Surface-Modified Porous Polymeric Membrane Using Vesicles

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If the surfaces of vesicles are chemically modified so that they can be dispersed in organic solvents, the application of vesicular colloids may be expanded. A polymerizable surfactant (BDAC) and nonpolymerizable bipolar surfactant (BPAS) were synthesized in multi-steps. Large vesicles composed of BDAC and BPAS with embedded a cross-linking agent (divinylbenzene) underwent a radical polymerization. BPAS was extracted out using methanol (skeletonization). The headgroup of BDAC was cleaved off via hydrolysis in an acidic condition to yield vesicles where surfaces were covered with -COOH groups. There was no significant change in the overall shape. The skeletonized vesicles appear to have many holes with diameters up to about 25 nm. The holes retained even after hydrolysis. The hydrolyzed vesicles were not dispersed in water and most organic solvents such as tetrahydrofuran and chloroform, but dispersed in methanol.

Key Words : Vesicle, Polymerizable surfactant, Skeletonization, Hydrolysis

Introduction

Lipid molecules can be spontaneously aggregated into spherically closed bilayers, *i.e.*, vesicles in water. These vesicular morphologies have found a multitude of applications in various scientific and applied fields in recent years. However, the stability of the vesicles is still a serious problem. One common approach for stabilizing vesicles is the polymerization of membrane components in the bilayers. The polymerizable groups in the lipid molecules have been incorporated in the head group or in the alkyl chain at different positions relative to the head group.[1,2]

A rather fascinating biomembrane process was observed in the death of a tumor cell that cannot escape from the immune system.[3] An activated macrophage attacked a tumor cell and drilled holes in the originally stable tumor cell membrane. As a result, the cytoplasmic interior leaked out, which caused the death of the tumor cell. In order to simulate the biological process, phase-separated vesicles were used initially by Rinsdorf and coworkers.[3] Tsuchida and coworkers also prepared skeletonized (drilled) hybrid liposomes using the phase separation which was induced upon polymerization of liposomes composed of polymerizable and non-polymerizable surfactants.[4]

The surfaces of reported drilled or skeletonized vesicles are hydrophilic. If the hydrophilic surfaces are chemically modified to be reasonably compatible with organic solvents, the application of vesicular colloids can be expanded since the porous particles may be dispersed in common organic media.

Recently, we reported vesicles composed of a polymerizable surfactant, a non-polymerizable lipid, and a cross-linking agent, which was N,N-bis[10-(4-vinylbenzyloxy)decanoyl-aminoethyl]-N,N-dimethylammonium chloride (BDAC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and divinylbenzene (DVB), respectively.[5] When the small unilamellar vesicles of BDAC and DPPC with embedded DVB were polymerized at 60 °C, most of the vesicles retained their initial spherical shapes, but a so-called parachute-like morphology was also occasionally observed. The parachute-like structure probably resulted from the polymerization of DVB proceeded in the bilayer in a latex-like fashion due to the phase separation between the DVB polymer and polymerized BDAC bilayer. When the polymerization was performed again with large multilamellar vesicles and at a lower temperature of 55 °C, the parachute-like morphology could not be observed.[6] However, the holes in the large multilamellar vesicle membranes, which were formed by the removal of DPPC from the polymerized membrane matrix, were too small to be studied in detail.

In this study, a 4,4'-biphenol-based tetraethylammonium salt (BPAS) was used instead of DPPC as a non-polymerizable component, as shown in Figure 1. The 4,4'-biphenol moiety of BPAS was expected to prevent the amphiphile from being vent (U-shaped) in the vesicle membranes because it is a rigid segment.[7] The reason for using the bipolar surfactant was that it can span the vesicle membranes, leading to the formation of holes which can also span the cross-linked membrane matrix on the removal of it. This paper describes the synthesis of BDAC and BPAS and some results on the surface modification of the vesicles prepared from the two different surfactants.

Experimental Section

10-(4-Vinylbenzyloxy)decan-1-ol (1-1). A solution of

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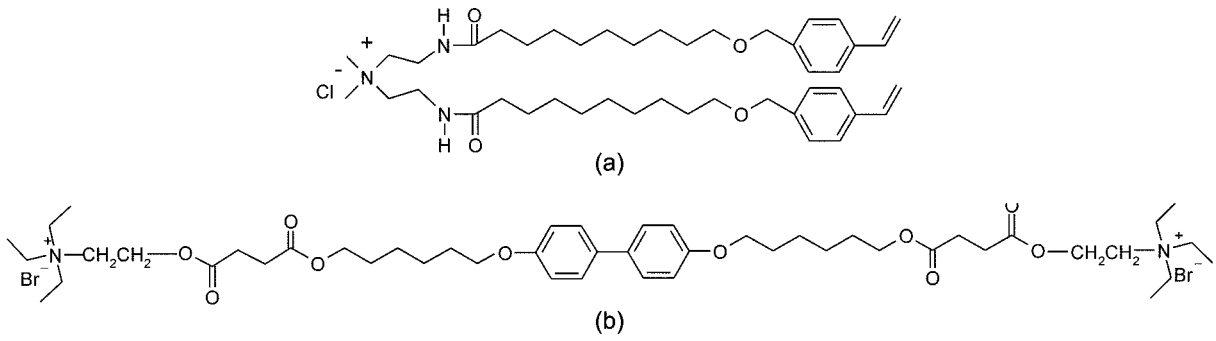


Figure 1. Chemical structures of (a) BDAC and (b) BPAS.

