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Ripening of Catanionic Aggregates upon Dialysis

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We have studied the dialysis of surfactant mixtures of two oppositely charged surfactants (catanionic mixture) by combining HPLC, neutron activation, confocal microscopy, and NMR. In mixtures of *n*-alkyl trimethylammonium halides and *n*-fatty acids, we have demonstrated the existence of a specific ratio between both surfactant contents (anionic/cationic ≈ 2:1) that determines the morphology, the elimination of ions, and the elimination of the soluble cationic surfactant upon dialysis. In mixtures prepared with lower anionic surfactant contents, ill-defined aggregates are formed, and dialysis quickly eliminates the ion pairs (H^+X^-) formed upon surfactant association and also the cationic surfactant until a limiting 2:1 ratio is reached. By contrast, mixtures prepared above the anionic/cationic 2:1 ratio form micrometer-sized vesicles resistant to dialysis. These closed aggregates retain a significant number of ions (30%) over 1000 hours, and dialysis is unable to eliminate the soluble surfactant. The interactions between surfactants have been estimated by measuring the partitioning of the CTA molecules between the catanionic bilayer, the bulk solution, and mixed micelles when they exist. The mean extraction free energy per CTA in the membrane has been found to increase by $1k_BT$ to $2k_BT$ as the soluble surfactant is depleted from the bilayer, which is enough to stop the dialysis. The vesicles produced above the anionic/cationic 2:1 ratio are formed by frozen bilayers and are resistant to extensive dialysis and therefore show an interesting potential for encapsulation as far as durability is concerned.

Introduction

Mixtures of oppositely charged surfactants, also called catanionic mixtures, form a wide range of morphologies depending on their composition in water and surfactants, such as mixed micelles,[1] lamellar phases,[2,3] cubosomes, hexosomes,[4] and vesicles.[5] In particular, it is now accepted that the catanionic vesicles form spontaneously and are stabilized by the spontaneous curvature and the entropy of mixing of the two surfactants in the bilayer.[6] The potential of the catanionic vesicles for encapsulation and release of water-soluble compounds has been evaluated, and it has been found that the permeability of the vesicle walls is generally comparable, and sometimes significantly lower, than that of vesicles prepared from liposomes (data in Table 1 calculated from refs 7−13). An alternative and now well-established way to use catanionic mixtures for drug delivery is to use a pharmaceutically active compound directly as one of the amphiphiles involved in the ion pair. In this case, the solute can be considered to be encapsulated inside the bilayer.[14−16]

In both contexts, it is important to understand how the ripening of catanionic aggregates may or may not occur. The simplest case is to consider a release driven by purely entropic phenomena (i.e., that the catanionic aggregate is in contact with an infinite volume of solvent). Despite the usual decrease in the critical micelle concentration of the catanionic mixtures as compared to that of the single-surfactant solutions,[17] this situation is eventually expected to lead to a significant loss of surfactant from the aggregate although it has been neglected in previous studies using the dialysis of catanionic mixtures.[12,18] In the first strategy (encapsulation inside a vesicle), loss of surfactant will determine the possible disruption of the vesicle or undesirable surfactant release. In the second strategy (encapsulation inside the bilayer), this directly determines the kinetics of release but also the final availability of the amphiphilic active compound.

In this article, we focus on the processes occurring during the dialysis of mixtures of myristic acid and cetyl trimethylammonium halides (bromide and chloride). The dialysis is expected to remove the ion pairs (H^+Br^- or H^+Cl^-) released upon surfactant association and also alter the surfactant composition in the catanionic mixture. We will examine how the interaction between

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Table 1. Permeability of Various Vesicles Calculated from Literature Data

vesicle composition	solute	vesicle diameter	half-life	vesicle permeability ($cm \cdot s^{-1}$)	calculated from ref
50% Span 60/50% fatty alcohol	carboxyfluorescein	~300 nm	~1−5 h	1.9×10^{-10}	7
Span/cholesterol/dicetylphosphate	carboxyfluorescein	~1 μm	~6−10 h	3.2×10^{-10}	8
25% PGDS/50% fatty acid or cholesterol/25% polysorbate 80	ketorolac tromethamine	~5 μm	~30 h	5.3×10^{-10}	9
70% POPC + 30% cholesterol	carboxyfluorescein	~100 nm	~30 h	1.1×10^{-11}	10
50% SOS/50% CTAB	glucose	unknown	< 2h		11
SDBS/CTAT, various compositions	glucose	~100 nm	~5 h	6.4×10^{-11}	12
CTAT 70% + SDBS 30%	carboxyfluorescein, lucifer yellow, rhodamine 6G, sulforhodamine 101, and doxorubicin hydrochloride	~100 nm	~84 days	1.6×10^{-13}	13

surfactants and in some cases the morphology of the aggregates determine the kinetics of release of ions and surfactants upon dialysis.

Materials and Methods

Preparation of the Catanionic Mixtures. Myristic acid (Fluka) was recrystallized twice from hot acetonitrile. All other products (cetyl trimethylammonium chloride, cetyl trimethylammonium bromide, myristyl trimethylammonium bromide, lauric acid, and palmitic acid) were purchased from Sigma and used as such. Solutions were prepared with Milli-Q water.

The catanionic mixtures have been prepared as follows: known amounts of fatty acid and cationic surfactant are mixed with water at a typical weight fraction of surfactant of 1% and heated to 50 ± 0.1°C for 3 days. The resulting mixture is dialyzed against a 100-fold volume of water (Pierce regenerated cellulose membranes, 3500 Da cutoff). In a typical experiment, the dialysis water is changed after 1, 4, 24, and 48 h and 5 days of dialysis. The results have been found to be independent of the number of dialysis water renewals.

HPLC. HPLC was performed using a C18 HyPurity column and a Sedex 75 refractometer in the 30−40 °C range under a nitrogen pressure of 2.9 bars. The continuous phase was a 70/15/15 (vol/vol/vol) mixture of acetone/methanol/water, acidified with 0.06% trifluoroacetic acid, and was injected at a rate of 1.5 mL/min. Under these conditions, alkyl trimethylammonium and fatty acid species give well-resolved signals at 3.5 and 6 min, respectively. Prior to analysis, the sample aliquots were dissolved in a 4-fold ethanol mass. The peak intensities were calibrated using reference solutions prepared in the same ethanol/water ratio.

