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Formation of rigid nanodiscs: edge formation and molecular separation

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Abstract We show that the control of size in catanionic nanodiscs relies on the balance between charge excess favoring the disc edge and frozen ion pair formation favoring rigid faces. In the absence of salt, average diameters in the range of 60 nm to 3 μm have been obtained by varying the molar ratio x (cationic/anionic + cationic) from $x = 0.39$ to 0.46. The cost in entropy of mixing associated with partial molecular separation inside each crystallized nanodisc is lower than the work associated with osmotic pressure in the region of the phase diagram where catanionic nanodiscs are produced.

Key words Catanionic surfactant · Nanodics · Osmotic pressure

Introduction

Catanionic mixtures of two amphiphilic molecules (myristic acid and cetyltrimethylammonium hydroxyde), with H^+ and OH^- as counter-ions, form swollen lamellar phases. The bilayers composing these lamellar phases are made of amphiphilic ion pairs. These infinite bilayers fragment upon dilution and form rigid nanodiscs in the absence of salt [1]. The large in-plane persistence length, exceeding one micrometer, is due to the sandwich structure of the nanodiscs: a two-dimensional alternated charge lattice is formed on each face. The core of the nanodiscs is made of frozen hydrocarbon chains [1]. Hence, the Young's modulus of this new type of colloidal object exceeds 10^8 Pa [2].

The formation of nanodiscs is reversible with temperature [1], since above the ion pair melting temperature (composition dependent, but of the order of 30 °C [3]), closed vesicles are formed. The high-temperature thermodynamically stable state is a dispersion of small unilamellar vesicles. This dispersion is similar to the equilibrium microstructure which has been described in the presence of excess salt when anionic and cationic components are mixed with their counter-ions

[4, 5, 6]. At concentrations of around 1% by weight, a two-phase regime is observed by electron microscopy where large crystallites of a lamellar phase are in equilibrium with a dispersions of discs (Fig. 1).

Statistics obtained after explicit counting of disc sizes in the electron microscopy pictures have demonstrated a large variation in disc size induced by only a small variation in composition [2]. The situation is similar to the ternary system which has been described as producing "bicelles" by Benedek and co-workers [7]. These authors used a combination of short-chain and long-chain neutral phospholipids. "Bicelles" are uncharged flat rigid discs, with curved edges [7]. In their classical work, Benedek and co-workers have shown that the available volume of short-chain phospholipids combined with the volume of long-chain phospholipids quantitatively explains the observed bicelle diameter, even in cases where nanodisc formation is not reversible and is only a metastable state in the ternary phase diagram.

The aim of the study presented here is to evaluate the charge separation needed to form disc edges and faces. This allows an evaluation of the molecular composition of both edges and faces. From the composition of these two
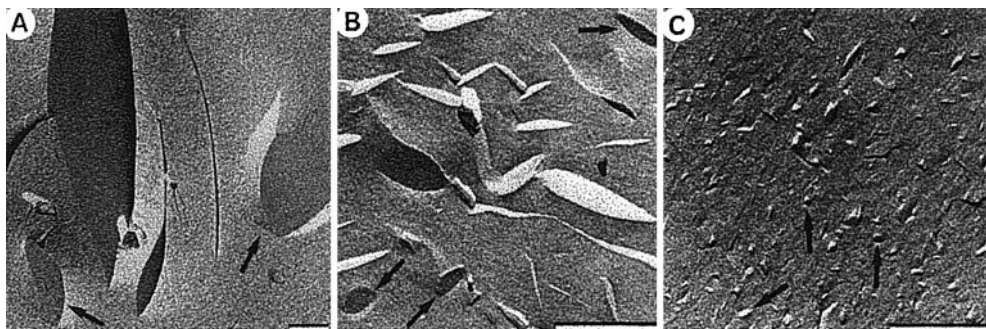
Fig. 1 Images obtained after cryofracture of nanodisc dispersions of different sizes. The initial weight content of the catanionic surfactant in solution is of the order of 10 g/l. With a molar ratio $x = 0.45$ on the basic side of the phase triangle, one obtains an average disc size of 2–3 μm (**A**); for $x = 0.43$ the average nanodisc size is 250 nm (**B**). In the case of a larger excess of cationic component ($x = 0.39$) the average nanodisc size is 30 nm (**C**). The *bar* represents 500 nm

parts of the discs, the cost in entropy can be compared to osmotic pressure due to electrostatic repulsions.

