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Thermodynamic and Physical Interactions between Novel Polymeric Surfactants and Lipids: Toward Designing Stable Polymer–Lipid Complexes

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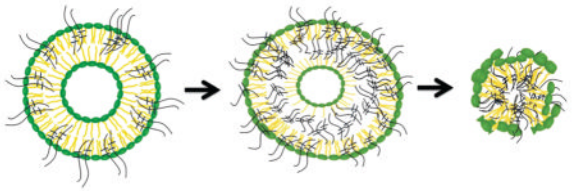
Abstract



Surfactant amphiphilic macromolecules (AMs) were complexed with a 1:1 ratio of 1,2-dioleoyl-3-trimethyl-ammonium-propane (DOTAP) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), either by a coevaporation (CE) or postaddition (PA) method, to form AM–lipid complexes with enhanced drug delivery applications. By characterizing the surfactant–lipid interactions, these heterogeneous drug delivery systems can be better controlled and engineered for optimal therapeutic outcomes. In this study, the physical interactions between DOPE:DOTAP liposomes and AM surfactants were investigated. Langmuir fllm balance and isothermal calorimetry studies showed cooperative intermolecular interactions between pure lipids and AM in monolayers and high thermostability of structure formed by the addition of AM micelles to DOTAP: DOPE vesicles in buffer solution respectively. Increasing the AM weight ratio in the complexes via the CE method led to complete vesicle solubilization—from lamellar aggregates, to a mixture of coexisting vesicles and micelles, to mixed micelles. Isothermal calorimetry evaluation of AM-lipid complexes shows that, at higher AM weight ratios, PA-produced complexes exhibit greater stability than complexes at lower AM weight ratios. Similar studies show that AM-lipid complexes produced by the CE methods display stronger interactions between AM-lipid components than complexes produced by the PA method. The results suggest that the PA method produces vesicles with AM molecules associated with its outer leaflet only (i.e., an AM-coated vesicle), while the CE method produces complexes ranging from mixed vesicles to mixed micelle in which the AM-lipid components are more intimately associated. These results will be helpful in the design of AM-lipid complexes as structurally defined, stable, and effective drug delivery systems.

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INTRODUCTION

The interaction of lipid membranes with surfactants is an extensively studied phenomenon.[1–6] These interactions have particular importance in biological research including biological membrane characterization,[1,5] regulation of cellular processes, and the activity and delivery of drugs.[5] Nanoscale unilamellar liposomes, composed of spherical lipid bilayer membranes, have been used as delivery systems for insoluble therapeutic agents.[7,8] Researchers have combined surfactants with drug-loaded liposomes to improve their therapeutic efficacy by increasing their structural and steric stability.[9–14] By characterizing the surfactant–lipid interactions, these heterogeneous drug delivery systems can be better controlled and engineered for optimal therapeutic outcomes.

Interaction of surfactants with liposomes can lead to the solubilization of the liposomes from a lipid bilayer vesicle to a mixed micelle (Figure 1). Solubilization is achieved by surfactant molecules partitioning into the lipid bilayer structure. The onset of solubilization, R^{sat}, occurs with saturation of the lipid bilayer structure and correlates with a morphological change from a lamellar structure to an intermediate structure. When a critical surfactant concentration in the lipid bilayer is reached (R^{sol}), complete solubilization is achieved. Complete solubilization correlates with a morphological change from an intermediate structure to a mixed micelle. This solubilization phenomenon occurs whether a surfactant solution is added to vesicle solution or the two components are combined in a common organic solvent.[2]

Distinct physicochemical and thermodynamic characteristics are associated with the interactions of lipid bilayers with surfactants. These lipid–surfactant interactions can be evaluated by the Langmuir film balance (LFB) technique, which determines if the surfactant and lipids are ideally mixing.[15] Similarly, the partitioning of a surfactant into a liposome bilayer is accompanied by thermodynamic changes that can be monitored by isothermal titration calorimetry (ITC).[16–18] Relevant thermodynamic parameters, including partitioning enthalpy, equilibrium constants, and stoichiometric coefficients, can be obtained by ITC analysis.[17] Another assessment of surfactant–lipid interactions is changes in size and turbidity. While both comprise amphiphiles, liposomes and surfactant micelles are distinctly different in structure, size, and turbidity.[2,4] In buffer, liposomes are turbid suspensions of spherical lipid bilayers with an aqueous core approximately 100 nm in diameter or greater. By comparison, micelles are transparent solutions of core–corona structures approximately 10 nm in diameter. When surfactants solubilize liposomes, the transition between lamellar, intermediate, and micellar aggregates are associated with decreases in size and turbidity. Therefore, by measuring surfactant–lipid aggregate size by dynamic light scattering (DLS) and turbidity by spectroscopy, insight can be gained to determine the structural state of the aggregates and extent of solubilization.[2,4]

