Surfactant Vesicles for High-Efficiency Capture and Separation of Charged Organic Solutes

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We demonstrate the unique ability of catanionic vesicles, formed by mixing single-tailed cationic and anionic surfactants, to capture ionic solutes with remarkable efficiency. In an initial study (Wang, X.; Danoff, E. J.; Sinkov, N. A.; Lee, J.-H.; Raghavan, S. R.; English, D. S. *Langmuir* **2006,** *22,* 6461) with vesicles formed from cetyl trimethylammonium tosylate (CTAT) and sodium dodecylbenzenesulfonate (SDBS), we showed that CTAT-rich (cationic) vesicles could capture the anionic solute carboxyfluorescein with high efficiency (22%) and that the solute was retained by the vesicles for very long times ($t_{1/2} = 84$ days). Here we expand on these findings by investigating the interactions of both anionic and cationic solutes, including the chemotherapeutic agent doxorubicin, with both CTAT-rich and SDBS-rich vesicles. The ability of these vesicles to capture and hold dyes is extremely efficient (>20%) when the excess charge of the vesicle bilayer is opposite that of the solute (i.e., for anionic solutes in CTAT-rich vesicles and for cationic solutes in SDBS-rich vesicles). This charge-dependent effect is strong enough to enable the use of vesicles to selectively capture and separate an oppositely charged solute from a mixture of solutes. Our results suggest that catanionic surfactant vesicles could be useful for a variety of separation and drug delivery applications because of their unique properties and long-term stability.

1. Introduction

Vesicles have long been of interest to the scientific community for their ability to encapsulate solute molecules such as drugs and proteins. Most studies on solute encapsulation have been carried out with vesicles made from two-tailed amphiphiles (lipids). However, *single-tailed* amphiphiles can also form vesicles,[1–4] and in particular, simple mixtures of cationic and anionic surfactants, often referred to as "catanionic" systems, can spontaneously give rise to unilamellar vesicles in water.[4] A variety of catanionic vesicle-forming systems have been studied with respect to their phase behavior,[5–12] but much less is known about the ability of these vesicles to capture, encapsulate, and retain organic molecules.

Recently, we published an initial report detailing some of the unique aspects of solute association with catanionic surfactant vesicles.[13] Specifically, we showed that catanionic vesicles with a molar excess of the cationic surfactant (CTAT) efficiently captured the anionic dye 5(6)-carboxyfluorescein (CF) and retained it for very long periods of time (half-life $t_{1/2}$ of 84 days).[13] In the present study, we expand on our initial investigation to include the anionic and cationic organic solutes shown in Figure 1. In addition, we also study vesicle interactions with the cationic anti-cancer drug doxorubicin. Our results show that surfactant vesicles can be highly efficient for the capture and long-term storage of organic solutes that have a charge *opposite* to that of the vesicles. Thus, there are strong, specific, charge-mediated interactions between vesicles and solutes, and we demonstrate how these interactions can be harnessed for the efficient separation of oppositely charged solutes from a solute mixture using only conventional, gravity-driven size-exclusion chromatography (SEC).

The catanionic vesicles that we focus on for this study are formed by combining the cationic surfactant cetyltrimethylammonium tosylate (CTAT) and the anionic surfactant sodium dodecylbenzenesulfonate (SDBS).[9,14] The CTAT/SDBS system has been the most studied catanionic system, and the vesicles are known to be unilamellar and fairly monodisperse, with radii of 60–80 nm.[9,13] Catanionic surfactant vesicles have been recognized to have several advantages over conventional phospholipid vesicles: they form spontaneously without the need for additional sonication or extrusion, they have an extremely long shelf life, and the raw materials are inexpensive compared to synthetic or purified phospholipids. More importantly, in this article we will demonstrate that catanionic vesicles have other advantages that have been hitherto unrecognized: they can efficiently capture and hold solutes that are of the opposite charge from the vesicles, and they retain these molecules for long periods of time.

2. Experimental Section

Materials. Surfactants CTAT, SDBS, and Triton X-100 were purchased from Aldrich Chemicals. Fluorescent dyes CF, sulfor-

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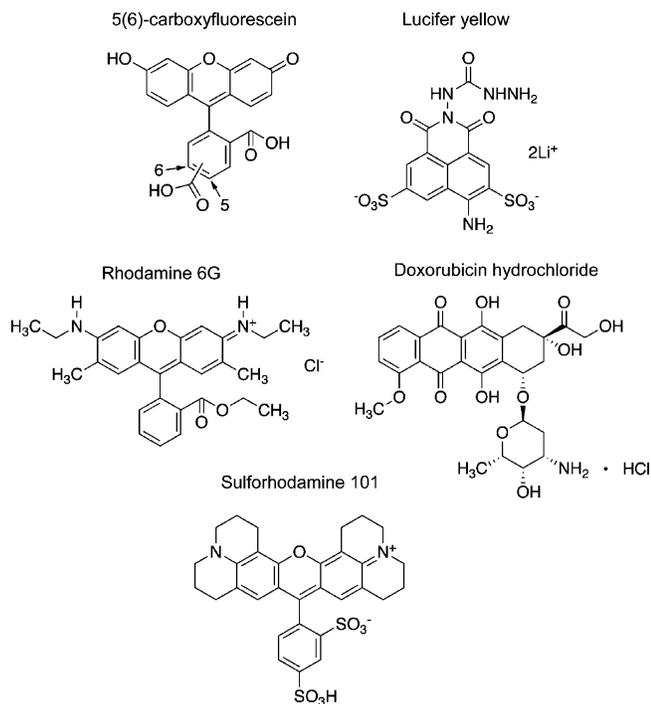
Figure 1. Structures of the five solutes used in these studies.

hodamine 101 (SR101), and Lucifer yellow (LY) were purchased from Molecular Probes, and the dye rhodamine 6G (R6G) and the chemotherapeutic drug doxorubicin hydrochloride (Dox) were purchased from Fluka. All materials were used without further purification. Dry surfactants CTAT and SDBS were stored in a desiccator to prevent water absorption.

