

## Microstructures in Aqueous Solutions of Mixed Dimeric Surfactants: Vesicle Transformation into Networks of Thread-Like Micelles

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The microstructures in mixtures of two dimeric (gemini) surfactants, the dimethylene-1,2- and eicosamethylene-1,20-bis(dimethyldodecylammonium bromide), referred to as 12-2-12 and 12-20-12, have been investigated at 25 °C by electrical conductivity, spectrophotometry, digital light microscopy (DLM), and transmission electron microscopy at cryogenic temperature (cryo-TEM). This mixture was selected because 12-20-12 forms vesicles in a wide range of concentration whereas 12-2-12 forms micelles that are spherical at low concentration then rapidly elongate, branch, or give rise to toroidal micelles (rings), and finally form a network of threadlike micelles at 2 wt %. The measurements were performed keeping the 12-20-12 concentration at 0.09 wt % and progressively increasing the 12-2-12 concentration from 0.1 to 2.0 wt %. The electrical conductivity data clearly showed that in the early stages of 12-2-12 addition to the 12-20-12 vesicles, 12-2-12 was strongly adsorbed by the vesicles. Spectrophotometry and DLM showed that, under the conditions used, the vesicles were nearly eliminated at above 0.7 wt % 12-2-12. The cryo-TEM observations were performed on samples vitrified two months after preparing the mixtures. The progressive increase of the 12-2-12 content in the mixture resulted first in vesicle growth (0.1 wt %), followed by vesicle breakage into smaller vesicles (0.26 wt %), the formation of disklike micelles (0.4–0.75 wt %), then of ring-like micelles and short elongated threadlike micelles (1 wt %), the growth of those threads (1.5 wt %), and finally the formation of a network (2 wt %), where threads and rings were interconnected. The network contained also a few isolated rings. The same structures were observed when the mixtures for cryo-TEM observation were vitrified 5–7 days or two months after mixture preparation, for the mixtures containing 0.5 wt % or less, and 1 wt % or more 12-2-12. Aging of the systems was observed for mixtures containing 0.65–0.75 wt % 12-2-12. When examined 5–7 days after preparation the mixtures showed long rigid rodlike micelles and irregular ribbons. Those structures disappeared when the mixtures were allowed to equilibrate for two months. The results are discussed and a model is presented to explain the observed behavior.

### Introduction

The vesicle-to-micelle transformation induced by the addition of a micelle-forming surfactant to systems containing vesicles made of phospholipids or surfactants has been much investigated.<sup>1</sup> Indeed, this process constitutes a good model for the solubilization of cell membranes for the recovery of specific cell components. In most instances the micelle-forming surfactants used were of the kind that form spherical or spheroidal micelles when present alone in aqueous solutions at the concentration used. Thus, octylglucoside,<sup>2</sup> sodium cholate,<sup>3</sup> C<sub>12</sub>E<sub>8</sub>,<sup>4</sup> Triton X-100,<sup>5</sup> cetyltrimethylammonium chloride,<sup>6</sup> and sodium alkyl sulfates,<sup>7</sup> to cite but a few, have been used in such studies. The reported results showed that the vesicle-to-micelle transformation involves the formation of intermediate lipid/surfactant mixed assemblies at different values of the surfactant-to-lipid concentration ratio. The concentration ratios at which microstructural changes occur and the type of mixed assemblies depend on the nature of the molecules involved in the solubilization process.<sup>1</sup> In the early studies, two intermediate structures between vesicles of pure lipid and mixed lipid/surfactant spheroidal micelles have been often observed using transmission

electron microscopy at cryogenic temperature (cryo-TEM): bilayer fragments and threadlike micelles.<sup>2–4,6</sup> Recent studies, however, reported the absence of the intermediate threadlike micellar structure.<sup>7–9</sup> These studies involved widely differing vesicle-forming pure or mixed chemicals: lecithin,<sup>7</sup> nonionic surfactant/cholesterol mixture,<sup>8</sup> and 12-20-12.<sup>9</sup> The last chemical is a *dimeric* (gemini) surfactant,<sup>10,11</sup> made up of two identical dodecyldimethylammonium bromide (amphiphilic) moieties, connected at the level of the ammonium headgroups by an eicosamethylene spacer group. When mixed with water, this surfactant forms vesicles upon heating and stirring.<sup>11</sup> The effect of the conventional surfactant dodecyltrimethylammonium bromide (DTAB) and of the dimeric surfactant 12-10-12 (decamethylene-1,10-bis(dodecyldimethylammonium bromide)), both of which form spherical micelles, on the 12-20-12 vesicles was investigated.<sup>9</sup> These surfactants were found to break the 12-20-12 vesicles into smaller ones, then into membrane fragments, and finally give rise to spheroidal mixed micelles with the 12-20-12, as their concentration was increased. As pointed out above, all previous studies involved the addition of spheroidal micelle-forming surfactants to vesicular systems. There has been no study of the effect of addition of a surfactant having a very strong tendency to form threadlike micelles at fairly low concentration (say below 2 wt %) to a vesicular

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system. This paper reports such a study. Thus the effect of addition of the dimeric surfactant 12-2-12 (dimethylene-1,2-bis(dodecyldimethylammonium bromide)) to 12-20-12 vesicles was investigated by cryo-TEM. This 12-2-12 surfactant forms spherical micelles at very low concentration (0.26 wt %). Upon increasing its concentration an increasing fraction of 12-2-12 makes up elongated micelles, and the density of the spherical micelles decreases (0.5–0.8 wt %).<sup>10–12</sup> At still higher 12-2-12 concentration the threadlike micelles become increasingly connected (branched), and annular micelles (rings) appear in the system. At a concentration of 1.5 wt % the system is in the form of a network, where almost all threadlike or ring-like micelles are interconnected.<sup>12</sup> This behavior of 12-2-12 led us to investigate the changes of microstructure that occur in a vesicular 12-20-12 system upon addition of increasing amounts of 12-2-12, as to cover the concentration range 0.1 to 2 wt % in which one goes from spherical 12-2-12 micelles to a network. In this study, we observed several microstructural intermediates between those two states (see below). We also showed that some microstructural transformations can be extremely slow, stretching over weeks or even months. The turbidity and electrical conductivity of the mixed 12-20-12/12-2-12 systems were also measured and the systems were examined by digital light microscopy. The observed variations were correlated with the changes of microstructure of the system observed by cryo-TEM.

## Experimental Section

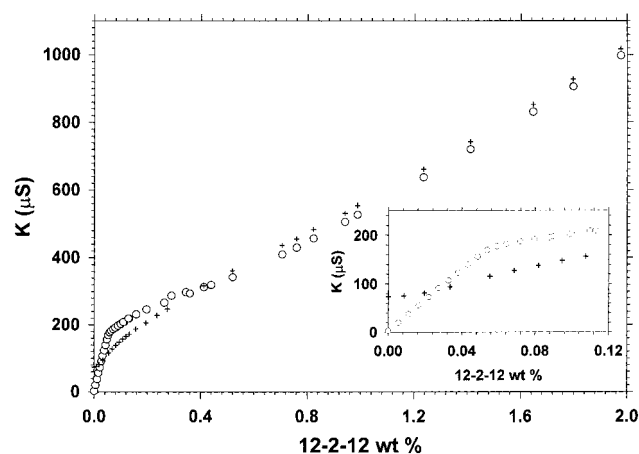
**Materials.** The synthesis and purification of the dimeric surfactants 12-2-12 and 12-20-12 have been described elsewhere.<sup>13</sup>

The conductivity measurements used two stock solutions: a 2 wt % 12-2-12 solution in water and a 2 wt % 12-2-12 solution in water + 0.09 wt % 12-20-12. The measurements in the high 12-2-12 concentration range were performed by progressively diluting the stock solution by water or water + 0.09 wt % 12-20-12. The low concentration range was investigated by successive additions of aliquots of either stock solution to water or water + 0.09 wt %.

