

## Notes

### Polymer-Induced Structural Effects on Catanionic Vesicles: Formation of Faceted Vesicles, Disks, and Cross-links

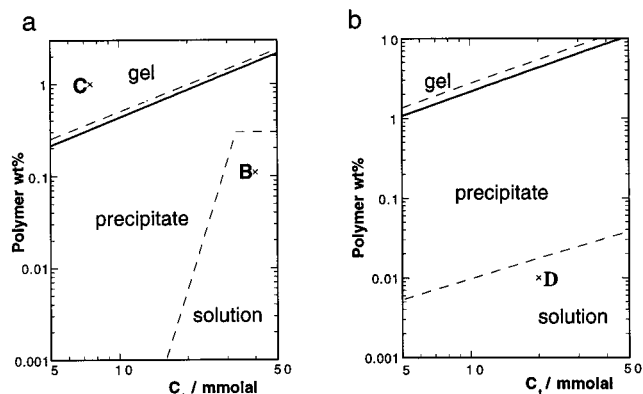
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#### Introduction

Studies on aqueous vesicle–polymer systems are a challenging area of colloid science, both from an experimental<sup>1–5</sup> and a theoretical<sup>6</sup> viewpoint. Vesicle–polymer systems constitute good model systems for living cells,<sup>7</sup> and they may also lead to important phase behavior phenomena, such as the formation of gels and networks,<sup>8–10</sup> with potential industrial applications. Polymers can also act as liposome stabilizers and controllers of membrane permeability in liposomal systems for drug delivery.<sup>11</sup> A first step in the understanding of the molecular interactions in these complex systems is to gain direct microstructural information. In this work, cryogenic transmission electron microscopy (cryo-TEM) is used as an imaging method of vesicle–polymer mixtures, in two regimes of polymer concentration  $c$  (in wt %), defined with respect to  $c^*$ , the critical association concentration: (i) at very low concentration ( $c \ll c^*$ ), where the polymer was seen to induce dramatic bilayer structural changes; (ii) at high concentration ( $c > c^*$ ), where gel formation occurs. The polymers used were the cationic polyelectrolytes JR400 and LM200 (hydrophobically modified polymer). The vesicles are composed of mixed anionic SDS (sodium dodecyl sulfate) and cationic DDAB (didecyldimethyl-



**Figure 1.** Phase maps of the polymer–vesicle systems investigated at 25 °C: (a) polymer JR400–vesicle; (b) polymer LM200–vesicle.  $C_t$  is the total surfactant concentration of the vesicle solution, which is composed of a mixture of SDS and DDAB at fixed  $X_{DDAB} = 0.29$ . The letters correspond to the compositions of the samples shown in Figure 2. The full line indicates charge neutrality in the polymer–surfactant system.

**Table 1. Relevant Parameters for the JR400 and LM200 Polymers**

polymer	MW	mean contour length (nm)	charge concn in a 1 wt % aqueous solution (mmolal)	mean contour length between charges (nm)
JR400	500 000	~1000	10	2
LM200	100 000	~200	2	10

ammonium bromide) surfactants at a fixed amount of SDS excess, thus bearing a net negative surface charge. These catanionic vesicles are particularly suitable for our goal, since they form spontaneously, remain stable with time, and are essentially small and unilamellar.<sup>12</sup> The polymers were initially expected to interact strongly with the vesicles, due to electrostatic and hydrophobic interactions (for LM200).