Vesicle Preparation. All samples were prepared at a total surfactant concentration of 1 wt.%. The surfactants were weighed and mixed with deionized water by gentle stirring and were then allowed to equilibrate at room temperature for at least 48 h.[9,15] Vesicle samples were prepared at two different surfactant compositions, 7:3 and 3:7 w/w CTAT/SDBS, which are denoted as V^+ and V^-, respectively. V^+ refers to the excess positive charge on the vesicle bilayers when there is excess of CTAT. In these samples, the concentrations of CTAT and SDBS are 15.4 and 8.6 mM, respectively. This corresponds to 6.8 mM excess CTAT or a 1.8-fold molar excess of cations. Likewise, V^- refers to vesicles with a net negative charge due to a 13.5 mM excess of SDBS (the samples contain 6.6 mM CTAT and 20.1 mM SDBS) or a 3.0-fold molar excess of anions.

Evaluation of Apparent Encapsulation Efficiency (ϵ). The apparent encapsulation efficiency, ϵ, describes the fraction of dye in a particular preparation that associates with the vesicle either through entrapment in the inner water pool or by association with the vesicle bilayer. The apparent encapsulation efficiencies of the two vesicle preparations, V^+ and V^-, were evaluated for all five solute molecules. In each case, vesicles were prepared using aqueous solutions of the solute at a concentration of 1 mM. In the case of CF, a pH of ~9 was required to dissolve the dye completely, and the stock solutions were adjusted accordingly. The solute/CTAT/SDBS mixtures were stirred for 30−60 min or overnight, and the resulting vesicle solutions were allowed to equilibrate in the dark at room temperature for at least 48 h. Thereafter, the samples were passed through a 25 mm syringe filter (0.45 μm mesh) to remove any impurities or large aggregates. Dynamic light scattering (described below) was conducted to confirm vesicle formation and to measure the average vesicle size.

To measure the apparent encapsulation efficiency ϵ, SEC was used to separate the free solute from that which is captured by the vesicles. A 1.0 mL aliquot of the vesicle−solute sample was run

through a 1.3 cm × 21 cm SEC column packed with Sephadex G50 resin (medium mesh, from Amersham Biosciences). During elution, 1.5 mL fractions were collected and analyzed, and a series of such fractions for a typical experiment is shown in Figure 2. (The solute here is CF.) Dynamic light scattering was used to determine which of the eluted fractions contained vesicles, and the vesicles were consistently found to elute at 5.5 mL total elution volume. The amount of solute in each fraction was determined using UV−vis spectroscopy (Hitachi U-3010 Spectrometer). The ϵ value is defined as the amount of vesicle-associated solute relative to the total initial amount of solute

$$\epsilon = \frac{V_f(A_{f1} + A_{f2} + ...)}{V_i A_i} \qquad (1)$$

where V and A are the volume and absorbance, respectively, i denotes initial values taken from the original preparation, and f denotes values taken from the fractions eluted from the SEC column shown by dynamic light scattering to contain vesicles. To avoid artifacts in UV−vis spectroscopy from light scattering or from solute aggregation inside the vesicles, the absorbance was determined after first disrupting the vesicles by adding Triton X-100 surfactant to each fraction. Note that ϵ reflects contributions from both the solute in the water pool inside the vesicle and the solute that is electrostatically adsorbed on the vesicle bilayers.

Long-Term Capture and Dye Release. To evaluate the ability of vesicles to retain solutes for long periods of time, the following procedure was adopted. First, the initial vesicle−solute mixture was purified using SEC (as described above) to remove the free solute. The sample was then checked for the release of solute from the vesicles over the course of several weeks. For this purpose, quick-spin columns prepacked with Sephadex G50 (fine) were used (column from Roche, additional beads for repacking the columns from Sigma). On a specific day, a 100 μL aliquot was run through a quick-spin column by centrifugation (3000 rpm, 15 s), and the eluted fraction was evaluated using UV−vis spectroscopy. Any solute that had been released from the vesicles was retained by the quick-spin column. Therefore, the amount of solute eluted by the column corresponded to the solute still associated with the vesicles. The UV−vis absorption value for the eluted sample was divided by the corresponding value obtained on day zero (immediately after SEC) to yield a fraction of solute that remains captured by the vesicles. The above procedure was repeated at various times to create a release curve (i.e., released solute vs time elapsed, as shown in Figure 3).

Dynamic Light Scattering (DLS). Vesicle sizes in solution were monitored using DLS on a Photocor-FC instrument. The light source was a 5 mW laser at 633 nm, and the scattering angle was 90°. A logarithmic correlator was used to obtain the autocorrelation function, which was analyzed by the method of cumulants to yield a diffusion coefficient.[16] The apparent hydrodynamic size of the vesicles was obtained from the diffusion coefficient through the Stokes−Einstein relationship. The intensity (total counts) of the signal was also recorded for each sample.

Small-Angle Neutron Scattering (SANS). SANS experiments were conducted on the neat vesicles as well as the vesicle−solute mixtures to probe whether there were any changes in vesicle size or bilayer integrity caused by the solutes. All samples for SANS experiments were prepared using deuterium oxide (99% D, from Cambridge Isotopes) in place of water. The measurements were made on the NG-7 (30 m) beamline at NIST in Gaithersburg, MD. Neutrons with a wavelength of 6 Å were selected. Two sample−detector distances of 1.33 and 13.2 m were used to probe a wide range of wave vectors from 0.004 − 0.4 Å^{-1}. Samples were studied in 2 mm quartz cells at 25 °C. The scattering spectra were corrected and placed on an absolute scale using calibration standards provided by NIST. The data are shown as the radially averaged intensity I (minus the background) versus the wave vector $q = (4\pi/\lambda) \sin(\theta/2)$, where λ is the wavelength of incident neutrons and θ is the scattering angle. Analysis was carried out as described previously.[17]