A series of 12-20-12/12-2-12 aqueous mixtures for light microscopy and cryo-TEM were prepared starting from stock solutions of the pure 12-2-12 (2.5 or 4 wt %) and 12-20-12 (0.2 wt %) surfactants. The stock solutions were prepared by mixing the required amount of Millipore water and surfactants, heating the flask to 50 °C, stirring for 1–2 h, and then cooling to room temperature. Appropriate amounts of the stock solutions and of Millipore water were mixed to a total volume of 2 mL, and to a final 12-20-12 concentration of 0.09 wt % (i.e., 1.14 mM). In those mixed solutions, the concentration of 12-2-12 ranged from 0.1 to 2.0 wt %. The mixtures were stirred for 2 h and left to equilibrate at room temperature. No changes (i.e., precipitation) were visually observed when the solutions were left for several weeks, even months, at room temperature (20–25 °C).

**Methods. Spectrophotometry.** The apparent absorbance (which is related to the turbidity) of the systems was measured with a UNICAM UV7 UV/VIS spectrophotometer at a wavelength of 400 nm. These measurements allowed us to locate the concentration range where the microstructural transformations took place.

**Electrical Conductivity.** The electrical conductance of the solutions was measured using a Wayne-Kerr conductivity bridge B905, operated at a frequency of 1 kHz, in conjunction with a Tacussel (France) conductivity cell TA100 with platinum electrodes embedded in glass. The solutions were contained in



**Figure 1.** Variation of the electrical conductivity of 12-2-12 solutions in water (O) and in water plus 0.09 wt % 12-20-12 (+) with the 12-2-12 concentration. The inset shows the results in the dilute concentration range.

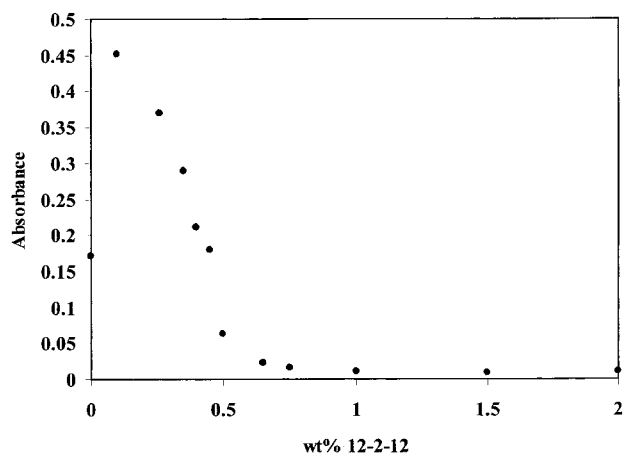
a double-walled cell that maintained the temperature of the system at  $25 \pm 0.1$  °C.

**Digital Light Microscopy (DLM).** Samples for light microscopy were prepared by placing a drop of the examined solution on a microscope slide, and covering it with a cover glass. The specimens were observed at 25 °C using an Olympus BH-2 light microscope, operated with Nomarski differential interference contrast optics, and connected to an Optronics LE-Digital Camera.

**Cryo-TEM.** The preparation of vitrified specimens for cryo-TEM was performed in a controlled environment vitrification chamber (CEVS).<sup>14</sup> All solutions were quenched from 25 °C and 100% relative humidity. Specimens were examined in a Philips CM120 microscope, operated at 120 kV, using an Oxford CT-3500 cryo-holder system. All specimens were observed in the microscope below  $-178$  °C. Images were recorded digitally by a Gatan 791 MultiScan CCD camera with the DigitalMicrograph software package. Images were prepared for publication by the Adobe Photoshop package.

## Results and Discussion

**Electrical Conductivity.** Figure 1 shows the variation of the electrical conductivity (K) with the 12-2-12 concentration in water and in water + 0.09 wt % 12-20-12. The results are qualitatively similar to those for the DTAB/12-20-12 system upon increasing DTAB concentration.<sup>9</sup> At concentrations above 0.6 wt % the two plots run nearly parallel. The higher conductivity of the system in the presence of 12-20-12 is due to the additional ions introduced by that surfactant. The plot in water shows a break at a concentration of 0.055 wt %, i.e., 0.89 mM, a value in good agreement with that reported for the CMC (critical micelle concentration) of 12-2-12.<sup>13</sup> The break disappears for the systems in the presence of 12-20-12, owing to the adsorption of the added 12-2-12 by the 12-20-12 vesicles. The ratio of the values of the slopes of the K vs concentration plots at concentration  $\rightarrow 0$ , in the absence and presence of 12-20-12 (see inset), is close to 10, indicating that the added 12-2-12 is indeed nearly totally adsorbed by the 12-20-12 vesicles. The increase of the slope of the K vs concentration plot at low concentration for the systems in the presence of 12-20-12 reflects an increased partitioning of 12-2-12 between the vesicles and the aqueous phase. The progressive breakup of the vesicles into smaller aggregates upon incorporation of 12-2-12 (see below) must also contribute to this change of slope. Indeed, at a given



**Figure 2.** Variation of the apparent absorbance of 12-2-12 solutions in water plus 0.09 wt % 12-20-12 with the 12-2-12 concentration. The absorbances were measured 5 days after preparation of the mixtures.

surfactant concentration, a system made up of a large number of small aggregates conducts electricity better than a system made up of a smaller number of larger aggregates.

An interesting effect was noted in the early stages of adding the 12-2-12 + 12-20-12 stock solution to the 0.09 wt % 12-20-12 solution. The conductivity increased rapidly immediately after the addition of the stock solution aliquot, then decreased slowly toward its equilibrium value. For instance, the addition of the first aliquot increased the 12-2-12 concentration from zero to 0.0085 wt % and resulted in a very rapid increase, in 5 to 10 s, of conductivity from 73.5  $\mu$ S to over 88  $\mu$ S. The conductivity then decreased slowly to 75.4  $\mu$ S in about 10 min. The amplitude of the slow decrease of conductivity decreased as the 12-2-12 concentration increased. This behavior was observed up to a 12-2-12 concentration of about 0.08 wt %. The slow decrease of conductivity observed at low concentration most likely reflects the progressive incorporation of the 12-2-12 into the vesicles. This explanation is supported by the fact that the slow change of conductivity is not observed when the experiments are performed in the absence of 12-20-12. At higher 12-2-12 concentrations only a relatively slow decrease of conductivity, of rather small amplitude, was still observed, and did not affect the results.

**Spectrophotometry.** For the measurements we used mixtures that had been left to equilibrate for 5 days. Figure 2 displays the variation of the apparent absorbance (which is related to the turbidity of the system) of a 0.09 wt % 12-20-12 vesicular system with the concentration of added 12-2-12. The absorbance is seen to go through a marked maximum at a rather low concentration of added 12-2-12 (around 0.1 wt %) and then to decrease monotonically upon increasing 12-2-12 concentration up to 0.75 wt %. Above that concentration and up to 2 wt % 12-2-12 no additional change of turbidity was detected. This may be taken as an indication for complete solubilization of the 12-20-12 vesicles. The presence of a maximum at low concentration of added surfactant in the plot absorbance vs concentration has been often reported in other vesicle solubilization studies.<sup>1-7</sup> It has been attributed to size increase of the particles present in the system. The maximum is, however, much more pronounced here than for the 12-20-12 /12-10-12 or the 12-20-12 /DTAB systems.<sup>9</sup> Nevertheless, the most important feature of the plot in Figure 2 is probably the relatively slow decrease of the apparent absorbance upon increasing 12-2-12 concentration. In similar studies involving phospholipids or 12-20-12 vesicles the decrease of absorbance was usually steeper

(an exception is given ref 4) and occurred over a narrower range of concentration of the spherical micelle-forming surfactant.

