottomed ampule was used.

Vesicle Polymerization. Photopolymerization of DODAM dispersions was carried out in quartz tubes equipped with no-air stoppers. Prior to irradiation, each sample was purged with a stream or argon for 10 min

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and the tube then placed into a constant temperature quartz vessel, located inside of the Rayonet reactor. In all cases, the irradiation time used was 2 h.

Isolation and Characterization of Poly(methacrylic acid). Typically, an aqueous dispersion of polymerized DODAM containing 306.7 mg (0.48 mmol) of surfactant (a combination of two separate preparations that had been polymerized via UV irradiation at 30 °C) was freeze-dried and the residue dispersed in 50 mL of absolute ethanol. After addition of 1 mL of concentrated HCl, the dispersion yielded a clear solution. The solution was then concentrated under reduced pressure, redissolved in 50 mL of ethanol, and reduced to dryness. This procedure was repeated 2 times and the residue then dried under high vacuum [25 °C, 12 h (0.05 mmHg)]. A minimum volume of ethanol (ca. 2 mL) was added to dissolve the residue, and *n*-hexane was then added in a dropwise fashion. During the addition of hexane, the mixture was gently shaken by hand, in order to precipitate poly(methacrylic acid). The crude yield of polymer was 39.8 mg (96%). Further purification of the polymer was carried out by repeating this precipitation procedure 3 times. The final product [33.2 mg (80%)] exhibited an IR spectrum which was identical with that of an authentic sample of poly(methacrylic acid). Using similar procedures, an aqueous dispersion containing 310 mg (0.49 mmol) of DODAM, which had been photopolymerized at 60 °C, afforded crude and purified yields of poly(methacrylic acid) of 33.4 mg (80%) and 23.5 mg (56%), respectively.

Attempted Polymerization of DODAM Using Radical Initiators in the Vesicle State, in Solution, and in the Bulk Phase. Addition of 5 mol % AIBN or ACVA to aqueous dispersions of DODAM (1 mg/mL), followed by brief sonication (2 min), heating (7 days, 60 °C), and freeze-drying afforded only starting monomer as indicated by its ^{1}H NMR spectrum in $CDCl_3$. Similarly, attempted polymerization of 644 mg of DODAM dissolved in 0.5 mL of absolute ethanol containing 8.2 mg (5 mol %) of AIBN, for 3 days at 60 °C, or of DODAM in the neat state (bulk polymerization), for 3 days at 75 °C, afforded only unreacted starting monomer.

Captured Volume and Permeability. Typically, a vesicle dispersion was prepared from 15 mg of DODAM in 2.25 mL of distilled water containing 15 μCi of sucrose by using procedures similar to those described above. After sonication, the dispersion was divided into three equal portions; one was photopolymerized at 30 °C and one at 60 °C. An aliquot of each portion (50 μL) was withdrawn and analyzed for nitrogen and sucrose content. Additional aliquots (0.5 mL) were then filtered through a 10 mm diameter column which was packed with water-swollen Sephadex G-50 (made from 2.0 g of dry Sephadex). Collected fractions (1.16 mL) were analyzed for radioactivity and nitrogen content. In all cases, a well-defined separation between free and vesicle-entrapped sucrose (void volume) was obtained. The ratio of entrapped sucrose/free sucrose (R) represents the counts per minute (cpm) in the void volume of the column divided by the total cpm added to the column. The apparent internal or captured volume (V) has been calculated by dividing R by the concentration of surfactant, [DODAM], in the vesicle state. The value of [DODAM] equals the moles of nitrogen/liter of the dispersion applied to the filtration column multiplied by the percentage of nitrogen recovered in the void volume. The extent of sucrose *adsorption* to preformed nonpolymerized and polymerized DODAM vesicles was determined by adding [^{14}C]sucrose to the appropriate vesicle dispersion, incubating the mixture for 0.5 h at room temperature, and then filtering the dispersion through a Sephadex G-50 column. The amount of adsorbed sucrose in the void volume was then expressed as an "adsorbed" captured volume (V_a). Specific V_a values obtained for nonpolymerized, 30°-polymerized, and 60°-polymerized vesicles are 0.22, 0.06, and 0.09 L/mol, respectively. The true captured volumes of these vesicles (V_{in}), defined as $V_{in} = V - V_a$, are reported in Table II. Dialysis experiments were carried out either at 30 or 60 °C, using procedures similar to those previously described.[13] Typically, 2.0 mL of a gel-filtered vesicle dispersion (immediately after filtration) was dialyzed against 200 mL of distilled water at the specified temperature.

Film-Balance Experiments. Force–area isotherms were recorded by using a computerized MGW Lauda film balance at 22 °C. Water that was used as a subphase was deionized and freshly distilled in an all-glass apparatus. Surfactants were spread onto the water surfaces (600 cm^2) from 9:1 hexane/ethanol solutions (ca. 0.2 mg/mL) by using a 10- or 50-μL glass syringe. Monolayers were compressed at a rate of 20 cm^2/min. Photopolymerization reactions were carried out by irradiating the monolayers with a single UV Rayonet lamp (254 nm) from a distance of 7 cm under an argon atmosphere.

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Registry No. DODAM, 7524-96-1; PDODAM, 89346-16-7; sucrose, 57-50-1.

Stoichiometry and Structural Effects in Alcohol Chemisorption/Temperature-Programmed Desorption on MoO_3†

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Abstract: The mechanism of alcohol oxidation over MoO_3 has been examined by using temperature-programmed desorption spectroscopy (TPD) with simultaneous microbalance and mass spectral detection. Two types of experiments are reported. The alcohol structure has been varied over the sequence methyl, ethyl, 2-propyl, *tert*-butyl, and systematic changes in the amount of chemisorption, the peak desorption temperature, and the nature of the products have been observed. The amount of water produced during chemisorption of ethyl alcohol on MoO_3 at room temperature has been measured. This determination makes it possible to estimate the coverage of alkoxy groups after the chemisorption stage. The fate of these ethoxy groups during subsequent TPD can be followed. A stoichiometric accounting of both acetaldehyde production and catalyst reduction can be made.

In a previous publication, we have described the use of temperature-programmed desorption spectroscopy (TPD) with simultaneous microbalance and mass spectral detection as a means of studying heterogeneous redox chemistry.[1] It was demonstrated that each of the essential elements of the overall process, adsorption of the substrate on the solid catalyst, chemical reaction of the adsorbate, and reoxidation of the catalyst, could be productively studied by using this instrumentation. In the particular case of methanol partial oxidation to formaldehyde over MoO_3, surface

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† In this paper the periodic group notation in parentheses is in accord with recent actions by IUPAC and ACS nomenclature committees. A and B notation is eliminated because of wide confusion. Groups IA and IIA become groups 1 and 2. The d-transition elements comprise groups 3 through 12, and the p-block elements comprise groups 13 through 18. (Note that the former Roman number designation is preserved in the last digit of the numbering: e.g., III → 3 and 13.)

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