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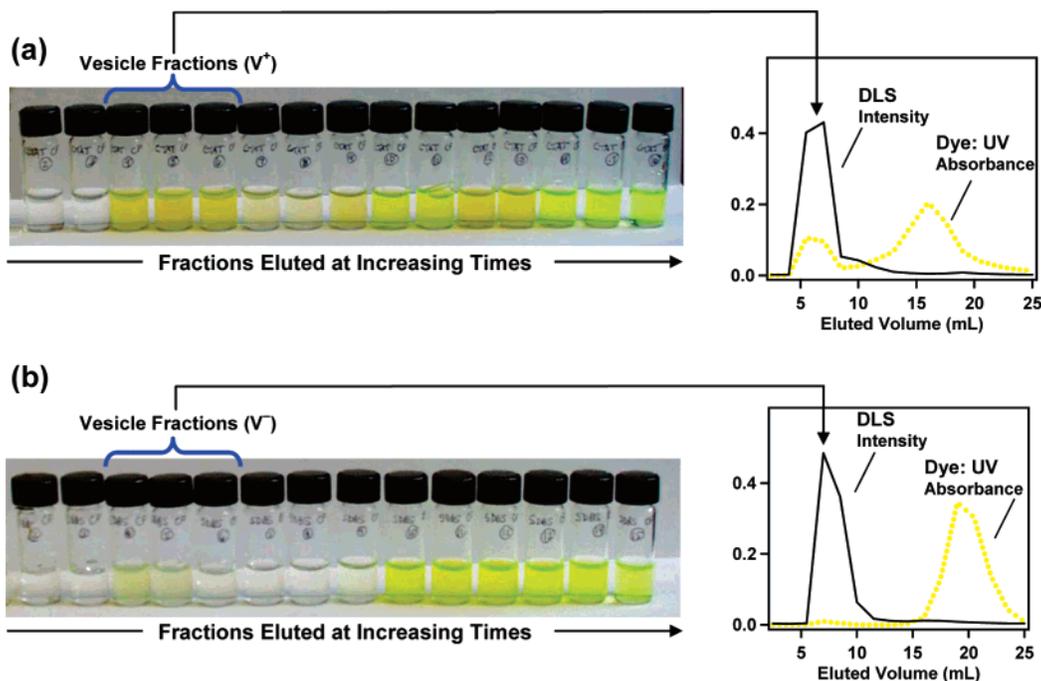(16) Koppel, D. E. *J. Chem. Phys.* **1972**, *57*, 4814.

Figure 2. Results from SEC of vesicles with the anionic dye CF: (a) V^+ vesicles and (b) V^- vesicles. The plots on the right show the DLS intensity, which is proportional to vesicle concentration, and the UV absorbance at 492 nm, which is proportional to CF concentration, as a function of eluted volume. The results show that a significant portion of the dye (23%) elutes with the V^+ vesicles, whereas only about 1.5% of the dye elutes with the V^- vesicles. This can be seen visually by the more intense yellowish hue of the vesicle fractions (vials 3–5) in the V^+ case.
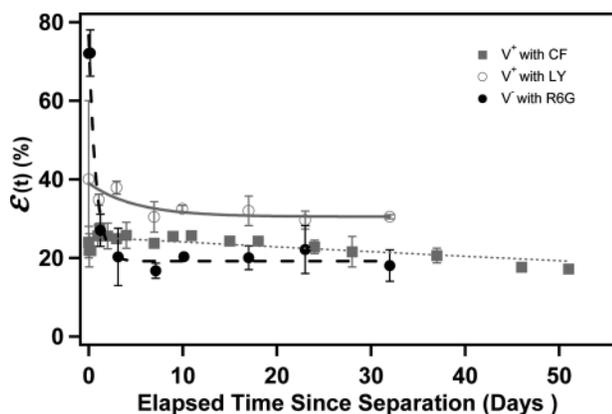

Figure 3. Dye release profiles. An evaluation of long-term release was achieved by monitoring ϵ as a function of time. Data were acquired for CF (■) and LY (○) in V^+ samples and R6G (●) in V^- samples. The corresponding lines are fits to an exponential decay. For CF in V^+, three trials were completed, and the error bars on the points are the standard deviations of the averages of the three data sets. For R6G and LY, two trials were run, and the error bars are the actual values for the two trials.

3. Results and Discussion

We have shown in our initial report[13] that the anionic dye CF can be efficiently sequestered in CTAT-rich vesicles (V^+) via two mechanisms: encapsulation in the inner water pool and electrostatic adsorption to the charged bilayer. The apparent encapsulation efficiency ϵ, measured by the procedure described above in the Experimental Section, was found to be about 22%. Electrostatic adsorption contributed about 75% of the ϵ value, as shown by experiments where the CF was added to preformed V^+ vesicles. Conversely, the ϵ for CF in SDBS-rich vesicles (V^-) was only ca. 1%, which is comparable to the ϵ for CF

encapsulation in neutral phospholipid (egg yolk–phosphatidyl choline, EYPC) vesicles. These observations confirmed that the unusually high apparent encapsulation efficiency in V^+ vesicles was likely due to electrostatic interactions of the dye with the vesicles. Studies in which dye adsorption decreases with increasing ionic strength (data not shown) also confirm that electrostatics plays a principle role. In addition to the apparent encapsulation efficiency, we also studied the time-dependent release of CF from the vesicles by utilizing the self-quenching of CF fluorescence. We found that the release rate from V^+ surfactant vesicles was at least 40 times slower than from EYPC vesicles.

In this article, we have expanded our studies to include two new anionic dyes, LY and SR101, as well as two cationic solutes, the dye R6G and the anti-cancer drug Dox. We have measured the initial value of ϵ for each of these solutes in both V^+ and V^- vesicles and have monitored ϵ as a function of time for three different solute/vesicle combinations. To demonstrate the strength and specificity of vesicle capture, we have also used the vesicles to separate an oppositely charged solute from a solute mixture. These studies indicate that surfactant vesicles are promising candidates for applications such as drug delivery and as separation media. An important requirement for realizing such applications will be to ensure the stability of vesicle–solute mixtures under a range of conditions. To investigate the issue of vesicle stability upon addition of solutes, we have conducted an initial set of studies using DLS and SANS, and these are reported in the last section of the article.