Neutron Activation. The amounts of chloride and bromide in the samples have been measured from the gamma emission spectrum after neutron activation. All samples irradiations were carried out at the Orphée nuclear reactor (CEA Saclay, France) at a thermal neutron flux of 1.2×10^{13} $n \cdot cm^{-2} \cdot s^{-1}$.[19] Because it is possible to recover the irradiated samples within a few minutes, short half-life radioisotopes, such as ^{80}Br ($\tau_{1/2} = 18$ min) and ^{38}Cl ($\tau_{1/2} = 37.3$ min), can be measured. About 80 mg of sample was sealed into a pure polyethylene container with 3 to 6 mg of IRMM-530 gold wire (Al/Au alloy containing certified 0.100% Au) as a neutron flux monitor and irradiated for 300 s. Irradiation was followed by four radioactivity countings at four different decay times (decay of 3 minutes, counting of 2 minutes; decay of 5 minutes, counting of 5 minutes; decay of 13 minutes, counting of 15 minutes; and decay of 5 to 10 h, countings of 1 h).

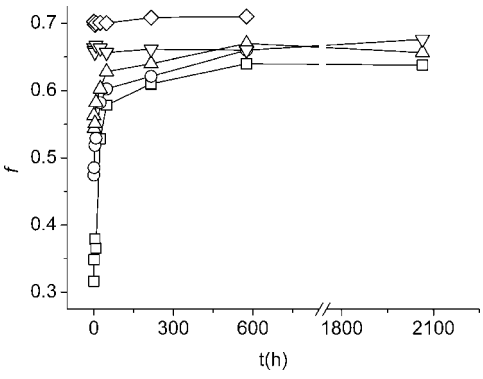

Figure 1. Evolution of the molar fraction of myristic acid f in the catanionic mixtures as a function of dialysis time, as evaluated from HPLC. The initial molar fractions f_0 of myristic acid are 0.35 (□), 0.55 (△), 0.66 (▽) and 0.70 (◇).

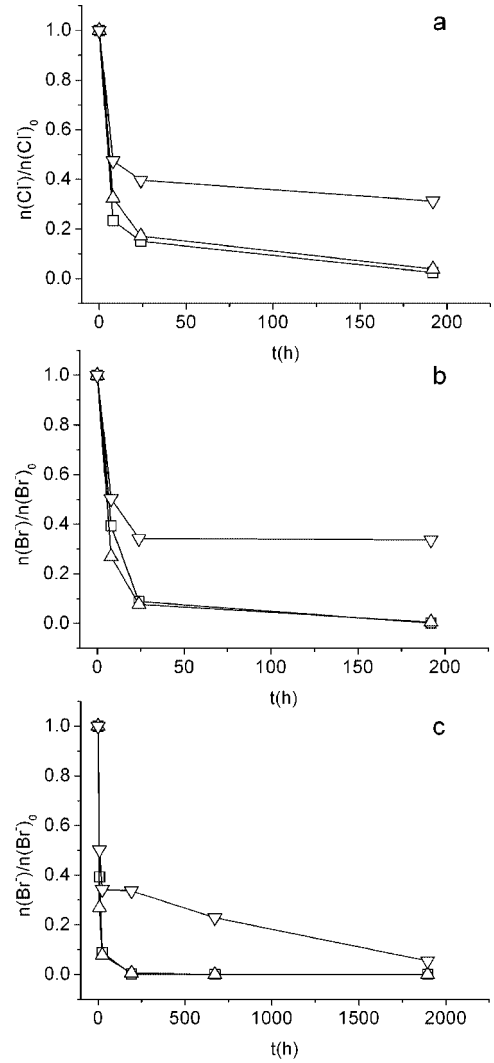

Figure 2. Residual counterion content in the (a) myristic acid/cetyl trimethylammonium chloride and (b and c) bromide mixtures as a function of dialysis time, as evaluated by neutron activation. The initial mixtures have myristic acid molar fractions f_0 of 0.35 (□), 0.55 (○), and 0.66 (▽). The ion contents are expressed as residual counterion fractions.

The γ emission was measured using a Canberra GX 2020 HPGe detector (coupled to a Canberra multichannel system) with 1.70 keV resolution as determined from the 1332 keV γ-ray peak of ^{60}Co. The analysis of γ-ray spectra was carried out using homemade software.[20] The compilation data (mean and standard deviation) were calculated from the average values, taking into account the number of results. In general, results are given with 3 times the standard deviation (3σ), corresponding to a confidence interval of 99%.

Confocal Microscopy. Confocal microscopy observations have been performed with an Olympus IX 81 microscope equipped with a Fluoview FV1000 confocal head. The samples have been labeled with Oregon green 488 isothiocyanate (Invitrogen) excited at 488 nm, and the emitted fluorescence has been collected through a 505−526 nm filter.

WAXS. Wide-angle X-ray scattering patterns (WAXS) were collected using a home-build pinhole-geometry camera at $\lambda = 0.71$ Å [21] and recorded on an image plate with a typical exposure time of 10 h. Subtraction of the empty sample cell (two kapton windows) was performed prior to radial averaging. Because of the high dilution of the sample, the crystalline peak of interest at $q = 1.51$ Å^{-1} is superimposed on the broad peak of water with a large relative intensity. No subtraction of the water signal has been performed to avoid any strong distortion of the pattern.

^{1}H NMR. The total number of CTA molecules in the form of micelles and monomers in coexistence with larger undetectable aggregates has been selectively measured with ^{1}H NMR at 500 MHz and 293 K on a Bruker Avance II spectrometer equipped with a *z*-gradient HXN 5 mm probe head. The samples were prepared in and dialyzed against H_2O with 10% D_2O for locking. A 3-9-19 Watergate pulse sequence[22] was used with an interpulse delay of 140 μs (next null at 3600 Hz from the carrier frequency) for attenuation of the water peak. The free induction decays have been apodized by a 2 Hz Lorenzian function before Fourier transformation. Manual phasing and baseline correction were then applied. The concentration of myristic acid has been extracted from the areas of the peaks at 2.11 and 1.53 ppm, calibrated with sodium myristate solutions at high pH. The concentration of CTAB has been extracted from the areas of the peaks at 3.4, 3.16, and 1.77 ppm. The selectivity of the measurement for the free surfactant molecules in bulk solution (as opposed to the molecules in the catanionic bilayers) has been tested as described below by monitoring the evolution of samples dialyzed against solutions of CTA at given concentrations.