1,10-decanediol (2.28 g, 13.1 mmol) and 4-vinylbenzyl chloride (2.0 g, 13.1 mmol) in DMF (20 mL) was refluxed for 24 h at 150 °C. The reaction mixture was poured into a separatory funnel containing 100 mL of water. The resulting mixture was extracted with diethyl ether (5 × 50 mL). The ether phase was washed with 50 mL of water (5 × 50 mL), dried over sodium sulfate, concentrated under a reduced pressure, and finally separated by flash column chromatography (hexane/ethyl acetate = 8/2. R_f = 0.3) to obtain 1.5 g of compound **1-1** as a solid (50%). IR (KBr, cm^{-1}) 3407, 1100. ^{1}H-NMR ($CDCl_3$) δ 1.23-1.90 (m, 16H, $(CH_2)_8$), 3.60 (t, 4H, CH_2O), 4.35 (s, 2H, $ArCH_2O$), 5.40 (d, 2H, CH_2=CH), 5.90 (d, 2H, CH_2=CH), 6.75 (dd, 1H, CH_2=CH-Ar), 7.45-7.60 (m, 4H, Ar).

10-(4-Vinylbenzyloxy)decanoic acid (1-2). Pyridinium dichromate (5.18 g, 14 mmol) was dissolved in 10 mL of DMF and cooled in an ice bath, followed by the addition of compound **1-1** (1.0 g, 3.5 mmol) in 2 mL of DMF. The resulting dark brown mixture was stirred for 16 h at room temperature, and poured into a separatory funnel containing 100 mL of 5% HCl aqueous solution. The mixture was extracted with diethyl ether (5 × 25 mL). The ether phase was washed with water (5 × 25 mL), dried over sodium sulfate, concentrated under a reduced pressure, and separated by flash column chromatography (hexane/ethyl acetate = 7/3, R_f = 0.3) to obtain 0.7 g of compound **1-2** as a solid (70%). IR (KBr, cm^{-1}) 3400-2400, 1710, 1100. ^{1}H-NMR ($CDCl_3$) δ 1.23-1.90 (m, 16H, (CH_2)-), 2.30 (t, 2H, CH_2CO_2), 3.60 (t, 2H, CH_2O), 4.35 (s, 2H, $ArCH_2O$), 5.40 (d, 2H, CH_2=CH), 5.90 (d, 2H, CH_2=CH), 6.75 (dd, 1H, CH_2=CH-Ar), 7.45-7.60 (m, 4H, Ar).

Methyl-bis[2-{10-(4-vinylbenzyloxy)decanoylamino}-ethyl]amine (1-3). A solution of compound **1-2** (1.0 g, 3.3 mmol), N-methyl-2,2'-diaminodiethylamine (0.20 g, 1.7 mmol), dicyclohexyldiimide (DCC) (0.80 g, 3.9 mmol), and N,N-dimethylaminopyridine (DMAP) (0.04 g, 0.33 mmol) in 10 mL of chloroform was stirred for 2 h at room temperature. Dicyclohexylurea was filtered off. The reaction mixture was concentrated under a reduced pressure and separated by flash column chromatography (CH_2Cl_2/MeOH = 20/1. R_f = 0.2) to obtain 0.80 g of compound **1-3** (73%). IR (KBr, cm^{-1}) 1640, 1100. ^{1}H-NMR ($CDCl_3$) δ 1.23-1.90 (m, 28H, $(CH_2)_7$), 2.30-2.50 (m, 11H, CH_3N, CH_2N, CH_2CO_2), 3.45-3.50 (m, 8H, CH_2NCO, CH_2O), 4.35 (s, 4H, $ArCH_2O$), 5.40 (d, 2H, CH_2=CH), 5.90 (d, 2H, CH_2=CH), 6.75 (dd, 1H, CH_2=CH-Ar), 7.45-7.60 (m, 4H, Ar).

BDAC. A solution of compound **1-3** (1.0 g, 1.4 mmol) and iodomethane (1.0 g, 7 mmol) in acetone was stirred at 20 °C for 4 h. Unreacted methyl iodide was removed under a reduced pressure. The resulting solution was crystallized from hexane to obtain the tetraalkylammonium iodide which was then dissolved in methanol and passed three times through a column packed with Amberlite IRA-400 (Cl), an ion-exchange resin. Methanol was removed under a reduced pressure to obtain 0.90 g of BDAC as a solid (86%). IR (KBr, cm^{-1}) 1640, 1100. ^{1}H-NMR ($CDCl_3$) δ 1.23-1.90 (m, 28H, (CH_2)-), 2.30-2.50 (t, 4H, CH_2CO_2), 3.28 (s, 6H, $(CH_3)N$), 3.43 (t, 4H, CH_2O), 3.74 (m, 8H, NCH_2CH_2N), 4.35 (s, 4H, $ArCH_2O$), 5.40 (d, 2H, CH_2=CH), 5.90 (d, 2H, CH_2=CH), 6.75 (dd, 1H, CH_2=CH-Ar), 7.45-7.60 (m, 4H, Ar). Elemental analysis (%); Calcd for $C_{41}H_{74}O_4NCl$: C 82.3; N 6.6; H 11.1. Found: C 81.8; N 6.5; H 11.7.

