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Polymer–vesicle association

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ABSTRACT

Mixed polymer–surfactant systems have been intensively investigated in the last two decades, with the main focus on surfactant micelles as the surfactant aggregate in interaction. The main types of phase behavior, driving forces and structural/rheological effects at stake are now fairly well understood. Polymer–vesicle systems, on the other hand, have received comparatively less attention from a physico-chemical perspective. In this review, our main goal has been to bridge this gap, taking a broad approach to cover a field that is in clear expansion, in view of its multiple implications for colloid and biological sciences and in applied areas. We start by a general background on amphiphile self-assembly and phase separation phenomena in mixed polymer–surfactant solutions. We then address vesicle formation, properties and stability not only in classic lipids, but also in various other surfactant systems, among which catanionic vesicles are highlighted. Traditionally, lipid and surfactant vesicles have been studied separately, with little cross-information and comparison, giving duplication of physico-chemical interpretations. This situation has changed in more recent times.

We then proceed to cover more in-depth the work done on different aspects of the associative behavior between vesicles (of different composition and type of stability) and different types of polymers, including polysaccharides, proteins and DNA. Thus, phase behavior features, effects of vesicle structure and stability, and the forces/mechanisms of vesicle–macromolecule interaction are addressed. Such association may generate gels with interesting rheological properties and high potential for applications. Finally, special focus is also given to DNA, a high charge polymer, and its interactions with surfactants, and vesicles, in particular, in the context of gene transfection studies.

Contents

Abbreviations: $C_{12}E_5$, Pentaethylene glycol monododecyl ether; $C_{12}E_4$, Tetraethylene glycol dodecylether; CdS, Cadmium Sulfide; Cryo-TEM, Cryogenic transmission electron microscopy; CTAB, Cetyltrimethylammonium bromide; CTAT, Cetyltrimethylammonium tosylate; DDAB, Didodecyldimethylammonium bromide; DNA, Deoxyribonucleic acid; DOPC, Dioleoylphosphatydilcholine; DOTAP, Dioleoyltrimethylammonium propane; DSC, Differential scanning calorimetry; DTAB, Dodecyltrimethylammonium bromide; HEC, Hydroxyethylcellulose; JR400, Hydroxyethylcellulose derivative with a charge concentration of 10 mM (1 wt.% polymer); LM200, Hydroxyethylcellulose derivative with a charge concentration of 2 mM (1 wt.% polymer) and 0.76% of hydrophobic modification; NaBr, Sodium Bromide; NaCl, Sodium Chloride; PEG, Poly(ethylene glycol); PPO, Poly(propylene oxide); PS-PEO, Polystyrene-poly(ethylene oxide); SDBS, Sodium dodecylbenzenesulfonate; SDS, Sodium dodecylsulphate; SOS, Sodium octylsulfate; TTAB, Tetradecyltrimethylammonium bromide.

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1. Introduction

The interactions between polar or amphiphilic polymers and low molecular weight amphiphilic compounds have far-reaching implications. Most industrial formulations, like water-based paints, detergents, personal care products, oil recovery fluids, contain both water-soluble polymer(s) and surfactant(s). In biotechnological applications and in biological systems, the interactions between polar lipids and different macromolecules, nucleic acids, proteins and polysaccharides, have a broad significance.

While the synergistic effects of water-soluble polymers and surfactants in applications have been realized for a considerable time, deeper fundamental studies took a long time to emerge. Early studies concerned, for example, the modification of surfactant surface activity exerted by a polymer and the influence of a surfactant on the thickening effect of a water-soluble polymer [1–6]. As the critical micelle concentration, cmc, has a central role in surfactant characterization, much early work was concerned with determining the surfactant cmc in the presence of a polymer [7], as exemplified in Fig. 1, for SDS in the presence of polyvinylpyrrolidone. On the other hand, the second important study object of surfactant systems, phase behaviour, received for a long time little attention.

Among the large number of surfactant self-assembly structures, micelles have received very much focus regarding polymer–surfactant interactions [8,9], while for example bilayer structures as found in lamellar liquid crystals and vesicles have received quite limited attention.

This situation is very different for polar lipids. Here both for historical reasons and for reasons of significance, vesicles have had a very prominent role. Thus phospholipids were the first vesicle systems investigated, well before vesicle studies became significant for surfactants. Furthermore, for many abundant polar lipids, the bilayer structure is the most common one. The fact that vesicles could be prepared from dispersions of phospholipid lamellar samples attracted immediately great interest; one reason being the suggestion that they could mimic cells since the bilayer structure is analogous in the two cases.

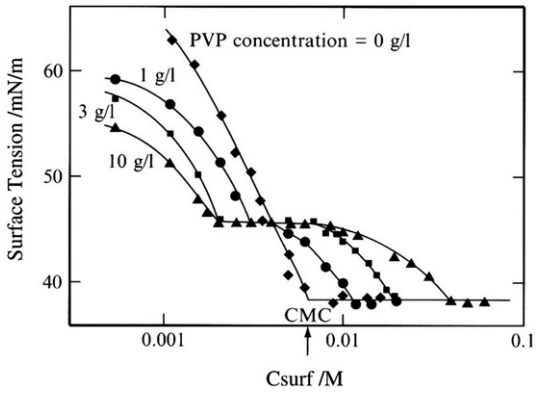

Fig. 1. Dependence of surface tension of SDS on surfactant concentration in the presence of different concentrations of polyvinylpyrrolidone (PVP). The polymer induces surfactant aggregation, as seen by a decrease of cmc. Redrawn from [7].

In the lipid vesicle field, the macromolecule–vesicle interactions are of interest primarily from two points of view; firstly, because of the mimicking of biological systems and, secondly, because of the need to stabilize the vesicles. Thus, an early focus for lipid vesicles was to use them in pharmaceutical and cosmetic formulations. However, being kinetically and not thermodynamically stable systems, the long-term stability was early an important issue much delaying acceptance in applications; the use of polymers as steric stabilizers of vesicles was seen as a remedy to this [10,11].

The fields of lipids and surfactants have traditionally been quite separated academically, with few contacts and little cross-fertilization. This has been natural, since surfactant science early was very much focused on applications, while lipid science dealt with biological and biotechnological aspects. The artificial separation is partly due to the fact that surfactant studies have very much focused on water-soluble amphiphiles, while the most studied lipids are water-insoluble. However, from a physico-chemical point of view, this separation has been quite unfortunate and lead to duplication of work. The general picture of amphiphile self-assembly was mainly established through surfactant work and many central concepts were only noted in the lipid field with considerable delay. The opposite is also true that important discoveries for lipid systems were not appropriately noted in the surfactant field. A notable example is that of vesicles, which were for a long time extensively studied for lipids. The large field of lipid vesicles, however, did not make much use of the knowledge of other amphiphile structures, mainly established in the surfactant field. On the other hand, surfactant vesicles did not receive much attention for a long time. It was only with the discovery of spontaneously forming and stable vesicles that interest on surfactant vesicles sparkled.

While mixed surfactant–polymer systems have received a considerable interest when the surfactant is in the micellar state, polymer-vesicle systems have only attracted broader attention much later. In view of a great potential in formulations, the interest can be expected to increase strongly. In view of this it was considered timely to review the field, thereby emphasizing the connection to the field of lipid–polymer interactions. Particularly in the latter case, putting the polymer–vesicle systems in the context of alternative amphiphile structures has often been neglected. Therefore, in this review we will start with a general background of amphiphile self-assembly and how a polymer may affect surfactant micellization. We will also discuss phase separation phenomena in mixed polymer–surfactant solutions. As already indicated, in considering these mixed systems we can either have a surfactant- or a polymer-centered view; this is illustrated by considering how a polymer influences an organized surfactant system and how a surfactant can affect a polymer network.

In reviewing our knowledge on surfactant and lipid vesicles, we note that while there are structurally close resemblances, there are, from a stability point of view, fundamental differences between different systems. In treating mixed polymer–vesicle systems, we emphasize how different interactions can have important influences on both phase stability and on vesicle structure; here the important influence of the polymer on the surfactant or lipid bilayer is only starting to be recognized. Mainly because of the special relevance, and some more deep-going analysis, we treat DNA-vesicle systems separately.

2. Polymer–surfactant systems

2.1. Polymer-induced surfactant self-assembly

As is well known, a water-soluble surfactant self-assembles cooperatively in water into nanosized aggregates with aggregation numbers typically in the range 50–100 [12]. These small micelles are spherical. For less polar surfactants, elongated "thread-like" micelles may form, the size increasing with increasing concentration. Less polar surfactants may be essentially water-insoluble, in which case addition of surfactant to water initially leads to the formation of a lamellar phase in equilibrium with excess water. We will initially consider mainly the micelle-forming surfactants.

If a surfactant is added to an aqueous solution containing a polymer, there may be different types of behaviour depending on the nature of the polymer and on the polymer–surfactant interactions. Considering first a homopolymer, there are two distinct types of behaviour. In one, the surfactant micellization appears unaffected by the polymer and the same cmc is found as without polymer; this suggests a repulsive polymer–surfactant interaction. In another, micelle formation is facilitated and we observe a lowering of the cmc, suggesting an attractive polymer–surfactant interaction.

A lowering of the cmc by homopolymers is essentially seen only for ionic surfactants [13]. A dramatic lowering may occur for oppositely charged surfactant and polymer; this lowering is reduced in the presence of electrolyte. A moderate lowering is typically seen for nonionic polymers, the lowering being more important for less polar polymers.

The binding isotherm for the association of the surfactant to the polymer indicates a high degree of cooperativity in the binding; alternatively, and preferably, we describe the situation in terms of a polymer-induced surfactant micellization.

For polymers with pronounced hydrophobic groups, like hydrophobically modified water-soluble polymers, the situation is rather different. Here the binding isotherm is composed of three parts, an initial non-cooperative binding followed by an anti-cooperative region; finally, we see a cooperative binding corresponding to binding to a polymer without the hydrophobic grafts [13,14].

For general references to dilute polymer–surfactant solutions see the reviews in Refs. [5,6,13,15].

2.2. Phase separation phenomena in mixed polymer–surfactant solutions

Phase diagrams of ternary systems of polymer, surfactant and water can be complex for several reasons. One reason is that there may be different types of phase separation phenomena depending on the molecular interactions, another that surfactant self-assembly can lead to several different structures and phases. A further aspect, of important relevance for the systems that interest us here, is that for mixtures of an ionic polymer with an oppositely charged surfactant, it is because of the different ways of pairing the ions, not possible to treat it as a ternary system.

On mixing two homogeneous solutions, one of a polymer, and one of a surfactant, one can encounter three types of behaviour. In the first, there is a homogeneous solution. In the second, when there is an effective repulsion between the two cosolutes, there will be a segregative phase separation, i.e. there is one phase enriched in polymer and one in (aggregated) surfactant. This will be expected for all cases without an attractive polymer–surfactant interaction Phase separation will be dictated by the weak entropic driving force of mixing for polymer and surfactant self-assemblies, phase separation increasing with increasing polymer molecular weight and micelle size. Examples of segregative phase separation include a combination of two nonionic cosolutes or two similarly charged ones, the latter case illustrated in Fig. 2.

In the case of a net attractive interaction, an associative phase separation, giving one concentrated phase enriched in both cosolutes coexisting with a water-rich phase, is expected. Associative phase separation could arise mainly because of electrostatic interactions between oppositely charged species or hydrophobic interactions. The simplest situation is to consider a nonionic polymer mixed with a nonionic surfactant. For less polar polymer and surfactant an associative phase separation will occur; for clouding polymers and surfactants, the phase separation can become much more accentuated at higher temperature.