Amphiphilic macromolecules (AMs) are novel, non-cytotoxic polymeric surfactants that self-assemble at low critical micelle concentrations (CMCs) to yield nanoscale micellar aggregates.[19–26] AMs are similar to conventional polymeric micelles, existing as nanoparticle aggregates with inherent thermodynamic instability.[27] However, unlike conventional polymeric micelles, they have high solution stability[25] and form significantly smaller micelles that are 10–20 nm in diameter.[26] As shown in Figure 2 below, AMs comprise a poly(ethylene glycol) (PEG) hydrophilic domain linked to a hydrophobic domain that consists of a mucic acid backbone ester-linked to four 12-carbon acyl chains.[26] The polymers have a molecular weight of 5.9 kDa, a melting point (T_m) of 56.4 °C, and a hydrophilic/lipophilic balance of 16.8.[26] In water, these AMs have a CMC of 1.25×10^{-7} M,[26] which is much lower than the CMC of most commercially available surfactants usually in the micromolar range.[25] Above this CMC, the unimers form micelles 10–20 nm in

diameter.[26] The free energy of micellization ($\Delta G°_M$) was −39.4 kJ/mol, much higher than that of most small molecule surfactants.[25]

In this work, AMs were complexed with a 1:1 ratio of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) and 1,2-dio-leoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) to form AM-lipid complexes for drug delivery applications.[28] The AM-lipid complexes exhibited a tunable zeta potential, steric stability, inherently low cytotoxicity, and ability to load and deliver insoluble chemotherapeutic agents.[28] *In vitro,* AM-lipid complexes exhibited a preferential uptake in a BT-20 carcinoma cell line over a normal cell line, demonstrating a passive targeting approach to deliver anticancer therapeutics.[28] The complexes were produced by the addition of preformed AM micelles in aqueous solution to preformed small unilamellar vesicles in suspension. Using this postaddition (PA) method, the complex size gradually increased from 100 nm at low AM-lipid weight ratios (1:10) to 180 nm at high AM-lipid weight ratios (10:1). On the basis of these results, AM-lipid complexes structurally existed as AM-coated liposomes.[28]

When surfactant and lipids are mixed in an organic solvent, the solvent removed by evaporation, and the heterogeneous fllm hydrated, the hydrophobic domains of the surfactants are localized and embedded into the lipid hydrocarbon chain bilayer during vesicle formation.[2] Surfactant–vesicle complexes produced by this coevaporation (CE) method were closer to equilibrium than other methods of assembly and may increase the stability of the complex when used as a drug delivery system.[2]

The goal of this study was to evaluate the physical interactions between DOPE:DOTAP liposomes and AM surfactants. Specifically, we measured the interactions of AMs with pure lipid monolayers as well as liposomes, AM partitioning into the liposome lipid bilayer, and the stability and phase state of AM-lipid complexes at various weight ratios and production methods. As an alternative approach to the postaddition (PA) method of production, coevaporation (CE) was also utilized to produce AM-lipid complexes. By investigating the effect that AM-lipid components have on their aggregate structure, various AM-lipid complexes can be engineered with optimal energetic stability. In addition, the ability to tailor AM-lipid complex structure may be advantageous in developing stable, effective drug delivery systems.

MATERIAL AND METHODS

Materials

1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were obtained from Avanti Polar Lipid (Alabaster, AL). Nonfunctionalized AMs were prepared as previously described.[26] All other solvents and reagents, unless otherwise stated, were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO) and used as received.

Preparation of AM-Lipid Complexes

AM-lipid complexes were prepared as weight-to-weight mixtures of AM and total weight of DOPE:DOTAP (1:1) lipids. The AM-lipid weight ratios assayed were 0:1, 1:10, 1:5, 1:2, 1:1, 2:1, 5:1, 10:1, and 1:0. AM-lipid complexes were created by either a coevaporation (CE)[29] or postaddition (PA) method (below).

In the CE method, separate stock solutions of lipids (33.6 mM DOPE and 35.8 mM DOTAP) in chloroform and AM (16.8 mM) in chloroform were prepared. Aliquots of lipids and AM at various weight ratios were codispensed into glass vials and solvent removed by rotary evaporation at room temperature. The remaining film was dried overnight under

vacuum at room temperature. The films were hydrated with 10 mM HEPES buffer (pH 7.2) and incubated on a rotary shaker overnight at room temperature. Samples were then processed through eight freeze–thaw cycles by immersion in a dry ice–methanol bath followed by immersion in a water bath at 60 °C. Solutions were then passed 27 times through a 100 nm filter using a mini-extruder (Avanti). Processed materials were stored at 4 °C until use. All samples were used within four to seven days of production.

In the PA method, DOPE:DOTAP (1:1) lipid vesicles were first formed using the CE method described above but without AMs present. Dried AM powder stock was hydrated in 10 mM HEPES. Concentrated aliquots of preformed lipid vesicles were added to concentrated aliquots of AM (25.2 mM) in10 mM HEPES at various weight ratios and brought to desired volume. Samples were statically incubated for at least 1 h at 25 °C. Processed materials were stored at 4 °C until use. All samples used within four to seven days of production.