4,4'-Bis(6-hydroxyhexyloxy)biphenyl (2-1). Potassium carbonate (3.0 g, 20 mmol) was added to a solution of 4,4-Biphenol (2.0 g, 1.07 mmol) and 6-chloro-hexan-1-ol (4.39 g, 3.2 mmol) in DMF and refluxed for 19 h at 120 °C under a nitrogen atmosphere. The mixture was filtered to remove potassium carbonate and the solvent was removed under a reduced pressure. The residue was dissolved in benzene and cooled to -30 °C, and crystallized to obtain 3.34 g of compound **2-1** as a white solid (81%). IR (KBr, cm^{-1}) 3300, 1100. ^{1}H-NMR ($CDCl_3$) : δ 1.38 (m, 8H, $CH_2CH_2CH_2CH_2O$), 1.57 (m, 4H, CH_2CH_2O), 1.71 (m, 4H, $ArOCH_2CH_2$), 3.50 (t, 4H, $ArOCH_2$), 3.98 (t, 4H, CH_2O), 6.9 (d, 4H, Ar), 7.49 (d, 4H, Ar).

4,4'-Bis(6-hemisuccinyloxyhexyloxy)biphenyl (2-2). Compound **2-1** (1.5 g, 0.38 mmol) and succinic anhydride (1.56 g, 1.55 mmol) were dissolved in toluene and refluxed for 3 h at 110 °C under a nitrogen atmosphere. The mixture was precipitated at room temperature and filtered. The precipitate was dissolved in THF, followed by the addition of ethyl acetate. The resulting solution was cooled to -10 °C to remove unreacted succinic anhydride which is soluble in ethyl acetate. The precipitate was isolated by vacuum filteration, and dried to yield 2.03 g of compound **2-2** as a white solid (90%). IR (KBr, cm^{-1}) 3400-2400, 1730, 1100. ^{1}H-NMR ($CDCl_3$) : δ 1.38 (m, 8H, $CH_2CH_2CH_2CH_2O$), 1.57

(m, 4H, $\underline{CH_2}CH_2O$), 1.71 (m, 4H, $ArOCH_2\underline{CH_2}$), 2.56 (t, 8H, $\underline{CH_2}CH_2CO_2$), 3.50 (t, 4H, $ArO\underline{CH_2}$), 3.98 (t, 4H, $\underline{CH_2}O$), 6.9 (d, 4H, Ar), 7.49 (d, 4H, Ar).

4,4'-Bis[6-{2-(N,N-diethylamino)ethyl}oxysuccinyloxyhexyloxy]biphenyl (2-3). Potassium carbonate (3.0 g, 20 mmol) was added to a solution of compound **2-2** (1.0 g, 0.17 mmol) and 2-diethylaminoethyl chloride hydrochloride (0.62 g, 0.36 mmol) in DMF and refluxed at 80 °C for 5 h under a nitrogen atmosphere. The mixture was filtered to remove potassium carbonate and evaporated to remove DMF under a reduced pressure. The residue was dissolved in benzene and washed twice with water. The benzene layer was dried over magnesium sulfate and evaporated to obtain 0.97 g of compound **2-3** as a colorless liquid (80%). IR (KBr, cm^{-1}) 1730, 1240, 1160. ^{1}H-NMR (DMSO-d_6) : δ 1.16 (t, 12H, $\underline{CH_3}CH_2N$), 1.38 (m, 8H, $\underline{CH_2}CH_2CH_2CH_2O$), 1.57 (m, 4H, $\underline{CH_2}CH_2O$), 1.71 (m, 4H, $ArOCH_2\underline{CH_2}$), 2.48 (q, 12H, $CH_3\underline{CH_2}N$), 2.56 (t, 4H, $\underline{CH_2}CH_2N$), 2.56 (t, 8H, $\underline{CH_2}CH_2CO_2$), 3.50 (t, 4H, $ArO\underline{CH_2}$), 3.98 (t, 4H, $\underline{CH_2}O$), 4.38 (t, 4H, $CH_2\underline{CH_2}N$), 6.9 (d, 4H, Ar), 7.49 (d, 4H, Ar).

BPAS. Compound **2-3** (0.97 g, 0.135 mmol) and bromoethane (2 mL) were dissolved in THF and refluxed at 70 °C for 24 h. The solvent and unreacted bromoethane were removed under a reduced pressure to obtain 1.07 g of BPAS as a white solid (85%). IR (KBr, cm^{-1}) 1730, 1240, 1160. ^{1}H-NMR (DMSO-d_6) : δ 1.16 (t, 18H, $\underline{CH_3}CH_2N$), 1.38 (m, 8H, $\underline{CH_2}CH_2CH_2CH_2O$), 1.57 (m, 4H, $\underline{CH_2}CH_2O$), 1.71 (m, 4H, $ArOCH_2\underline{CH_2}$), 2.48 (q, 12H, $CH_3\underline{CH_2}N$), 2.56 (t, 4H, $\underline{CH_2}CH_2N$), 2.56 (t, 8H, $\underline{CH_2}CH_2CO_2$), 3.50 (t, 4H, $ArO\underline{CH_2}$), 3.98 (t, 4H, $\underline{CH_2}O$), 4.38 (t, 4H, $CH_2\underline{CH_2}N$), 6.9 (d, 4H, Ar), 7.49 (d, 4H, Ar). Elemental analysis (%); Calcd for $C_{48}H_{78}O_{10}N_2Br_2H_2O$: C 56.5; H 7.9; N 2.7. Found: C 56.3; H 8.2; N 2.5.

Preparation of vesicles. BDAC and BPAS in a 3 : 1 molar ratio were dissolved in chloroform/methanol (3/1), followed by the addition of a 2,2'-azobisisobutyrnitrile (AIBN) solution (10 mol%) in chloroform. The resulting solution was slowly evaporated to obtain a thin film of the surfactant mixture on the inner wall of the vial, and dried further under a high vacuum for at least 1 h. The dried film was then hydrated with the repeated freeze-thaw cycles (-70 °C~50 °C).