Results and Discussion

Heating to 50°C allows the progressive dissolution of myristic acid crystals by cetyl trimethylammonium halide, leading to a white homogeneous solution. However, as already reported, this mixture is unstable at room temperature and forms a white solid present either as a supernatant or a precipitate, depending on composition.[23] The formation of the mixed surfactant bilayer indeed releases inorganic ions (HBr or HCl) that screen out the electrostatic interactions and leads to the aggregation of the bilayers into hydrated crystals. However, this process is slow enough (several days) to allow treatment by dialysis before aggregation occurs. On one hand, the dialysis is expected to eliminate the ion pairs (HBr or HCl) before flocculation occurs and to stabilize the catanionic aggregates. However, on the other hand, it is also expected that soluble surfactant molecules will be eliminated, leading to an alteration of the composition of the surfactant. The evolution of the composition of the catanionic mixture upon dialysis at room temperature has therefore been monitored with time using HPLC (Figure 1).

Evolution of Surfactant Composition. HPLC has been used to measure the evolution of the concentration of each surfactant in the catanionic mixture during dialysis. From these concentrations, it is possible to measure the evolution of the molar fraction f of myristic acid with respect to the total amount of surfactant, as defined by

$$f = \frac{c(\text{myristic})}{c(\text{myristic}) + c(\text{CTA})} \quad (1)$$

It is observed that the general behavior of f depends on the initial composition of the catanionic mixture, prior to any dialysis. In catanionic mixtures prepared with initial molar fractions (f_0) below 0.63 (Figure 1, squares, circles, and upward triangles), f increases with time but finally reaches a plateau value in the 0.60−0.66 range within 200−300 h. This increase in f corresponds to an enrichment in myristic acid. This reflects the progressive elimination of CTA by dialysis, whereas the fatty acid is retained in the aggregates because of its low solubility in water. Very strikingly, the molar fraction f tends towards an asymptotic value close to 0.66, independent on the initial composition. Additional changes in the dialysis bath against fresh water have no effect on this final surfactant composition, up to total dialysis times of over 2000 h. The elimination of soluble surfactant is therefore kinetically blocked at this peculiar molar fraction instead of reaching a value of $f = 1$ that would correspond to the complete elimination of the soluble surfactant. This complete elimination of CTA could not be observed experimentally either because the amounts eliminated after each dialysis bath renewal are too low or because the equilibration times finally reach values that are significantly higher than the experimental times (several months).

Accordingly, in catanionic mixtures prepared with an initial molar fraction above this critical value of 0.60−0.66 (Figure 1, downward triangles and diamonds), the surfactant composition remains unchanged upon dialysis. Because of its poor solubility in water, no elimination of myristic acid is possible upon dialysis. Therefore, f cannot decrease to reach the limiting value of 0.66 and remains unchanged. We have therefore demonstrated the existence of a critical molar fraction of myristic acid $f_{\text{critical}} \approx 0.66$ (corresponding to myristic acid/CTA 2:1) with the following properties: preparations richer in CTA ($f_0 < f_{\text{critical}}$) will lose CTA molecules upon dialysis until f_{critical} is reached. In preparations richer in myristic acid ($f_0 > f_{\text{critical}}$), the surfactant composition will not change upon dialysis.

Evolution of Ion Content. Dialysis is also expected to remove completely the inorganic ion pairs (HBr or HCl) released upon formation of the catanionic bilayers, but quantitative chemical analysis of the halide content has demonstrated a more subtle behavior. The residual concentrations of counterions Cl^- and Br^- as a function of dialysis duration have been evaluated with neutron activation. For mixtures prepared with initial compositions of $f_0 < 0.66$, the counterions are quickly removed by dialysis (Figure 2a,b, squares and upward triangles): half of the ions are washed out within a few hours, 90% of the ions are eliminated within 24 hours, and only traces are detected after 200 hours. The removal of counterions from mixtures prepared with initial compositions of $f_0 = 0.66$ show a drastically different path: first, about 70% of the ions are quickly eliminated from the mixture (Figure 2a,b, downward triangles). This first step occurs with an extrapolated half-life of a few hours. Subsequently, the remaining 30% of ions are slowly eliminated with a characteristic half-life of 1000 h (Figure 2c, downward triangles). We emphasize that

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this two-step ion elimination is observed only in surfactant mixtures prepared with an initial molar fraction of myristic acid of $f_0 \geq 0.66$.

Chemical analysis of the halide content therefore demonstrates that the elimination of ions from the catanionic mixtures by dialysis is not as trivial as expected. The critical molar fraction of myristic acid $f_{critical} \approx 0.66$ (corresponding to myristic acid/CTA 2:1) observed above also determines how the ions are eliminated: preparations initially richer in CTA ($f_0 < f_{critical}$) will quickly and completely eliminate all of the Br^- or Cl^- ions released upon surfactant association. By contrast, in preparations initially richer in myristic acid ($f_0 > f_{critical}$), about 30% of the ions are retained in the mixture over significant amounts of time (half-life of 1000 h).

Morphology of the Catanionic Aggregates. The difference in the ion elimination process can be correlated with the morphology of the catanionic aggregates as monitored by confocal microscopy (Figure 3). The added anionic fluorescent probe (Oregon Green 488) shows preferential adsorption onto the catanionic aggregates that are known to be positively charged in the final state.[24] The morphology of the aggregates has been observed after stabilization of the surfactant composition as evidenced by HPLC (i.e., for dialysis times >24 h). For initial molar fractions of $f_0 < 0.66$, the dialysis yields ill-defined aggregates (Figure 3a). By contrast, the samples prepared at $f_0 \geq 0.66$ yield vesicles with a typical diameter of $5-10\,\mu$m (Figure 3b). The distance between vesicles is significantly shorter than the diameter, producing a close-packed appearance. Dialysis after a 3-fold dilution of the initial 1% myristic acid/CTAX solution yields well-individualized vesicles (Figure 3c). The difference in morphology of ill-defined aggregates or vesicles depends only on the initial molar fraction of myristic acid f_0 but is uncorrelated with the final molar fraction, which is close to 0.66 in all cases.