For reference, the weight ratios for the complexes were converted into moles for comparison:

AM-lipid (w/w)	AM-lipid (mol/mol)
1:0	n/a
10:1	1.2:1
5:1	1:1.6
2:1	1:4
1:1	1:8
1:2	1:16
1:5	1:41
1:10	1:82
0:1	n/a

Langmuir Monolayers

To compare the stability of AM-lipid complexes, compression isotherms of several binary monomolecular films comprising DOPE:DOTAP and increasing weight fractions of AM were measured at the air–water interface using a Nima 302 M Langmuir-type surface balance (Coventry, U.K.) at ambient temperature (~22 °C). Water for the subphase was obtained by filtration through a Milli-Q water purification system (Millipore, Molsheim, France) to a resistivity of ≥18 MΩ · cm. Mixtures were prepared by combining appropriate volumes of 1:1 lipid and AM (w/w) from stock solutions in HPLC-grade chloroform (Fisher). Monolayers were produced by applying 10–15 μL of the various mixtures to the aqueous surface using a Hamilton digital syringe (Reno, NV). Compressions, at a rate of 10 cm^2/min, were initiated after a 15 min delay to allow for solvent evaporation. The ability of AM to condense lipid films were evaluated directly from force–area data collected using software provided by *Nima* (v 615) and analyzed with *Origin*. Deviations of experimentally measured molecular area from that predicted by assuming ideal additivity (eq 1) of the components indicate either attractive (negative deviation) or repulsive (positive deviation) forces between AM and lipid.

$$A_\pi=(M_1A_1)+(M_2A_2)_\pi \quad (1)$$

M_1 and M_2 are lipid and AM mole fractions, respectively, converted to masses using the known molecular weights of AM and the lipid mixture, and A_1 and A_2 are the respective molecular areas of the pure components at surface pressure π.

Complex Size by Dynamic Light Scattering (DLS)

The sizes of AM-lipid complexes prepared by CE methods were determined by dynamic light scattering using a NanoZS90 Instrument (Malvern Instruments, Malvern, U.K.). Samples in 10 mM HEPES were evaluated at room temperature with the lipid content for all samples fixed at 5 mg/mL, which is above the 1 mg/mL detection limit of DLS instrument.

Turbidity

The turbidity of AM-lipid complexes prepared by both methods was determined by measuring percent transmission using an UV/vis spectrophotometer (Perkin-Elmer Lambda XLS, Beaconsfield, U.K.). 400–600 nm wavelength scans were performed with percent transmission at 500 nm.[4,30] Samples in 10 mM HEPES were evaluated at room temperature with the lipid content for all samples fixed at 5 mg/mL. 10 mM HEPES was used as a blank prior to sample analysis.

Isothermal Titration Calorimetry (ITC)

ITC experiments were performed using a Microcal VP-ITC (GE Healthcare) and data were collected and analyzed using Microcal's proprietary *Origin* software (v 7, Northamton, MA). Thermostability of AM-lipid complexes was compared by titrating complexes comprising various AM-lipid ratios, prepared using either the PA or CE method, into the calorimeter cell containing pure HEPES buffer at 30 °C. In short, 6 mM solutions (total AM plus 1:1 mixtures (w/w) of DOTAP and DOPE) were loaded into the injection syringe and the cell was filled with 10 mM HEPES after thorough degassing. Once a stable baseline was achieved with stirring at 307 rpm, an initial injection of 1 μL was made (the heat signal of which was not included in the subsequent data analysis at the recommendation of the manufacturer). Thereafter, 5 μL aliquots were injected into the sample cell at 240 s intervals until the heat signals asymptotically approached a constant value. The peaks were integrated and the resulting thermograms analyzed using the model in Microcal's ITC version of *Origin*, from which K_{eq} values and enthalpy changes were obtained. In this analysis, AM-lipid complex equilibrium constants (K_{eq}) and relative enthalpy changes from dilution of complexes were obtained by least-squares fit to a simple partition model as previously described.[31] Calculated values were used to compare the stability of AM-lipid complexes, where higher (less negative) enthalpy changes were taken to indicate more stable mixtures.

To obtain thermodynamic parameters of AM-lipid interactions, a second set of ITC experiments was conducted in which three concentrations of preformed small unilamellar vesicles (0.5, 1, and 1.5 mg/mL total lipid) were titrated at 360 s intervals with micellar solutions (6 mg/mL) of AM. Heat signals thus obtained were normalized to account for dilution and cell overflow and breakpoints in the resulting curves, which were plotted using a slightly modified version of the *Excel* spreadsheet available by download from Heerklotz et al., were assigned.[18] The results yield a phase diagram whose boundaries are straight lines described by eqs 2 and 3:

$$[\mathrm{AM}]^{\mathrm{sat}} = R^{\mathrm{sat}}\left([\mathrm{Lipid}] + \frac{1}{K}\right) \quad (2)$$

$$[\mathrm{AM}]^{\mathrm{sol}} = R^{\mathrm{sol}}\left([\mathrm{Lipid}] + \frac{\mathrm{CMC}}{1+R^{\mathrm{sol}}}\right) \quad (3)$$

These models generate AM-lipid ratios required for onset (R^{Sat}) and completion (R^{Sol}) of vesicle solubilization, K_{eq} (equilibrium constant between micelle and vesicle), and the AM critical micelle concentration, assuming ideal behavior. The lines obtained were extrapolated to include ratios beyond those tested in these experiments.