Capture of Charged Solutes by Vesicles. The chemical structures of the five different solutes studied here are shown in Figure 1. CF is a trianionic fluorescent dye at pH above 6.9,[18] and LY is dianionic in water and SR101 is monanionic. R6G possesses a quaternary amine, is cationic at all pH values, and was chosen for its structural similarities with CF. Dox is a cationic

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drug with a pK_a of ~7.6[19] that has been used to treat a variety of cancers.[20–22] In fact, the toxic side effects of Dox have been shown to be reduced if it is delivered using liposomes.[20]

The apparent encapsulation efficiency, ϵ, for each of the above solutes was determined for both V$^+$ and V$^-$ vesicles using the procedure described in the Experimental Section. It is important to point out that a solute concentration of 1 mM was used in all cases. In an earlier study, Caillet et al. attempted to encapsulate CF in V$^+$ catanionic vesicles, but their attempt failed because of the high CF concentration (50 mM) used.[23] It has been well documented that the addition of polyelectrolytes to oppositely charged surfactant vesicles can destabilize vesicles, leading to changes in bilayer and vesicle structure as well as precipitation.[24,25] Consistent with these findings, we have found that high concentrations of CF (and, similarly, other solutes) tend to disrupt the vesicles and lead to precipitation over time. Vesicle stability appears to be unaffected when the solute concentration is kept below 5 mM, and at these concentrations, solute capture does occur. The results of experiments using 1 mM CF in V$^+$ and V$^-$ vesicles are shown in Figure 2. The photographs show successive eluted fractions (1.5 mL each) from the SEC column for V$^+$ vesicles (Figure 2a) and V$^-$ vesicles (Figure 2b). The vesicle-containing fractions are in vials 3–5 (fractions 4–6) in both cases, and this is evident from the high DLS intensity for these samples (plotted as a solid line in the graphs). In addition, the fraction of CF in each vial (from UV–vis) is also plotted as a yellow dotted line. Note that vials 3–5 in the case of V$^+$ have a strong yellowish tinge, confirming that these vesicles contain an appreciable fraction of CF (23%). However, vials 3–5 in the case of V$^-$ vesicles have a much lower dye content (1.5%). Thus, the anionic CF is efficiently incorporated into the V$^+$ vesicles but not into the V$^-$ ones.

Similar results (highly efficient capture in V$^+$, weak encapsulation in V$^-$) were obtained for the other two anionic dyes (LY and SR101) as well. For the cationic solutes (R6G and Dox), the results were switched, and these solutes are efficiently captured in V$^-$ samples and weakly captured in V$^+$ samples. Counterparts to Figure 2 in the form of photographs, DLS intensity, and UV–vis absorbance data for each of the solutes are provided in Supporting Information. Table 1 shows the ϵ values (calculated using eq 1) for each solute in both V$^+$ and V$^-$ vesicles. It is clear from this data that ionic solutes are efficiently captured in catanionic vesicles having an opposite net charge.

One interesting observation from Table 1 is that the ϵ values for cationic solutes in V$^-$ vesicles are remarkably high: ϵ is 72% for R6G and 55% for Dox. These values are much higher than those for the anionic solutes in V$^+$ vesicles and may stem from the larger excess charge present in V$^-$ (i.e., 13.5 vs 6.8 mM). In addition, this difference may also lie in the relative lipophilicities of the counterions for the two surfactants, with these being tosylate in the case of CTAT and sodium in the case of SDBS. Tosylate (*p*-toluene sulfonate) is a hydrophobic counterion and will mostly (>90%) remain bound to the trimethylammonium headgroup in CTAT, with the aromatic ring of tosylate intercalating into the vesicle bilayer.[9,14] The bound tosylate counterions will reduce the cationic charge of the bilayer, and

Table 1. Apparent Encapsulation Efficiencies and Vesicle Radii[a]

probe molecule	ϵ (apparent encapsulation efficiency)		vesicle radius after SEC by DLS (nm)	
	CTAT-rich V$^+$	SDBS-rich V$^-$	CTAT-rich V$^+$ (81 ± 13)	SDBS-rich V$^-$ (98 ± 6)
CF	24 ± 4%	1.0 ± 0.4%	87 ± 5	91 ± 8
LY	40 ± 20%	4%	208 ± 18	96 ± 3
SR101	32.8%	8.2%	122 ± 38	84 ± 7
R6G	0.07 ± 0.1%	72 ± 3%	156 ± 24	109 ± 16
Dox	0%	55%	143 ± 32	93 ± 4

[a] The ϵ values were determined as described in the Experimental Section. ϵ includes contributions from probe molecules that are both encapsulated in the inner water pool and adsorbed at the vesicle bilayer. Vesicle radii are reported for the vesicle-containing SEC fractions only. Vesicle radii were determined by DLS, and the radii for neat vesicles (no probe molecules) obtained after SEC are given in parentheses in the column headings. Radii for V$^+$ and V$^-$ samples before SEC are 74 and 70 nm, respectively.

in turn, the strength of interactions between anionic solutes and the bilayer will be reduced. In comparison, the sodium counterions in SDBS will be largely dissociated; therefore, the sulfonate headgroups will present a strongly negative bilayer surface for the electrostatic binding of cationic moieties.