In our discussion of polymer–vesicle association, we will be very much interested in another situation of associative phase separation, i.e. that between oppositely charged species. The nature of the concentrated phase for such associative systems can vary depending on surfactant and on polymer–surfactant interactions; cases include a concentrated solution, a liquid crystalline phase and a solid precipitate. As illustrated in Fig. 3, phase separation will be strongly affected by the presence of electrolyte effectively screening the polyion–surfactant attraction; for sufficiently strong screening, a segregative phase separation, analogous to that obtained for two nonionic species, is found.

It is important to recall, that, as illustrated in Fig. 4, the latter type of polymer–surfactant mixture cannot be described in the conventional simple ternary representation.

Systems illustrated here concern the most studied situation, where the surfactant forms micellar aggregates. Phase separation phenomena for mixed polymer–vesicle systems have so far only been investigated to a limited extent, but are expected to be quite analogous. Since the aggregation number of a vesicle is much larger than for a micelle, phase separation phenomena are expected to be more pronounced with vesicles.

For general references to phase behaviour of polymer–surfactant mixtures see refs. [6,16–23].

2.3. Polymer–surfactant gelation

The rheological effects seen for mixed solutions of hydrophobically modified water-soluble polymers and surfactant micelles are the basis of important applications based on synergistic thickening effects; as

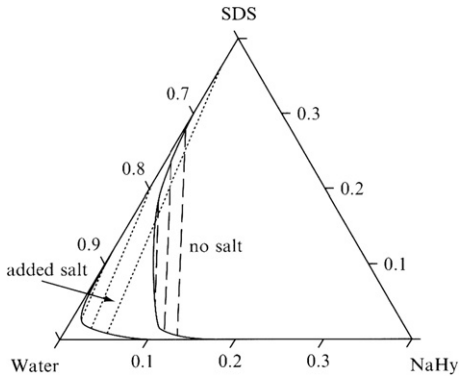

Fig. 2. Segregative phase separation in a system of an anionic polymer (sodium hyaluronate) and an anionic surfactant (sodium dodecylsulfate). As salt is added, phase separation becomes more pronounced, which one can attribute to a common electrolyte-induced micellar growth [16].

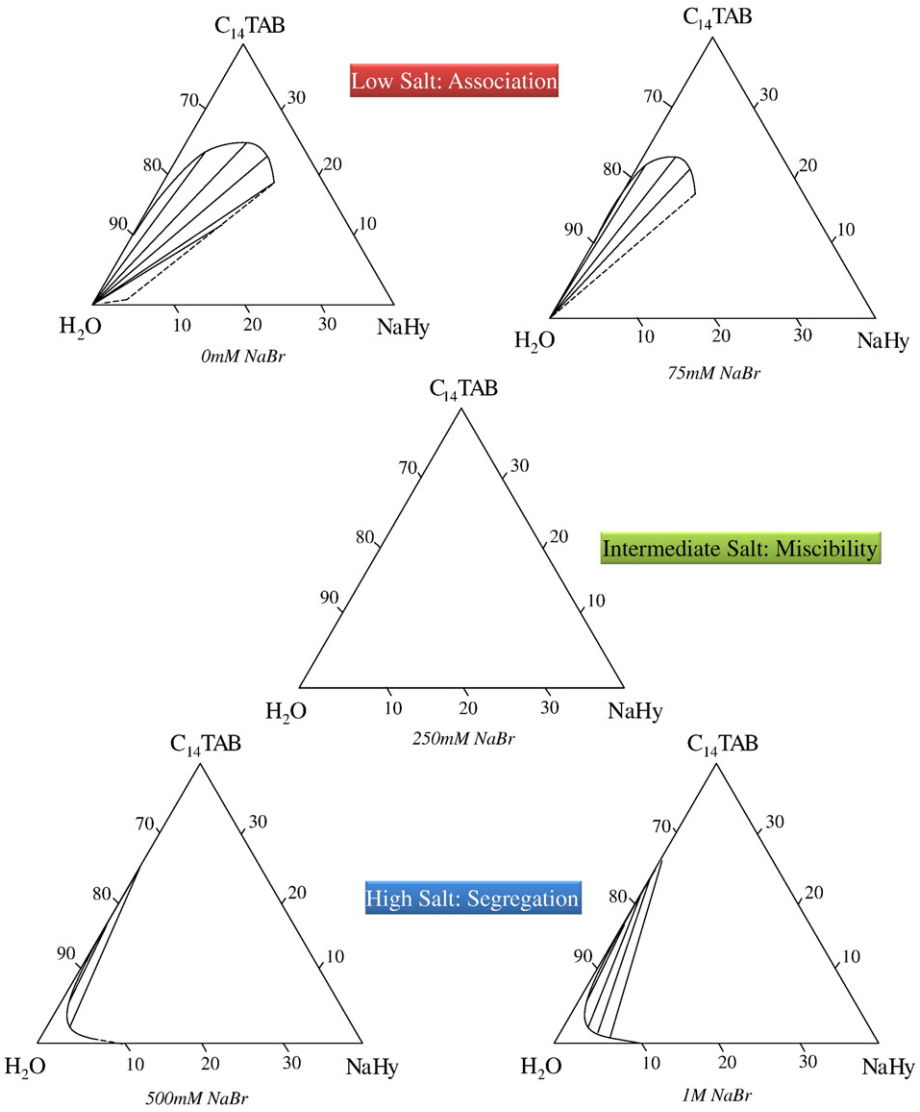

Fig. 3. Polyelectrolyte and oppositely charged surfactant typically display an associative phase separation, with a concentrated phase of the two cosolutes in equilibrium with a dilute solution. Addition of salt may at lower concentrations induce miscibility, at higher a segregative phase separation. Adapted from [17].

these have their counterpart for mixed polymer–vesicle solutions they deserve a special attention.

The generic behaviour observed when a micelle-forming surfactant is progressively added to a semi-dilute solution (concentration of ca. 1% by weight) of such a type of associating polymer is shown in Fig. 5. The hydrophobic modification of the polymer gives itself an increase in viscosity by an order of magnitude or so relative to the unmodified polymer; this is due hydrophobic interpolymer associations into small micellar-like aggregates giving rise to a three-dimensional network. Added surfactant at low concentration binds non-cooperatively into this network (cf. above). This increases the size and aggregation number of the cross-linking aggregates; this also increases the life-times of the aggregates and thus of the physical cross-links, in turn leading to the increased viscosity.

The magnitude of this viscosity increase varies strongly with surfactant chain length and polymer–surfactant interaction, but can amount to several orders of magnitude and thus lead to an effective gelation. However, as the surfactant concentration is progressively increased the viscosity reaches a maximum and decreases strongly; in fact it decreases to a level, which is much below that of the surfactant-free polymer solution. The reason for this decrease is to be found in the stoichiometry between polymer hydrophobes and micelles; at higher micelle concentrations cross-linking is eliminated since there is only one polymer hydrophobe per micelle. The correlation between viscosity and micelle concentration is illustrated in Fig. 6 for the case of a surfactant forming small spherical micelles.

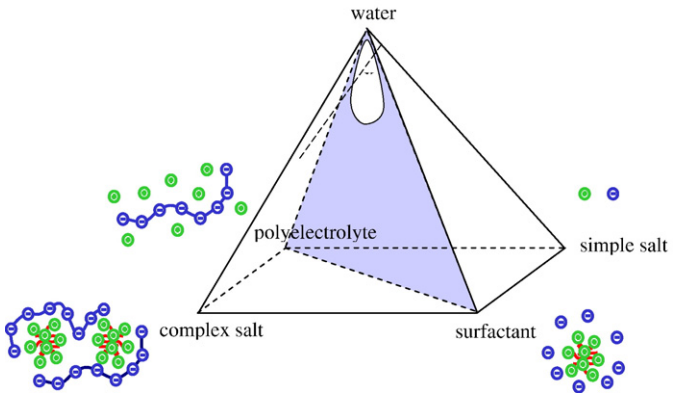

Fig. 4. The phase behaviour of a mixture of water and two electrolytes, without a common ion, cannot be described as a ternary system. For a polyelectrolyte–surfactant system, one has, in addition to the polyion with its counterion and the surfactant ion with its counterion, to consider the combination of polyion and surfactant ion (the "complex salt") and the "simple salt"[6]. (By courtesy of Lennart Piculell).

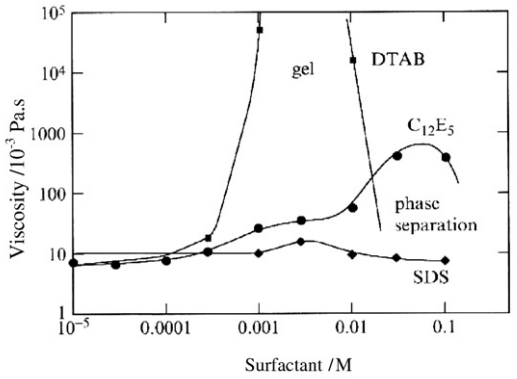

Fig. 5. The viscosity as a function of surfactant concentration for hydrophobically modified polyacrylate mixed with three different surfactants, one anionic (SDS), one cationic (DTAB) and one nonionic ($C_{12}E_5$) [24].

A completely analogous rheological behaviour can be expected for other surfactant aggregates than spherical micelles. However, noting the importance of the relative number of surfactant aggregates and hydrophobic groups on the polymer molecules, the relevant surfactant concentrations expected for the viscosity maximum can be very different. This has been very well illustrated for the case of thread-like or rod-like aggregates. Thus for the case of a surfactant forming rod micelles, with a high aggregation number, we may not observe the decrease in viscosity since it would occur at unattainably high concentrations.

As will be illustrated below, vesicles follow the same type of behaviour: We do observe major viscosity increases, with gel formation in some cases, as a vesicle-forming surfactant is added to a solution of a hydrophobically modified water-soluble polymer. However, in view of the much higher aggregation number of a vesicle compared to a spherical micelle, conditions corresponding to a larger number of vesicles than polymer hydrophobes are typically not reached.

2.4. Polymers in lamellar phases

Both micelles and vesicles are discrete surfactant self-assemblies and they will, therefore, display important analogies. However, with the vesicle having a bilayer structure, another most relevant reference is the lamellar liquid crystalline phase.

Considering the effect of adding a water-soluble polymer to a lamellar surfactant phase, we can focus on two important parameters, the polymer–surfactant interaction and the polymer molecular weight.

In the case of repulsive polymer–surfactant interactions, the polymer is only expected to enter the aqueous layers of the lamellar phase if it has a sufficiently low molecular weight; if the diameter of gyration of the polymer coils is larger than the thickness of the water layers, we expect the entropic penalty of reducing the available polymer conformations to be unfavourable for dissolving the polymer in the lamellar phase. Thus polymer addition will lead to an excess polymer aqueous phase, the amount of which will increase with polymer concentration; as a consequence, the thickness of the water layers will decrease as can be inferred from, for example, X-ray diffraction studies.

For the case of an attractive interaction, an entry of the polymer in the water layers will be facilitated. This leads to an increased water swelling of the lamellar phase compared to the cases with no attractive interactions. This can be for reasons of polymer hydration and because of an osmotic swelling for a polyelectrolyte, and a polymer conformational entropy effect, the latter becoming more important for a higher polymer molecular weight. There is, however, a maximum in the swelling in such cases. The swelling limit of the lamellar phase corresponds to a balance of repulsive and attractive interactions[26,27]. Salt addition may result in the collapse of the lamellar phase due to the concomitant screening of electrostatic repulsion and possibility of polymer bridging[27].

Fig. 7 illustrates the different situations.

3. Vesicles as a self-assembled structure

3.1. Structure, dynamics and composition of vesicles

Prior to addressing mixed polymer–vesicle systems, it is relevant to review the main properties of vesicles, covering the variety of systems where this type of aggregates has been found, beyond the classic polar lipid dispersions.