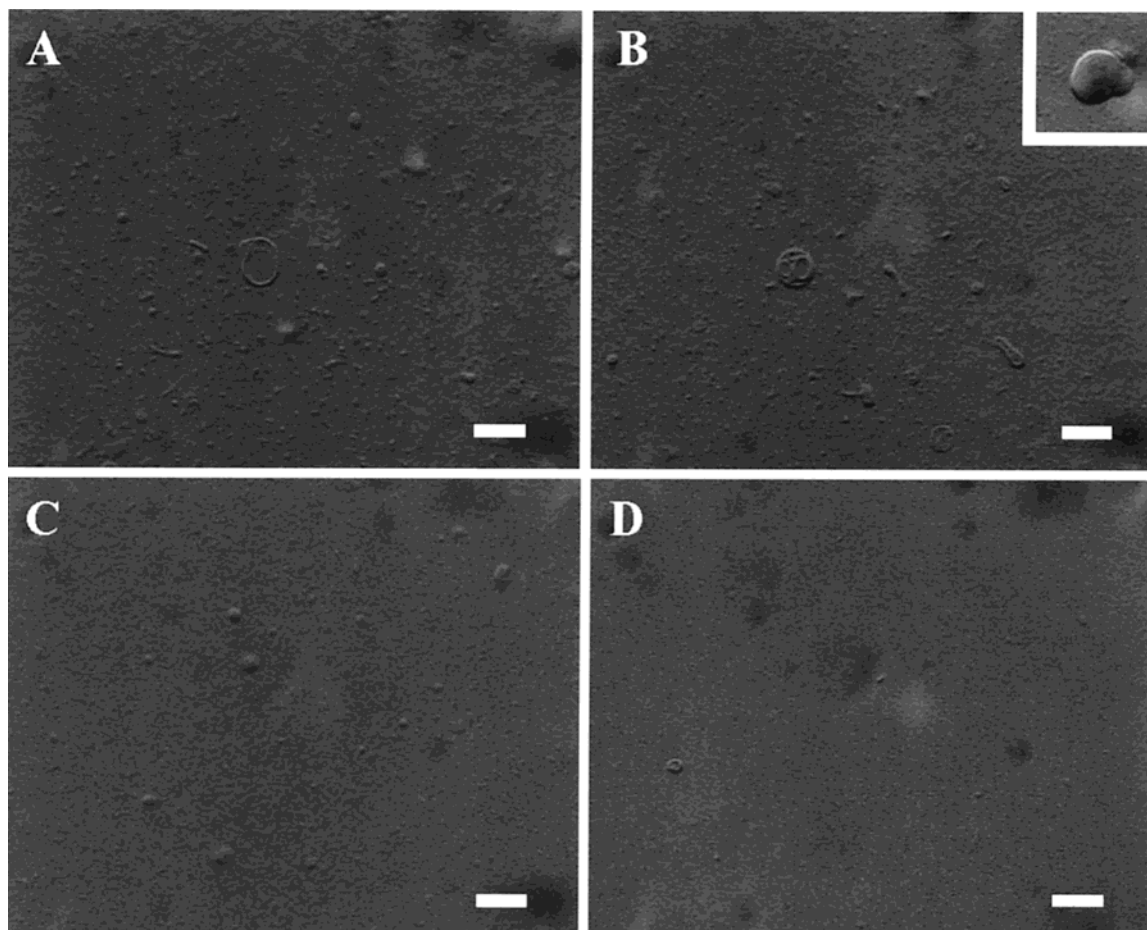
**Digital Light Microscopy.** The 12-20-12 /12-2-12 mixtures were examined by digital light microscopy two months after preparation. Recall that this technique allows visualization of particles in the range 1–500  $\mu$ m. Representative images are shown in Figure 3. In the pure 0.09 wt % 12-20-12 system (Figure 3A) a wide population of spherical vesicles is observed. Most of the vesicles have diameters smaller than 20  $\mu$ m. A few larger ones of diameter around 40  $\mu$ m and some tubular vesicles are also present. The addition of 0.1 wt % 12-2-12 (Figure 3B and inset) induces vesicular growth and large vesicles are frequently seen. The growth in vesicle size explains the increase of the turbidity (intensity of the scattered light) for this surfactant concentration noted in Figure 2. Indeed, at a given solute concentration, large objects scatter light much more than small objects. The increase of vesicle size is obviously due to the 12-2-12 incorporation into the 12-20-12 vesicles revealed by the conductivity measurements in Figure 1. This growth of the vesicles may arise from the relaxation of the tension in the vesicle. Further addition of 12-2-12 caused decrease in both the size and number of the vesicles (Figure 3, parts C and D). At 0.45 wt % 12-2-12 very few vesicles were still observed in Figure 3D. The complete disappearance of particles visible by light microscopy was reached at a 12-2-12 content of about 0.65 wt % (not shown).

**Cryo-TEM.** *Systems Equilibrated for Two Months.* The electron micrographs in Figure 4, parts A and B, illustrate the types of microstructure present in the pure 0.09 wt % 12-20-12 system. Unilamellar vesicles, some of them very elongated and in the form of tubules with swollen ends, constitute the main structure. Thus cryo-TEM reveals structures very similar to those seen by DLM but of smaller size. Some bilayer fragments are also seen. In Figure 4B, black arrows mark openings in the vesicle bilayers. Recall that our previous study of 12-20-12 vesicular systems at a concentration of 1.4 wt % also showed evidence for large vesicles, many of them of double-lamellae.<sup>9</sup> 12-20-12 systems at concentrations between 0.09 and 1.21 wt % were examined as part of the present work. Up to a concentration of about 0.7 wt % (not shown) the micrographs revealed the same structures as for the 0.09 wt % system. At 0.7 wt % a few double-lamellae vesicles were observed, coexisting with unilamellar vesicles and also tubes with swollen ends. Double- and multilamellar vesicles and tubes were observed at 1.21 wt %. The stirring of the system prior to specimen preparation and vitrification resulted in structures that were generally smaller than without stirring.

The addition of 12-2-12 at a level of 0.1 wt % resulted in nearly no change in the type of microstructures present in the system (see Figure 4, parts C and D). However, the average vesicle size increased and ranged typically between 0.02 and 1  $\mu$ m. This growth was due to the incorporation of the major fraction of the added 12-2-12 into the vesicles, indicated by the conductivity data. In general the vesicles were unilamellar, but a few double-lamellar vesicles were also observed. The majority of vesicles had smooth curved bilayers. Some faceted vesicles were also seen (Figure 4C).