RESULTS AND DISCUSSION

Langmuir Monolayers

The interaction of AMs with DOPE: DOTAP liposomes was assessed by the AMs' ability to condense lipid monolayers at the air–water interface. On the basis of the molecular area of pure AM and pure lipid at a given surface pressure, the molecular area of a given mixture ratio can be predicted for that surface pressure. However, this prediction is based on the absence of intermolecular interactions. Experimentally obtained molecular areas that deviate from the predicted area indicate the presence of either attractive or repulsive forces.[32] Experimental molecular areas that deviate negatively from the predicted area indicate condensation and suggest intermolecular accommodation between the mixed monolayer components.[33] Figure 3 (top panel) shows compression isotherms of monolayers comprising pure AM, pure 1:1 DOTAP:DOPE, and three selected combinations. The molecular area surface pressure of the AM alone monolayer is much greater than that of the lipid alone monolayer. This result is due to the increased molecular weight of the AM (5.9 kDa) as compared to the lipid alone (0.7 kDa). As the mass of AM in the sample is gradually replaced with lipid, both the molecular area and the surface pressure decrease. The data from these isotherms are used to generate an area–compression curve that represents a pseudophase diagram(Figure 3, lower panel). On the basis of the isotherms in Figure 3 (top panel), the molecular areas of each sample at a surface pressure of 20 mN/m are plotted. The dotted line between the pure AM monolayer area (3540 $Å^2$) and the pure lipid monolayer area (5.9 $Å^2$) represents the predicted molecular area of a given monolayer assuming no intermolecular interactions. A significant degree of condensation, as exhibited by a negative deviation in measured monolayer area verses predicted behavior, was observed at all mixtures tested (Figure 3, lower panel) inferring a cooperative intermolecular interaction. Additionally, collapse pressures were higher for all mixtures than for either pure AM or pure DOTAP: DOPE (data not shown), demonstrating the stabilizing effect of the AMs.[15]

Isothermal Titration Calorimetry—Titration of Vesicles with AM

Changes in aggregation state that occur when the AMs are added to preformed vesicles (i.e., postaddition) were followed by injecting 6 mg/mL AM into the calorimeter cell containing 0.5, 1.0, and 1.5 mg/mL lipid vesicles (Figure 4, top panel). The large exothermic heat changes observed indicate a high thermostability of the structure formed by the addition of AMs to DOTAP:DOPE vesicles. Titrations of the AM solution into each vesicle solution yield a local minimum, followed by an abrupt rise in the heat signal. The curve break points associated with the local minimal and abrupt rise in heat signal correlate with the phase transitions from vesicles, to micelles and vesicles, to mixed micelles. The AM amount required to reach these break points increases with increasing vesicle concentration, and all samples are completely solubilized at the experiments' conclusions. Plotting the AM concentration versus the lipid concentrations at each break point for the three lipid concentrations tested allows for the construction of the phase diagram shown in Figure 4 (lower panel), which illustrates the relative amounts of AM and lipid at each phase transition

point. By extrapolating the phase state boundaries from the data generated, the phase state can be determined for a given AM-lipid weight ratio. Additional experiments at other lipid concentrations in the cell are currently being considered.

AM-Lipid Size and Turbidity

As stated earlier, the solubilization of lipid bilayers to mixed micelles is associated with distinct changes in size and turbidity. DLS histograms for all samples yielded a single peak curve with low standard deviation (representative histograms shown in Figure 5, top panel), indicating that the AM and lipid combined to form a distinct, single structure. Figure 5 (bottom panel) shows the size of the AM-lipid complexes produced by the PA[28] (previously reported and included for comparison) and CE method as assayed by DLS. The size of the pure AM in solution (~20 nm) was consistent with previous data[26] and the size of the pure lipid liposomes (~100 nm) was consistent with the 100 nm filter used in the extrusion process. The complex sizes produced by both methods were consistent with an extruded liposome at low AM concentrations. However, as the AM-lipid ratio was increased to 5:1, the size of complexes produced by the CE methods sharply decreased to a size consistent with that of the AM micelle alone (Figure 5, bottom panel).

Figure 6 compares the turbidity of the AM-lipid complexes produced by both PA and CE methods expressed by percent transmission at 500 nm as measured by UV/vis spectroscopy. The turbidity values of the lipid alone (~45%) and AM alone (~95%) are consistent with the turbidity characteristics of liposomes and micelles respectively.[4] AM-lipid complexes produced by PA were consistent with the pure lipid sample. Complexes produced by the CE method maintained a turbidity that was consistent with lipid alone at low AM concentrations, with a slight increase in turbidity noted with increased AM concentration. These data correspond with the known concept that surfactants induce and increase liposome turbidity prior to complete solubilization to mixed micelles.[1,4] As the relative AM amount increased to an AM-lipid ratio of 5:1, complex size sharply decreased to a turbidity consistent with the AM micelle alone. On the basis of the size and turbidity data, it appears that AM-lipid complexes at AM-lipid weight ratios above 2:1 produced by the CE method exist as mixed micelles. These data are largely consistent with the pseudophase diagram shown in Figure 4 (bottom panel).

Isothermal Titration Calorimetry—Formed AM-Lipid Complexes

The partitioning of AM into the lipid bilayer may affect the stability of the overall complex. Therefore, the stability of formed AM-lipid complexes was assessed by ITC. Suspensions of AM-lipid complexes, at various mass ratios of each component and above their CMCs, were titrated into buffer at 30 °C. As the complex is diluted in the calorimetry cell, partitioning between the AM-lipid aggregate and the aqueous environment generates changes in the feedback heat signal; thermally stable complexes are expected to evolve less heat as stronger interactions decrease the degree of dissociation. The ITC experiments thus provide a comparative measure of the complex stability. The integrated heat signals from a representative thermogram of one such experiment (10:1 AM-lipid complex), prepared by the PA method, is shown in Figure 7 (top panel); the bottom panel contains a summary of the data from a series of titrations of various AM-lipid complex compositions.