Composition of edge and faces

A schematic view of a nanodisc of thickness $2t = 43$ Å (measured) and radius R is shown in Fig. 2. The two faces (total surfaces S_f) contains N_f surfactant molecules of composition x_f, each occupying the surface $\sigma_f = 25$ Å^2 (area per headgroup for frozen chains involved in ion pairs): $S_f = 2\pi R^2 = N_f \sigma_f$. The edge (surface S_e) contains N_e surfactant molecules of composition x_e with a area per chain $\sigma_e \approx 50$ Å^2 (twice the previous value for molecules in excess): $S_e = 2\pi^2 Rt = N_e \sigma_e$. The total number of

molecules per disc is $N = N_f + N_e$ of global composition $x = (N_f x_f + N_e x_e)/N$. The disc size is a function of the three compositions, $R(x_f, x_e, x)$. Assuming fixed values for the composition x_f, x_e (independant of the total composition), the best fit of the experimental data $R(x)$ are obtained with $x_f = 0.454$ and $x_e = 0.18$ (Fig. 3). Table 1 gives the different parameters describing the disc for each measured size.

The faces are close to equimolar equilibrium, which ensures that the faces are less charged than the edge, a physical situation opposite to the case of clay particles, where faces are more hydrophilic than the edge. The composition of the edge is consistent with the molar ratio found in the neighboring phase of giant cylindrical micelles [8]. Indeed, the disc edge shape is close to a long wormlike micelle cut in half. We find, by fitting, a disc edge composition (0.18) close to the ratio where the single phase of large wormlike micelles has the lowest free energy of formation. The molar ratio where cylindrical micelles are observed is between 0 and 0.3 for myristic acid. Therefore, it is consistent to find a disc edge composition of 0.18, which is in the middle of the L$_1$ phase region.