As the 12-2-12 concentration was increased above 0.1 wt %, the average size of the microstructures decreased (Figure 5), in agreement with the DLM observations. In addition, spheroidal micelles were observed. At 0.26 wt % 12-2-12 (Figure 5A) unilamellar vesicles larger than 300 nm were no longer seen. A small population of larger unilamellar vesicles with diameters of about 100 nm coexisted with a fairly large population of





**Figure 3.** Digital light microscopy images of mixtures containing 0.09 wt % 12-20-12 and increasing amounts of 12-2-12: (A) no added 12-2-12; (B) 0.1 wt %; (C) 0.26 wt %; (D) 0.45 wt %. Bar: 40  $\mu\text{m}$ . The mixtures were examined 2 months after preparation.

small vesicles, most of them with diameters ranging between 20 and 30 nm. Spheroidal and small disk-shaped micelles can also be seen in Figure 5A. These assemblies are probably mixed, with variable proportions of 12-2-12 and 12-20-12, as reflected by their shape. Figure 5B shows that a further increase in the 12-2-12 concentration to 0.4 wt % results in the nearly complete solubilization of the vesicles. Small unilamellar vesicles are still present, but their number and size (diameter between 10 and 30 nm) are significantly reduced. Many bilayer fragments (or disk-shaped mixed micelles) and some spheroidal micelles can also be seen. Some vesicles in Figures 5A and 5B are unusually small (10 to 20 nm). Similarly small vesicles with diameters of about 20 nm have been reported to exist in more complex systems,<sup>15–17</sup> as for instance the water/*n*-octanol/Triton X-100/cetylpyridinium chloride system.<sup>15</sup>

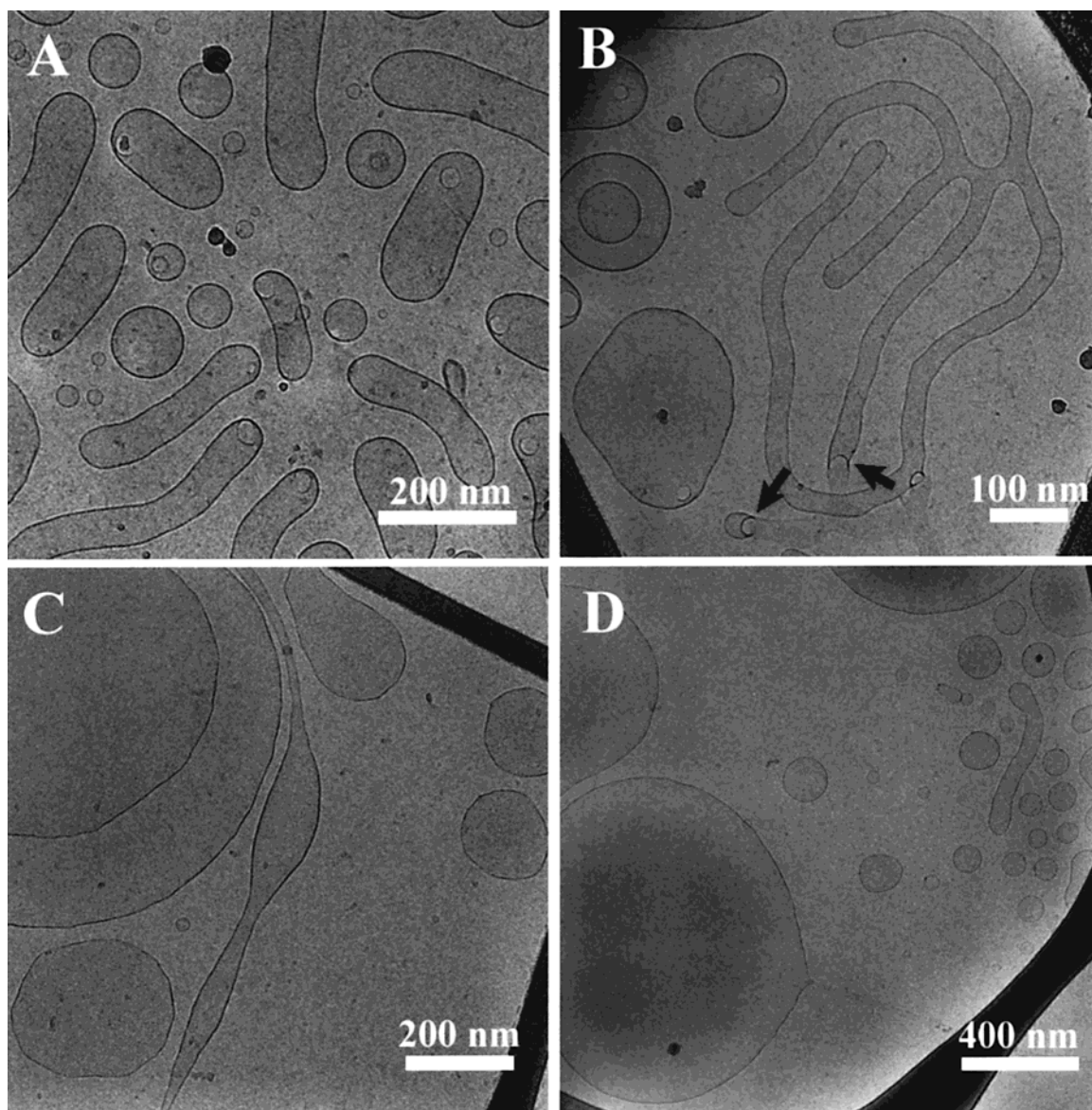
Figure 6A shows that vesicles have been almost completely solubilized at 0.75 wt % 12-2-12. Only very few of the smallest vesicles are still present, coexisting with many disks (10 nm or less in diameter) and short threadlike micelles a few tens of nanometers long.

Vesicles were no longer observed at above 0.75 wt % 12-2-12 (see below). The complete solubilization of vesicles at that 12-2-12 content is in agreement with the turbidity measurements in Figure 2 that show only very small variations in apparent absorbance above that concentration. Note that at concentrations above 0.75 wt % the system is predominantly made up of 12-2-12. One is then observing how the small and constant amount of 12-20-12 present in the system affects the microstructures of 12-2-12 solutions of increasing concentration. The effect of

the 12-20-12 is expected to diminish as the 12-2-12 concentration is increased.

Figure 7 shows cryo-TEM images of three systems at increasing 12-2-12 concentration. At 1 wt % 12-2-12 (Figure 7A) the main microstructures observed are closed rings (diameter 20–50 nm) and threadlike micelles shorter than 150 nm. Some spheroidal micelles are also seen, but disklike micelles were seldom found in this mixture. At 1.5 wt %, 12-2-12 very long threadlike micelles (Figure 7B) and many individual rings, occasionally connected to other rings (Figure 7C), can be seen. The rings are apparently of larger average diameter than in the 1 wt % mixture. A well-developed network is seen at 2 wt % 12-2-12 (Figure 7D), similar to that reported for a 1.5 wt % solution of pure 12-2-12,<sup>12</sup> with 3-fold junctions (branching) and overlapping threadlike micelles. Rings connected to other rings (inset), in addition to few disconnected rings, are also present.

It is interesting to compare the microstructure in the pure 12-2-12 solutions and in the 12-2-12/12-20-12 mixtures at the same 12-2-12 concentration. It is clear that the effect of the presence of 12-20-12 on the microstructure of the mixture is very strong even at a 12-2-12 concentration of about 0.7 wt %, that is when the mole fraction of 12-20-12 in the mixture is only 0.083. Indeed, at such a concentration 12-2-12 alone already forms very elongated threadlike micelles that are the most prominent microstructural feature of this surfactant. Such micelles are still completely absent in the mixture containing 0.75 wt % 12-2-12. In most instances the microstructures seen in pure 12-2-12 solutions are observed at a higher 12-2-12



**Figure 4.** (A and B) Cryo-TEM micrographs of mixtures containing 0.09 wt % 12-20-12 and no 12-2-12 vitrified two months after mixture preparation: mostly unilamellar vesicles are observed, with a broad distribution of size and shape. Branched tubular vesicles are seen in Figure 4B. Arrows mark openings in vesicle bilayers (B). (C and D): Cryo-TEM micrographs of mixtures containing 0.09 wt % 12-20-12 and 0.1 wt % 12-2-12 vitrified two months after mixture preparation: the vesicles are larger. Some have faceted walls (e.g., vesicles at the left side of Figure 4C).