Strikingly, the absence of vesicles for preparations at $f_0 < f_{critical}$ coincides with the quick elimination of ions described above, whereas the presence of vesicles at $f_0 > f_{critical}$ coincides with the occurrence of the two-step slow counterion elimination. The first, quick ion elimination is therefore interpreted as the diffusion of ions through the solution that are outside the vesicles. The second, slow step is assigned to the diffusion of ions inside the vesicles, which is hindered by permeation through the vesicle bilayer. Accordingly, this second process is not observed if no vesicles are produced (i.e., at initial surfactant compositions of $f_0 < 0.66$). Instead, a significant loss in surfactant from the membrane (e.g., around one CTA surfactant molecule out of two for $f_0 = 0.50$) creates a large number of defects in the bilayers. Because the bilayers become rigid as the ion content decreases,[23,25] these defects may trigger mechanical constraints that lead to ill-defined, open morphologies. This suggests indirectly that the vesicles produced at $f_0 > f_{critical}$ are able to encapsulate the halide ions released upon ion pairing. However, more straightforward evidence for this encapsulation inside the aqueous compartment of the vesicles is required because the anions could simply be adsorbed to the vesicle walls. The direct location of the halides in the vesicles (encapsulation vs adsorption) will be presented in a separate contribution.

Morphologies encountered in this work, using ripening via slow extraction at room temperature of CTA from surfactant mixtures initially heated to 50°C, differ profoundly from shapes and features obtained from the same surfactants but with another preparation path. Indeed, slow dissolution at room temperature of surfactants with no HBr or HCl, followed by short temperature cycles (< 1 min at 70°C) produces either

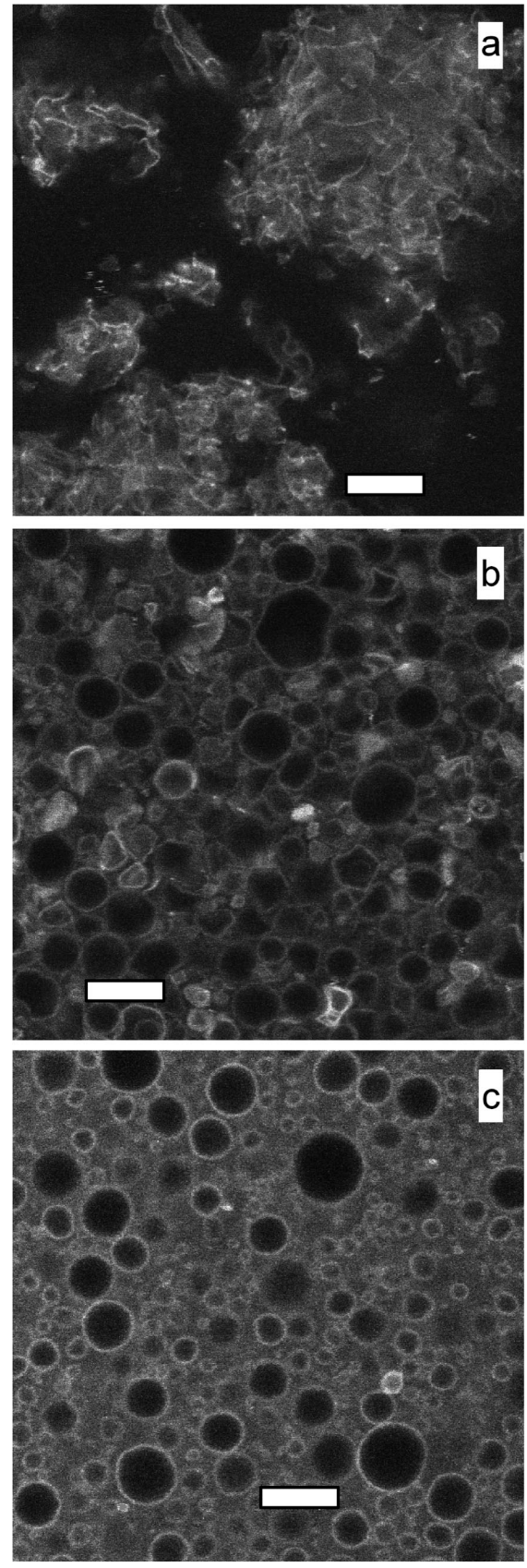

Figure 3. Confocal microscopy pictures of 1% w/w myristic acid/cetyl trimethylammonium bromide mixtures in water, dialysed against water for 600 h and labeled with anionic Oregon green. (a) $f_0 = 0.35$ ($f = 0.64$), (b) $f_0 = 0.66$ ($f = 0.66$), and (c) $f_0 = 0.66$ ($f = 0.66$) diluted 3-fold prior to dialysis. The scale bars are 10 μm.

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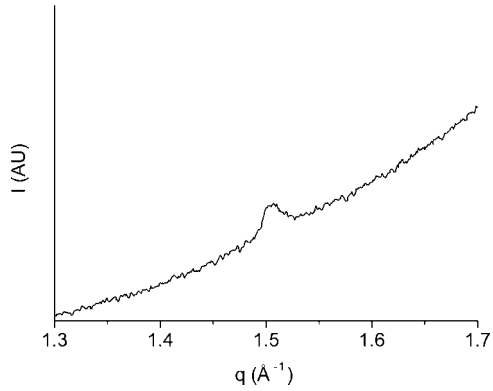

Figure 4. Wide-angle x-ray scattering of an $f_0 = 0.66$ myristic acid/cetyl trimethylammonium bromide mixture (1% w/w) in water, dialysed against water for 7 days.

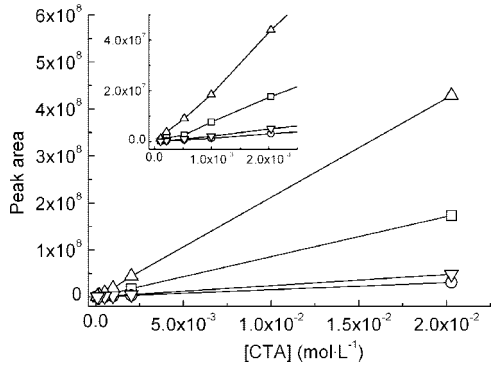

Figure 5. Calibration of monomer titration: peak areas of the ^{1}H NMR spectra of CTAB solutions as a function of CTAB concentration: (△) sum of the multiplet at 3.29 ppm and the singlet at 3.09 ppm, (□) multiplet at 1.75 ppm, (▽) sum of the singlets at 1.34 and 1.26 ppm, and (○) multiplet at 0.85 ppm.