It is well known that bilayer-based structures, such as vesicles, are the most widespread and relevant form of surfactant self-assembly in nature, since they form the structural basis of biological membranes. Vesicles are discrete colloidal structures, typically of spherical shape, composed of a bilayer which folds over itself and entrap part of the solvent. Thus, a vesicle has an inner and outer surfactant leaflet and an internal solvent pool, usually aqueous, where solutes can be present. An illustration of a spherical unilamellar vesicle is shown in Fig. 8. Water-soluble molecules, if charged, can adsorb electrostatically onto the vesicle surface or partially anchor to the bilayer if they have a non-polar segment; nonpolar molecules can be solubilized in the hydrophobic core of the bilayer.

This structural versatility of vesicles allows them many functional uses. Their structure is compatible with the major roles of biological membranes, such as compartmentalization, permeability control of solutes, recognition and signaling, and support matrix for proteins; hence, they are extensively used as cell mimetic systems in biosciences [29–31], e.g. in protein reconstitution studies. They also find important use in pharmacology as drug (anticancer drugs, vaccines etc.) [32–35] and gene-delivery [36–38] agents, in water-based systems for cosmetic use [39,40], in food science [41], in micro-reactor chemistry [42], to cite a few technical applications.

Any typical vesicle solution presents a certain degree of polydispersity. Normal or log-normal distributions are common, with the mean diameter varying enormously between different systems, from about 5–50 nm in small unilamellar vesicles to tens of μm in giant vesicles and multilamellar vesicles (composed of many concentric bilayers and also known as spherulites or onions). While micelles form above the Krafft temperature of the surfactant, for vesicles the characteristic temperature is the gel-to-liquid crystal temperature (T_m), also termed chain melting transition. At this temperature, the surfactant chains change from a frozen quasi-crystalline state to a fully fluid one, with conformational disorder of the chains; concomitantly, changes occur in the headgroup hydration [43]. Fluid-state vesicles are typically spherical but below T_m, in their gel state, they may undergo shape deformations due to surfactant chain crystallization. Thus,

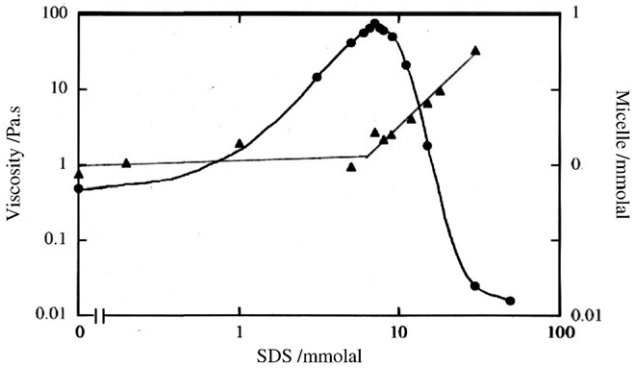

Fig. 6. The viscosity as a function of surfactant concentration for solutions of hydrophobically modified ethyl hydroxyethyl cellulose (1 wt.%) to which sodium dodecyl sulfate is added (circles). The rheological behaviour is compared with the concentration of micelles in the solution (triangles) [25].

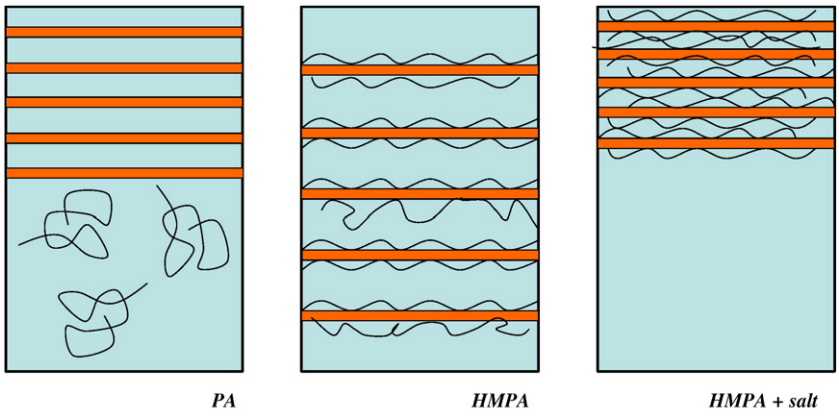

Fig. 7. Schematic representation of the attractive and repulsive interactions between a polymer and a nonionic surfactant lamellar phase. While polyacrylate (PA) is nonadsorbing, hydrophobically modified polyacrylate (HMPA) adsorbs to the surfactant film by the hydrophobic stickers. The lamellar phase with HMPA collapses when adding salt [27,28]. (By courtesy of Ulf Olsson).

several non-spheroidal shapes have been reported, such as disks, planar bilayer fragments, lens-shaped vesicles, regular polygon-shaped vesicles and irregularly faceted vesicles [44–48].

In many systems, micelles and vesicles are closely related—for instance, a micelle-vesicle transition (or vice versa) can be induced upon change of a controllable variable, such as temperature, composition or pH. Therefore, some useful comparison can be made between micellar and vesicular dynamics [12,29,49]. In common micellar solutions, the surfactant monomer solubility is in the 0.01–10 mmol dm^{-3} range (depending essentially on amphiphile charge and alkyl chain length), while for vesicle solutions the values are several decades lower, typically in sub-μmol dm^{-3} range. Consequently, vesicular characteristic time scales are much bigger than micellar ones. The monomer residence time is of the order of μs–ms for micelles, whereas for vesicles they lie within tenths of minutes to several hours. A micelle has a lifetime of the order of 1–100 ms, as compared with days to years for vesicles. Within the fluid bilayer (above T_m) some characteristic motions are fast, such as the lateral diffusion of the amphiphile or molecular rotation; however, the exchange rate of amphiphiles between the two leaflets of the bilayer (flip-flop) is extremely slow.

It was Bangham and co-workers who originally reported in the early 60s that liposomes can be formed by the energetic dispersion of phospholipids in water [50]. Since then, a myriad of other vesicle-forming amphiphilic systems has been discovered or designed. A simple rule-of-thumb is that any amphiphile with a geometric packing parameter close to unity will tend to form bilayer structures and, under appropriate conditions, these bilayers will adopt discrete spherical shapes. This essentially implies molecules with a small headgroup area and a bulky nonpolar part, such as double-chained amphiphiles or non-ionic surfactants of the polyoxyethylene type with short headgroup (e.g. $C_{12}E_4$) [12,49]. Any mixtures where an effective packing parameter lies close to unity will also follow the trend.

In terms of chemical compositions, vesicles can be classically produced from single-component phospholipids or their mixtures—being then designated as liposomes—or double-tailed surfactants, such as dialkyldimethyl ammonium surfactants with hydroxide, acetate or halide counterions [51–55]. Dilute mixtures of oppositely charged surfactants can also give rise to vesicles (catanionic vesicles), having emerged in the last fifteen years as a relevant and exciting new class of vesicle-forming systems [56–70]. Vesicles are also found in many-component mixtures of the type non-ionic surfactant/co-surfactant/water (or brine) [71–73], in some cases also with added small amounts of ionic surfactant [74]. Amphiphilic macromolecules are also reported to form vesicular aggregates—block co-polymers with flexible or rigid blocks in selected solvents and some grafted copolymer in mixtures with surfactants [75–77].

Another interesting type of structures are the so-called reverse vesicles, in which the bulk and the vesicle pool contain an organic solvent and the surfactant polar headgroups contact a thin water layer [78–82]. Here the separating layer is formed by the polar media (surfactant headgroups plus water). They have been reported, e.g. in sucrose monoalkanoate/hexaethylene glycol hexadecyl ether/decane/water systems [78–82] and in some PEG-based diblock and triblock copolymers in heptane [83].

In the last two decades, the number and type of vesicle-forming systems has expanded from the classic systems of double-chained amphiphiles (lipids and synthetic surfactants) to increasingly more diverse and "exotic" ones [63,68–70,84].

3.2. Vesicle formation and stability

The vast majority of vesicles used in fundamental studies and practical formulations are not the equilibrium state for the system at the specified composition [29,85,86]. This type of vesicle is classically composed of swelling phospholipids or bilayer-forming double-chained synthetic surfactants. They are metastable structures of long-term stability but with time they evolve back to their true equilibrium state—a lamellar dispersion, i.e. a biphasic region of the phase diagram where a lamellar phase is dispersed in the excess solvent phase.

The flat bilayer is usually the lowest free energy aggregation state and, therefore, the formation of vesicles in general requires non-spontaneous methods, typically involving high energy input to break and disperse the lamellae in the form of smaller spherical aggregates. The methods can be essentially divided into two [87]: (i) mechanical fragmentation of pre-formed bilayer structures, e.g. through vortexing, sonication and extrusion; (ii) induction of bilayer curvature by

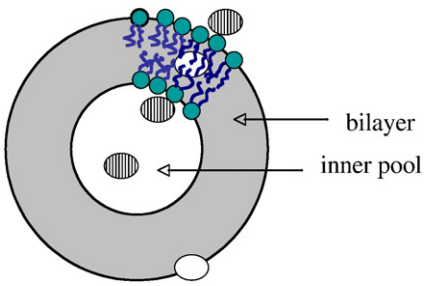

Fig. 8. Schematic representation of a spherical unilamellar vesicle, evidencing the type of association with different solutes: water-soluble molecules can be encapsulated in the inner pool or bound to the bilayer, e.g. by electrostatic interactions in the case of ionic co-solutes; amphiphilic molecules can anchor at the bilayer polar–nonpolar interface, whereas hydrophobic molecules can dissolve in the hydrophobic core.

breaking the symmetry of the flat bilayer, e.g. by intercalation of hydrophilic counterions or macromolecules in the outer layer. Solutions can then be readily obtained with different size, controlled polydispersity and variable kinetic stability, depending on the different methods used. The kinetics of vesicle formation play an important role in the characteristics of the vesicles formed. Typical parameters for non-spontaneous vesicles are the rate of surfactant depletion in mixed phospholipid/surfactant micelles, intensity and duration of the shaking of dry lipid films in water, time and intensity of sonication of lipid dispersions [29,88]. Depending on the amount of energy used or generally the sample history, the system may fall into different metastable states. The change from one local free energy minimum to another may involve a high energy barrier. Thus, the system may remain in higher (non-equilibrium) free energy states indefinitely or for a long time, until the excess free energy is dissipated and the lowest free energy state is found.

We will now consider the factors governing vesicle stability. In the overall free energy of forming a vesicle phase from an infinite bilayer the dominating contributions are the curvature energy, i.e. the energy required to bend a planar bilayer into a vesicle; and the translational entropy [89]. Within this approach the formation of vesicles is essentially a competition between the curvature energy (usually unfavorable) and the translational entropy (favorable).

It has been shown that the curvature free energy of forming a vesicle from a flat bilayer—for which the spontaneous curvature, c_0, is equal to 0—is given by [89]:

$$f_c = 2k_c(c-c_0)^2 + \bar{k}_c C \tag{3.1}$$

where k_c is the mean curvature modulus, c is the mean curvature at a given point ($c=1/2(c_1+c_2)$), $\bar{k}_c$ is the Gaussian curvature (or saddle-splay) modulus and C is the Gaussian curvature ($C=c_1c_2$). The curvature moduli are usually expressed in terms of k_BT units (where k_B=Boltzmann constant). For the planar membrane $f_c=0$ and the conditions for planar stability are $k_c+\bar{k}_c/2>0$ and $\bar{k}<0$. The term $k_c+\bar{k}_c/2$ can also be designated as the bending constant K.