#### Experimental Section

The vesicle–polymer samples were prepared by weight from stock solutions of surfactant vesicles and polymer. The vesicular solution was obtained by thoroughly mixing a SDS solution and a DDAB homogenized lamellar dispersion, with a fixed molar fraction of DDAB in the final mixture of  $X_{DDAB} = C_{DDAB}/(C_{DDAB} + C_{SDS}) = 0.29$ , where  $C_{DDAB}$  and  $C_{SDS}$  are, respectively, the DDAB and the SDS concentration in millimolal. This composition of the surfactant mixture lies midway in the range of composition for which a solution of stable, SDS-rich vesicles occurs.<sup>12</sup> The total surfactant concentration in the polymer–vesicle samples is varied in the range  $C_t = C_{DDAB} + C_{SDS} = 5–50$  mmolal (cf. Figure 1). The two cationic polyelectrolytes used, JR400<sup>13</sup> and Quatrisoft LM200,<sup>13,14</sup> are derivatives of hydroxyethyl cellulose (HEC) in the chloride salt form. In both polymers, the charge is placed in a quaternary ammonium group attached to the

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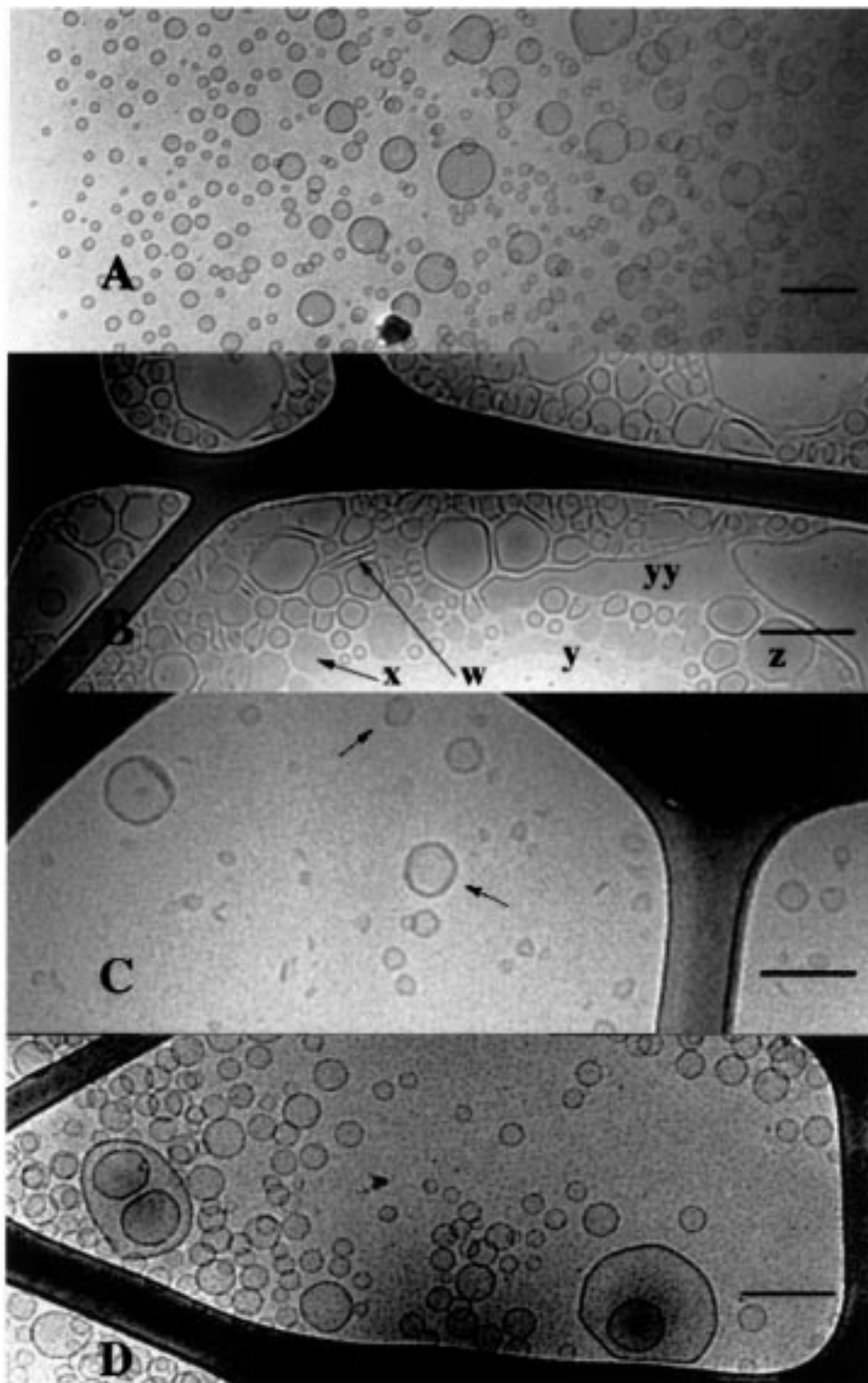
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**Figure 2.** Cryo-TEM micrographs of the polyelectrolyte-vesicle mixtures at 25 °C: (A) Mixed SDS-DDAB vesicles (polymer-free solution) at  $X_{\text{DDAB}} = 0.3$  and  $C_t = 48$  mmolal. (B) JR400 (0.11 wt %) and  $C_t = 40$  mmolal, in the solution region; disks in a face-on (x) or edge-on (w) orientation, bilayer fragments—face-on (y) and partially edge-on (yy), and opened vesicles (z). (C) JR400 (1 wt %) and  $C_t = 7.5$  mmolal, in the gel region. Mostly faceted vesicles are seen (arrow) and also some bilayer fragments. (D) LM200 (0.01 wt %) and  $C_t = 20$  mmolal, in the solution region. Faceted and round vesicles; clusters of vesicles due to polymer cross-linking.

