DNA compaction at interfaces, an application of mixed colloid systems.

Björn Lindman

Marité Cardenas

Tommy Nylander

Physical Chemistry 1, Chemical Center,
Lund University, POB 124,
22100 Lund, Sweden.

COLLOIDIAL SYSTEMS

Colloid science is a mature field. It received a lot of attention in the beginning of the 20th century with seminal contributions due to Einstein, Perrin, Svedberg and others. After a strong period of fundamental work, the applications received more attention and much of research was performed in industrial research laboratories or by academics with an interest in applications. The progress in the field meant a revolution for formulations with particularly strong applications for pharmaceutical formulations, paper industry, cosmetics, paints, coatings, foods and detergents. However, there is no exaggeration to state that there are very few branches of industry, which do not use colloids in a significant way.

While early work was often based on biological aspects and problems, this was largely forgotten with the strong development of molecular biology. However, if we want to learn about organization and interactions in biology, molecular biology must be complemented with *colloidal biology.*

Colloidal systems were traditionally classified into 1) macromolecular solutions, 2) association colloids, and 3) disperse systems, where the first two are thermodynamically stable one-phase systems of polymers, or other macromolecules, and of self-assemblies, respectively, and the third describes kinetically stable dispersions of one phase in another, for example. Recent years have seen the borders between the three classes to become more diffuse and complex.

Recently, there has been a very strong revival of fundamental work on colloids. This has several reasons. One is the feasibility of theoretical studies of more complex systems, with computer simulations constituting an important part. Secondly, experimental techniques have revolutionized our ability to probe detailed structure and kinetics of larger molecules and self-assemblies. Finally, traditional areas of applications have become much stronger at the same time as many new ones have been developed.

This enlargement of interest has much extended the academic disciplines involved. While initially, colloids were most extensively studied by physical chemists, it now concerns several fields notably physics, chemical engineering and several of the life sciences. As a consequence also many new names have been added for the field like nanoscience/technology, soft matter, complex fluids etc.

We will here indicate some recent progress in the field, first reminding of simple amphiphilic systems and then addressing amphiphilic polymer self-assembly. Polymer-surfactant systems in bulk and at interfaces will be introduced and exemplified with surfactant-induced DNA compaction.

SMALL AMPHIPHILIC MOLECULES

Association colloids, originally comprising surfactants and polar lipids, are amphiphilic, i.e. they are built of distinct polar and non-polar parts. In any aqueous system the combined hydrophilic-hydrophobic nature leads to an ambivalent behaviour: the polar parts interact favourably with water while the non-polar ones are not accepted. This has two consequences, adsorption at polar or nonpolar surfaces and self-assembly. Adsorption isotherms on hydrophilic and hydrophobic surfaces are very different as illustrated in **Figure 1**[1].

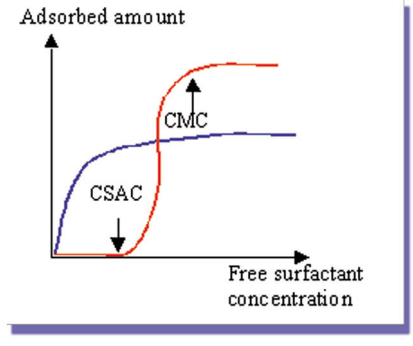

Fig 1. *Typical adsorption isotherms for an ionic surfactant on a hydrophilic (red curve) and hydrophobic (blue curve) surface.*