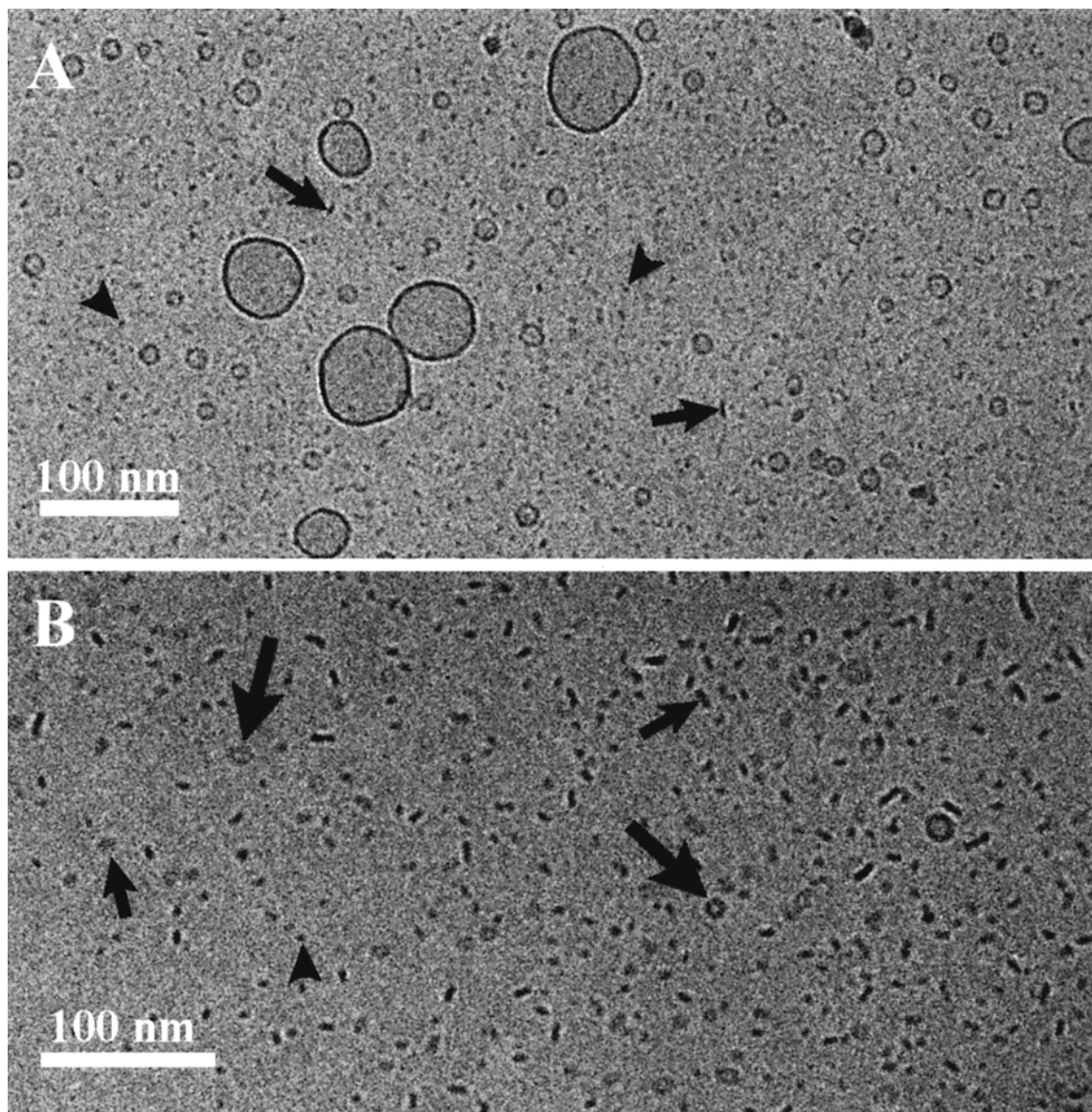
content in the mixtures. Thus, very long threadlike micelles are present in the 0.5–0.74 wt % pure 12-2-12 solutions, whereas only rather short threadlike micelles ( $\leq 150$  nm) are seen in the mixture containing 1 wt % 12-2-12. The very long threadlike micelles were observed only at 1.5 wt %. Likewise, branched networks are observed at 1.5 wt % with the pure 12-2-12, but only at 2 wt % 12-2-12 in the mixture. On the contrary, the ring-like micelles are apparently more easily formed in the mixture than in the pure 12-2-12. Thus individual rings are present in large numbers in the 1 wt % mixture, but in very small numbers in pure 12-2-12 solutions. The ring-like micelles are the most frequently seen structure in the 1.5 wt % mixture. At this concentration the pure 12-2-12 solution shows only few individual rings dispersed in a network formed by rings and threadlike micelles.

**Effect of Aging of the Systems.** Most of the 12-20-12 /12-2-12 mixtures were also observed by cryo-TEM just 5 to 7 days after being prepared. No difference between “fresh” and “aged” solution was noted in the dilute systems, with 12-2-12 concentrations up to 0.5 wt %, and in the concentrated ones, with

concentrations equal to or above 1 wt %. However an interesting effect of aging was observed for the mixtures containing 0.65 and 0.75 wt % 12-2-12. Figure 6B shows a typical micrograph of the 0.75 wt % mixture vitrified 7 days after mixture preparation. This micrograph shows the same microstructures as those seen in the mixture equilibrated for two months, namely, a few small vesicles, many disklike micelles and short threadlike micelles (Figure 6A). Two additional microstructures can be seen in micrograph in Figure 6B: very long and linear, i.e., rigid, rodlike micelles spanning the whole micrograph and bilayer fragments connected by threadlike micelles that also extend over very long distances. The latter structures look occasionally like irregular twisted ribbons because the width of the connected bilayer fragments varies much. These ribbons are obviously intermediate, probably nonequilibrium, structures.

The 0.65 wt % mixture (Figure 8) showed the same features as the 0.75 wt % one, namely, the six-day old mixture showed very long and fairly linear threadlike micelles and irregular ribbons (Figure 8A). The main difference between the 0.65 and 0.75 wt % mixtures was that the former did not appear to have





**Figure 5.** Cryo-TEM micrographs of mixtures of 0.09 wt % 12-20-12 and 0.26 wt % (5A) or 0.4 wt % (5B) 12-2-12 vitrified two months after mixture preparation. (A) Many small unilamellar vesicles (diameter  $\sim 20\text{--}30$  nm) coexisting with larger vesicles (diameter  $\sim 100$  nm) are seen. Disklike (smaller arrows) and spheroidal (arrowheads) mixed micelles are also seen. (B) Small unilamellar vesicles (large arrows) in smaller number are still seen in addition to disklike (smaller arrows) and spheroidal mixed micelles (arrowhead).

reached equilibrium even after two months. Indeed, the micrograph (Figure 8B) of the 0.65 wt % mixture two months after its preparation still showed some fairly long threads which sometimes looked like twisted ribbons. This does not fit in the microstructural evolution pattern upon increasing 12-2-12 content, described above for the “aged” mixtures.