Fig. 2 Schematic view of a nanodisc: thickness of bilayer is 2t, disc radius is R. Edge composition with a higher molar ratio of the excess cationic component is the proposed explanation for disc size control via composition
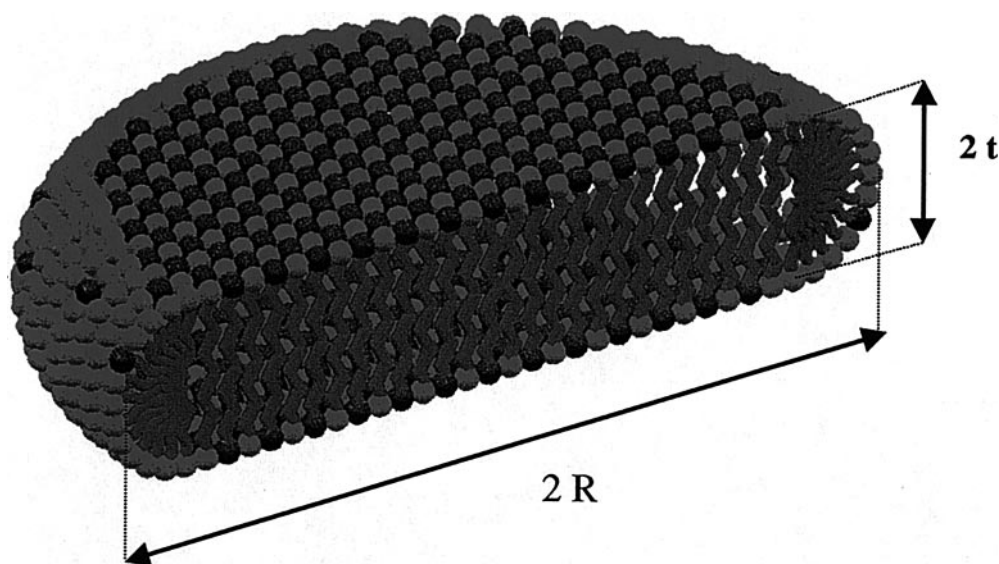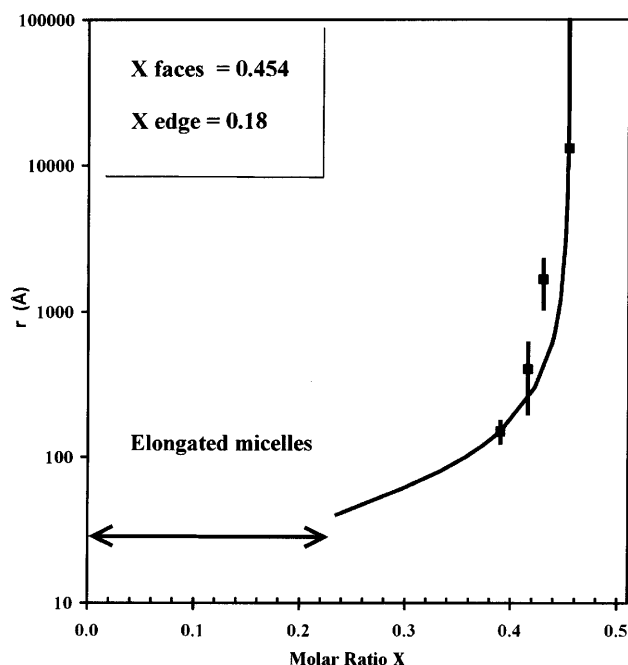
Fig. 3 Observed equilibrium radii of nanodiscs compared to the evolution expected (*line*) if the composition of edges and faces are 0.18 and 0.454, respectively. The region where micelles are found instead of nanodiscs is indicated

Neglecting charge effects, the cost in free energy associated with the entropy of mixing per molecule is derived from:

$$-\frac{\Delta S}{k} = \frac{N_e}{N}[x_e \ln x_e + (1 - x_e)\ln(1 - x_e)]$$
$$+ \frac{N_f}{N}[x_f \ln x_f + (1 - x_f)\ln(1 - x_f)]$$
$$- [x \ln x + (1 - x)\ln(1 - x)] \tag{1}$$

This quantity can be calculated from the values of Table 1. The result is shown in Fig. 4, where the free energy of mixing is plotted versus the molar ratio of the sample, i.e. the macroscopic value, averaged over edge and faces. In the range where nanodiscs form, the order
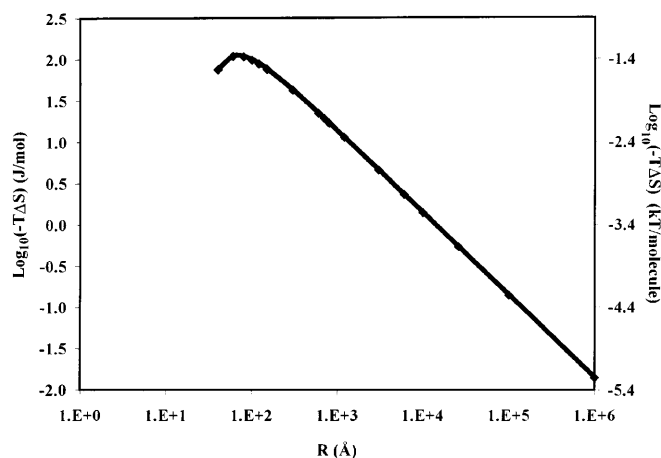

Fig. 4 Free energy cost of molecular separation $T\Delta S$ needed to form edges and faces of different molar ratio, as a function of nanodisc diameter R

of magnitude of the free energy cost of molecular separation is less than 0.1 kJ/mol.