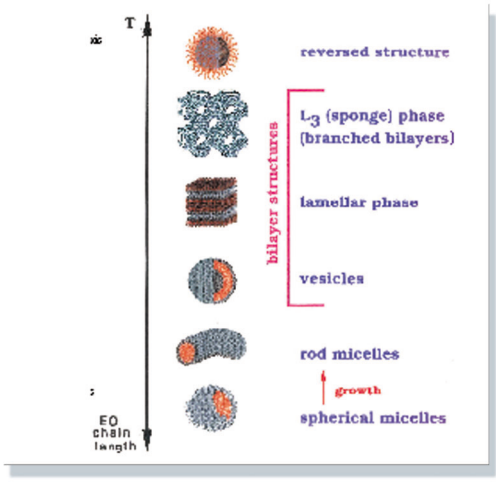

Fig. 2 *Self-assembly structures for small amphiphilic molecules.*

Self-assembly leads for more polar amphiphiles, like single alkyl chain ionic surfactants, to spherical micelles at low concentrations and to different liquid crystalline structures at higher concentrations[1,2]. For less polar amphiphiles, like phospholipids and monoglycerides, a lamellar or a cubic liquid crystalline phase is the result of the self-assembly process. Several types of self-assembly structures are known as illustrated in **Fig. 2**. The actual structure form depends mainly on the chemical structure of the amphiphile, in particular on the balance between the polar and the non-polar parts, although it is influenced by the external parameters. For ionic surfactant, electrolyte addition will make the surfactant effectively less polar. For the most common nonionic surfactants, those where the polar part is an oligo oxyethylene chain, temperature and head group size are competing factors. An oxyethylene chain changes conformation with increasing temperature from more polar to less polar states; this makes the head-group less polar with increasing temperature.

Adsorption and self-assembly are not unrelated effects. Rather they have the same driving forces and adsorption is often best described as a surface-induced self-assembly process. On a hydrophobic surface, adsorption typically leads to a monolayer, or for more weakly amphiphilic molecules, to "hemi-micelles", as illustrated in **Figure 3**[3-5].

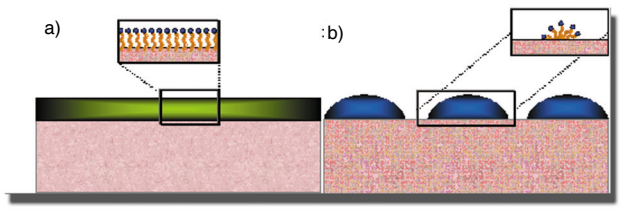

Fig. 3 *Adsorption structures for small amphiphilic molecules on non-polar surface where a a) monolayer or b) hemimicelle is formed*

On a hydrophilic surface, adsorption does not lead to traditionally discussed monolayer and bilayer structure-but instead to aggregates similar to those formed in the bulk; for example discrete globular micelles and long rod micelles **(Fig. 4)**[3-5]. All these structures are dynamic equilibrium structures and change with conditions, for example if the surface is taken out of water into air; then a more favourable structure is one with the nonpolar parts on the exterior in contact with air.

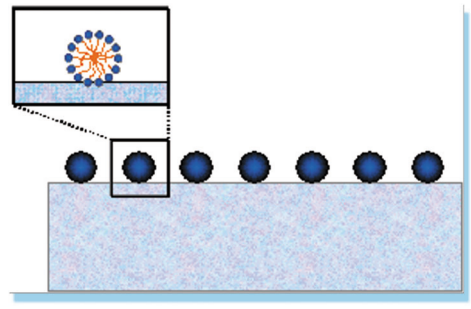

Figure 4. *Adsorption structures for small amphiphilic molecules on a hydrophilic surface.*

LARGE AMPHIPHILIC MOLECULES - ASSOCIATING POLYMERS

The dynamic character of the low molecular weight amphiphiles (traditional surfactants) is one reason for replacing them with macromolecular compounds: these give much more long-lived aggregates both in bulk and at interfaces, of large significance in many applications like stabilizing a particle suspension or an emulsion. However, amphiphilic polymers or polymeric surfactants have a much broader significance and numerous applications. The chemical structure of an amphiphilic polymer can obviously be highly variable[1]. We can, however, conveniently distinguish between block and graft copolymers. Di-block copolymers have a general structure similar to that of a conventional surfactant and show a very similar self-assembly behaviour. For reasons of more simple synthesis, triblock copolymers are more common in applications and also received more attention in fundamental work. An important feature is that there appears to be little difference between di- and triblock compounds[6].

The self-assembly behaviour for polymers is much richer, although very analogous, to that of surfactants, and becomes richer the higher the molecular weight. This is illustrated in **Fig. 5** for a triblock copolymer of poly oxyethylene and poly oxypropylene in a mixture of two solvents, each a good solvent for one of the blocks.