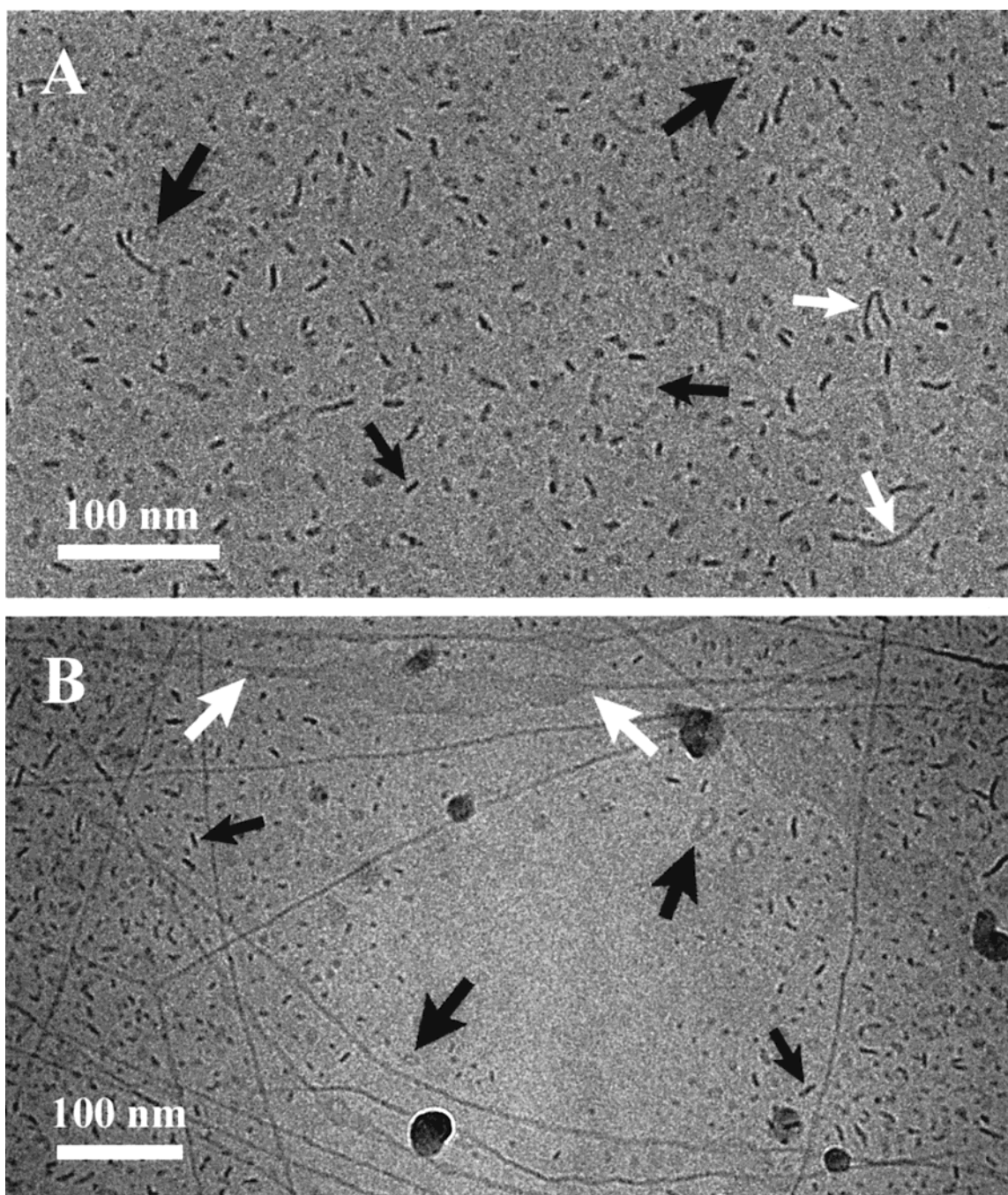
Finally we would like to discuss the overall microstructural evolution of the 12-20-12/12-2-12 mixtures with increasing 12-2-12 concentration for the mixtures equilibrated two months after preparation. The effect of aging is briefly discussed at the end of this section.

The conductivity results show that the added 12-2-12 is quite rapidly adsorbed by the existing vesicles, in tens of minutes. This time is much shorter than the time the systems were allowed to equilibrate, 5 days to two months. Thus it can be assumed that the mixing of the two surfactants in the mixed aggregates is fully achieved, and that the microstructures observed after two months represent equilibrium microstructures. Nevertheless, it is realized that the vesicles present in the pure 0.09 wt % initial 12-20-12 system and also in the mixtures at

low content of 12-2-12 may not be true equilibrium structures. However the observed microstructures did not significantly change over a long time period (7 days to two months) and the term “equilibrium structures” refers to this time scale. We shall also implicitly accept that in systems showing various coexisting structures the vesicles and bilayer fragments are rich in 12-20-12, whereas the spheroidal or threadlike micelles are rich in 12-2-12, because these structures are the ones encountered in the pure 12-20-12 and 12-2-12 systems, respectively.

When considering only equilibrium structures the sequence of microstructures observed in this study is the one that can be expected when mixing a vesicle- or bilayer-forming surfactant (12-20-12) with a threadlike micelle-forming surfactant (12-2-12). Thus starting from a pure vesicular 12-20-12 system, the addition of a small amount of 12-2-12 (about 0.1 wt %) only brings about an increase of the vesicle size by the effects discussed elsewhere.<sup>1</sup> Further additions of 12-2-12 (up to 0.26, 0.4, and 0.75 wt %) result in a progressive transformation of the large vesicles into smaller ones, and in the occurrence of disklike micelles rich in 12-20-12 (but locally rich in 12-2-12