How does this figure compare to the main interaction energy in these solutions in the absence of salt? We consider now the case where discs are found in equilibrium with a partially swollen charged catanionic lamellar phase. Using the two control variables, i.e. surface charge controlled by molar ratio and weight fraction controlling the interlayer distance, the molar ratio phase diagram can be remapped in terms of water layer thickness between bilayers and area per charge. This is shown in Fig. 5. On the same diagram are drawn isobar lines of constant osmotic pressure, calculated assuming parallel lamellae or parallel cylinders geometries. In order to allow comparison, the molecular volume is identical in both geometries while the area per head group is twice as large in cylinders than in plans. In terms of free energy associated with $\Pi \Delta V_m$, where V_m is the molar volume, the interaction term is predominant over charge separation effects, as can be seen by comparing Figs. 4 and 5.

From the comparison of the location of the discs and lamellar phase in the phase diagram and the ab initio

Table 1 Characterization of nanodiscs with a molar ratio of 0.454 on the faces and 0.18 on the edge (see text for the notation)

	Disc radius (Å)			
	150	400	1650	13000
Surf. faces (Å²)	1.0×10^5	9.0×10^5	1.7×10^7	1.1×10^9
Surf. edge (Å²)	6.4×10^4	1.7×10^5	7.0×10^5	5.5×10^6
N_f	4.1×10^3	3.6×10^4	6.7×10^5	4.2×10^7
N_e	1.3×10^3	3.4×10^3	1.4×10^4	1.1×10^5
$N = N_f + N_e$	5.4×10^3	3.9×10^4	6.8×10^5	4.2×10^7
X calculated	0.390	0.430	0.448	0.453
X measured	0.390	0.416	0.430	0.454