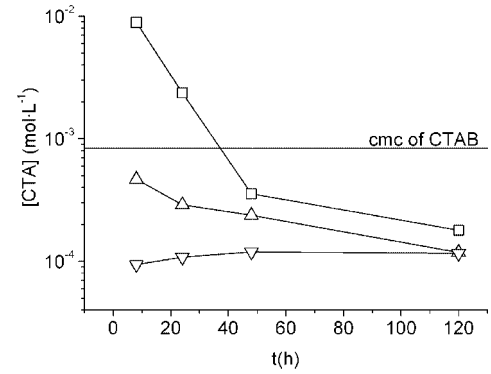

Figure 6. Total concentration of CTA present as monomers and small aggregates vs dialysis time in myristic acid/CTAB mixtures, as evaluated from ^{1}H NMR spectroscopy: (△) $f_0 = 0.35$, (▽) $f_0 = 0.55$, and (□) $f_0 = 0.66$.

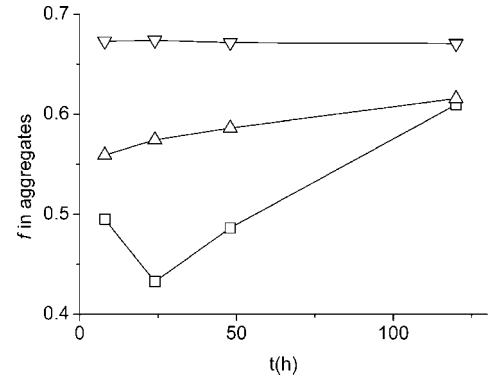

Figure 7. Molar fraction of myristic acid in the catanionic membrane, as evaluated from the HPLC and NMR data: (△) $f_0 = 0.66$, (▽) $f_0 = 0.55$, and (□) $f_0 = 0.35$.

punctuated planes, facetted closed objects,[26] or discs.[27] Both procedures reach similar final compositions but do not lead to the same final morphologies. This indicates that at least one of the two preparations path yields off-equilibrium structures. However, it is usually claimed that catanionic vesicles are in thermodynamic equilibrium and spontaneously form independently of the preparation path. We suggest that this is not the case in the current system because the surfactant bilayers are in the frozen state: this has already been demonstrated in concentrated (10% w/w) solutions prepared from myristic acid and mixtures of CTAOH and CTACl or CTABr.[23] This is also directly evidenced by wide-angle X-ray scattering on 1% w/w mixtures of myristic acid and CTABr at $f_0 = 0.66$ dialysed for 7 days (Figure 4): a sharp peak at $q = 1.51$ Å^{-1} is superimposed on the very broad diffusion peak of water. The sharp peak is characteristic of the packing of frozen alkyl chains in the plane of the bilayers and is assigned to the 110 reflection, degenerated with the 020 of a hexagonal lattice. Such a hexagonal arrangement of surfactants with frozen chains is characteristic of a gel phase with local orthorhombic order.[28]

Full Description of Dialysis. The above-mentioned results from the HPLC, halide quotation and confocal microscopy give the total picture of the evolution of the catanionic mixture upon dialysis. The two most important observations are that the catanionic mixture gets enriched in fatty acid upon dialysis until a fraction of anionic/(anionic + cationic) of around $f_{\text{critical}} = 0.66$ is reached and that the inorganic ion pairs are not always straightforwardly eliminated. The ions initially present are eliminated either directly in the form of CTA^+X^- or H^+X^-, resulting from the association of myristic acid and CTAX. However, the elimination of CTA is blocked by the preferential interaction between surfactants once f_{critical} is reached. Similar kinetic blockings have been reported in the dialysis of lipid/detergent systems[29] but are accompanied by a micelle-to-vesicle transition upon detergent elimination, which has not been observed in our system. As a consequence of this kinetic blocking, in preparations at an initial molar fraction of $f_0 = 0.66$, only inorganic H^+X^- ion pairs are eliminated, whereas at $f_0 = 0.35$, 90% of the X^- ions are eliminated in the form of CTA^+X^- and only H^+X^- is eliminated at the end of dialysis. Understanding this ripening under nonequilibrium conditions is of prime importance as far as the use of catanionic aggregates in encapsulation or vectorization applications is concerned, depending on whether loss or preservation of the composition or morphology is required.

In the case where the sample consists of vesicles ($f_0 \geq 0.66$), the apparent permeability (P) of the surfactant bilayer forming the vesicles to X^- ions can be evaluated using Overton's rule[30] and Fick's law of diffusion from (derivation

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in Appendix)

$$P = \frac{\ln 2}{3}\frac{R}{t_{1/2}} \quad (2)$$

where R is the radius of the vesicle and $t_{1/2}$ is the leakage half-time.

From the diameter of the vesicles (5 μm) and the half-time of the second step (1000 h), the permeability was found to be 1.6×10^{-11} cm·s^{-1}. This value is comparable to the usual permeability of ions through liposome bilayers of opposite charge.[31] In our case, additional phenomena may contribute to the apparent permeability because the H^+X^- pair is created only if both anionic and cationic surfactants approach at a distance that is sufficiently small to allow charge separation. This contribution could involve diffusion phenomena of the surfactants within the membrane.