The magnitude of the heat change is sensitive to the relative AM-lipid amounts (Figure 7, lower panel, right axis). Complexes comprising high AM-lipid ratios (10:1 and 5:1) evolved less heat than those containing lower relative AM amounts, indicating greater stability. With the exception of the 10:1 AM-lipid mixture, partition coefficients correspond to enthalpy changes (left axis), where greater values of the former coincide with more negative values of the latter. According to these results, complexes comprising 2:1, 1:1, and 1:5 AM-lipid

ratios are the least stable and those containing high AM-lipid (10:1 and 5:1) display greater stability.

Complexes prepared by the CE method were titrated into buffer (representative isotherm is shown in Figure 8). Titration of CE-produced AM-lipid complexes resulted in considerably lower heat changes (compare the scales of ordinates in Figures 7 (top panel) and Figure 8) at all compositions tested, suggesting that coevaporation yields more stable complexes, suggesting stronger interactions among complex components. Qualitatively, the curves differ significantly from PA titration curves and cannot be fitted by a simple model. The heat changes are at first exothermic, then become endothermic before again reversing sign, and the experiment ends with a nonzero negative enthalpy. These results suggest a more complex series of events when CE-produced complexes are diluted. The thermodynamic pattern is most likely the result of initial separation of loosely associated AM molecules from the complex, followed by disintegration and finally, reaggregation of mixed AM-lipid and pure AM micelles. Thus, we cannot apply eq 2 to generate K_{eq} and DH date as performed on the PA-generated systems, where the data of Figure 7 (top panel) was converted and shown in Figure 7 (bottom panel). Taken together, the results indicate that the PA method, in which the AMs are added to preformed lipid vesicles, produces AM-coated vesicles. In contrast, the CE method produces complexes with more intimately associated components such that further dilution disrupts these attractive forces in favor of newly formed mixed aggregates. Future studies are being discussed to directly compare specific features of titration curves (breakpoints, e.g.) between dilution of complexes prepared by CE and PA methods.

CONCLUSIONS

In this study, the interactions between AM and DOPE: DOTAP monolayers and liposomes were evaluated using multiple experimental techniques. LFB studies showed cooperative intermolecular interactions between the lipids and AMs that stabilize lipid monolayers. Isothermal calorimetry of lipid suspensions titrated with AMs displayed exothermic heat changes indicating a high thermostability of the structure formed by the addition of AMs to DOTAP:DOPE vesicles. Increased AM addition led to the complete solubilization of the vesicle, the data yielding a phase diagram that proposed AM-lipid complex transitions from lamellar aggregates, to a mixture of coexisting vesicles and micelles, to mixed micelles. DLS and spectroscopy data substantiated the proposed phase diagram with sharp decreases in the size and turbidity of CE produced AM-lipids at weight ratios of 5:1 or above. ITC evaluation of preformed complexes showed that at or above a 5:1 weight ratio, PA-produced complexes evolved less heat than those containing lower relative amounts of lipid, suggesting greater stability. Complementary ITC experiments of CE-produced AM-lipid complexes showed overall lower heat changes as compared to PA-produced complexes, indicating stronger interactions between AM-lipid components with the CE method. Taken together, the results strongly suggest that the PA method produces vesicles with AM molecules associated with its outer leaflet only (i.e., an AM-coated vesicle). On the other hand, the CE method produces complexes in which the AM-lipid components are more intimately associated. The multiple experimental techniques in this study were successfully used to define surfactant-liposome interactions. These results will be helpful in the design of AM-lipid complexes as structurally defined, stable, and effective drug delivery systems.

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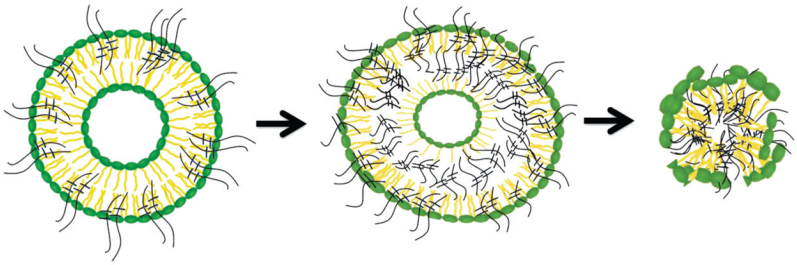

Figure 1.
Surfactant solubilization of lipid bilayer vesicle: structural transition from a lamellar structure (left) to an intermediate structure (middle) to a mixed micelle (right).