Graft copolymers are also very significant in applications in particular for rheology control. These polymers have as a backbone a water-soluble polymer and onto this has been grafted a relatively small number of hydrophobic groups like alkyl chains; typically one or a few percent of the monomer units are modified. These hydrophobically modified water-soluble polymers can

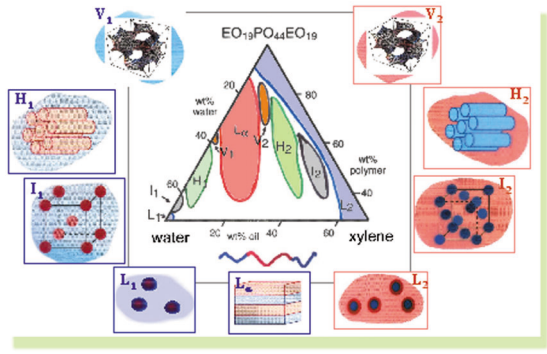

Fig. 5 *A record-rich phase behaviour.*[7-9]

be considered as polymer-modified surfactants and, as any surfactant, there is a driving force for hydrophobic association. The polar group, the hydrophilic backbone, strongly counteracts aggregation due to losses of conformational freedom; therefore, only small globular aggregates form. However, this formation of hydrophobic microdomains or micelles leads to a three-dimensional network, as illustrated in **Fig. 6**, and leads to a major increase in viscosity; typically there is an increase in viscosity by one order of magnitude or more on hydrophobic modification for 1 wt % solutions.

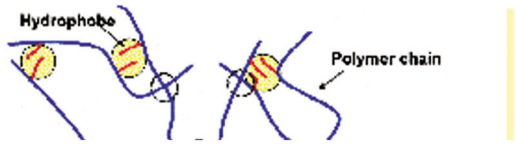

Fig. 6 *Network formation due to a hydrophobically modified water-soluble polymer.*[10-12]

POLYELECTROLITES

A polyelectrolyte is composed of polyions and counterions. The counterions will largely determine the properties of a polyelectrolyte. For example, they confer to a polyelectrolyte a high solubility and miscibility in aqueous systems; the entropic penalty of phase separating the large number of counterions is very large. Another consequence is that a polyion will be very extended, thus showing a large persistence length and rigidity. DNA is a polyelectrolyte with a very high charge density and illustrates the properties of a high rigidity and high solubility in water.

DNA is an amphiphilic random copolymer. Therefore, single stranded DNA, which exposes the hydrophobic bases, has a large tendency to self-assemble. The self-assembly is rather special in this case because of the distribution of bases and leads to the well-known double helix structure; while the driving force is the hydrophobic interaction the double helix is further stabilized by hydrogen-bonding between complementary bases[13] (ver **Fig. 7**).

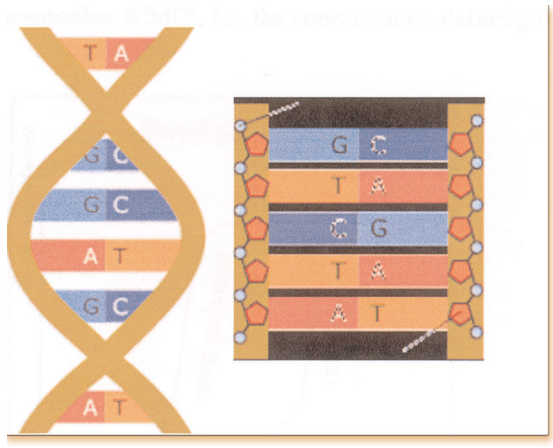

Fig 7. *The DNA molecule and the base pairing in the double helix.*

The transition between single and double stranded DNA is strongly affected by temperature and electrolyte concentration. At higher temperatures double helix DNA is transformed into single stranded, often referred to as DNA melting or denaturation. Double helix DNA has a very high charge density and attracts, therefore, a large number of counterions[14,15] . The associated entropic penalty destabilizes the double helix conformation, which dissociates at high dilution[16] On the other hand, there is an electrolyte-induced stabilization similar to that for ionic micelles[17].