polymer backbone. LM200 differs from JR400 (cf. Table 1) in that it has lower molecular weight and lower charge density. Moreover, while JR400 is a homopolymer, LM200 is a hydrophobically modified polymer, having a dodecyl hydrophobic side chain attached to the backbone through the charged quaternary ammonium group. The vesicle-polymer samples were carefully mixed and left to equilibrate for at least 1 week until the phase behavior was examined at 25 °C. The phase maps obtained for

the two vesicle-polymer mixtures are shown in Figure 1, where the main phase regions are identified. Samples in the solution and gel region were imaged by cryo-TEM. The samples were prepared according to the usual procedure, described in previous work.<sup>15</sup> A Philips CM 120 cryo-dedicated Bio-twin microscope

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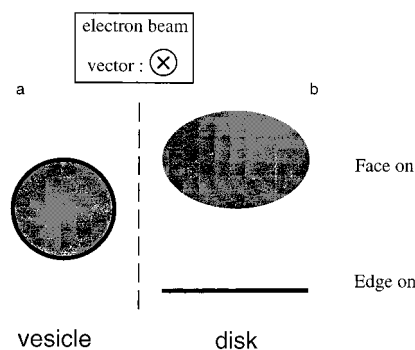
was used, operating at 120 kV in the conventional TEM mode with a nominal underfocus of 5  $\mu\text{m}$  and an exposure time of 1 s.

## Results and Discussion

Cationic polyelectrolyte was added to surfactant solutions containing self-assembled, large aggregates consisting of mixed SDS–DDAB vesicles. A polymer-free SDS-rich vesicular solution at  $C_t = 48$  mmolal was imaged as a “reference state” (Figure 2A), and the results confirm those previously reported.<sup>12</sup> As can be seen in the cryo-TEM micrograph in Figure 2A, essentially relatively small and polydisperse unilamellar vesicles are found, with diameters in the range 20–70 nm and an average size of approximately 30 nm. Also apparent in the figure is the spherical shape of the vesicles and the smooth, integral appearance of the bilayer. Giant vesicular aggregates (1–40  $\mu\text{m}$ ), mainly giant unilamellar vesicles below 2  $\mu\text{m}$ , are also present in very low concentration in these solutions, as reported previously (not shown here). Dilution within the vesicle region ( $C_t = 5$ –50 mmolal) does not induce significant structural changes in the shape and size of the vesicles.<sup>12</sup>