A curvature instability is produced if one of these conditions is violated. If $K=k_c+\bar{k}_c/2<0$, the formation of a vesicle is favored since $f_c<0$; if $\bar{k}_c>0$, the formation of a sponge phase is favored. So, the curvature stabilization of a vesicle lies in the sign of K. Normally k_c assumes large values for phospholipids and double-chained surfactants, in the range of $k_c=10$–$40\ k_BT$, and these molecules are said to form *rigid* bilayers; the $\bar{k}_c$ values are usually low (only a few k_BT) and negative [90]. Therefore, since $k>>|\bar{k}_c|/2$ and solely on the basis of curvature energy, the formation of vesicles is unfavored for these systems.

There is an entropy increase associated with the formation of many finite-sized vesicles from an infinite bilayer. Entropy favors thus a large number of small vesicles. The total curvature energy is proportional to the number of vesicles and with each vesicle fusion it is reduced by 1/2; therefore larger vesicles are energetically favored. While f_c is in the order of 50–300 k_BT per vesicle, the entropy contribution is in the order of only a few k_BT—consequently, vesiculation is overall unfavored. Nevertheless, the formation of equilibrium vesicles should be theoretically feasible: (i) in the case of soft bilayers where k is extremely low, $k<<|\bar{k}|/2$, and $f_c\approx k_BT$) or if the surfactant concentration is very low, in which case the entropic factor dominates—entropic stabilization; (ii) the bilayer has non-zero spontaneous curvature. i.e. $c_0\neq 0$—curvature energy stabilization. In principle, the latter case requires the presence of more than one component in the membrane.

There is a vast number of systems reported in the literature in which vesiculation occurs spontaneously; such spontaneously formed vesicles may or may not be the equilibrium state [85]. Experimental studies have provided in some cases strong evidence for the formation of equilibrium vesicles. In principle, thermodynamically stable vesicles should [57,91]: (i) be generated spontaneously, i.e. with minimal shearing forces (e.g. gentle mixing of components in solution), and in a controlled, reversible way; (ii) be stable in time with respect to size and shape; (iii) have size distributions and stability independent of formation path; (iv) be found in equilibrium with other phases, such as solids and lamellar phases, in appropriate multiphase regions. Not all of these criteria are always easily accessed experimentally, but if vesicles form spontaneous and reversibly, attain an invariant size distribution and remain stable, they have essentially the features of equilibrium vesicles.

Reports on the formation of equilibrium vesicles in aqueous mixtures of lipids (fatty acids, fatty acids/phospholipids, bile salts/phospholipids) have appeared regularly in the past two decades [85]. Equilibrium vesicles have also been reported for some dialkyldimethylammonium surfactants in water [51–55], the single-chain ganglioside G_{M3} [92] surfactant in water, mixtures of ionic surfactant/cosurfactant (alcohol) in water or NaCl solution [71–73] and mixtures of non-ionic/cosurfactant in the presence of doping amounts of ionic surfactant [74]. Mixtures of oppositely charged surfactants (catanionic systems) have also made an outstanding and prolific contribution and will be analyzed in more detail below.

3.3. Lipid and block co-polymer vesicles

Vesicle-forming surfactants include the vast class of natural or synthetic double-chained lipids which swell in water, forming lamellar phases where the packing parameter P_s is close to 1. As discussed above, in most cases the vesicular solutions and dispersions obtained from these surfactants alone in water, or in mixtures with other components, are non-equilibrium systems. The phase diagram of lecithin in water is illustrated in Fig. 9 it shows where these classical vesicles are prepared: in the dilute part of the two-phase region lamellar phase+(excess water) solution. These vesicle dispersions are thus not single-phase systems and are intrinsically unstable. In these vesicles, the outer and inner monolayers have curvatures opposite in sign: $c_{out}\approx -c_{in}$. Thus, a curvature frustration occurs and, as a result, the vesicle is not energetically stable reverting with time to the zero-curvature bilayer state, where $c=c_0=0$.

Most preparation methods involve high magnitude shear forces. A common method is sonication (by bath or tip methods) of a lipid dispersion, yielding unstable and relatively polydisperse vesicles. Methods such as high-pressure extrusion, dry lipid film hydration, detergent dialysis and reverse-evaporation yield also metastable vesicles with controllable size and polydispersity [87]. If the symmetry of the lipid bilayer is broken, for instance, by the presence

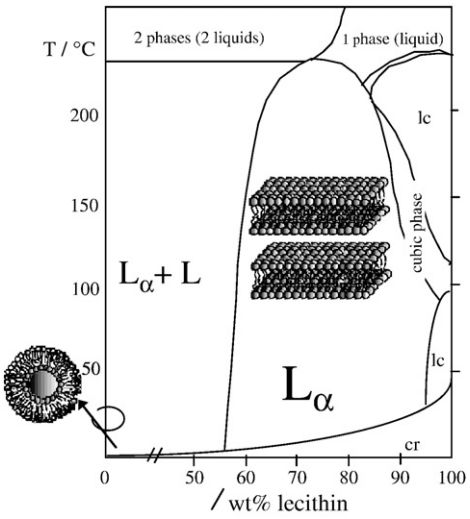

Fig. 9. Phase diagram of the lecithin–water system, where the two-phase region lamellar liquid crystalline phase (Lα)+monomer solution (L) can be seen, from where metastable liposome dispersions are typically prepared. Adapted from [93].

of a PEG-lipid molecule in the outer layer, fairly monodisperse vesicles can be made with long-term stability [94].

A relatively new class of vesicle-forming systems are block copolymers, either diblock or multiblock copolymers in dilute solutions of different solvents [75–77]. Following a simple packing parameter rationale, if the hydrophobic block in the copolymer is made enough long or very rigid, the system will tend to form bilayers, from which vesicles can be spontaneously or non-spontaneously formed. For instance, spontaneous unilamellar vesicles have been found for $PEO_5PPO_{68}PEO_5$, a triblock copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) [95]. Metastable vesicles of polystyrene-poly(acrylic acid) (PS-PAA) and PS-PEO diblock copolymers in water have been prepared by dialysis, where the water-insoluble PS block is in a glassy state at ambient temperatures [96,97]. However, vesicles with a non-glassy state of the hydrophobic block have also been found in the polybutadiene-PAA copolymer/water system [98].

3.4. Catanionic vesicles

Experimental investigations of dilute cationic/anionic surfactant mixtures, now known as catanionic mixtures [99,100], have made a key contribution to the field of vesicle formation in surfactant systems. Since the original observation by Hargreaves and Deamer [101] and the groundbreaking work of Kaler's group [56], catanionic vesicles have been reported in mixtures of: sodium alkyl benzene sulphonates with alkyltrimethylammonium tosylate/halides [56,57,101]; sodium alkylsulphates with quaternary alkyl ammonium halides [58,60,61,91,102]; sodium alkyl carboxylates with quaternary alkyl ammonium bromides [103,104]; ionic amino acid-based surfactants [64,105–107]; single-chained fluorocarbon surfactants [108]; fluorocarbon/hydrocarbon surfactants [104,109,110]; cationic gemini surfactants of the alkane-diyl-α,ω-bis(alkyldimethylammonium) type with alkylsulfates [111,112].

The catanionic vesicles found in different systems are usually spherical and the mean sizes and polydispersity can vary markedly, providing some hint to the mechanism behind their thermodynamic stabilization (where this one applies). Charge neutrality in the system usually leads to phase separation, with formation of crystalline precipitates [57,59,113–118] or, less commonly, liquid–liquid separation [119,120]. A small excess of either ionic surfactant is usually enough to lead to resolubilization and for a narrow range of mixing ratios, a solution phase with stable vesicles is formed. A typical phase diagram for these systems is schematically depicted in Fig. 10 As the molar mixing ratio of the two surfactants or the total surfactant concentration is varied, different phase transitions involving the vesicles are found: micelle–vesicle, vesicle–lamellar phase, vesicle–solid phase transitions.

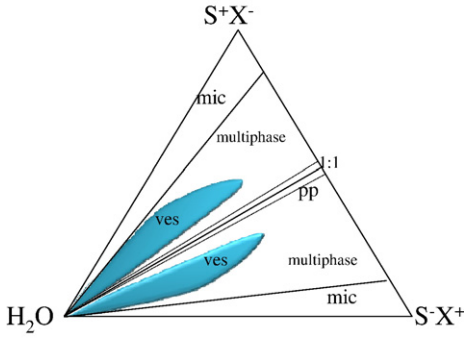

Fig. 10. A schematic triangular phase diagram of a catanionic mixture, at constant temperature and pressure, where the main phase regions are highlighted. In and around the charge neutrality line (*1:1 line*), a precipitate (*pp*) is usually formed, but excess charge in the system usually leads to vesicle stabilization (*ves* regions), that may occur in both sides or not. The micelle (*mic*)-vesicle transition may be continuous (single solution phase) or involve phase separation (two solution phases). Multiphase regions often involve a lamellar phase occurring for higher concentrations.

It has been shown that an increase in steric hindrance at the level of headgroups can decrease vesicle stability [64]; however, if the interaction between cationic/anionic surfactant is too strong, association in the form of precipitation will dominate entirely at the expense of vesiculation [121]. An asymmetry in chain length between the two ionic surfactants is also compatible with vesicle formation [57]. The vesicle region in the phase diagrams is more extended in the side of excess short-chained surfactant than in the long-chained one. This is observed for the systems DTAB, TTAB or CTAB with SDBS. The bilayers rich in short-chain surfactant should have higher curvature, leading to smaller vesicles and therefore extension of the vesicle region toward higher surfactant concentration.

The micelle-to-vesicle transition upon increase of the minority surfactant in the mixture (Fig. 10) has been seen to occur either continuously, via a single macroscopic phase of coexisting micelles and vesicles (if there is limited micellar growth) [60,105], or via a first order phase transition, if there is substantial micellar growth, in which case the large micelles phase-separate from the vesicles [58,103]. These changes can often be accounted for by considering electrostatic screening effects and geometric packing constraints as the surfactant mixing ratio is changed. Other variables can induce, at fixed mixing ratio, a micelle-to-vesicle transition, namely temperature [122], salt [123] and organic solutes [124]. Temperature has been shown to be important for the case of strongly interacting pairs and where large micelles form, in a striking similarity to nonionic surfactants [122]. Specific alkali cation effects (in accordance to the Hofmeister series) are found for the transition from SDS/DTAB rodlike micelles to vesicles [123]. The effect of salt directly on catanionic vesicles is also interesting and often not obvious. A "salting out" effect is generally expected (transition to lamellar phase or even precipitation), as observed for instance in the DTAB/SDS system [58]; however, in the CTAB/SOS system, addition of salt induces a transition from vesicles to small micelles, in view of the "salting in" effect on the short-chained, more soluble surfactant (SOS) [125].

Another interesting case to analyse is that of salt-free catanionic surfactants, for which spontaneous vesiculation in zero salt concentration and without excess of ionic surfactant has also been reported to occur [46,126–135]. These systems have the further advantage that the effect of electrostatic interactions can be studied in a controlled way by the addition of salt. Examples include the acid–base pairs tetradecyltrimethylammonium hydroxide (TTAOH) with different fatty acids [127] and hexadecyltrimethylammonium hydroxide/tetradecanoic acid [130]. The salt-free surfactant hexadecyltrimethylammonium octylsulfonate has the peculiarity of forming a lamellar phase with a miscibility gap and, in the dilute regime, a temperature-induced vesicle-to-micelle transition [131]. In this case, however, the observed effects can be rationalized if one considers that the higher solubility of the short chain pair (octylsulfonate, which then acts a large "counterion") effectively induces the formation of charged aggregates. Stable and spontaneous reverse vesicles can also be prepared from these amphiphiles, as shown for tetradecyltrimethylammonium laurate in the presence of toluene, tert-butylbenzene, and cyclohexane and small amounts of water [132,135].