Next, we turn briefly to the issue of solute adsorption. In our previous paper, we reported that electrostatic adsorption of CF to the V$^+$ vesicle bilayer made a significant contribution to the apparent encapsulation value, ϵ. The contribution from electrostatics was obtained by adding CF to preformed V$^+$ vesicles and then measuring the apparent encapsulation. This resulted in an ϵ value that was 75% of that measured by the conventional method. In the present study, we have conducted similar experiments with the cationic R6G dye and found that if the dye is added to premade V$^-$ vesicles we obtain an ϵ that is ca. 85% of the value reported in Table 1. Thus, the electrostatic contribution to solute binding is crucial for both V$^+$ and V$^-$ vesicles. Dye adsorption at the vesicle bilayer is being investigated systematically and will be the subject of a separate paper.[26]

Long-Term Solute Release from Vesicles. In our previous paper, we used the self-quenching properties of CF fluorescence to evaluate the release rate of CF from V$^+$ vesicles.[13] This is a well-established method of studying solute release from vesicles,[27–30] but it can be applied only to fluorescent solutes that show the self-quenching phenomenon. Here, we measure release rates using a more general procedure based on SEC that can be applied to a wide range of solutes, including nonfluorescent ones. The details of the procedure are described in the Experimental Section. Briefly, we start with a batch of solute-bearing vesicles, with the free dye removed using SEC. The amount of solute remaining in the vesicles is evaluated at a later time by removing an aliquot and performing a small-scale separation using a quick-spin column. Our method directly yields the apparent encapsulation, ϵ, as a function of time.

Figure 3 shows ϵ versus time data for three different solute/vesicle combinations. The data for CF in V$^+$ vesicles (red squares) are quite comparable to our previous results for the same CF/V$^+$ system using the self-quenching of CF. The new data give a half-life for CF in the vesicles of 114 days, whereas previously we had estimated an 84 day half-life for the same system from a more limited data set. Also shown in Figure 3 are the results

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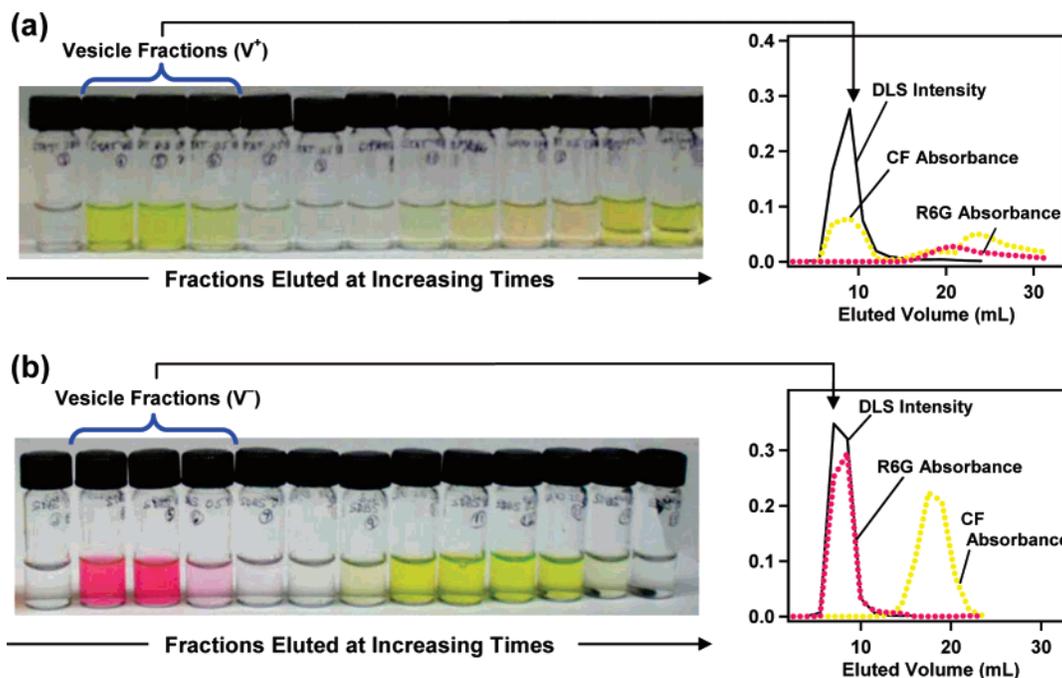(30) Rex, S. *Biophys. Chem.* **1996**, *58*, 75.

Figure 4. Vesicle-mediated separation of mixtures of R6G and CF dyes using (a) V^+ vesicles and (b) V^- vesicles. The plots on the right show the DLS intensity, which is proportional to vesicle concentration, and the UV absorbances, which are proportional to the concentrations of R6G and CF, as a function of eluted volume. The results show that the V^+ vesicle fractions contain 31% of the initial amount of CF with no detectable R6G whereas the V^- vesicle fractions contain 88% of the initial amount of R6G with no detectable CF. This can be seen visually by the yellowish hue of the V^+ vesicle fractions compared to the pinkish hue of the V^- vesicle fractions.

for LY in V^+ vesicles (open red circles) and R6G in V^- vesicles (black circles). The ϵ values for both LY and R6G start out significantly higher than that of CF in V^+ but decay over the course of a few days to a comparable value of ϵ (from 0.2 to 0.3). R6G has the largest initial rate of dye leakage, which could be because it is captured to a much greater extent than the other two dyes (Table 1). On the whole, our new results confirm that oppositely charged solutes can be held for very long periods of time by catanionic vesicles. For comparison, the half-life for CF in EYPC liposomes is only about 2 days,[13] which means that the surfactant vesicles retain dye for about 40−60 times as long.

Separation of Oppositely Charged Solutes by Vesicles. The strong electrostatic interactions between catanionic vesicles and ionic solutes may be harnessed for an interesting potential application: separation of an oppositely charged solute from a solute mixture. To test this possibility, we prepared vesicles with equimolar mixtures of two solutes, one cationic (R6G) and the other anionic (CF). The total solute concentration was maintained at either 0.5 or 1.0 mM, and the experiments were done with both V^+ and V^- vesicles. Experiments with these solute mixtures were performed and analyzed in exactly the same way as the determination of ϵ. To account for the overlapping of the dye spectra, we subtracted a scaled spectrum of pure R6G from the total spectrum in order to find the peak absorbance of CF.