Since electrostatic interactions are at play in the polymer–vesicle systems investigated here, the phase behavior can be followed in terms of the mole fraction of polymer charge in the mixture, defined as  $f_p^+ = C_p^+ / (C_p^+ + C_s^-)$ , where  $C_p^+$  is the molal concentration of polymer charge and  $C_s^-$  is the molal concentration of net surfactant charge (i.e.  $C_{\text{SDS}} - C_{\text{DDAB}}$ ). The full straight lines drawn in the phase maps in Figure 1 correspond to charge neutrality in the system ( $f_p^+ = 0.50$ ). Upon polymer addition to surfactant solution, three main regions can be found in the two phase maps (Figure 1): a solution phase, which can only take up a small amount of polymer; a wide region of phase separation, containing a precipitate and a solution, present up to a slight excess of polymer charge in the system; and a gel region, which appears to form when a given constant excess of polymer charge is attained in the system.

The micrograph in Figure 2B shows the microstructure of sample B (Figure 1) in the solution region of the JR400-vesicle system (40 mmolal surfactant, 0.11 wt % polymer). The effect of the polyelectrolyte on the vesicle structure, at the value  $f_p^+ = 0.059$ , is dramatic. Smooth-shaped spherical vesicles are practically absent, and they are replaced with faceted, polyhedron-like vesicles, some of which show membrane discontinuities (z), that is, ruptured vesicles. In addition to these structures, aggregates of disklike shape (x) are clearly seen in Figure 2B. Several projections of the disks with respect to the electron beam are visible:<sup>12,16,17</sup> the edge-on (w), the face-on (x), and the tilted (elliptical) projection. A schematic view of the different disk orientations is shown in Figure 3. Note the difference between the disk and vesicle projections in the TEM micrograph: whereas the vesicle shows an edge contour due to mass thickness contrast, the disk appears with uniform contrast and thus no edge contour.<sup>12</sup> Some bilayer fragments (y) are seen as well. The black contour line on some of these fragments (yy) indicates that they are partially viewed in an edge-on orientation (yy), a situation which reflects the fact that the membrane sheet is a flexible surface. Disklike micelles have been previously reported for the polymer-free SDS–DDAB system for values of  $X_{\text{DDAB}} = 0.13$ –0.25.<sup>12</sup> The appearance of the



**Figure 3.** Schematic view of the 2D projection of a disk and a vesicle seen in cryo-TEM micrographs: (a) vesicle; (b) disk in an edge-on and a face-on orientation, with respect to the direction of an electron beam perpendicular to the page.

bilayer in the faceted vesicles observed here is noticeably similar to that which is present in the gel phase of double-chained lipids in water.<sup>11</sup> The latter occurs at temperatures below the lipid chain-melting temperature. However, the presence of the faceted vesicles here is probably of a different origin. The bilayer deformation could result from a tight vesicle packing due to a polymer-induced attractive force between the vesicles. More samples in the solution region were imaged (not shown here), so that the polymer effects could be monitored. Samples within the range  $f_p^+ = 0.02$ –0.06 showed the following microstructure: at low polymer concentration, faceted vesicles appear as the dominant aggregate form; as the polymer concentration increases toward phase separation, disks and faceted vesicles coexist.