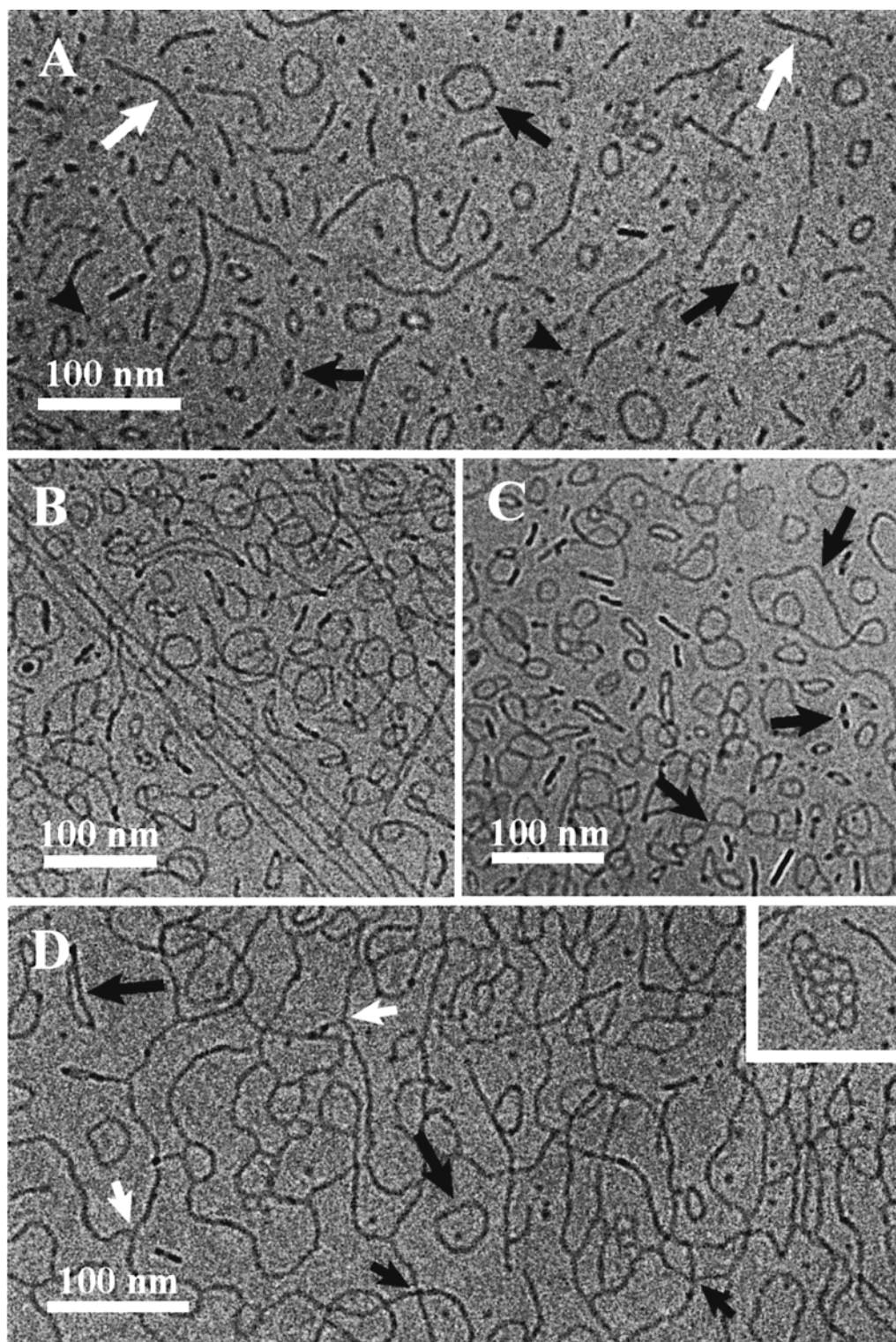


**Figure 6.** Cryo-TEM micrographs of mixtures made up of 0.09 wt % 12-20-12 and 0.75 wt % 12-2-12 vitrified two months (A) and 7 days (B) after preparation. (A) Many small unilamellar vesicles (diameter  $\sim 10$ – $20$  nm) marked by large black arrows are seen. Disklike (small black arrows) and short threadlike mixed micelles (white arrows) are also seen. (B) Very long rigid rodlike micelles and irregular ribbons are observed. Large white arrows mark ribbon opening-up. Black arrows mark disklike micelles, and the large black arrows mark small vesicles.

in their rims) and of spheroidal micelles rich in 12-2-12. All intermediate species between the latter two types of microstructures are present in the systems, in variable proportions depending on the 12-2-12 concentration. The presence of spheroidal micelles is not surprising. Indeed, they are also seen in pure 12-2-12 solutions even at a concentration as high as 0.74 wt %. As expected, the progressively increasing content of the threadlike micelle-forming 12-2-12 in the presence of a constant amount of 12-20-12 results in the progressive breakup of the vesicles and occurrence of threadlike micelles. This is seen at 1 wt % 12-2-12. At this concentration the first ring-like micelles are also observed as in the pure 12-2-12 solutions, but in a much larger proportion. At a still higher concentration (1.5 and 2 wt %) the observed structures look very much like those

seen with the pure 12-2-12 solutions: first ring-like and threadlike micelles, then a network. As expected, at high 12-2-12 content (2 wt %) the microstructures present in the mixtures are very similar to those seen with the pure 12-2-12 at a lower concentration. Thus even the presence of a very small amount of 12-20-12 can significantly shift the formation of the very long threadlike micelles to higher 12-2-12 concentration and favor the formation of ring-like micelles. The first effect is easily understood. Indeed the mixed aggregates tend to have a curvature intermediate between that adopted by the pure 12-2-12 and that adopted by the pure 12-20-12 resulting in the small disklike micelles at low 12-2-12 concentration and shorter elongated micelles at higher concentration. The increased stability of ring-like micelles in the presence of 12-20-12



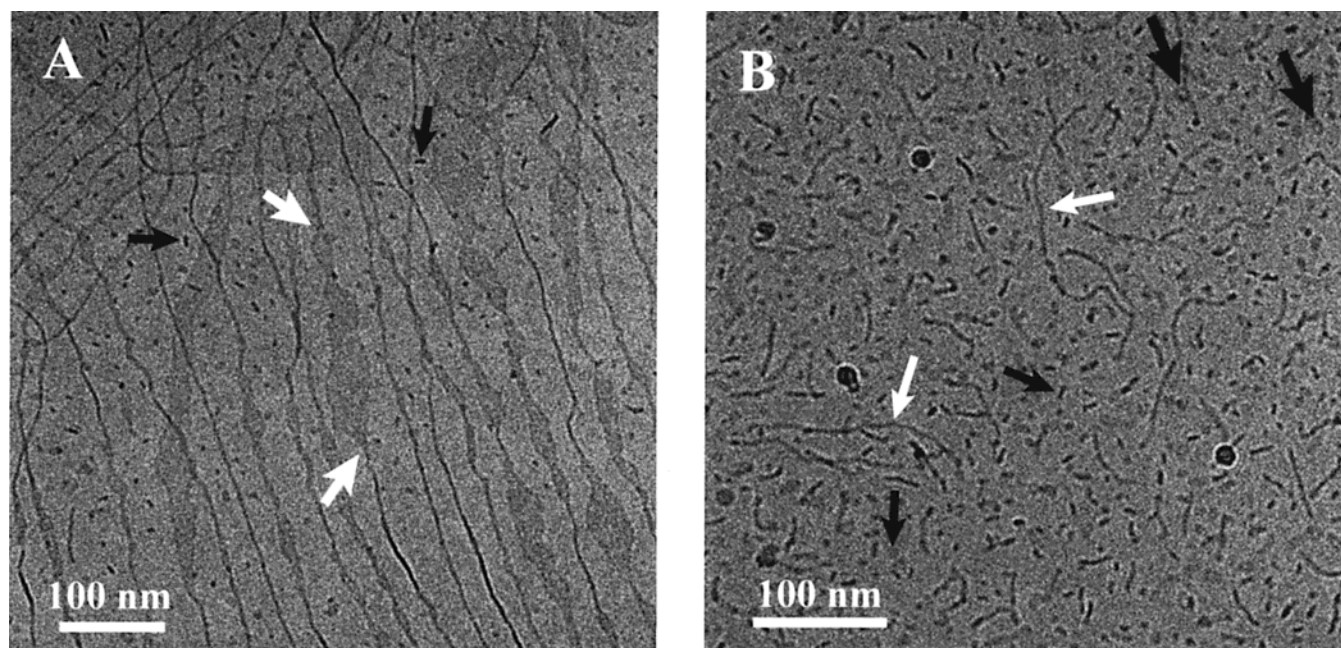


**Figure 7.** Cryo-TEM micrographs of mixtures made up of 0.09 wt % 12-20-12 and 1 wt % (7A), 1.5 wt % (7B), and (7C), and 2 wt % (7D) 12-2-12 vitrified two months after mixture preparation. (A) Short threadlike micelles (white arrows) and closed rings (black arrows) coexist. For rings which are parallel to the vitrified film, the contrast is about uniform along their circumference. For rings that are tilted with respect to the film, the contrast varies along the circumference (When the ring is perpendicular to the film it is seen as two black dots connected by a gray line). Spheroidal micelles are also seen (black arrowheads). (B and C) Very long threadlike micelles coexist with many individual rings, occasionally connected to each other (black arrows). The average diameter of the rings is larger than in system (A). (D) A network of branched cylindrical micelles (black arrows). Small white arrows mark 3-fold junctions (branching) and small black arrows indicate overlapping micelles. Rings connected to other rings (see inset), in addition to few disconnected rings (large black arrows), are also seen.