Results from an equimolar mixture of CF and R6G, at a total dye concentration of 0.5 mM, in V^+ vesicles are shown in Figure 4a. While 31% of the anionic CF is carried through the SEC column within the V^+ vesicle band, no detectable R6G emerges with the vesicles. In short, the V^+ vesicles are able to capture the anionic dye selectively and thereby separate it from the dye mixture. The opposite behavior is observed for the same dye mixture in V^- vesicles (Figure 4b). In this case, the V^- vesicle band emerging out of the SEC column contains 88% of the R6G, but the amount of CF in this band is negligible. Thus, the V^- vesicles are able to bind and separate the cationic dye from the dye mixture. To our knowledge, this is the first demonstration

of using surfactant vesicles as a means to separate ionic compounds. We conducted the same experiments with a total dye concentration of 1.0 mM CF and R6G and obtained similar results. We have also conducted separation experiments using the anionic dye LY and the cationic drug Dox, and we again observed very efficient separation using vesicles, much as in Figure 4. The results for LY and Dox are given in Supporting Information. These results demonstrate that charged surfactant vesicles can be used as separation media for similarly sized and oppositely charged molecules. The use of surfactants in separations science is not new. For example, micelle-containing mobile phases in liquid chromatography were pioneered by Armstrong and Henry,[31] and since then, micellar liquid chromatography has been widely used for a variety of applications including the evaluation of drug candidates.[32,33] Surfactant vesicles themselves have also been used as pseudostationary phases for electrokinetic chromatography with good results.[34]

Effects of Solutes on Vesicle Stability. It is clear from the above data that catanionic vesicles have the remarkable capability of binding and slowly releasing oppositely charged solutes, but for these vesicles to be used in applications, certain questions relating to vesicle stability need to be answered. For example, what effect, if any, does the solute have on vesicle size and stability? Why is it important to use low solute concentrations (<5 mM)? In other words, what happens to the vesicles when higher concentrations of solute are added? Also, for many biological applications, the pH and ionic strength of the external solution have to be strictly controlled. How will pH and ionic strength affect vesicle stability, and more importantly, how will they influence the electrostatic binding of solutes to vesicles? Many of these aspects are being studied in detail in our laboratories

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and will be addressed in future papers. In the present study, we briefly examine using SANS and DLS the effect on vesicle stability upon addition of solute.

As noted previously, we used a low solute concentration (1 mM) to ensure the stability of our vesicle formulations. At concentrations above 5 mM, the solutes seemed to compromise the integrity of the vesicles, as revealed by large changes in vesicle size (from DLS) and/or by the formation of a precipitate over time. Even at a concentration of 1 mM, some solutes may have a large effect on vesicle morphology. To study these aspects in some detail, we have used DLS and SANS. First, we performed DLS on the purified vesicles obtained from the SEC column (after removing all of the free solute) and compared their sizes to those for neat vesicles (no solute). DLS gave radii of 74 nm for neat V^+ vesicles and 70 nm for neat V^- vesicles. Passing these neat vesicles through an SEC column changed their sizes slightly, and the new radii were 81 nm for V^+ and 98 nm for V^- vesicles. The incorporation of 1 mM solute had a negligible effect on vesicle size in some cases but a large effect in others (Table 1). For example, both V^+ and V^- vesicle radii were essentially unchanged by 1 mM CF. However, whereas 1 mM LY anionic solute had no effect on V^- vesicles, it induced a 2.5-fold increase in the radii of V^+ vesicles. Interestingly, the effects on vesicle size seem to be more significant for V^+ vesicles than for V^-, and this is true for both cationic and anionic solutes. Changes in hydrodynamic radii are an important indicator that structural changes are occurring in the presence of ionic solutes. Results from our laboratory indicate that these vesicles are stable at elevated salt concentrations (Supporting Information). Thus, it is unlikely that the size increases are due to simple flocculation brought about by increasing ionic strength upon addition of solute. Direct imaging techniques such as cryogenic electron microscopy (cryo-TEM) may be able to reveal the solute-induced changes indicated by DLS. However, at this time, we do not have access to cryo-TEM and instead have turned to SANS, which is a sensitive probe of nanoscale structure.

SANS data are reported in Figure 5 for two mixtures of vesicles and oppositely charged solutes: V^+/CF and V^-/R6G. Figure 5a shows data for the neat V^+ vesicles with no solute and for the same vesicles prepared with 1 mM CF and purified by SEC. Additionally, data are shown for a sample of the same vesicles with 1 mM CF added after preparation (i.e., with the dye adsorbed on the bilayers), followed by purification via SEC. Passing the vesicles through an SEC column lowers the vesicle concentration, which is why the latter two data sets show a lower intensity. Nevertheless, all three curves have approximately the same shape, and all show a limiting slope of −2 at low q, which is indicative of scattering from vesicle bilayers.[35,36] Similar observations also hold for Figure 5b, which shows data for neat V^- vesicles and for the same vesicles with 1 mM R6G followed by SEC. Again, the intensity levels are lower because of SEC purification, but the −2 slope is maintained. Thus, SANS confirms that all of these samples contain intact unilamellar vesicles. In all cases, there appear to be subtle differences in vesicle size and polydispersity upon incorporation of solute. Further analysis of the SANS data is beyond the scope of the present article. However, we are putting together an expanded study of solute/vesicle interactions using SANS, accompanied by detailed modeling, and this will be communicated in the future. The combined observations from DLS, SANS, and SEC experiments strongly
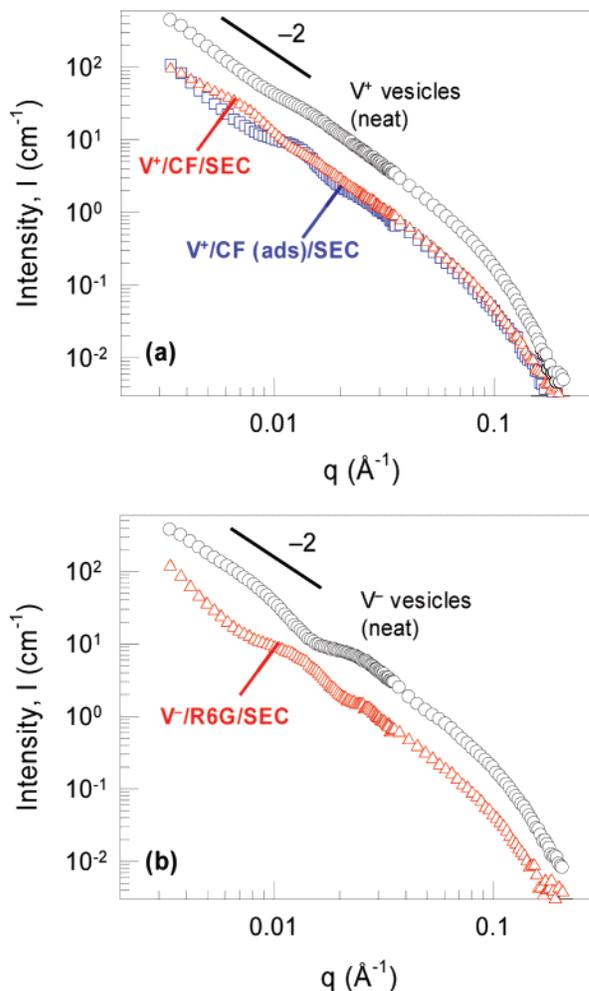