As demonstrated for the SDS/DDAB [136] and hexadecyltrimethylammonium hydroxide/tetradecanoic acid [130] systems, catanionic vesicles may have a chain melting transition temperature, similar to double-chained amphiphile vesicles, with the difference that T_m is highly dependent on salt and total surfactant concentration [136]. Above the transition temperature, all vesicles irrespective of size have a spheroidal shape but below T_m the larger ones appear with a faceted shape, an effect directly resulting from the chain crystallization [137]. The stability of these faceted aggregates could result from the existence of a neutral cationic/anionic stoichiometry in the flat areas, whereas the excess anionic surfactant could accumulate in the edges

where the curvature is significantly higher. In the fluid vesicles, there is complete in-plane miscibility of the two components and faceting is absent. Such reversible segregation, concomitant with chain crystallization, has been proposed for some salt-free catanionic vesicles [46].

Within the confines of the elastic curvature energy model for vesicle stability described above, for catanionic systems also the stabilization is thought to stem from one of two cases [90]. (*Case 1*) From a favorable curvature energy, giving rise to small vesicles of low polydispersity, if the mean curvature modulus is high ($k_c >> k_BT$). This should be the case of catanionic bilayers that are charged and relatively rigid. It implies that only narrow mixing ratio ranges are optimal and any deviations from this ratio give rise to an unfavorable bending energy. This is in agreement with experimental observations which show that vesicles occur for narrow mixing ratio between the two amphiphiles, slightly away from equimolarity. An example of this kind of stabilization is thought to be provided by the 12Lys12/DTAB vesicles shown in Fig. 11 where 12Lys12 is a synthetic double-chained lysine-based surfactant [105]. (*Case 2*) From translational entropy, if the bending constant $K=2k+\bar{k}$ is relatively low (of the order of k_BT), i.e. for relatively soft catanionic bilayers, in which case large and polydisperse vesicles form.

Spontaneous curvature values different from zero can, for example, be attained when asymmetry in the bilayer constitution occurs, as proposed by Safran et al. [138]. This is feasible in catanionic systems, where an uneven distribution of the two surfactants between inner and outer layer could result in the reduction of the curvature energy, even for systems with high bending constant *K*, favoring the spherically curved aggregates over flat bilayers. For the catanionic systems, such non-ideal mixing could result from the electrostatic interactions between oppositely charged headgroups and further mixing entropy term resulting from the release of the small counterions. Vesicles with a well defined size and bilayer composition could then constitute the lowest free energy state for the given mixing ratio.

An alternative microscopic model for equilibrium catanionic vesicles based on charge density inhomogeneities between the two monolayers has been proposed by Duque et al., with good qualitative success when applied to the CTAB/SOS system [139]. Yuet and Blankschtein have introduced a comprehensive molecular-thermodynamic model for vesiculation in catanionic vesicles [140,141]. In this model the central quantity is the free energy of forming a vesicle from the monomers in solution, which is decomposed into different terms. The model is able to predict vesicle properties such as size, composition and surface charge density, successfully accounting for some of the experimentally observed properties of catanionic vesicles [140].

A number of studies have also addressed the relevant problem of the kinetics of catanionic vesicle formation and break-up [142–144]. It has been shown that vesicle formation rates are highly dependent on the difference between the initial surfactant composition and the optimal one that leads to the spontaneous curvature for the bilayer, while, in contrast, vesicle breakup to mixed micelles appears to be a rapid single-step process (ms/s range, depending on systems) [142]. Furthermore, a two-step model has been proposed for CTAB/SOS vesicles in which initial nonequilibrium vesicles rapidly formed upon mixing of the surfactant solutions, followed by vesicle growth due to slower vesicle fusion [143].

The use of catanionic vesicles for application-oriented goals is clearly a field of great interest and in rapid expansion, in view of their spontaneous formation and, in many cases, intrinsic stability. For instance, hexadecylbenzenesulfonic acid/CTAB vesicles have been used as micro-reactor agents for the synthesis of magnetic nanoparticles [145,146]. CTAB/SOS vesicles have also been successfully used in electrokinetic separation methods for organic solutes [147]. Different catanionic vesicles have been used as templates for the synthesis of silica or polymeric hollow spheres [148–150] and CdS nanoparticles [151]. They have also been reported to possess good encapsulation properties for a variety of molecules, including not only probe molecules [152–155] but also some currently used therapeutic drugs [155]. Furthermore, as will be shown in detail in the next sections, catanionic vesicles have been successfully used in the formation of vesicle-crosslinked gels with polycations [137,156,157] and in compaction/decompaction studies with DNA [107,158–160].

4. Polymer–vesicle systems

4.1. Interaction forces and mechanisms

The interactions between polymer and surfactant vesicles or polymer and lipid vesicles have essentially the same driving forces as those found in polymer–surfactant, polymer–polymer and surfactant–surfactant systems in general. Depending on the mutual interactions, phase behavior can be manifested as either miscibility or phase separation of two natures: segregative or associative [161–163]. Polymer–vesicle association can be controlled by a number of forces:

Evidence for *electrostatic bridging* has been found for the association between cationic polymers (e.g. polycations derived from poly(4-vinylpyridine)) and small unilamellar vesicles (e.g. negative vesicles composed of diphosphatidylglycerol and phosphatidylcholine).

Hydrophobic interactions constitute a most effective attractive interaction due to the penetration of the polymer groups into the vesicle bilayers. The hydrophobic nature of the association with polymers bearing *n*-alkyl side groups was demonstrated by using fluorescent side groups in the polymers [164–169]. Vesicle—hydrophobically modified polymer binding is expected to occur via the insertion of polymer hydrophobes into the vesicle bilayer. Fig. 12 illustrates this association.

Hydrogen-bond interactions have been discussed, for instance, for the complex formation of translocase proteins and phosphocholine

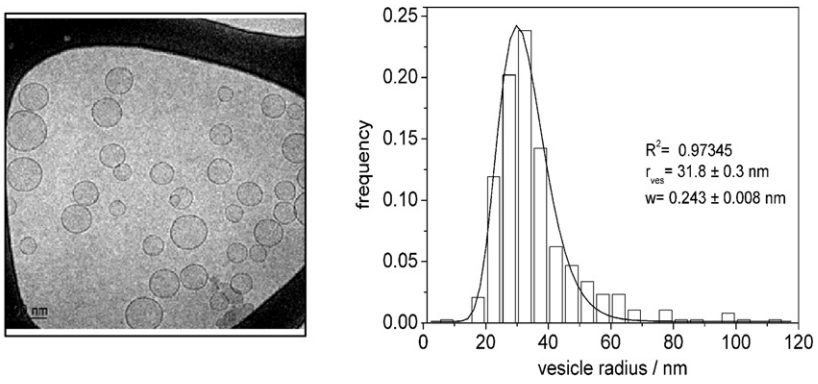

Fig. 11. Catanionic vesicles found for the system 12Lys12/dodecyltrimethyl-ammonium bromide. The corresponding log-normal size distribution (right hand side) is shown, obtained from the cryo-TEM micrographs (left hand side) [105].

headgroups in lipid vesicles [170]. It has also been suggested that hydrogen binding drives the adsorption of poly(*N*-isopropylacrylamide-co-glycidylacrylamide) in ethyleneoxide based vesicles [165,166]. However, it is unlikely that hydrogen-bonding is the driving force for association in water-rich systems; on the other hand, it can control the local molecular arrangements on the vesicle surface.

Hydrophobic modification in the polymer backbone increases the polymer association with vesicles in general. Hydrophobic association rapidly overcomes other interactions, including strong coulombic repulsions. The binding strength is calculated by the average free energy of transfer of a methylene group from water to apolar solvents (ca. 3 kJ mol^{-1} of CH_2 group) and the free energy of ca. 1 k_BT per ion entering in the Debye layer of the vesicle (with k_B the Boltzmann constant and T the temperature). Long hydrophobic groups provide stable anchoring. However, the polymer–vesicle binding can also be seen in relatively short alkyl groups, such as ethyl, if present at high density in the macromolecule [49,166].

Although the oppositely charged hydrophobically modified polymer–vesicle mixtures share essentially the same features of associative phase separation with polyelectrolyte-oppositely charged surfactant mixtures in general, the range of phase separation becomes strongly reduced for the former. Such difference can be referred to the stoichiometry of the formed polymer–surfactant complexes [14]; the hydrophobic association leads to a strong tendency to form non-stoichiometric complexes, i.e., water soluble complexes with a net charge. When the vesicles and hydrophobically modified polymers are similarly charged, the precipitation is eliminated and the systems may display gel properties at high enough vesicle and/or polyelectrolyte concentration, as shown for the SDS/DDAB and SDBS/CTAT vesicles with hydrophobically modified sodium polyacrylate [171].

When a segment of the polymer chain has enough affinity for the membrane, this brings the rest of the chains to the vicinity of the membrane irrespective of the monomer–vesicle interaction. This leads to tight polymer adsorption if a few monomers have affinity for a lipid surface [166].

Concerning catanionic vesicles, when an aqueous dispersion of such aggregates is mixed with a polymer of opposite charge, the system displays both phase separation and miscibility, depending on the composition [18,19,156]. A monophasic gel region occurs at excess polymer and a monophasic low viscous solution appears at very low polymer concentration, where there is enough excess of surfactant in solution to dissolve the precipitated complexes. Generally, this may be due to the formation of non-stoichiometric soluble complexes or to a "salt effect", induced by the excess charged surfactant, which screens the attraction between the polyelectrolyte and the surfactant [172]. A phase separation of associative type occurs at excess of surfactant charges, where a coacervation complex of surfactant and polymer with a (near) zero net charge is in equilibrium with a bluish solution.

The driving force behind the polyelectrolyte-oppositely charged vesicle associative phase separation is the release of the counterions. This is why, for example, polycations strongly adhere to the negative lipid membranes [166,173–175] and, similarly, DNA binds to cationic vesicles [159,176]. Surrounding a charged vesicle and a polyelectrolyte there is a diffuse layer of spatially confined counterions which screens long range electrostatic interactions. However, both the polymer and the surfactant prefer to associate with multivalent counterions rather than with monovalent. The outcome of the interaction depends on surfactant aggregate size and concentration, the flexibility of the polyelectrolyte backbone, its charge density and the ionic strength of the medium. The polyelectrolyte adsorption onto the vesicle of opposite charge may result in a polyion-coated aggregate or in charge inversion if more polyions collapse in the charged surface than those necessary to neutralize it [177] The polymer adsorption onto the vesicles may be tuned or abruptly switched by pH, temperature, salt concentration, or even by competition with a soluble polyelectrolyte of opposite charge. The two latter constitute common ways to completely remove a protein from the surface of a charged membrane [166,174,178]. SDS/CTAB negatively charged vesicles have been used in the presence of lysozyme [179]. The protein release from the vesicles depends on the polymer–vesicle interactions and the protein retains its native conformation upon release, which can be suitable for reversible protein binding onto vesicles for transfection technologies.

Coating of a liposome surface with polyelectrolytes and covalently crosslinking the polyelectrolyte multilayers has been a strategy to form surfactant stable nanocapsules [180]. Most of the liposome nanocapsules have been developed by polymerization of the lipids and studies focus on their stabilization against rupture below the critical micelle concentration or when other amphiphiles are present [181]. Such entities are also being studied for novel biomedical applications, namely as nanocarriers for drug delivery [182,183].

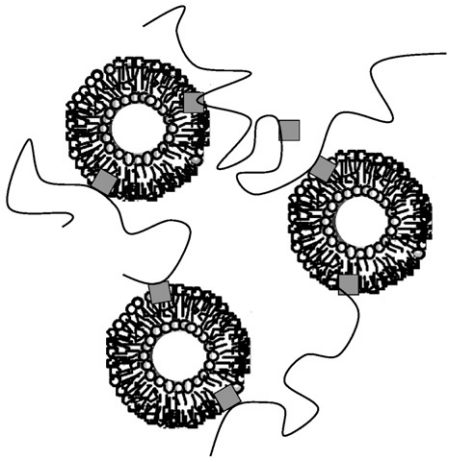

Fig. 12. Schematic model of the association between hydrophobically modified polymers and vesicles. The hydrophobic grafts of the polymer anchor to the hydrophobic interior of the bilayer.