Coexisiting Free Surfactant and Micelles. The asymptotic surfactant composition reached independently of the initial composition is a property determined by the interaction between surfactants. This interaction can be quantified by measuring the concentration of free surfactant molecules in equilibrium with the surfactant aggregates. ^{1}H NMR with the elimination of the signal from H_2O has been used to measure selectively the concentration of surfactant molecules that are not inserted into the bilayers, as opposed to the concentration of surfactant molecules forming the bilayers. The NMR signal of the larger catanionic colloidal aggregates is expected to vanish because of their slow tumbling motions inducing fast transverse relaxation, so only the concentration c_{fast} of the most mobile surfactant molecules is measured. The experiments have been calibrated using solutions of CTABr in the absence of myristic acid. The peak areas at 3.4, 3.16, and 1.77 ppm (Figure 5) show a linear dependence on the concentration of surfactant in the $10^{-4}-10^{-2}$ mol·L^{-1} range, even far above the critical micelle concentration (0.8−0.9 mmol·L^{-1}, [32]). This demonstrates that NMR allows the measurement of the total number of CTA molecules in the form of both monomers and micelles when they exist. The concentration in one given surfactant evaluated from NMR is therefore assigned to the sums of concentrations in monomers and molecules forming micelles:

$$c_{NMR} = c_{fast} = c_{micelles} + c_{monomers} \quad (3)$$

Comparison with the total concentration of each surfactant as measured by HPLC allows the determination of the concentration of surfactant forming the large catanionic aggregates:

$$c_{aggregates} = c_{HPLC} - c_{NMR} \quad (4)$$

Comparing both results for each surfactant finally allows the determination of the molar fraction of myristic acid in the aggregates, as opposed to the fraction in the whole sample:

$$f_{aggregates} = \frac{c_{HPLC}(\text{myristic}) - c_{NMR}(\text{myristic})}{c_{HPLC}(\text{myristic} + \text{CTA}) - c_{NMR}(\text{myristic} + \text{CTA})} \quad (5)$$

For mixtures prepared at molar fractions in myristic acid $f_0 = 0.35$ (Figure 6, squares) and at dialysis times shorter than 24 h, small amounts of free myristic acid are detectable by NMR ($(2-7) \times 10^{-4}$ mol·L^{-1}). More strikingly, the total amount of detectable CTA ($(2-9) \times 10^{-3}$ mol·L^{-1}) is significantly above the critical micelle concentration of CTAB alone. This suggests that the catanionic aggregates coexist with mixed micelles in solution. In catanionic mixtures with high total CTA concentrations ($f < 0.50$), up to 50% of the CTA molecules are excluded from the larger catanionic aggregates with composition $f_{membrane} = 0.45-0.53$ (Figure 7, squares). These molecules form CTA-rich micelles with a molar fraction of $f_{micelles} = 0.0-0.10$. This coexistence between micelles and larger aggregates has already been characterized in aqueous solutions of more soluble single-surfactant molecules. For instance, didodecyl dimethylammonium acetate forms vesicles in which micelles spontaneously accumulate.[33] This so-called supra-self-assembly accounts for different physical parameters between the inner compartment of vesicles and the external solution. At longer dialysis times, the concentration of CTA molecules coexisting with the larger aggregates finally becomes smaller than the cmc of CTA alone and reaches values in the 10^{-4} mol·L^{-1} range. Extensive dialysis therefore eliminates the mixed micelles initially observed in the preparations at $f_0 = 0.35$.

In samples prepared at higher myristic acid contents ($f_0 = 0.55$ and 0.66), the concentration of myristic acid in coexistence with the larger aggregates is below the detection threshold of NMR. This is coherent with the absence of elimination of myristic acid by dialysis, allowing only an increase in f with time (Figure 1). The concentration of CTA as determined by NMR is below the cmc of CTA during the entire dialysis period (Figure 6, upward and downward triangles). This suggests that mainly monomers, instead of micelles, coexist with the catanionic aggregates. The amount of surfactant coexisting with the bilayers is negligible, and the aggregates show a composition $f_{aggregate}$ equal to the effective composition f of the sample (Figure 7, upward and downward triangles).

This analysis relies on the hypothesis that all signals detectable by ^{1}H NMR originate from the surfactants in the bulk solution and that no other mobile species in the bilayers would contribute to the signal, leading to an additional term in eq 3. The validity of this assertion has been checked with a reverse experiment: various catanionic preparations have been dialyzed against solutions of CTABr with concentrations equal to the values obtained from the NMR experiments. Under these conditions, HPLC measurements indicate that the composition in surfactants in the samples remains unchanged within experimental error, which confirms that the CTA concentration as measured by NMR corresponds to the equilibrium concentration of surfactants in solution.

Very strikingly, the concentration of CTA molecules in coexistence with the catanionic bilayers tends toward identical values for $c_{final} \approx 10^{-4}$ mol·L^{-1}, independent of the initial composition f_0 (Figure 6). Moreover, the final compositions of the aggregates $f \approx f_{aggregates}$ also tend toward similar values (Figure 1), which strongly suggests that c_{final} is the signature of a thermodynamic equilibrium that is identically reached in all cases, independently of the initial composition and the morphology of the catanionic aggregate. This final value will now be used to evaluate the interaction between surfactants.

Evaluation of the Interaction between Surfactants. As the composition of the catanionic bilayer gets closer to the critical molar fraction $f_{critical} = 0.66$, the concentration of surfactant in equilibrium with the aggregates $c_{monomers} \approx c_{NMR}$ decreases. This demonstrates that the transfer of one surfactant molecule from the bulk solution to the aggregate becomes progressively more favorable energetically as the composition approaches the critical molar fraction $f_{critical}$. The free energy of transfer can be evaluated from the experimental data by writing the equality of the chemical

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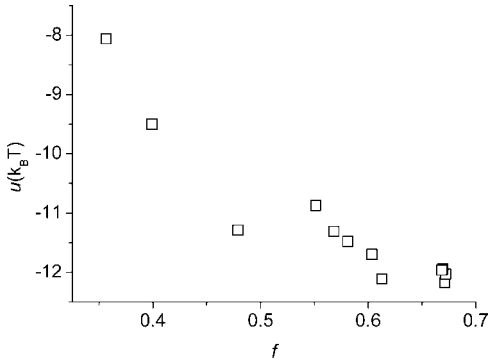

Figure 8. Evolution of the free energy of transfer u of one CTA molecule from the monomer phase to the surfactant bilayers calculated from eq 6.