suggests that the incorporation of 12-20-12 molecules into 12-2-12 micelles induces an increase of the end-cap energy,  $E_c$ , the energy needed to create two new chain ends.<sup>18</sup> In fact, the probability of ring-like micelle formation was shown to increase with increasing  $E_c$ .<sup>19</sup> The system can gain entropy by fragment-

ing very long chains (for large  $E_c$ ) into numerous smaller rings. Such rings pay a conformational entropy penalty for closure, but it is lower for shorter micelles. This, of course, increases the probability of closure of those micelles into rings, that gain entropy of mixing without having to pay the high free energy





**Figure 8.** Cryo-TEM micrographs of mixtures made up of 0.09 wt % 12-20-12 and 0.65 wt % vitrified 6 days (A) and two months (B) after mixture preparation. (A) Very long and fairly linear threadlike micelles and irregular ribbons are observed, similar to those observed at 0.75 wt % (Figure 6A). White arrows mark ribbon opening-up; black arrows show disklike micelles. (B) White arrows indicate threads; black arrows mark disklike micelles, and large black arrows show small vesicles.

cost for creating two ends.<sup>20</sup> Ring-like micelles have been observed in solutions of a cationic tetrameric surfactant,<sup>21</sup> and in several other systems.<sup>22–24</sup>

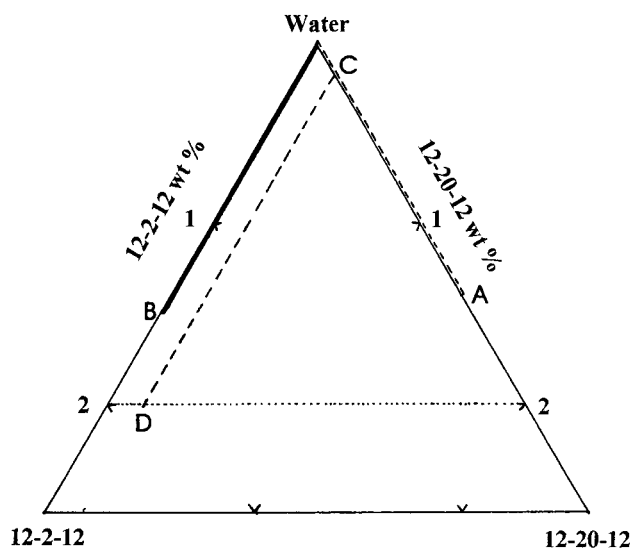
Turning now to the effect of aging, it is important to recall that the mixtures investigated by spectrophotometry, DLM, and cryo-TEM were all prepared by mixing stock solutions of 12-20-12 and 12-2-12. The 0.2 wt % stock solution of 12-20-12 contained vesicles whereas in the 2.5 and 4 wt % 12-2-12 stock solutions the surfactant formed a network of connected rings and threads.<sup>9</sup> When the two stock solutions are mixed, new structures are formed, following the progressive redistribution of the two surfactants. This redistribution cannot occur through collisions between initially present assemblies with merging of the colliding assemblies. This is so because the assemblies in the 12-20-12 /12-2-12 mixtures are electrically charged and strongly repel each other. The redistribution must therefore occur through surfactant exchange via the solution surrounding the assemblies: surfactants dissociate from assemblies, diffuse in the solution, and incorporate into other assemblies. This process is expected to involve essentially 12-2-12. Indeed, the residence time of 12-20-12 in its assemblies is much longer than that of 12-2-12.<sup>25,26</sup> Thus redistribution via 12-20-12 exchange can be neglected. Besides, the residence time of 12-2-12 into the branched network present in the stock solution is quite long. Last, the rate of incorporation of a free 12-2-12 by a 12-20-12 vesicle is probably slower than for a diffusion-controlled process, contrary to what happens in micellar solutions.<sup>20,21</sup> On this basis the redistribution of the surfactants and thus the formation of the equilibrium structures is expected to be very slow. It is also expected that when a 12-2-12 surfactant ion adsorbs onto a membrane fragment or disklike micelle rich in 12-20-12, it will locate at the rim of these assemblies, since the rim can be considered as half of a threadlike micelle that 12-2-12 tends to form. Likewise, the adsorbed 12-2-12 will locate at the structural defects of the 12-20-12 -rich vesicles (for instance the openings seen in some vesicles in Figure 4B). As more 12-2-12 slowly dissociates from the 12-2-12 assemblies and adsorbs on 12-20-12-rich assemblies, the latter progressively

break up into structures of higher curvature. The disklike micelles represent equilibrium structures, whereas the rigid rods and the irregular ribbons are intermediate nonequilibrium structures still richer in 12-20-12 than the overall composition of the system. These structures will slowly evolve toward the equilibrium structures because the processes discussed above, by which this equilibrium is reached, are slow.

## Conclusions

This study focused on the microstructure of aqueous mixtures of the vesicle-forming 12-20-12 dimeric surfactant and of the threadlike micelle-forming 12-2-12 dimeric surfactant. The mixtures were investigated by means of electrical conductivity, spectrophotometry, digital light microscopy, and cryo-TEM. We studied by cryo-TEM samples vitrified two months after the preparation of the mixtures, at a constant 12-20-12 concentration of 0.09 wt %. The progressive increase of the 12-2-12 content in the mixture resulted first in vesicle growth (0.1 wt %), followed by vesicle breakage into smaller vesicles (0.26 wt %), the formation of disklike micelles (0.4 wt %), that of ring-like micelles and short elongated threadlike micelles (1 wt %), extensive growth of those threads (1.5 wt %), and finally formation of a network (2 wt %), where the threads and rings are interconnected. The network contained also a few isolated rings. Similar structures were observed when vitrifying the mixtures for cryo-TEM 5–7 days or two months after mixture preparation in the case of mixtures containing up to 0.5 or 1 wt % and more 12-2-12. Aging of the systems was observed for mixtures in the intermediate range of 12-2-12 content (0.65 and 0.75 wt %). When examined 5–7 days after preparation these mixtures showed long rigid rodlike micelles and irregular twisted ribbons. These structures nearly or completely disappeared when the mixtures were allowed to equilibrate for two months. A model has been presented to explain the observed behavior.

At the end of this paper it is worth pointing out that our study has probed only a very small part of the phase diagram of the ternary system 12-2-12/12-20-12 /water. A partial phase diagram



**Figure 9.** Partial phase diagram of the dilute range of the ternary mixture water/12-20-12 /12-2-12. See text.

is shown in Figure 9. The bulk of the present study concerned systems located on the straight line CD (systems at 0.09 wt % 12-20-12 content) with vesicles observed close to point C and a branched network observed close to point D. A few systems on the line from the water apex to point A (aqueous systems with a maximum 12-20-12 content of 1.21 wt %) have been investigated and all showed unilamellar or multilamellar vesicles of varied shapes. Last, the systems between the water apex and point B correspond to the 12-2-12 aqueous solutions previously investigated.<sup>12</sup> It would be worthwhile in a future study to examine systems where the total surfactant concentration in the mixture is fixed, as for instance those with compositions on the dotted horizontal line at 2 wt %. Systems at constant ratio of 12-2-12 and 12-20-12 concentrations also deserve consideration (located on a line going from the water apex to a fixed point on the 12-2-12/12-20-12 axis). Such studies may reveal unexpected microstructures.

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