Dynamic Light Scattering (DLS). The vesicle size was estimated with DLS measurements. All measurements (10 mg surfactant/2 mL water) were performed at 25 °C and 90 °C using a BI 8000-AT (Brookhaven Instruments Corp.). The mean diameter of vesicles was calculated by multiple mathematical procedures.

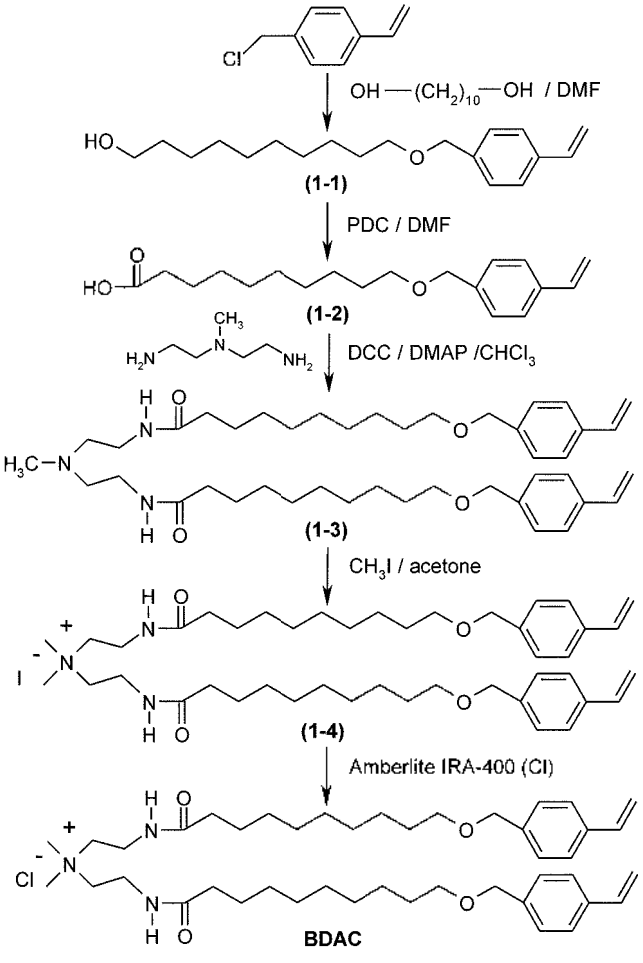
Polymerization of vesicles. Vesicles composed of BDAC/BPAS (3/1) containing AIBN were prepared as described above (BDAC/AIBN = 5/2). DVB was injected into the vesicle dispersion using a microliter syringe, and the resulting mixture was stirred for 2 days at room temperature to ensure a complete incorporation of the cross-linking agent into the lipid bilayers (BDAC/DVB = 1/1). The dispersion was stirred at room temperature for 48 h, and then stirred at 50 °C for at least 24 h. Nitrogen was bubbled through the solution to eliminate oxygen in the samples.

Skeletonization and hydrolysis of polymerized vesicles. The polymerized vesicle suspensions were dried, and suspended in 10 mL of methanol in order to remove BPAS. The resulting mixtures were centrifugated at 13000 rpm, and decanted to remove the supernatant. The same procedure was repeated twice. A concentrated HCl aqueous solution was added to the skeletonized vesicle suspension until the final HCl concentration attained was 12 wt%. The resulting suspension was then stirred at 50 °C for 48 h, followed by centrifugation. The supernatant was decanted off, and the precipitate was resuspended with water, and then centrifugated. This overall procedure was repeated thrice in order to remove any byproducts formed during the hydrolysis.

Transmission Electron Microscopy (TEM). The samples were stained with 2 wt% phosphotungstic acid (pH 6.8 with 1 N KOH) for 1 h on a parafilm (vesicle sample/phosphotungstic acid = 1 : 1, v/v), covered formvar/carbon grid for 1 min. The residual sample was removed from the grid with filter paper. The samples were washed with water and dried in vaccum. TEM images were obtained using a transmission electron microscope (EM10CR, Carlzeiss Co.) at 60.0 kV.

Results and Discussion

Synthesis. BDAC was synthesized in five consecutive



Scheme 1. Synthetic route to BDAC.