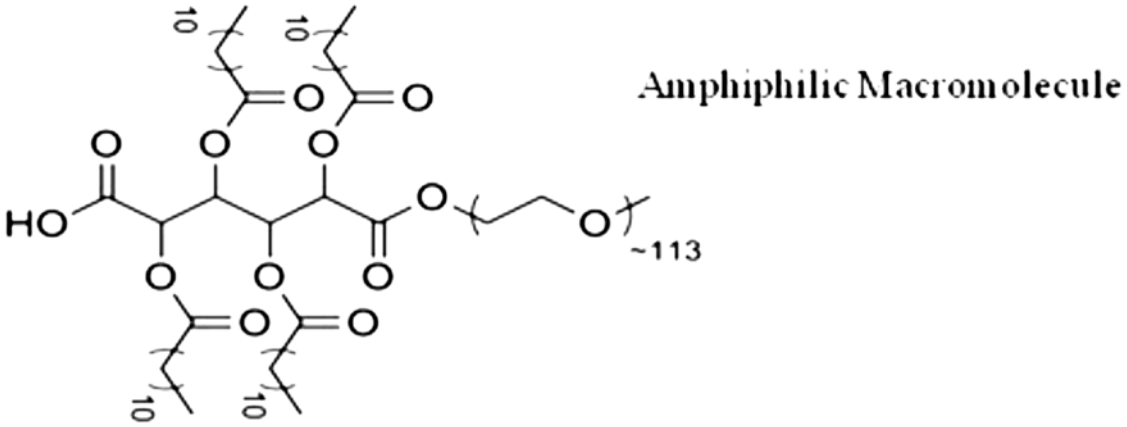

Figure 2.
AM comprising mucic acid, 12-carbon acyl chains, and PEG.[26]

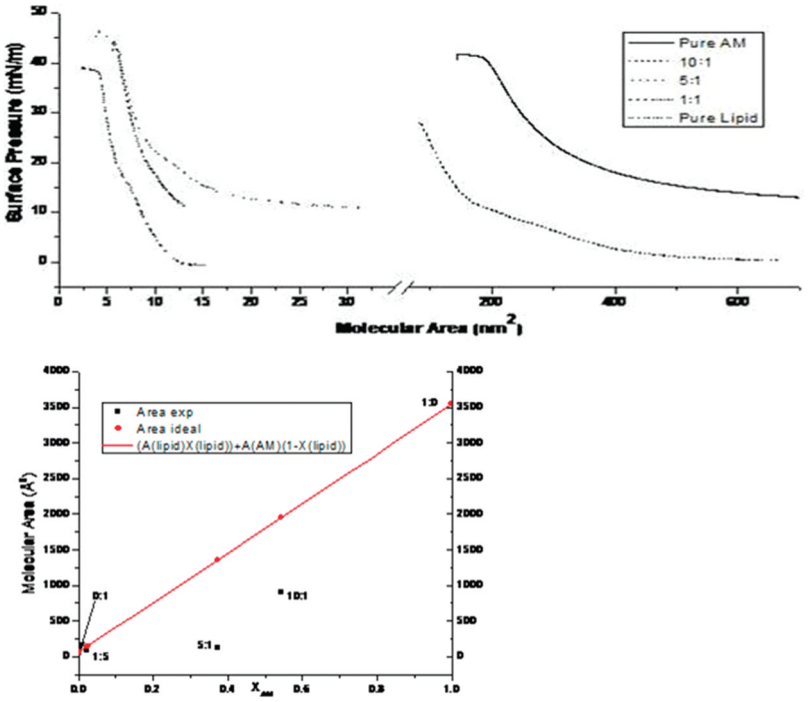

Figure 3.

Top: Compression isotherms at the air–water interface of pure AM, pure DOTAP:DOPE, and selected mixtures of the two measured at 22 °C. Bottom: Area–composition curves at 20 mN/m constructed from the data in the top panel. Composition values on the *x*-axis are molar ratios converted from the molecular weights of AM and the lipid mixture.

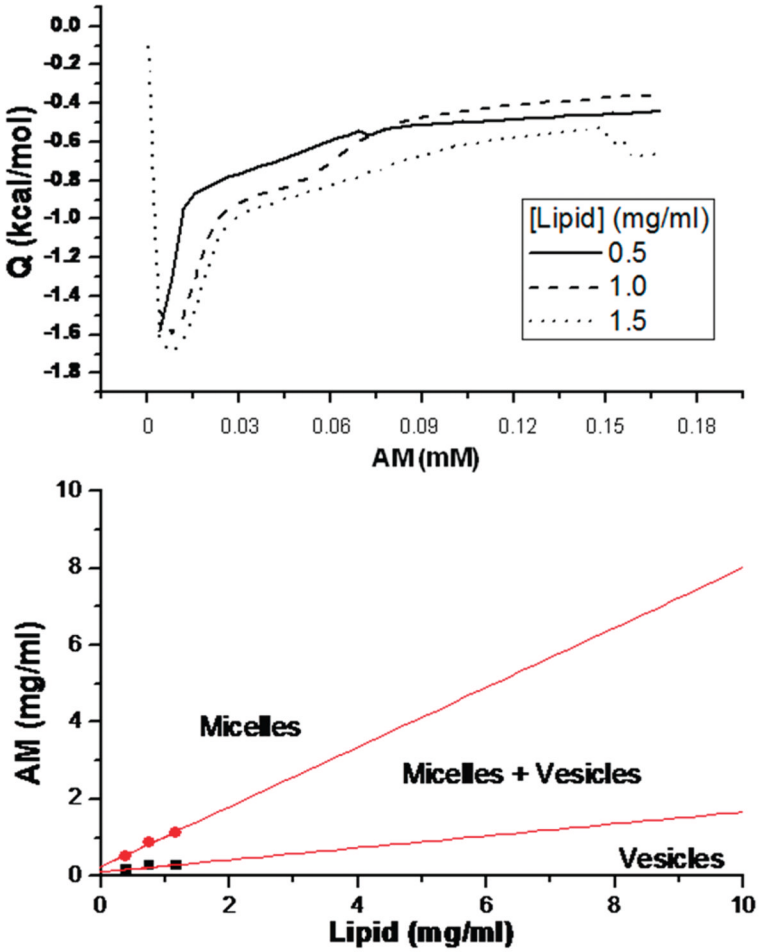

Figure 4.