4.2. Networks and rheological behavior

The interactions of associative polymers with catanionic vesicles described above can result in steric stabilization of the vesicles or polymer–vesicle networks. Thus the polymer can prevent vesicle coalescence into a lamellar phase (this strategy has been used to stabilize liposomes and control the membrane permeability of liposomal drug delivery vehicles [29,171]), but the associative polymers can also bridge vesicles to form aggregated structures and develop novel vesicle gel networks with high viscosity [171,184–187]. For instance, liposome-loaded gels are powerful candidates for the encapsulation and the controlled release of drugs [188,189]. So far, studies on vesicle gels have largely been carried out using hydrophobically modified polymers, e.g. hm-poly (ethylene oxide), hm-polyacrylamide, hm-ethylhydroxyethylcellulose, hm-carboxymethylcellulose, among others [137,171,184–187]. What is commonly observed is a changeover from dominating viscous effects in polymer-free solutions to dominating elastic properties at sufficiently high polymer concentration. The onset of network formation is usually below the polymer overlap concentration, if vesicles are present. The formation of a robust associative polymer–surfactant gel network requires the presence of vesicles in solution instead of normal or wormlike micelles; e.g., adding a hydrophobically modified chitosan to a viscous sample containing CTAT-SDBS wormlike micelles increases the viscosity further but does not give rise to a gel-like response [185], which is only obtained in the vesicle region. This requirement is largely due to the typical high volume fraction of vesicles, compared to micelles. Fig. 13 illustrates the rheological differences between a vesicle system, a hydrophobically modified polymer system, and a mixed hydrophobically modified

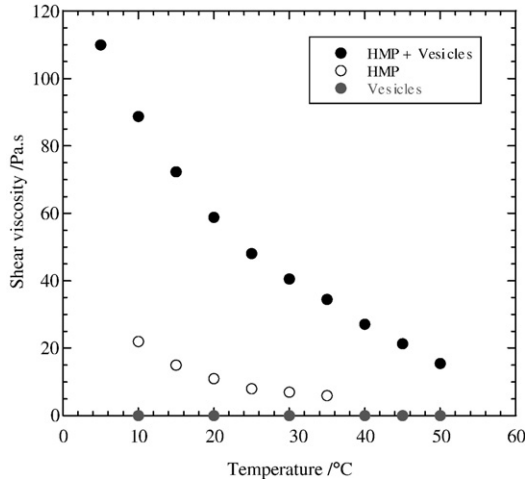

Fig. 13. Shear viscosity, measured at different temperatures, of a mixed solution of a hydrophobically modified polycation and an anionic vesicle dispersion, compared to the shear viscosity of the individual solutions. Adapted from [137].

polymer–vesicle system. While the vesicle dispersion exhibits a viscosity similar to that of water, the mixed polymer–surfactant system is a strong gel. This is due to the formation of polymer–vesicle crosslinks.

Water soluble polymers carrying a net charge opposite to that of the catanionic vesicles can thus give rise to physical gels, as indicated above. This happens when the amount of polymer chains is enough to bridge adjacent vesicles and to create a three-dimensional network. The type of network is, however, dependent on the polymer charge density, molecular weight, and hydrophobic chain length, among other parameters. Higher charge densities lead to more long lived polymer–vesicle crosslinks. Fig. 14 illustrates an example of such an effect—the rheological response of the interaction between SDS/DDAB catanionic vesicles (with an excess of negative charges) and two cellulose derivatives with different charge densities, LM200 and JR400 [137].

We will consider this system as a case-study. LM200, the polyion with lower charge density, is also hydrophobically modified and it shows a pronounced viscoelastic behavior, with liquid-like response at low frequencies and a solid-like response at high frequencies. The storage modulus at high frequencies, which gives information about the number of active links or bridges [190], shows a large magnitude, while the relaxation time is around 8 s. When the polymer charge density increases, by substituting LM200 by JR400, the storage modulus gets higher than the one for the system composed of the lower charged polycation. However, G' exceeds G″ over the entire frequency range; i.e., the cross-link lifetimes are very long. The less charged polymer can thus be seen to give mixtures with higher viscosity as compared to the more highly charged polymer that gives more elastic mixtures (more long lived cross-links). The more charged polymer can efficiently match the vesicle surface charge density while the situation is less optimal for the interaction of the less charged polymer. The strength of the network can also be tuned by increasing the surfactant concentration, due to shorter inter-vesicle distances, and, therefore, to a larger number of bridges.

The viscosity of a mixed polymer-oppositely charged catanionic vesicle system displays a strong increase on lowering the temperature below T_m. As indicated above, vesicles, while alone, are in a fluid state above the chain melting transition, T_m; the amphiphile has fluid disordered alkyl chains and vesicles typically exhibit a spheroidal shape. Cooling below T_m induces chain rigidity, where bilayers are less flexible than in the liquid crystalline state (due to the arrested dynamics of the alkyl chains). The strong increase in viscosity below T_m is connected with the reduction in surfactant flexibility at the molecular level when chains are in a *quasi*-crystalline state. This is illustrated in Fig. 15 Very high relaxation times were detected in these systems, below T_m. Another aspect is that while cooling, the viscosity increases within a broad region, instead of a steep increase. This is usually found in polydisperse vesicle systems, with larger vesicles melting at higher temperatures than the smaller ones [137].

4.3. Polymer effects on vesicle microstructure

Polymers that can influence the properties of membranes are of broad fundamental and practical interest; for example the ability of the liposomes to promote transmembrane delivery may be tuned by the presence of macromolecules.

The curvature energy is much higher in gel state, below T_m of the vesicle. This leads to the formation of nonspheroidal aggregates [44,45,47,48]. Segregation has been shown to occur in the frozen state of vesicles in general and to be responsible for the observed polygonal-

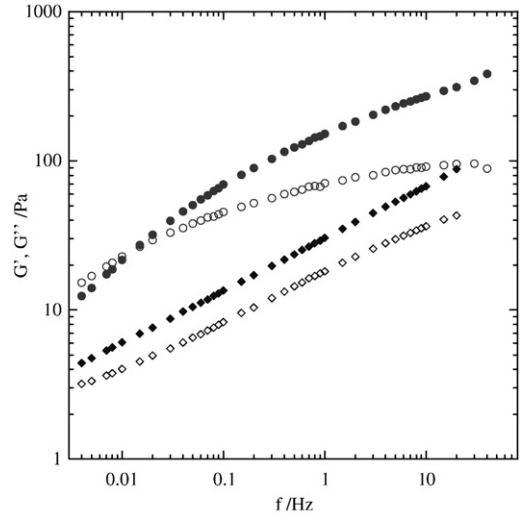

Fig. 14. Mechanical spectra for SDS/DDAB vesicles and two oppositely charged polyelectrolytes: with low charge density and hydrophobic groups (spheres) or with high charge density (squares). Storage and loss moduli are represented by filled and open symbols, respectively [184].

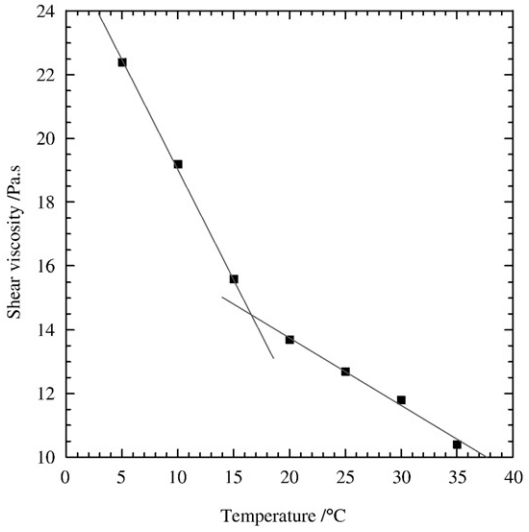

Fig. 15. Dependence of shear viscosity on temperature for a polyelectrolyte—oppositely charged catanionic vesicle system. The T_m of the surfactant aggregate is, at the conditions of the figure, c. a. 15 °C. Adapted from [137,191].

shaped vesicles [192]. In the fluid vesicles, there is complete in-plane miscibility of the two components and the faceting is removed.

Anchored polymers may induce several changes in the shapes and fluidity of bilayer membranes: they can induce shifts in the gel-to-liquid crystal transition temperature of the vesicles [137,193,194], shape changes [195] or breakage of the vesicles [196]. An example comes from the interaction between a positively charged peptide (poly-L-lysine) and liposomes, where changes in the membrane lipid distribution were observed upon lipid–protein agglutination. Addition of polylysine induces the formation of crystalline patches of bound phosphatidic acid and increases the transition temperature of the lipid by c. a. 12 °C [193].

There is evidence that, yet, at low coverage, anchored polymers increase the bending rigidity of the lipid membrane [166,197,198]. At high coverage, the polymers control the formation of lateral lipid domains [199] and of pores [200,201], or also induce asymmetry in the bilayer composition [173].

Addition of a polyelectrolyte to a charged bilayer has also been experimentally [137,159,202] and theoretically [203] shown to promote lateral segregation of charges. This phenomenon is illustrated by the results obtained in the study of the interaction between the polyelectrolytes described above, LM200 and JR400, and SDS/DDAB catanionic vesicles (with excess of SDS). These vesicles are above T_m and spherical at room temperature. However, faceting occurs when LM200 is added to the surfactant solution at room temperature. This is clearly associated with a polymer-induced crystallization of the surfactant alkyl chains because as the temperature increases, the chains melt and the faceting is removed. This signifies that the crystalline state of the vesicles can be stabilized by a polymer with respect to the fluid state. It has been shown that the increase of tension due to polymer adsorption in spherical vesicles favors multiple faceting, 4–6 facets being the most common [166]. DSC results suggest, further, that the mechanism behind the T_m increase is