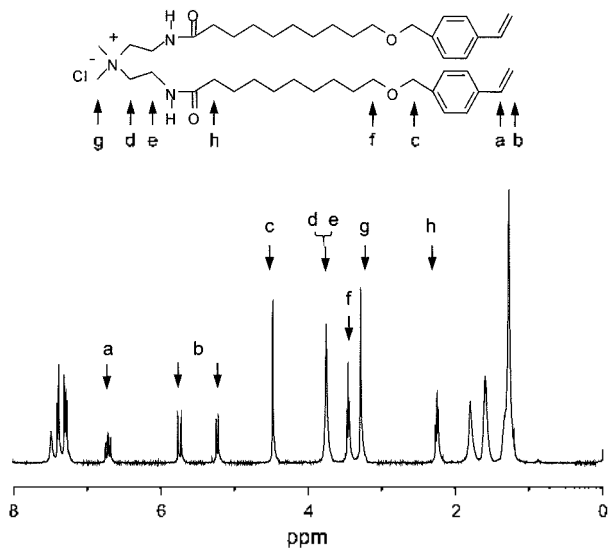
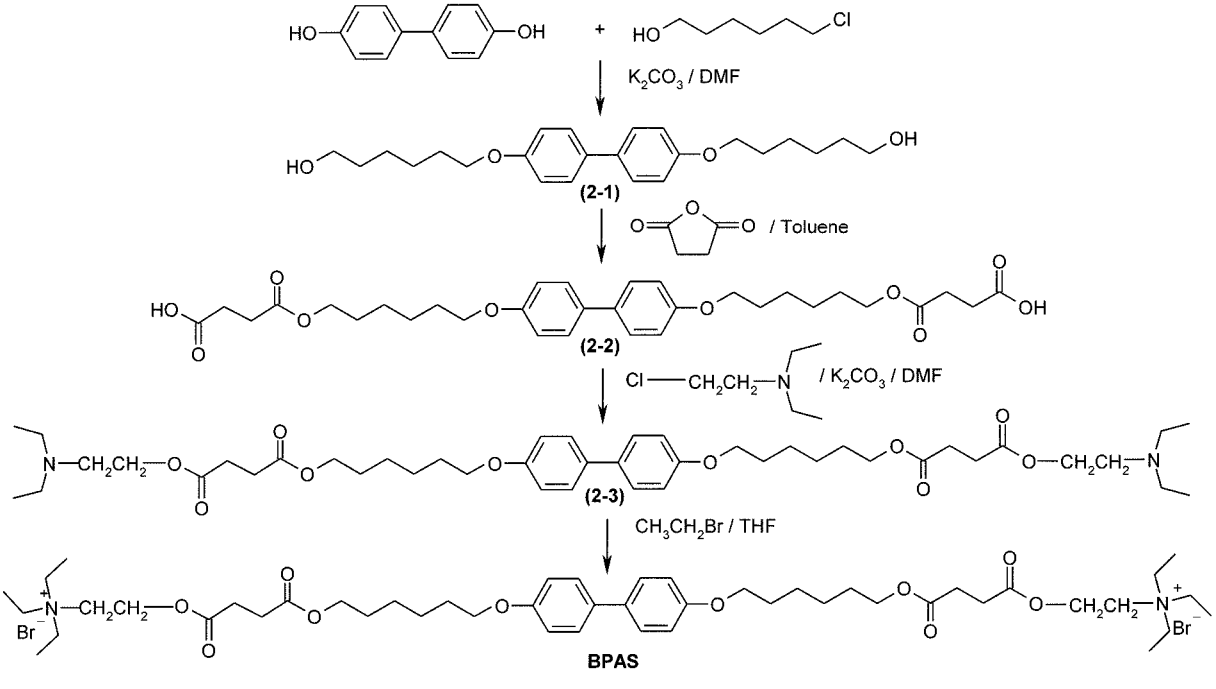


Figure 2. ^{1}H-NMR ($CDCl_3$) spectrum of BDAC.

steps, as shown in scheme 1. 4-Vinylbenzyl chloride was reacted with 1,10-decanediol to yield compound **1-1** which was oxidized to give compound **1-2**. The carboxylic acid was then amidized with N-methyl-2,2`-diaminodiethylamine in the presence of DCC to yield compound **1-3**. The tertiary amine was quaternarized with excess iodomethane. The resulting tetraalkylammonium iodide (**1-4**) was ion-exchanged using an ion exchange resin, Amberlite IRA-400(Cl), to obtain BDAC as a white solid. Even though the synthesis consisted of five steps from 4-vinylbenzyl chloride and 1,10-decanediol, most of the reactions were efficient and the overall yield was about 20%.

The FT-IR spectrum of BDAC showed strong absorption peaks at 3300 and 1640 cm^{-1}, which correspond to the N-H and carbonyl group of the amide bonds, respectively. The proton NMR spectrum of BDAC was interpreted, as shown in Figure 2. The most characteristic resonance peaks are those of vinyl protons at 5.40-6.75 ppm, methylene protons next to carbonyl at 3.28 ppm, and dimethyl protons at 3.43 ppm. Thus, the FT-IR and NMR spectra confirmed that the isolated compound had the expected chemical structure of BDAC. No impurity peak was detected in the NMR spectrum. The elemental analysis data along with the NMR spectrum confirmed that the isolated compound was pure enough for the next experiments.

BPAS, a nonpolymerizable bipolar surfactant, was synthesized by the reactions shown in Scheme 2. The synthesis was accomplished in four different steps. 4,4-Biphenol was reacted with 6-chlorohexan-1-ol in DMF to yield compound **2-1** which was coupled with succinic anhydride in toluene to obtain compound **2-2**. The dioic acid was coupled further with N,N-diethylaminoethyl chloride hydrochloride in DMF to yield compound **2-3** which was reacted with bromoethane in THF to yield compound **2**. The overall yield was about 50%. The FT-IR spectrum of BPAS showed a peak at 1730 cm^{-1}, which corresponds to the ester carbonyl group. The proton NMR spectrum of BPAS is shown in Figure 3. The most characteristic resonance peaks are those for methylene protons next to ester carbonyl group (triplet at 2.56 ppm) and another methylene protons on carbons linked to the ether oxygen atom (triplet at 3.50 ppm). Thus, the FT-IR and NMR spectra confirmed that the isolated compound had the expected chemical structure of BPAS. The elemental analysis data suggests that the isolated each molecule was hydrated with one molecule of water, which is not uncommon for hygroscopic salts.



Scheme 2. Synthetic route to BPAS.