Fig. 5 Using total surfactant volume fraction and surface per charge (controlled by the composition) as variables, this phase diagram superposes the location of the three states found for nanodiscs: U is the unbound dispersion of interacting nanodiscs; L_β is the lamellar phase with frozen chains; L_1 is a solution of wormlike charged micelles. Isobar lines of constant osmotic pressure (labeled in Pa) are drawn on the same diagram for flat bilayers (*thick line*) and for parallel cylinders of same molar volume and double area per headgroup (*dashed lines*)
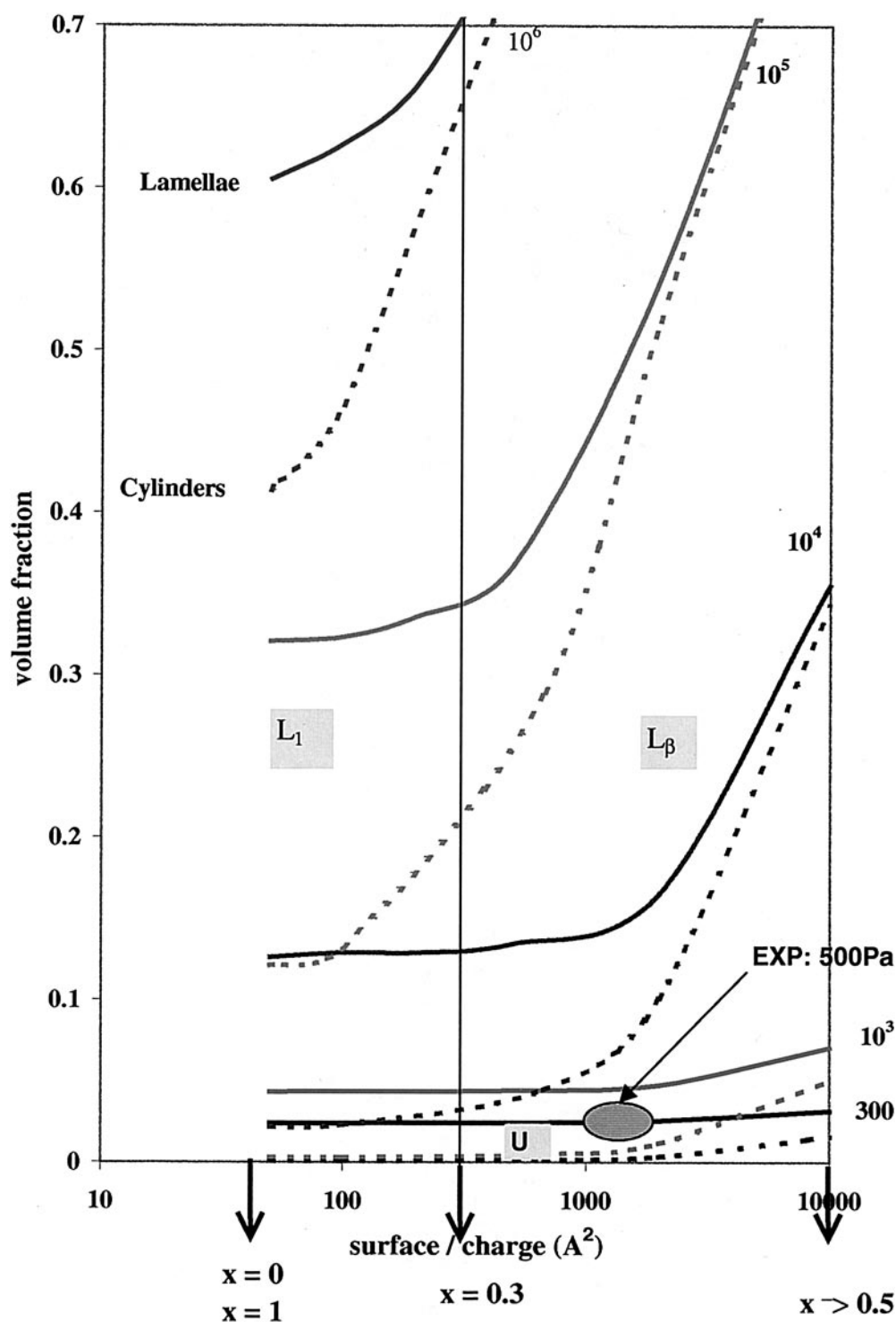
evaluations of the free energy, one expects that the bilayers fragment into discs when the electrostatic term of the free energy corresponds to a pressure of the order of 500 Pa. At a mole fraction $x = 0.45$ and a global concentration of 1%, the sample spontaneously microphase separates into stacks of lamellae as shown in Fig. 1. The osmotic pressure of this sample, as measured by a conventional osmometer, is between 400 and 600 Pa. The relatively low precision is due to partial permeability of the membrane to the catanionic mixture;

therefore the osmotic pressure has to be determined via successive comparisons.

In Fig. 5 we can see that in the region where the previously studied nanodiscs of controlled size coexist with a lamellar phase, the osmotic pressure is evaluated to be less than 300 Pa. Moreover, an experimental evaluation using a membrane osmometer of the pressure of disc-lamellar phase coexistence region has been made. The pressure range is 500 ± 200 Pa, well correlated with the theoretical expectation in the region where coexistence is found (dashed region in Fig. 5).

From the known area per charge and residual ionic strength of solution, we derive an estimation of the surface potential of the faces of 200 mV using a mole fraction for the faces of $x = 0.45$. From the molar ratio of the faces, which constitutes the major part of the disc volume, we expect a new type of packing of the hydrocarbon chains and therefore that the position of the large angle peak corresponding to frozen chains is in unconventional position and shape, since ion pairing should induce shifts towards large q or a superstructure at lower q. Investigations of this point are in progress.

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