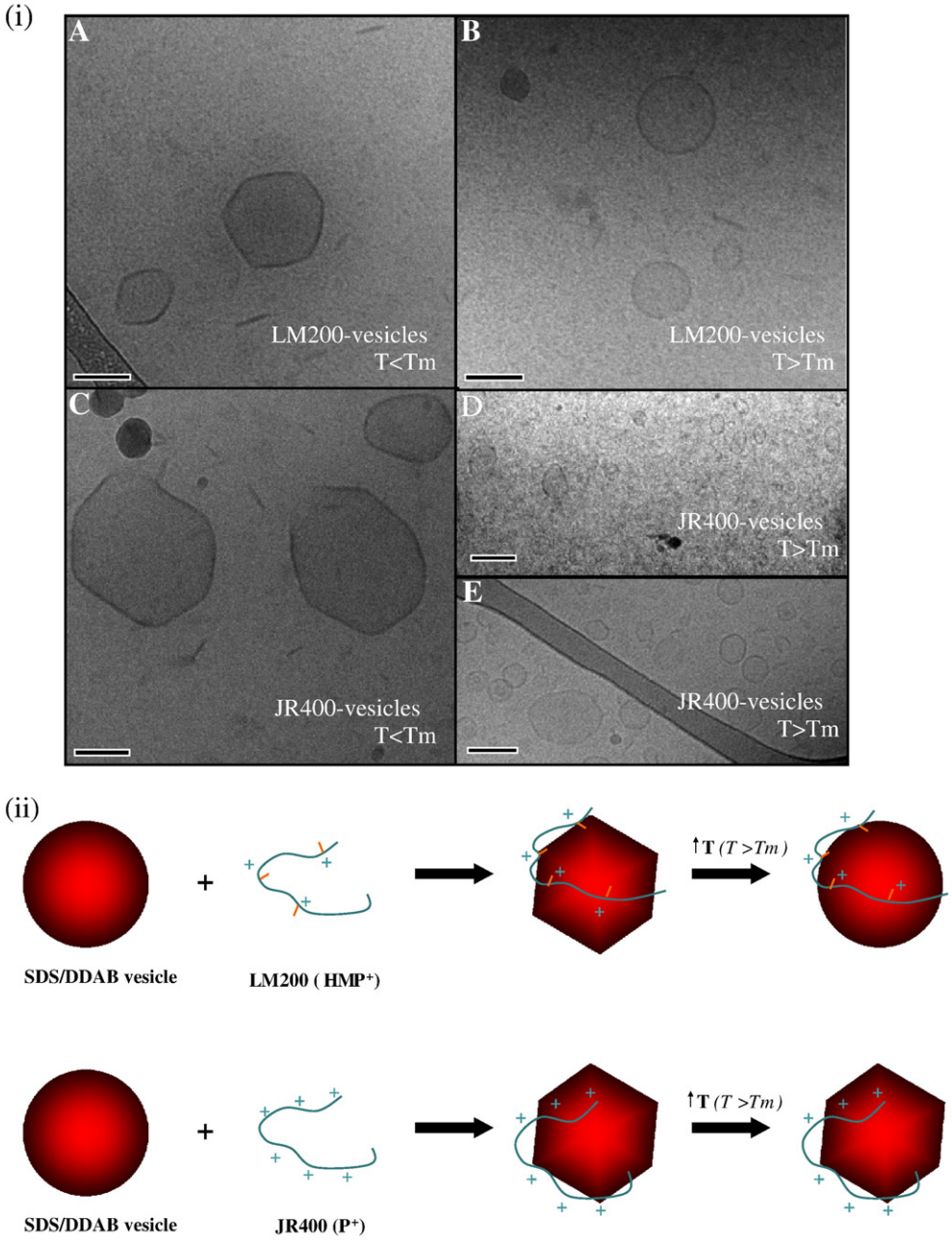

Fig. 16. (i) Cryo-TEM imaging of vesicles at temperatures below and above their chain melting transition. A) and B): vesicle–polymer with low charge density (LM200). C) and D) vesicle–polymer with high charge density (JR400). Adapted from [137]. (ii) Schematic representation of the polycation effect on SDS/DDAB vesicle shape.

not related primarily with the simple anchorage of the polymer hydrophobic grafts in the bilayer, since a polymer with uncharged grafts (like hm-HEC) does not induce crystallization effects. The grafts per se may induce vesicle bridging and a dramatic viscosity increase in the system, but it is the associated charge which is responsible for the crystallization effect and hence the reshaping and a concomitant further viscosity increase.

On the other hand, JR400 decreases the chain melting temperature with respect to the neat vesicles, to values similar to those of neat DDAB vesicles. Furthermore, the faceted deformation occurs here both for vesicles below and above their T_m. This indicates that the polymer induces DDAB-rich regions. A complete separation into SDS micelles and DDAB vesicles does not occur once DDAB vesicles are typically larger that the ones found in the cryo-TEM images; also they remain spherical above T_m. Fig. 16 shows how the shape of the vesicles respond to changes in temperature, for both polymer systems.

The most likely explanation for the observations for the JR400 system is related to polymer-induced charge polarization of the membrane and, thus, in-plane segregation of the two vesicle components. The addition of such a polycation of high charge density can, therefore, induce changes in a catanionic vesicle based on charge polarization, i.e., formation of domains rich in anionic surfactant in the bilayer, where the polymer is strongly associated, due to strong electrostatic attractions and favorable polymer counterion entropy, and polymer-depleted domains rich in cationic surfactant. The high cross-link lifetimes reported for the JR400-vesicle system above T_m could also be explained by the existence of regions of strong polycation–surfactant association. Further support for this mechanism comes from Monte Carlo simulations, which have shown that adsorption of a flexible polyion can promote spatial polarization of weakly charged or neutral catanionic membranes, for the case of freely mobile charges [203].

The type of shape effects and the interaction mechanism for the negatively charged catanionic vesicles may, therefore, depend on polycation charge density. For low enough charge density, there is evidence of surfactant crystallization in the bilayer. For high enough charge density, polarization effects in the fluid state take place, where faceting would not occur above T_m.

Other studies indicate that strong hydrophobic and/or electrostatic polymer–vesicle associations induce the formation of domains markedly different from the rest of the membrane [204,205]. Concerning electrostatics, the polymer–liposome association depends critically on whether the surfactant alkyl chains are below or above their melting temperature, especially at low polymer charge densities [166,206].

Wang et al. proposed a general approach for predicting shapes of fluid vesicles with anchored polymer chains. The idea combines the Helfrich curvature elasticity theory for fluid membranes and the self-consistent field theory for polymers, to determine stable and metastable shapes of the vesicle–polymer systems, as well as the segment distributions of the anchored chains [207].

5. DNA–vesicle systems

5.1. Overview and interest

The largest literature in the field of polymer–vesicle systems concerns DNA. Thus the complexation of DNA through the use of vesicle- or liposome-based systems has received a very large attention [208,209], especially due to the potential of these systems as gene delivery vectors in gene therapy. The interest in the area has, because of complications arising in alternative viral vectors, experienced a renewed interest in recent years. The delivery of genes to the cell nucleus is a complex processes, with many obstacles. The interaction of a lipid–DNA complex with cells contains several steps, including binding and internalization of DNA, escape of DNA into the cytoplasm, and entry into the cell nucleus. From the schematic illustration in Fig. 17 we can infer the importance of packaging of DNA for transfer through the cell membrane and its release to the cell nucleus.

The compaction of DNA outside the cell and its decompaction inside the cell are considered important steps in a successful gene transfection process. Because of the high charge density of DNA, in addition to a very high molecular weight, cationic cosolutes are considered essential in this process and both cationic amphiphiles and polymers are intensely investigated as ingredients of transfection formulations. Cationic lipids and surfactants are indeed efficient compaction agents and have also in a

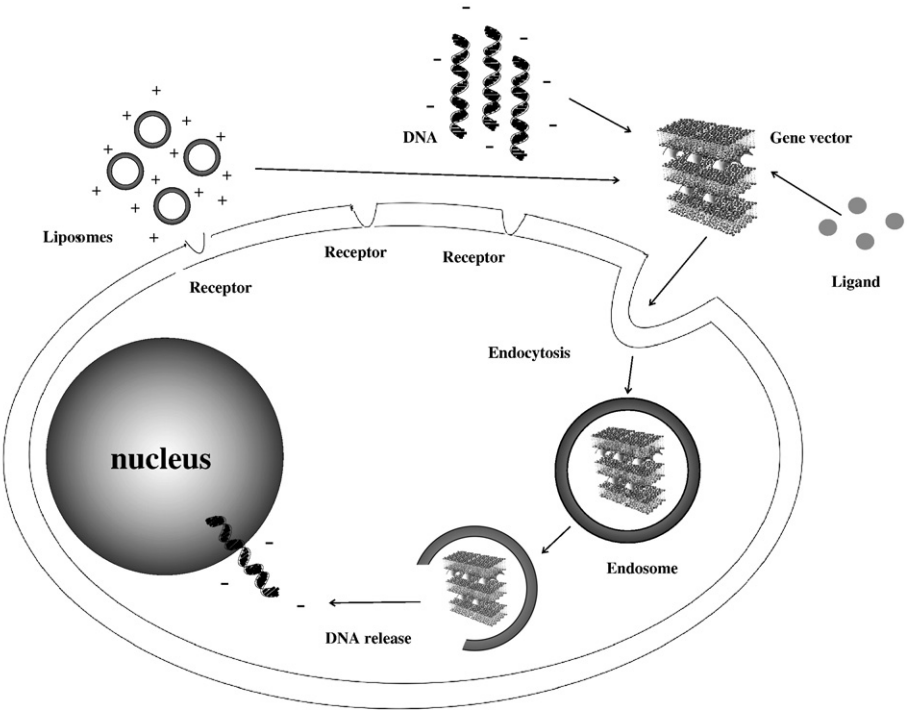

Fig. 17. A schematic illustration of gene delivery based on cationic liposomes or vesicles (from [212]).

number of cases been found to promote transfection. A common procedure makes use of cationic surfactant or lipid vesicles or a doping of phospholipid vesicles by a cationic surfactant or lipid. The mechanisms behind a successful surfactant induced or surfactant promoted transfection remain to be established [210]. Several factors can be determining, and in particular we can consider the nature of the hydrophilic and hydrophobic parts of the surfactant, the microstructure of the DNA–surfactant complex, and the size of the particles delivered. Dramatic changes in transfection efficiency on minute changes in headgroup have been observed [211], but it is not straightforward to establish if this is related, for example, to interactions on the individual molecular level, or it is an indirect effect associated with surfactant packing.

It is not the purpose of this review to discuss more biological and biotechnological aspects of mixed systems of DNA and vesicles. On the other hand, we will describe mixed DNA–vesicle systems from a more general physico-chemical point of view and show that they well illustrate basic aspects of polyelectrolyte–vesicle interactions.

The discussion is here centered on the interactions between DNA and catanionic vesicles. For natural reasons, studies have very much focused on the biologically most relevant form of DNA, the double-helix B form. We recall that this double-stranded (ds) DNA is only stable at lower temperatures and in the presence of a sufficiently high electrolyte concentration; in a salt-free medium and at higher temperature, ds DNA dissociates into two single strands, a process described as DNA melting or denaturation. There are many observations that demonstrate that single-stranded (ss) DNA associates more strongly with cationic surfactants than does ds DNA [213]. Such observations suggest that a simple electrostatic explanation is not sufficient—ds DNA has a much higher linear charge density than ss DNA—but that DNA chain flexibility and amphiphilicity play an important role.

Mixtures of ds DNA with several types of catanionic vesicles, with different combinations of cationic and anionic surfactants, will be described in the next sections.

5.2. DNA–vesicle association

While vesicles with a positive net charge show strong association to DNA, we note that vesicles with a net negative charge showed no indication of association [158].

The effects observed when the charge ratio of DNA to the net charge of positively charged vesicles is varied are basically the same for all systems investigated. At low amounts of DNA, intact vesicles are observed. For larger amounts of DNA, phase separation occurs. Due to the association between vesicles and DNA, the vesicles are disrupted with possible formation of multi-lamellar structured particles [107,158]. A lamellar structure is confirmed by the small angle X-ray studies, as illustrated in Fig. 18.

There are a few further significant points to be made in this connection. Firstly, the lamellar spacing does not change with the amount of DNA incorporated, showing that DNA incorporates in the water layers without perturbing notably the surfactant packing. In the SAXS diffractograms we can, in addition to the peaks demonstrating the lamellar packing, observe additional peaks, which are associated with a regular DNA–DNA spacing in the water layers. A notable feature of these catanionic systems is that they have a very large capacity for encapsulation of DNA. The reason for this is that, in contrast to conventional systems based on a mixture of a cationic and a nonionic lipid, the catanionic systems are charge regulating: as DNA is incorporated, there is a release of anionic surfactant (typically forming micelles). The cationic surfactant illustrated in these studies, is based on the amino-acid arginine; it is characterized by a low cytotoxicity and shows good transfection [107,214]. The association behaviour described is the same for conventional quaternary surfactants (which are highly cytotoxic).

Apart from the internal structure of the particles and their composition, it is also important to consider their size as a function of overall solution composition. As DNA is added to the catanionic vesicle solution, small complexes start to form at low concentrations; at slightly higher concentrations these initial complexes aggregate into larger clusters, reaching a maximum around the point of charge neutrality. Above the isoelectric point, the clusters become smaller and the complexes begin to repel each other leading to cluster disruption. Then, the fraction of smaller particles increases considerably.