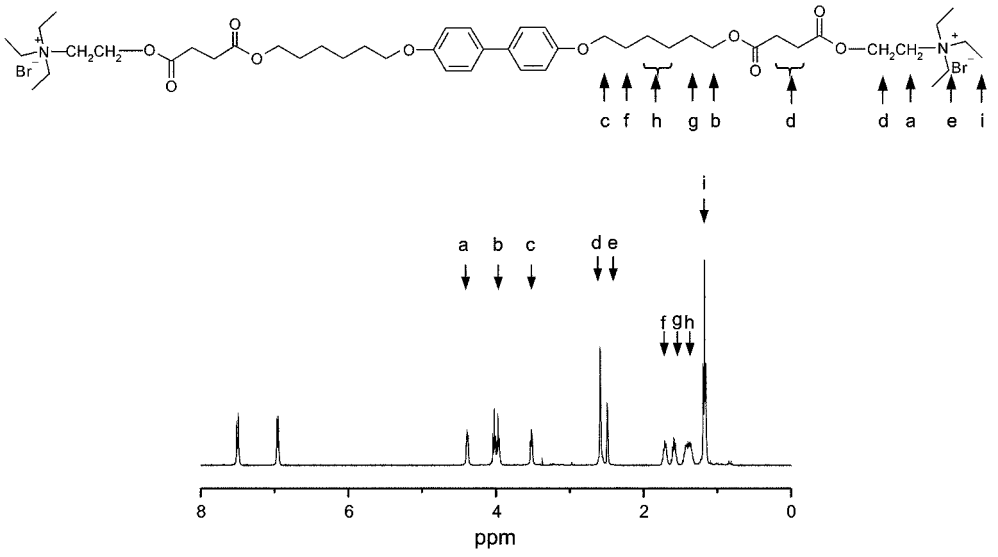


Figure 3. ^{1}H-NMR (DMSO-d_6) spectrum of BPAS.

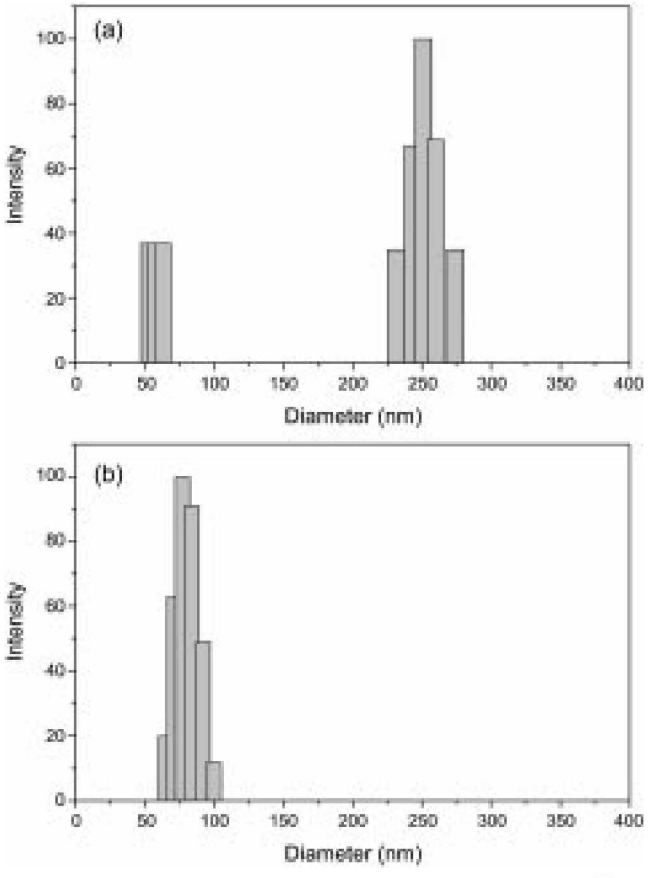


Figure 4. Size distributions of vesicles in water prepared from BPAS (a) before and (b) after ultrasonication.

General properties. BDAC did not form vesicles by itself. A surfactant of ammonium salt whose chemical structure is very similar to that of BDAC was also reported not to form vesicles.[8] Probably, the bulky phenyl group at each hydrophobic chain terminus may prevent the molecule from assembling in a bilayer. On the other hand, even before ultrasonication, the hydrated BPAS did form vesicles whose size distribution was bimodal with mean diameters of about 60 nm and 250 nm, respectively, as shown in Figure 4. However, after ultrasonication, the distribution became unimodal with mean diameter of about 80 nm. Some bipolar surfactants were reported to also form vesicles by themselves on hydration, but some others needed a second component such as cholesterol to form vesicles.[9-11]

Mixtures of BDAC and BPAS in proper molar ratios formed large vesicles with mean diameter of about 500 nm on hydration. Vesicles prepared from BDAC and BPAS in a 3 : 1 molar ratio exhibited a unimodal distribution with mean diameter of 130 nm on ultrasonication. The small vesicles prepared from BDAC and BPAS were stable for weeks since there was no precipitate formed and its transparency was not changed during the storage of the dispersions at room temperature.

Differential scanning calorimetry (DSC) experiments were performed in order to know the chain-melting temperature (T_m) of respective surfactant bilayer above where polymerization reaction usually occurs (2910 TA Instrument). Any phase transition was not observed either from BDAC or BPAS dispersions in the temperature range from 0 °C to 90 °C, as shown in Figure 5. The absence of melting transition for the BDAC dispersions is understandable because BDAC did not form vesicle membranes by itself, as mentioned above. On the other hand, vesicles prepared from DPPC and BPAS underwent a melting transition at 48.8 °C. The transition corresponds to the melting transition of DPPC bilayers since pure DPPC bilayers underwent the transition at about 43 °C according to our experiment.[6] A slight higher T_m of DPPC domains may be due to closer packing of DPPC molecules in the presence of BPAS. The DSC curve does not show any other melting transition, indicating that BPAS domians do not undergo a melting transition in the temperature range studied. The absence of the melting transition of BPAS domains suggests that the bipolar membranes may be in a