The rigidity of polyelectolytes has several biological implications, including lubricating and structure-forming effects for ionic polysaccharides. The DNA rigidity, as well as its high charge density, severally limits the possibility to have it transported through membranes into the cell nucleus as required in gene therapy. One possibility to facilitate gene transfection is to transform DNA into compacted states[18-21].

Interactions in systems of highly charged polymers and surfaces in general are strongly dependent on electrolyte concentration and, in particular, on counterion valency[22,23]. With monovalent counterions there is a strongly repulsive interaction due to the counterion entropy. With divalent and higher valent counterions this entropic effect is much reduced and the net interaction even becomes attractive due to ion correlation effects. Thus on approach of two similarly charged surfaces there is an attraction with multivalent counterions since the ion distributions become correlated. This has consequences for the conformation of polymer chain. While in the presence of monovalent counterions, charging up of a polymer leads to more expanded states, there is a transformation to more compacted states with multivalent counterions.

POLYMER - SURFACTANT ASSOCIATION

The combined use of polymers and surfactants is ubiquitous in pharmaceutical, cosmetic and industrial formulations. The interaction between low molecular weight amphiphiles and macromolecules is also of central relevance in all biological systems and in foods. In the simplest case, a polymer can facilitate the surfactant self-assembly, and thus lower the critical micelle concentration (CMC), i.e. the concentration defining the onset of micelle formation. (**Fig. 8**).

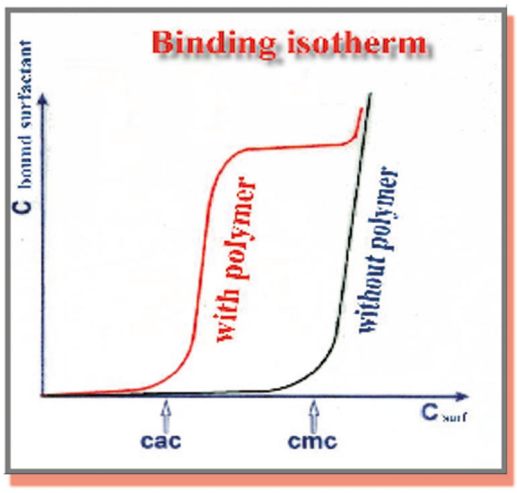

Fig. 8. *A polymer may facilitate micelle formation of a surfactant.*

Polymer-surfactant association is generally significant for ionic surfactants. However, it does not occur for nonionic surfactants and homopolymers. Micelle formation of ionic surfactants leads to highly charged aggregates, which attract by Coulombic forces a large number of counterions. This leads to a strongly uneven counterion distribution, since the concentration is much higher on the micelle surface than in the bulk. The associated entropy decrease explains the relative instability of ionic micelles as compared to nonionics, as illustrated by a typically two orders of magnitude higher CMC for ionic micelles. The presence of either a polyelectrolyte or a nonionic polymer molecule, can significant affect this entropic penalty. A polyion can lower the CMC by associating to the micelle and thereby liberating a large number of counterions. A nonionic polymer will be located at the micelle surface and thus reduce the charge density; in this way the number of counterions associated to the micelle is reduced and thus the entropic penalty in forming micelles. In both cases, the shape and size of the micelles are quite similar to those formed without polymer and the association is best regarded as a stabilization of the micelles by the polymer and thus a lowering of the CMC. On the other hand, amphiphilic polymers like the hydrophobically modified water-soluble polymers behave differently. Here the polymer itself self-assembles and the polymer-surfactant association is best regarded as a mixed micelle formation between two amphiphiles.

POLYMER - SURFACTANT COMPLEXES AT INTERFACES

The adsorption of a polymer on a solid surface is very different for different polymers and different surfaces. There can be an attraction between the polymer and the surface but this is not necessarily a requirement for adsorption since there is a complex interplay involving also polymer-solvent and surface-solvent interactions. Solvency effects play a very important role for aqueous solutions: for nonionic polymers these can be affected by temperature and various ionic and nonionic cosolutes, for ionic polymers electrolyte addition and counterion valency have a determining effect.