5.3. Striking reversibility

The interaction between catanionic vesicles and DNA shows reversibility in formation of these vesicles. Dias et al. [215] compacted DNA by a cationic surfactant and then anionic surfactant was added. On addition of anionic surfactant, DNA decompacts and attains the extended natural conformation. This demonstrates that the surfactant–surfactant association is stronger than that between DNA and cationic surfactant. As can also be seen, the cationic surfactant released from DNA associates with the added anionic surfactant to form vesicles spontaneously, exactly as on adding the two surfactants together directly. The system thus responds reversibly on changing the conditions both with respect to DNA conformation and surfactant self-assembly, and adds further support for the thermodynamic stability of catanionic vesicles.

5.4. DNA compaction on the surface of a vesicle

Another phenomenon encountered when mixing catanionic vesicles with ds-DNA is illustrated in Fig. 19. As can be inferred, DNA changes from the extended “coil” state to a compact “globule” state on contacting the vesicle surface. This is in contrast to our general picture of polyelectrolyte

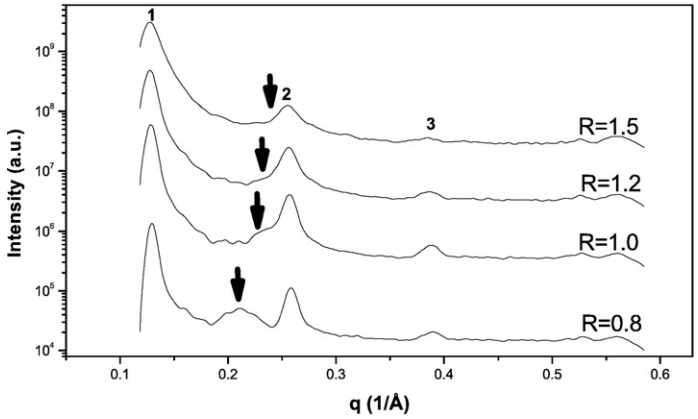

Fig. 18. SAXS diffractograms for a dispersion obtained by mixing catanionic vesicles formed by an arginine-based cationic surfactant and sodium octyl sulfate, and DNA, for different charge ratios between DNA and catanionic vesicles. The 1:2:3 ratios of the scattering vector signify a lamellar structure. The arrows indicate the peaks due to DNA–DNA organization. With increasing amount of DNA, the DNA–DNA distance is seen to decrease, while there is no shift in the surfactant bilayer spacing (from [107]).

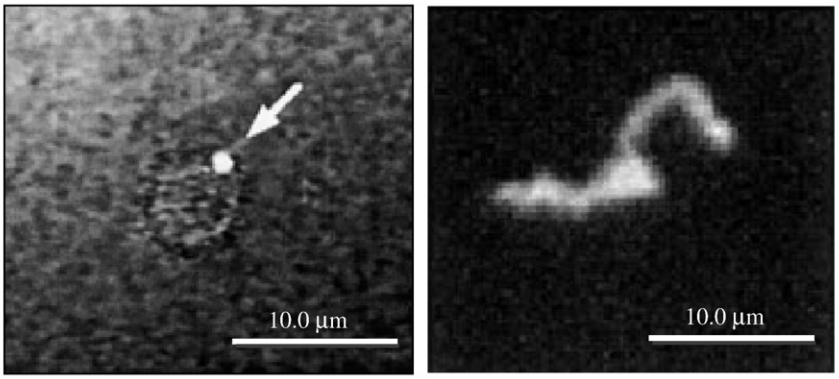

Fig. 19. DNA adsorbs in a compacted conformation of the surfaces of CTAB/SOS catanionic vesicles having a net positive charge [158] (left). DNA adsorbs in an extended conformation on a positively charged DOTAP–DOPC bilayer on a glass substrate (right). Adapted from [216].

adsorption on oppositely charged surfaces. Thus we expect a flat extended conformation, as indeed observed previously for DNA adsorption onto glass surfaces doped with cationic lipids. So, what is going on? To understand this we have to note the fundamental difference between a vesicle surface in the liquid state and a solid surface. In a vesicle in dynamic equilibrium, there are several motions taking place that do not occur in the case of the solid surface, as has been worked out by Dias et al. [203].Thus in a liquid-state bilayer, the surfactant molecules are mobile and will, therefore, adapt to any changes in the environment. Firstly, we note that there are lateral motions giving the possibility for an in-plane redistribution of the surfactant molecules. Secondly, there are perpendicular motions allowing for molecular protrusions and the creation of a non-planar surface.

As demonstrated by the Monte Carlo simulations, both processes play a role in the association of DNA to a catanionic vesicle and, in fact, for any mixed amphiphile vesicle [217]. At the location where a DNA molecule approaches the vesicle surface, there is a strong polarization of the bilayer, corresponding to an accumulation of cationic surfactant molecules and a depletion of anionic ones. Adaptation of the surface by protrusion effects will strengthen further the association between the vesicle surface and the compacted DNA molecule, as demonstrated by the very high relaxation times observed for the association between a cellulose based polyelectrolyte and SDS/DDAB vesicles [184].

5.5. Transfection studies

A brief description of DNA–vesicle transfection will be made. Fig. 20 relates to precompaction of DNA using an arginine-based cationic surfactant and then using a conventional liposome formulation. An important aspect is that introduction of this surfactant into the lipoplexes can lead to a very important increase in transfection efficiency. The figure shows the transfection efficiency as a function of the cationic surfactant-to-DNA charge ratio. For further information regarding DNA transfection see references [218,219].

6. Conclusions and outlook

In this review, we have started by broadly describing the main features and analyzing the underlying forces/mechanisms of polymer–surfactant systems, from one side. Bilayer vesicles as self-

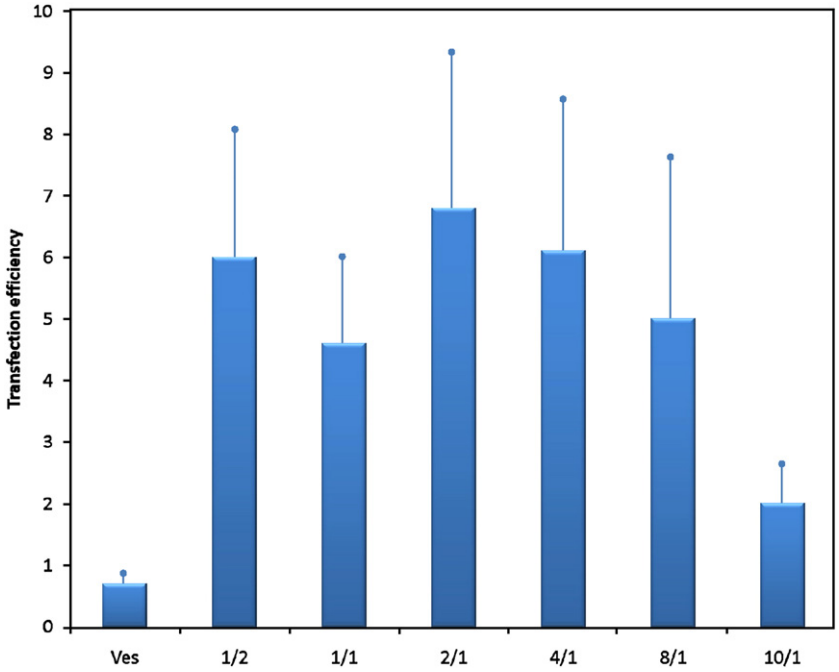

Fig. 20. Addition of an arginine-based cationic surfactant to conventional liposome formulations can significantly increase transfection. Transfection efficiency is presented for different charge ratios between surfactant and DNA. Adapted from [214].

organized systems made up from various types of amphiphiles have also been reviewed, from another side. With these backgrounds, it was then a natural step to establish the bridge and address the main features of polymer–vesicle association, also including here proteins and DNA as interacting macromolecules (in the latter case, with more specific aspects).

As described, miscibility or phase separation of segregative/associative type can take place in polymer–surfactant systems, depending on the type of interactions (attractive or repulsive) between those entities. These interactions, often of electrostatic and/or hydrophobic nature, can be modulated by a number of factors, such as presence and degree of charge of the co-solutes, salt, polymer molecular weight and hydrophobic modification. Charge and polymer hydrophobe/aggregate stoichiometric ratios can also largely influence connectivity and viscosity of these mixtures. As has become clear, many of these solid concepts can be usefully conveyed to polymer–vesicle mixtures. We have also seen that beyond classic and often metastable lipid vesicles, there are now at hand other types of model vesicle systems (e.g. catanionic and block copolymer vesicles). Structurally, the latter are entirely analogous to liposomes, but their spontaneous formation and long-term stability, as well controlled charge regulation/size in some instances, offer new possibilities. This is the case of catanionic vesicles, which have been extensively considered here.

Polymer–vesicle association can thus be understood from the viewpoint of either electrostatic or hydrophobic interactions at stake. The interactions can result in steric stabilization of the vesicle or, more significantly for our purpose here, on the formation of highly interconnected polymer–vesicle networks. Hydrophobic modification in the polymer backbone greatly enhances gelation and hydrophobic association rapidly dominates over other interactions, including repulsive electrostatic ones. We have focused here more, however, on the case of oppositely charged polyelectrolytes and catanionic vesicles as model bilayer systems, in order to illustrate some general principles.

Miscibility, phase separation phenomena and rheological behavior largely depend on polymer hydrophobic modification and charge density, and the stoichiometric ratios between polymer binding sites and vesicles, but also on the chain melting transition temperature of the vesicles (a novel feature, as compared to micellar systems). From a more vesicle-centered perspective, we have also seen that strongly adsorbed polymers may induce different changes in the shape and fluidity of the vesicle bilayer, from shifts in the chain melting transition temperature, to shape deformations or simply breakage of the vesicles. The type of shape effects and interaction mechanisms are also dependent on polycation charge density and hydrophobic modification. Work on several types of systems has also shown that strong hydrophobic and/or electrostatic interactions can, for instance, induce the formation of bilayer domains markedly different from the rest of the membrane, a feature quite distinctive from micelles or solid surfaces as adsorbing sites.

We have also addressed mixed DNA–vesicle systems from a general colloid-point of view and shown that they illustrate basic aspects of polyelectrolyte–vesicle interactions. While in the past cationic amphiphiles and polymers have been intensely investigated as tools for gene transfection formulations, we have now at hand the possibility to expand views by using oppositely charged catanionic vesicles. As seen, a prominent feature of the latter is their large ability to compact/decompact DNA upon charge regulation. Adsorption, phase separation and formation of multilamellar structures occur upon increasing DNA concentration. Less obvious is the adsorption of DNA in a compact globule form onto a charged membrane, a feature arising from the mobility and segregation of charges and quite distinctive from solid surfaces.

It is our conviction that the field of polymer–vesicle systems will expand considerably in the near future, both at a more fundamental level, where there is still much to investigate and understand, and in close relation to practical implications. For this, model catanionic and polymeric vesicles made up with biofriendly amphiphiles are increasingly at our disposal. From this side, the control of charge, size, stability and stimuli–response is attaining ever more sophisticated levels. From the polymer side, macromolecules with controlled charge density and controlled hydrophobic modification are available, not to mention of course proteins and DNA as seen as polyelectrolytes. Both factors thus allow for a careful design of polymer–vesicle networks in order to test and understand trends and general principles. We thus foresee a continuous exploration of vesicle–polymer networks, in close relation to application goals, such as drug and gene delivery, cosmetic formulations, rheological control and micro-reactor chemistry.

Acknowledgements

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