Top: Heat traces from titrations of 0.5, 1.0, and 1.5 mg/mL DOPE:DOTAP (total lipid concentration) with 6 mg/mL AM at 30 °C. The *x*-axis was converted to molar units to better illustrate molecular interactions. Bottom: Composition diagram for aggregation state changes of lipid vesicles titrated with AMs. Data points are from the titrations shown in the upper panels and straight lines were derived as described in Materials and Methods.

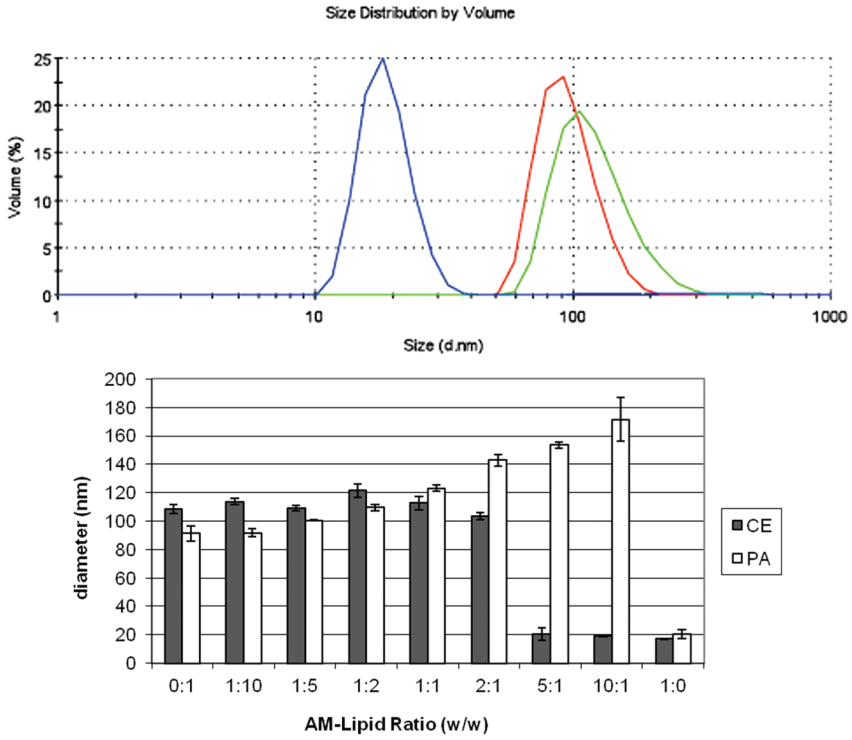

Figure 5.

(top) Representative DLS histograms: blue, AM alone; red, DOPE:DOTAP alone; green, 1:1 AM-lipid complex. (bottom) AM-lipid complex in 10 mM HEPES buffer produced by CE and PA methods as assayed for size by DLS. (PA data previously reported[28] and included here for comparison.) All complexes were assayed within 2–4 h after production. Similar results are seen for complexes assayed up to seven days after production. Lipid content for all samples was fixed at 5 mg/mL. CE - coevaporation, PA - postaddition.

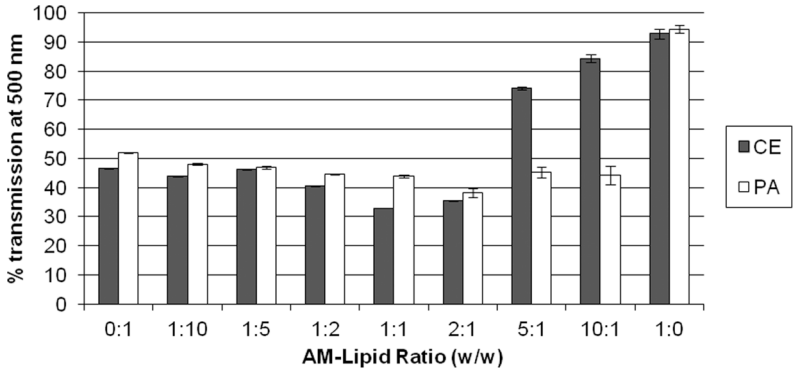

Figure 6.

AM-lipid complex in 10 mM HEPES buffer produced by CE and PA methods as assayed for turbidity by UV/vis spectroscopy. All complexes were assayed within 2–4 h after production. Similar results are seen for complexes assayed up to seven days after production. Lipid content for all samples was fixed at 5 mg/mL. CE - coevaporation, PA - postaddition.