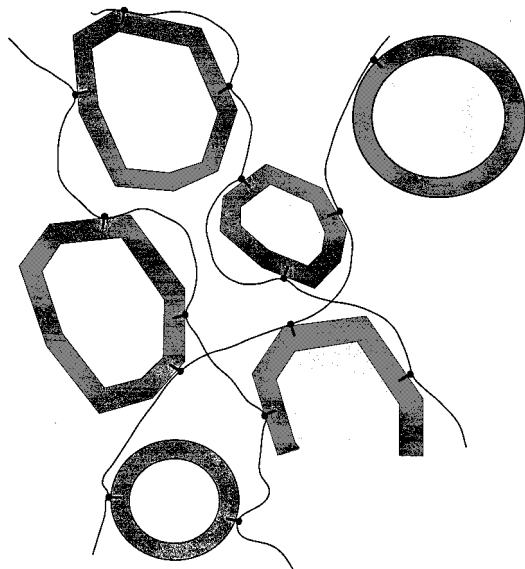
In Figure 2C, a micrograph of sample C lying in the gel region (Figure 1) is presented (7.5 mmolal surfactant, 1 wt % polymer). Macroscopically, this sample is intensely bluish and very viscous and is best described as gel-like. Excess polymer charge is present for this composition ( $f_p^+ = 0.76$ ). The cryo-TEM image shows essentially the presence of vesicles of small size, 20–30 nm in diameter and mostly intact; again the vesicle wall is in most cases polygon-like. The smaller number density of the vesicles, as compared with those in Figure 2A, reflects the lower surfactant concentration for this sample. It is striking to observe that at high polymer concentration, above the neutralization line, the precipitate disappears and gel-like homogeneous samples are found, where the vesicular structure is re-formed. It is thus likely that vesicles interact with the polymer to yield a network responsible for the viscoelastic properties observed for the samples in this region (rheology data not shown). The rheological characterization of the gels formed in this system is currently being studied.

The solution region of the hydrophobically modified polymer LM200 was also imaged by cryo-TEM. There are some similarities with JR400, with respect to the effect of the polyelectrolyte on the vesicle membrane. At a value of  $f_p^+$  as low as 0.0012, faceted vesicles can already be observed (not shown). In Figure 2D, a micrograph of sample D (see Figure 1b) is shown, at 20 mmolal surfactant and 0.01 wt % polymer concentration ( $f_p^+ = 0.0023$ ). Similar to the JR400 case, some faceted vesicles can be observed. However, a significant novel feature is present with LM200. The vesicles do not crowd in the thicker (darker) part of the film,<sup>18</sup> like in Figure 2A, but instead appear to agglomerate in clusters of relatively large size containing many vesicles. This observation suggests that the vesicles are closely interconnected. In a large number

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**Figure 4.** Schematic view of the possible structure for the polymer LM200-vesicle network, where both faceted and round vesicles are shown.

of these clusters one can distinguish essentially highly deformed faceted vesicles, some of which are broken (not shown here). The strong polymer-vesicle interactions in this mixture are of both hydrophobic and electrostatic origin. The hydrophobic side chains of the polymer, bearing a positive charge close to the backbone, can favorably anchor in the vesicle bilayer (which bears a net negatively charged surface), thus opening the possibility to vesicle cross-linking by the polymer. Since the mean contour length between the hydrophobes, approximately 10 nm, is of the order of the diameter of the small vesicles, 20–30 nm, it is reasonable to picture that even one polymer molecule can make cross-links between several small

vesicles. Such a possible type of network is shown in Figure 4, where both faceted and round vesicles are seen interconnected by the polymer chains. We are unable to see the polymer chain itself in the TEM micrographs, since its diameter is below the resolution of our imaging technique.<sup>19</sup>

Since the gels obtained for the LM200 system have higher viscosity, as compared to that of the previous system, they do not form a thin enough film on the TEM grid and therefore it was impossible to image them by the cryo-TEM technique. It is likely that the gels derive their high viscosity from strong cross-linking effects resulting from the hydrophobic interactions between polymer and surfactant aggregates.

### Summary

JR400 thus interacts with the vesicles so as to induce, prior to phase separation, two effects: (i) breakage of vesicles into small disklike aggregates; (ii) formation of faceted vesicles. The formation of disk aggregates prior to phase separation seems to be less favored in the LM200 system just before phase separation. Two main effects are induced by this polymer: (i) the formation of ruptured, faceted vesicles; (ii) the formation of clusters of vesicles and other bilayer structures due to polymer cross-linking. The presence of the hydrophobic side chain is clearly in the origin of the last effect.

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