Figure 5. SANS data for neat and dye-containing V^+ and V^- samples. (a) Scattering data for V^+ samples. A −2 slope characteristic of bilayer scattering is observed for neat vesicles (○) and when CF is added before (△) or after (□) vesicles are formed. (b) Scattering data for neat V^- samples (○) and for V^- loaded with R6G (△). As in plot a, the data from both samples are consistent with the presence of vesicles. The neat vesicle samples (○) were run as-prepared with 1% total surfactant concentration. The other samples (□, △) were diluted by approximately 4-fold as a result of undergoing SEC.

indicate that the vesicles remain intact in the presence of dyes, although precipitation has been observed when dye concentrations exceed 5 mM.

Implications for Applications Involving Surfactant Vesicles. As discussed in the Introduction, important potential applications for vesicles are in storage or controlled release applications (e.g., in drug delivery, agrochemicals, and cosmetics). This is an area of great promise, as evidenced by the success of the liposome-based delivery of the chemotherapeutic drug doxorubicin.[37] So far, most of the research in this area has focused on phospholipid vesicles (liposomes). Researchers working with liposomal encapsulation technologies have discovered many improvements in solute loading efficiency and long-term solute retention. For instance, there have been numerous strides toward enhancing the long-term retention capabilities of liposomes by varying the lipid composition of the bilayer or by adding cholesterol to the bilayer.[38−40] Similarly, there have been successful attempts to reach extremely high loading efficiency of drugs such as

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doxorubicin by employing chemical gradients.[41] Loading of DNA into vesicles can be greatly improved with the addition of cationic lipids[42] or by using micrometer-sized vesicles.[43] Such advances in liposomal preparations have led to important advances in chemotherapy. Here, we have shown that catanionic vesicles may show promise as a simple alternative to more expensive and complex liposomal-based approaches. In short, catanionic vesicles could be an attractive alternative to phospholipid vesicles (liposomes) for many controlled-release applications. For therapeutic applications, a range of toxicological studies will first need to be conducted with these catanionic vesicles. In this regard, recent studies by Kuo et al. are promising in that they show catanionic vesicles to be nontoxic toward mouse fibroblast and liver cells.[44]

4. Conclusions

In this study, we have measured the apparent encapsulation of five different charged solutes in catanionic CTAT/SDBS vesicles and have used these vesicles to separate an oppositely charged solute from a solute mixture. We have shown that solutes can be weakly encapsulated by like-charged vesicles but are captured much more efficiently in oppositely charged vesicles. Efficient containment in vesicles of opposite charge is due to strong electrostatic interactions between the solute and bilayer. At 1 mM solute concentration, apparent encapsulation values range from 24 to 72%. Long-term solute release kinetics were monitored for three vesicle/solute preparations. Release profiles show that all dyes are held for long periods of time but that both R6G and, to a lesser extent, LY have an initial rapid dye release that brings them close to the initial value for CF. Highly efficient separations of mixtures of similarly sized but oppositely charged probe molecules were performed by using vesicles to control the elution time of ionic probe molecules in SEC. Results from DLS and SANS experiments are also included to measure the effects of solute loading on vesicle integrity and stability. DLS results show that V^+ samples appear to undergo an increase in radius when solutes are added at a concentration of 1 mM but that the effect on V^- vesicles is negligible. SANS experiments confirm that vesicles remain intact when loaded with strongly interacting probes. The effects of ionic strength and pH are being examined in detail and will be the subject of a separate paper.

Supporting Information Available: Additional SEC and separations results and effects of salt concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

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Data and Images from SEC Experiments for Evaluation of Apparent Encapsulation Efficiency (ε). The four figures below are counterparts to Figure 2 of the main paper with photographs, DLS intensity (solid line), and UV-vis absorbance data (dotted line), for each of the solutes studied. The encapsulation values for each solute are reported in Table 1 of the paper.