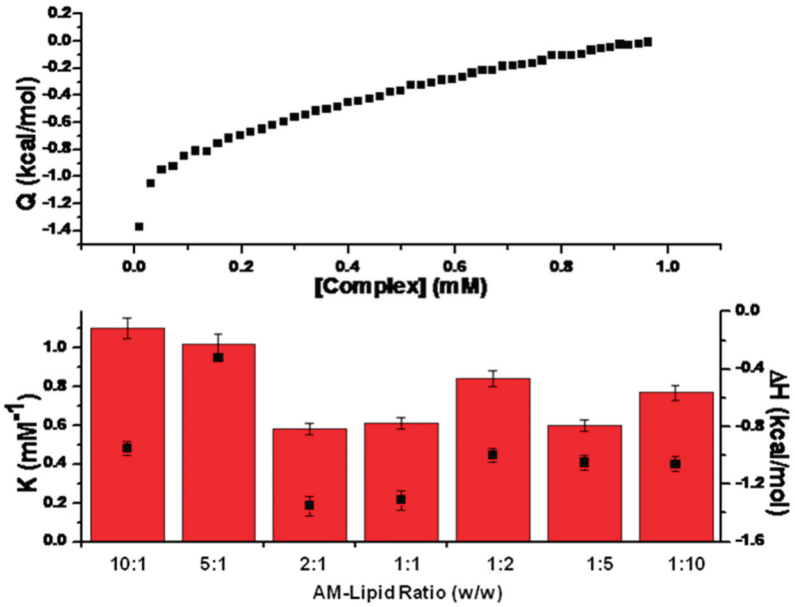

Figure 7.

Top panel: Integrated heat signal from titration of 6 mM 10:1 (w/w) AM-lipid complex, prepared using the PA method, into HEPES buffer at 30 °C. Bottom panel: Summary of equilibrium constants, K_{eq}, (left axis, black squares) and enthalpy changes (right axis, red bars) obtained from the best fit of the data for titration of various AM-lipid complexes into HEPES buffer at 30 °C. See Materials and Methods for experimental details and data analysis routine.

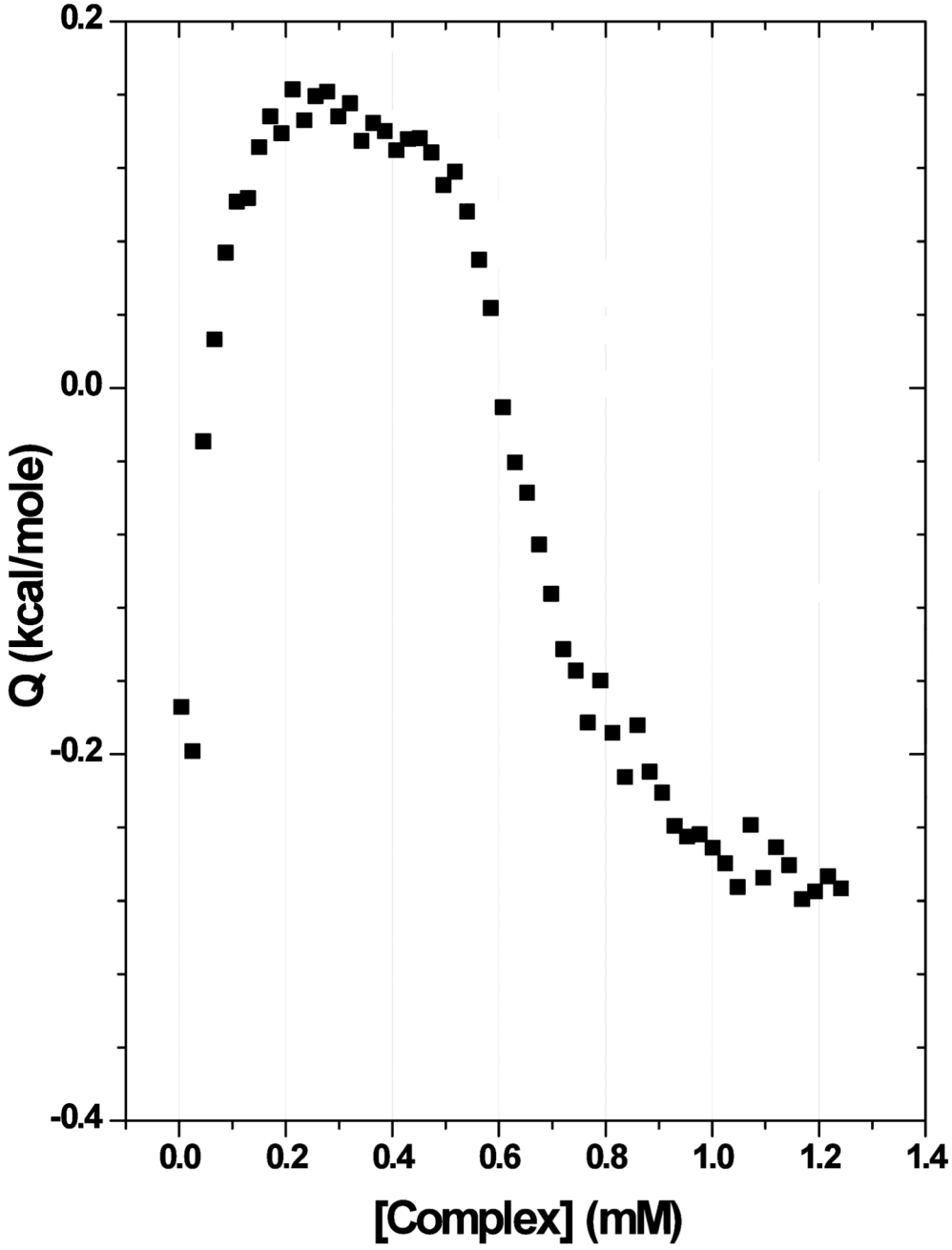

Figure 8.
Integrated heat signals from a representative experiment in which 6 mM CE-prepared AM-lipid (10:1 (w/w) complexes were titrated into HEPES buffer at 30 °C.