Polymer adsorption is often characterized by a high affinity and an effective irreversibility. Contacting a polymer-coated surface with a surfactant solution can lead to very different effects depending on surfactant-surface and polymer-surfactant interactions as well as on the solubility of polymer-surfactant complexes, as illustrated in **Fig. 9**. An adsorbed polymer can be removed from a surface by two principal mechanisms. Firstly, there may be competitive adsorption, as example for hydrophobic surfaces, where all surfactants adsorb strongly. Secondly, surfactant association to the polymer may lead to a highly soluble complex; this is a typical phenomenon when an ionic surfactant is added to an adsorbed layer of a nonionic polymer.

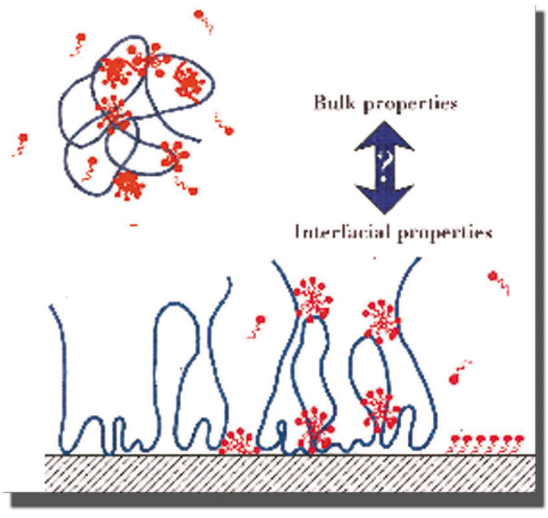

Fig. 9 *Possible situation for polymer-surfactant complexes in the bulk and on surfaces*[24].

A surfactant and a polymer may also coadsorb and form an interfacial complex; this may lead to major rearrangements in the polymer layer. An important example is shown in **Fig 10** where a nonionic cellulose ether is adsorbed on a hydrophilic surface; it adsorbs strongly into a rather compact layer. An anionic surfactant that binds to the polymer layer causes large swelling of the adsorbed layer. By associating to the nonionic polymer (as micelles), the adsorbed layer becomes ionic and gives an important osmotic swelling.

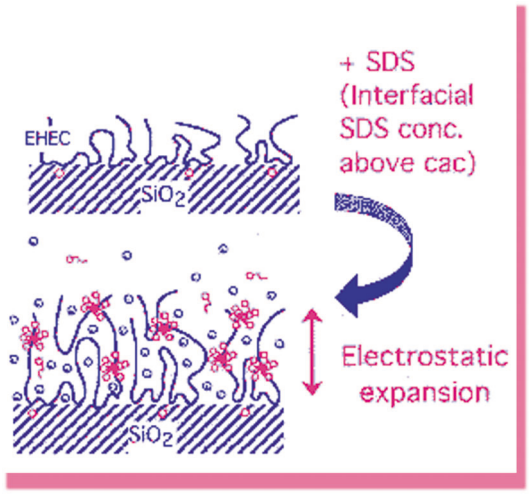

Fig 10. *Effect of an anionic surfactant on the structure of an adsorbed layer from a nonionic polymer*[25,26].

SURFACTANT - INDUCED DNA COMPACTION

Cationic surfactant binding to DNA is, at low surfactant concentrations, weak and proportional to surfactant concentration due to a simple ion exchange mechanism. As the cationic surfactant concentration at DNA reaches the CMC, a strongly cooperative binding results, leading to the formation of surfactant self-assembly aggregates; as seen in figure 8 the interaction is so strong so that normally other structures than simple spherical micelles form.