R6G

A — V⁺

C — V⁻

B

D

Dox

A — V⁺

C — V⁻

B

D
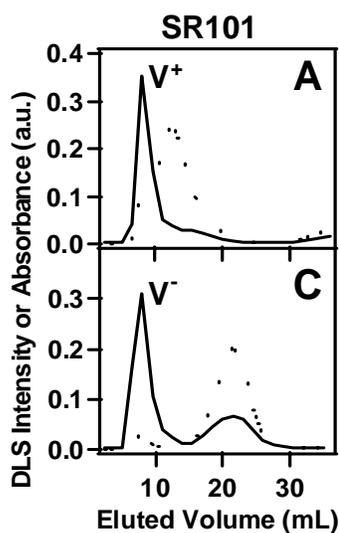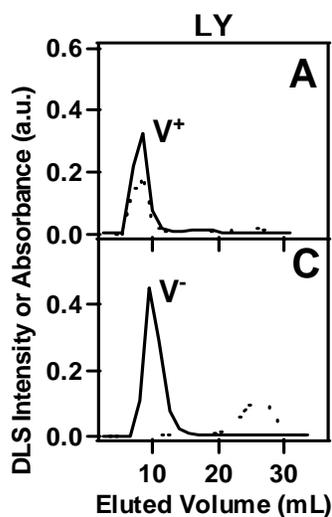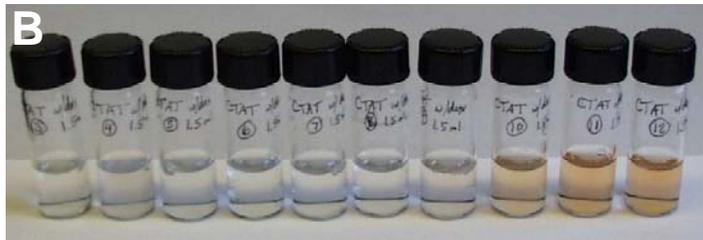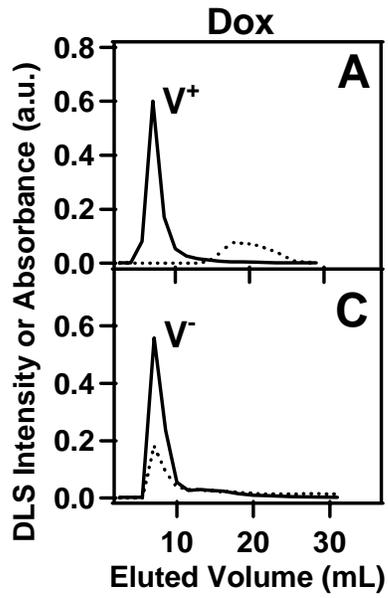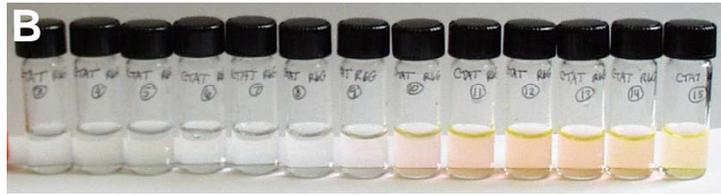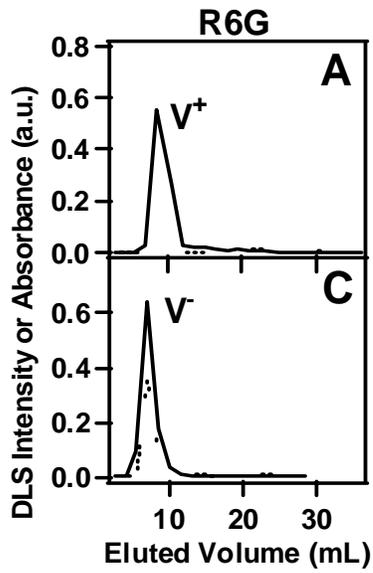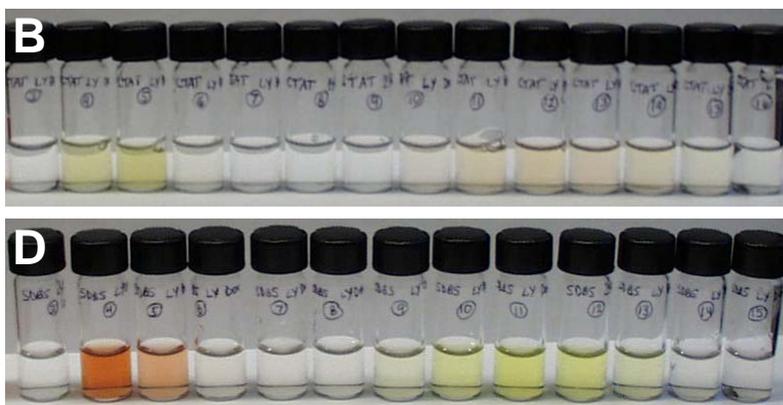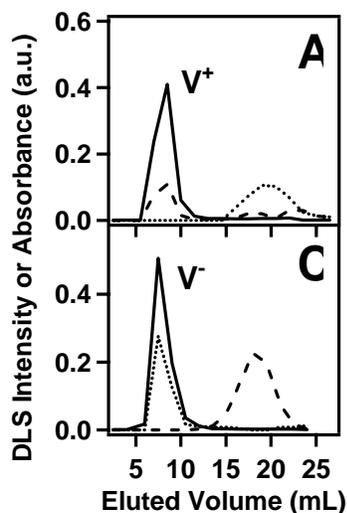
Separations Performed on a Mixture of LY and Dox. In addition to using catanionic vesicles to separate the dyes CF and R6G, we also conducted separation experiments using the anionic dye, LY and the cationic drug, Dox. The separation illustrated below was performed on an equimolar solution of the two dyes. The total dye concentration was 1 mM. We again observed very efficient separation using vesicles, much like in Figure 4 of the main paper. The figure below shows the DLS intensity (solid line) and UV-vis absorbance of LY (dashed line) and Dox (dotted line) for each eluted fraction. The vesicle band for the V^+ sample contained 30% of the LY with no detectable Dox, while the vesicle band for the V^- sample contained 63% of the Dox with no detectable LY.

Effect of Salt Concentration on Vesicle Stability. Preliminary studies of vesicle stability as a function of salt concentration were conducted in both NaCl and $CaCl_2$ solutions. The results are documented in the tables below.

CTAT-rich with NaCl

Sample	Radius (nm)
1M	99
1.5M	68
2.0M	60
2.5M	63
3.0M	73
3.5M	78
3.75M	84

CTAT-rich with $CaCl_2$

Sample	Radius (nm)
1M	101
1.5M	92
2.0M	77
2.5M	81
3.0M	120
3.5M	130
3.75M	100

SDBS-rich with NaCl

Sample	Radius (nm)
0.5M	60
0.75M	67
0.8M	70

SDBS-rich with $CaCl_2$

Sample	Radius (nm)
0.001M	81

For SDBS-rich vesicles, precipitate was observed at NaCl concentrations above 0.8 M and at $CaCl_2$ concentrations above 1.0 mM.