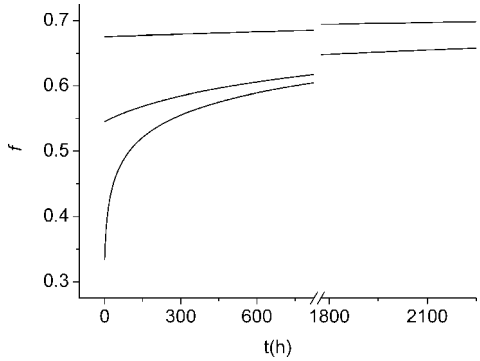

Figure 9. Evolution of the molar fraction f as a function of time as estimated from a modified first-order reaction for the extraction of CTA (eq 9).

potentials of all species throughout the sample. In particular, considering that the CTA molecules are distributed between two phases, the monomers (M) and the aggregates (A), leads to the following expression for the free energy of transfer of one CTA molecule from the monomers to the aggregates

$$u = \mu^{\circ}_{\mathrm{CTA,(A)}} - \mu^{\circ}_{\mathrm{CTA,(M)}} = k_{\mathrm{B}}T \ln \frac{N^{\mathrm{M}}_{\mathrm{CTA}}}{N^{\mathrm{M}}_{\mathrm{CTA}} + N_{\mathrm{w}}} - k_{\mathrm{B}}T \ln \frac{N^{\mathrm{A}}_{\mathrm{CTA}}}{N^{\mathrm{A}}_{\mathrm{CTA}} + N^{\mathrm{A}}_{\mathrm{Myr}}} \quad (6)$$

where N_{w} is the number of water molecules, $N^{\mathrm{A}}_{\mathrm{Myr}}$ is the number of myristic acid molecules (assumed to be exclusively present in the vesicles), $N^{\mathrm{M}}_{\mathrm{CTA}}$ and $N^{\mathrm{A}}_{\mathrm{CTA}}$ are the number of CTA molecules in the monomer and vesicle phase, respectively. The first term stands for the entropy of mixing of the free CTA monomers with the solvent and has been evaluated from c_{NMR}(CTA) as described above. The second term describes the entropy of mixing of CTA in the bilayers and has been evaluated from $f_{\mathrm{aggregates}}$ as described above. Note that the term u implicitly contains all other contributions to the free energy of transfer from the monomer solution to the aggregates, including the variations in electrostatic interactions, in the van der Waals interactions, in the entropy of mixing of the aggregates with the solvent, etc. The evaluation of u using the experimental data gives an estimate for the free energies of transfer on the order of $-8\ k_{\mathrm{B}}T$ to $-12 k_{\mathrm{B}}T$ in favor of transfer to the aggregates (Figure 8). The highest free energies of transfer of one surfactant molecule to the aggregates, achieved when the aggregates are in the most stable form ($f = f_{\mathrm{critical}}$, $c_{\mathrm{NMR}}(\mathrm{CTA}) = 10^{-4}$ mol·L^{-1}), are comparable to the values reported in lipid/detergent systems.[34,35] It is remarkable to note that u decreases by $1.5 k_{\mathrm{B}}T$ only as the molar fraction of the whole sample varies from 0.55 to 0.66 but that this slight change is related to drastic collective effects such as the arrest of dialysis at a given molar fraction.

Possible Origins of the Limiting Molar Fraction. The existence and value of the limiting fraction of $f_{\mathrm{critical}} = 0.66$ may be determined either by thermodynamic or kinetic arguments. The first, most obvious interpretation is to assume that both surfactants co-crystallize into a preferential compound of composition $f = 0.66$ and that the excess surfactant is rejected from this compound and is eliminated by dialysis if it is soluble. The coexistence of such 2D crystalline fragments with excess surfactant has been assumed to explain the faceting of catanionic mixtures.[26,36] Preferential 1:3 molar fractions have also been reported in surfactant mixtures.[37] It was proposed that these peculiar fractions result from the formation of superstructures in the hexagonal packing of the surfactant in the bilayers.[38] However, there are an infinite number of fractions where a superstructure in a binary distribution onto a hexagonal network can be found. Purely topological arguments are therefore not sufficient, and the full energy minimization must be taken into account to understand why particular fractions are selected.

In the case of dialysis, a limiting molar fraction may be observed, not necessarily because of energetically favorable co-crystallization but also because of strictly kinetic phenomena. This can be qualitatively understood as follows: if we consider that the extraction of the surfactant molecules during the dialysis is a first-order reaction

$$\mathrm{Myr}_{Nf}\mathrm{CTA}_{N(1-f)} \xrightarrow{k} \mathrm{Myr}_{Nf}\mathrm{CTA}_{N(1-f)} + \mathrm{CTA}$$

with N being the total number of surfactant molecules in the aggregates, then

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -kC \quad (7)$$

with C being the concentration of CTA molecules in the aggregates. The constant of reaction k is given by the law of Arrhenius

$$k = A\mathrm{e}^{\Delta G^*/k_{\mathrm{B}}T} \quad (8)$$

where ΔG^* is the activation energy for the extraction of one surfactant molecule from the membrane. The evolution of the molar fraction f of myristic acid in the membrane is therefore given by the following equation

$$\frac{\mathrm{d}f}{\mathrm{d}t} = Af(1-f)\mathrm{e}^{-(\Delta G^*(f)/k_{\mathrm{B}}T)} \quad (9)$$

where the dependence of ΔG^* on f has been explicitly written. As a first approximation, we may consider the activation energy ΔG^* of extracting a molecule from the membrane to scale with $|u|$, which is the free energy cost to extract one molecule from the membrane. For instance, $\Delta G^* = 2|u|$, with u varying as indicated in Figure 8 and $A = 2000$ s^{-1}, gives an evolution of f similar to that observed experimentally (Figure 9). All samples tend asymptotically toward a composition $f = 1$, but the time

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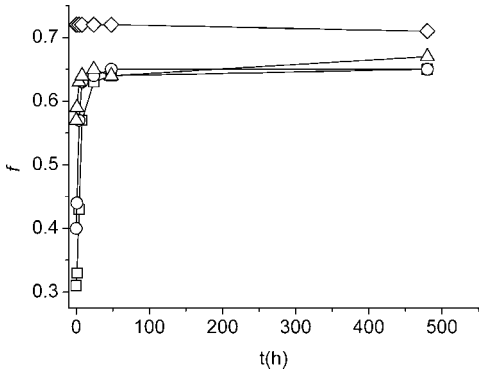

Figure 10. Evolution of the molar fraction of palmitic acid f in catanionic mixtures of palmitic acid and myristyl trimethylammonium bromide as a function of dialysis time.