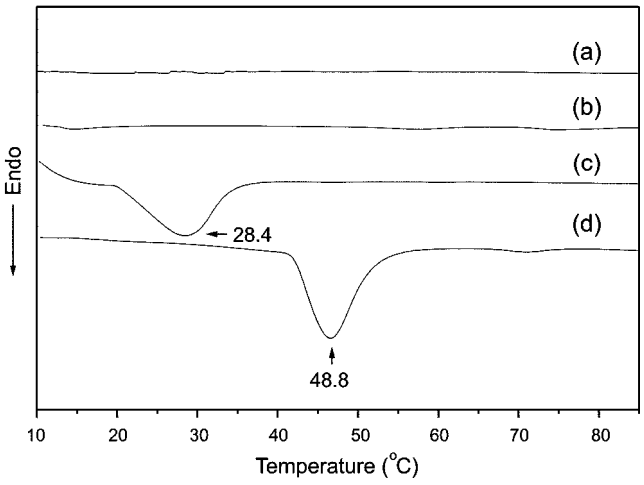


Figure 5. DSC thermograms of (a) BDAC, (b) BPAS, (c) BDAC/BPAS (3/1), and (d) BPAS/DPPC (1/1) dispersions.

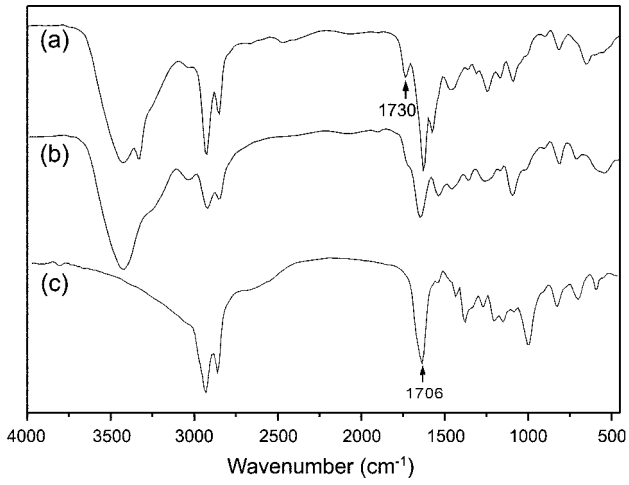


Figure 7. FT-IR spectra of large vesicles after (a) polymerization, (b) skeletonization, and (c) hydrolysis.

gel phase in the temperature range due to the rigid biphenyl segment and relatively long chain length.

However, vesicles prepared from BDAC and BPAS in a 3 : 1 molar ratio exhibited a melting transition with T_m 28.4 °C. Based on the experiment with vesicles prepared from DPPC and BPAS, the BPAS membrane does not undergo melting transition in the temperature range. Thus this DSC result suggests that BDAC molecules in the mixed membranes undergo melting transition and change into a liquid-like state above 28.4 °C, indicating that polymerization of the membranes can be easily performed using a radical initiator such as AIBN.

Polymerization, skeletonization, and hydrolysis of vesicles. Large vesicle (~500 nm) dispersions prepared from BDAC, BPAS, and DVB in a 3 : 1 : 1 molar ratio were polymerized at 50 °C in the presence of AIBN (BDAC/AIBN = 5/2) under bubbling of nitrogen gas for 24 h. The proton NMR spectrum of the polymerized sample dried in a vacuum oven revealed that the vinyl protons of the polymerizable groups in BDAC and DVB had disappeared, as shown in Figure 6. This result indicates that BDAC and DVB were almost completely reacted.

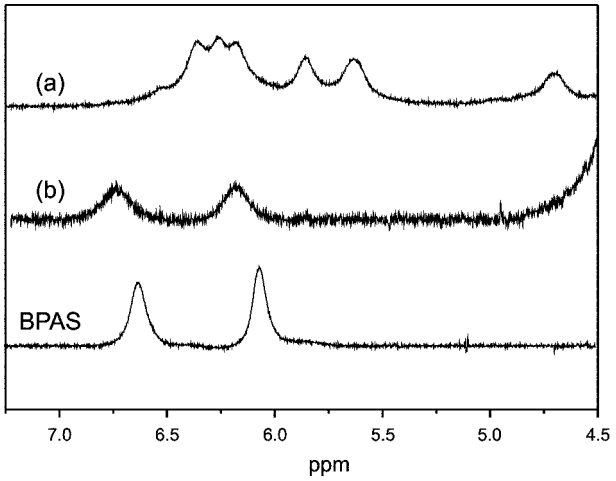


Figure 6. ^{1}H-NMR spectra of sonicated vesicles prepared from BDAC/BPAS embedded with DVB (a) before polymerization and (b) after polymerization and BPAS (conc. 20 mg/mL of D_2O).

The polymerized vesicle suspensions were dried and washed with methanol in order to remove BPAS from the vesicle membranes since BPAS is well soluble in methanol. The FT-IR spectrum of the resulting dried vesicles revealed that BPAS molecules were removed since characteristic FT-IR peaks for ester carbonyl groups of BPAS disappeared, as shown in Figure 7b. This result suggests that the polymerized vesicles might be skeletonized by the loss of BPAS if any significant size of BPAS domains had been induced by the radical polymerization.

A concentrated HCl solution was added to the skeletonized vesicle suspension until the final concentration of HCl in the dispersion became 12 wt%. The resulting dispersion was stirred at 50 °C for 48 h, and centrifugated at 13000 rpm for 5 min, and then finally a white precipitate was obtained. The precipitate was purified further by repeating the centrifugation procedure. Figure 7c is a FT-IR spectrum of the purified precipitate. The absorption peaks of amide group at about 3400 and 1654 cm^{-1} disappeared and those of carboxyl group at about 3500-2400 cm^{-1} and 1706 cm^{-1} emerged quite clearly. This result indicates that the quaternary ammonium head groups of the polymerized BDAC units were completely removed via hydrolysis.