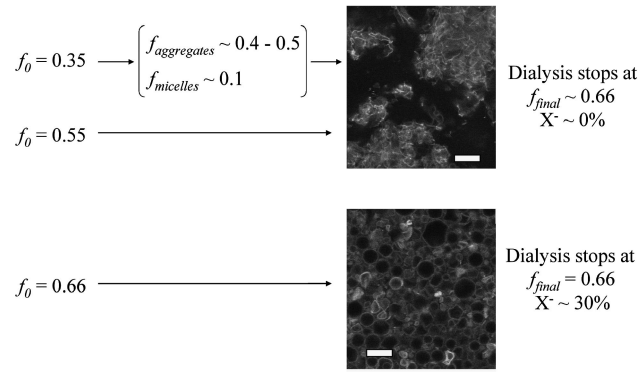

Figure 11. Summary of the main results. The elimination of soluble surfactant stops at $f_{final} \approx 0.66$ in all cases. The formation of ill-defined aggregates vs vesicles during dialysis is determined by the initial molar fraction of fatty acid f_0, not the final fraction f_{final}. Micelles are present only in preparations very rich in CTA, and halide ions are retained only if vesicles are present.

scale is large enough to suggest the presence of a limiting molar fraction around $f = 0.66$, independent of the initial molar fraction or within the error. The corresponding rates of reaction k vary in the $10^{-5}-10^{-8}$ s^{-1} range, which is orders of magnitudes lower than the typical values reported for the release of one surfactant molecule from a micelle of an ionic surfactant with a comparable chain length ($k = 10^4$ s^{-1}).[39] Such slow kinetics of exchange could originate from the frozen state of the alkyl chains in our catanionic systems or from a local electrostatic interaction between the CTA heads and the oppositely charged myristic acid head groups. Although this rough evaluation is not complete, it is sufficient to illustrate that the apparent limiting molar value of $f = 0.66$ can originate from the kinetic arrest of dialysis and is not necessarily related to the formation of a preferential compound with a given surfactant composition. Direct structural and calorimetric experiments will be reported that allow discrimination between both possibilities.

Generality of the Observation: Other Catanionic Systems. The properties described above are general enough to have been observed with various analogue surfactant mixtures. For instance, the molar fraction of palmitic acid in mixtures of palmitic acid and myristyl trimethylammonium bromide has been measured as a function of time during dialysis. The behavior is extremely similar to the mixture of myristic acid and cetyl trimethyl ammonium bromide: if the initial molar fraction of palmitic acid f_0 is below a critical value around $f_{critical} = 0.66$, then f reaches $f_{critical}$ independently of the initial composition f_0 (Figure 10, squares, circles, and triangles), and the resulting aggregates show no well-defined morphologies. If f_0 is chosen above $f_{critical}$, then f does not evolve during dialysis (Figure 10, diamonds), and the mixture yields vesicles with a diameter in the 5 μm range. The only noticeable difference is that the critical fraction $f_{critical}$ is reached in significantly shorter characteristic times than in the previous myristic acid/cetyl trimethyl ammonium bromide mixtures (a few hours against hundreds of hours, Figure 10). This quicker elimination of the cationic surfactant is attributed to the shorter chain of the soluble surfactant (C14 against C16), which allows both a higher diffusion coefficient and higher solubility in water. The features summarized in Figure 11 are therefore expected to be generally observed at least in the fatty acid/alkyltrimethyl ammonium halide mixtures: the arrest of dialysis at a given molar fraction, the correlation of this molar fraction with the production of large vesicles, and the retention of a significant number of ions in the interior of the vesicles.

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Conclusions

In summary, we propose an overall behavior for mixtures of myristic acid and cetyltrimethyl ammonium halides (CTACl and CTABr) in which the inorganic ions are removed by dialysis. The CTA molecules have limited solubility in the catanionic membrane: if the molar fraction of myristic acid is smaller than 0.50, then excess CTA and part of the anionic surfactant are expelled from the membrane in the form of mixed micelles. Above a fraction of 0.50, CTA remains incorporated in the catanionic membrane, from which it can be extracted by dialysis. However, the elimination of CTA from the catanionic membrane becomes impossible when the molar fraction of the aggregates reaches a value of around 0.66. The samples prepared directly at a molar fraction above 0.66 will not lose CTA molecules. They also spontaneously form vesicles instead of ill-defined morphologies and therefore hinder the elimination of ions by dialysis. Work is in progress to evaluate the relative contributions of all interactions to the specific fraction observed. However, our experimental evidence suggests that this effect is driven by the tail of the surfactants because the same effects are observed in analogue surfactants. Finally, this work opens perspectives in the understanding of the out-of-equilibrium loss of surfactant in catanionic aggregates but also of a broader class of self-assembling molecules, such as acid−soap assemblies and surfactants forming so-called supra-self-assemblies.[33]

Acknowledgment. We thank Alain Valleix for technical help with HPLC and Jean Daillant (LIONS, CEA/Saclay) for fruitful scientific discussions.

Appendix: Derivation of Permeability

Permeability through a membrane can be expressed as

$$P = \frac{KD}{l} \tag{10}$$

where D is the diffusion coefficient of the solute in the membrane and l is the thickness of the vesicle membrane. K is the partition coefficient of the solute between the membrane and water, which is defined as

$$K = \frac{c_{membrane}}{c_{in}} \tag{11}$$

where $c_{membrane}$ and c_{in} are the concentrations of solute in the membrane and inside the vesicle, respectively. In the case where

the solute concentration outside the vesicle is zero (e.g., during dialysis against an ideal reservoir of pure water), the diffusion law of Fick yields an exponentially decaying concentration of solute in the membrane

$$c_{\text{membrane}} = c^{\circ}_{\text{membrane}} \exp\left(-\frac{t}{\tau}\right) \tag{12}$$

with

$$\tau = \frac{Vl}{KDS} = \frac{V}{SP} = \frac{1}{k} \tag{13}$$

In this equation, V and S are respectively the volume and the surface of vesicles and k is the rate constant (s^{-1}) defined as

$$k = \frac{\ln 2}{t_{1/2}} \tag{14}$$

where $t_{1/2}$ is the leakage half-time.

Using Fick's law, one finds an exponentially decaying concentration, and the permeability can be expressed as

$$P = \frac{\ln 2}{3}\frac{R}{t_{1/2}} \tag{15}$$

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