Transmission electron microscopy (TEM) experiments were performed in order to visualize the vesicles. TEM micrographs of the vesicles are shown in Figure 8. The approximate spherical vesicle shape retained even after skeletonization and hydrolysis. The skeletonized vesicles appear to have many holes with diameters up to about 25 nm. The holes retained even after hydrolysis. This result indicates that most vesicles were sufficiently stabilized by the cross-linking process during polymerization. It seems to be that small vesicular particles are coexisting with regular vesicles after hydrolysis to some more extent. The formation of the additional small assemblies or aggregates may be due to cleavage of some polymer main chains in uncross-linked vesicles during hydrolysis under the such strong acidic

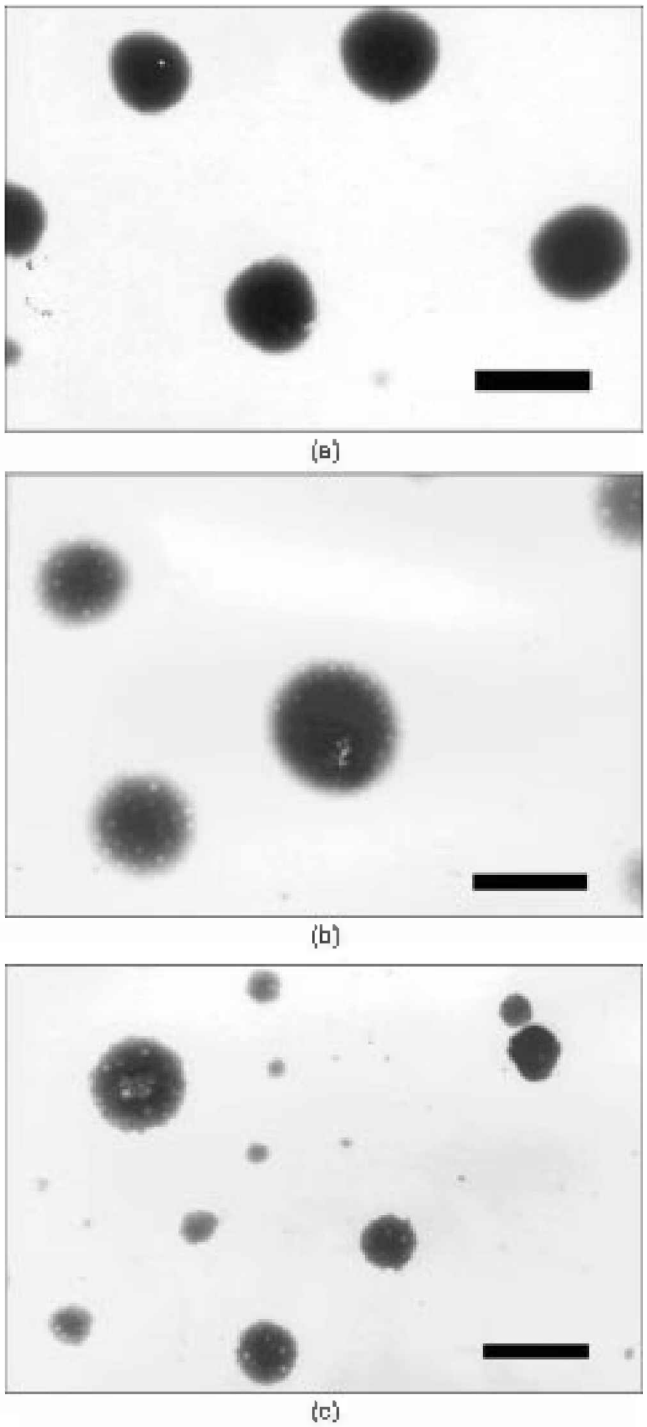


Figure 8. TEM micrographs of large vesicles after (a) polymerization, (b) skeletonization, and (c) hydrolysis. The bar indicates 500 nm.

conditions.

The hydrolyzed vesicles were not dispersed in water any longer, but precipitated. This result indicates that the polarity of the hydrolyzed vesicle surfaces was significantly reduced and their compatibility with water was greatly reduced accordingly. The hydrolyzed vesicles were attempted to be dispersed in organic solvents such as THF and chloroform, but they did not disperse. However, a very stable milky suspension of the hydrolyzed vesicles formed in methanol. This result indicates that the hydrolyzed vesicle surfaces are much less polar than the initial ones, but still too polar to be dispersed in such common organic solvents.

Conclusion

BDAC and BPAS were successfully synthesized in multi-steps. BDAC did not form vesicles by itself while BPAS did form vesicles. Large vesicles composed of BDAC and BPAS with embedded DVB underwent radical polymerization. BPAS was removed from the cross-linked vesicles using methanol. The headgroup of BDAC was removed *via* hydrolysis in the acidic condition. According to TEM micrographs, the overall spherical shape retained after hydrolysis even though much smaller particles emerged to some more extent. Many holes with diameters up to 25 nm were observed from the skeletonized and hydrolyzed vesicles, and they retained even after hydrolysis. The hydrolyzed vesicles did not disperse in water, but dispersed in methanol. They were not dispersed either in organic solvents such as THF and chloroform. The hydrolyzed vesicle surfaces will be further modified so that the resulting porous vesicles can be dispersed in common organic solvents and the result will be reported in near future.

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