DNA addition to a cationic surfactant solution lowers the CMC by orders of magnitude. The large self-assembly structures formed by the cationic surfactant act as multivalent counterions to DNA. As mentioned above, the presence of multivalent counterions in systems of highly charged polyions leads to attractive interactions due to ion-correlation effects between different polyion chains. In this case, an inhomogeneous distribution results that ultimately leads to phase separation, as for DNA solutions that are not very dilute. At very high dilution, where intermolecular interactions are less significant, there will be an intramolecular association instead: different parts of a DNA chain will attract each other. This leads to compaction of the DNA molecule. Both macroscopic phase separation and compaction of individual DNA molecules are well documented; the latter is illustrated in **Fig. 11**. There are two important and non-trivial observations in this figure. Firstly, DNA compaction is not gradual, but rather it is an all-or-none process. Thus, compacted DNA molecules occur even for small additions of surfactant and there is a wide concentration range with a coexistence of extended DNA molecules and compact globules. Secondly, subsequent addition of anionic surfactant reverses the process, again with a broad coexistence region. DNA compaction is clearly reversible and the association between cationic and anionic surfactant is stronger than between cationic surfactant and DNA.

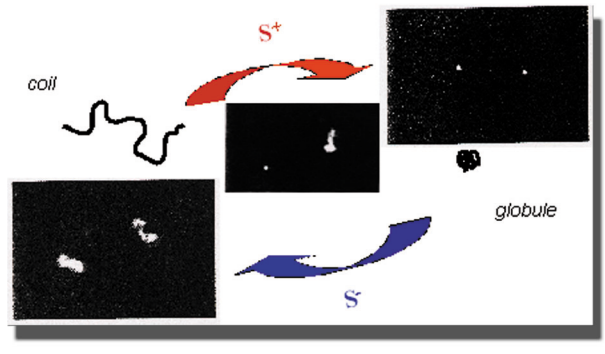

Fig 11. *Fluorescence microscopy images for DNA compaction by cationic surfactant, where the coexistence between coils and globules is shown. The DNA globules are destablished by the addition of an ionic surfactant.*[27-29].

DIFFERENT DNA - SURFACTANT COMPLEXES

Addition of cationic surfactant leads for individual DNA molecules, as observed in fluorescence microscopy experiments, to separate globules of compacted DNA and for more concentrated cases to bundles of DNA molecules or macroscopic precipitation. In all cases, surfactant molecules are associated to DNA in a self-assembled state and the microstructure can be expected to be the same independent of the mode of preparation since equilibrium states are expected. On the other hand, the surfactant packing in the precipitate can be predicted to depend strongly on the surfactant used, in particular on how polar it is. For example we expect a reverse hexa-gonal or lamellar packing for a double-chain surfactant or lipid, while a normal hexagonal phase would form for a single-chain surfactant.

The three different structures for the precipitate have been identified so far. A lamellar phase is found for a mixture of a zwitterionic lipid and a double-chain cationic surfactant (**Figure 12a**). According to expectation the reversed hexagonal phase was found for a double-chain cationic lipid (**Figure 12b**), while the normal hexagonal was found for single-chain surfactant (**Figure 12c**). Furthermore, combining a single-chain surfactant with a long-chain alcohol can induce an expected transition from lamellar phase to a reversed hexagonal.

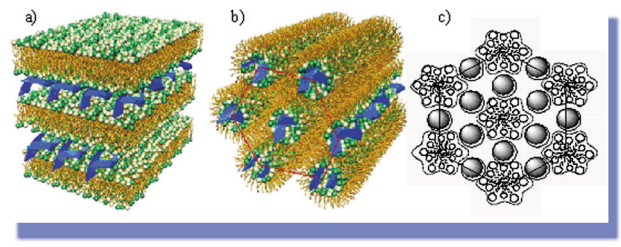

Fig 12. *Liquid crystalline phases for different lipid/surfactant-DNA complexes*[30-33].

INTERFACIAL DNA COMPACTION

Double helix DNA adsorbs to polar surfaces only if they have a net positive charge but not to negatively charged surfaces. Furthermore, it adsorbs weakly to hydrophobic surfaces due to its amphiphilic character; there it forms very extended structures characterized by a layer thickness of a few hundred Ångströms. As expected in view of the larger amphiphilicity, single stranded DNA adsorbs more extensively to hydrophobic surfaces. As is typical for a high molecular weight polymer, adsorption shows a marked irreversibility; on rinsing with water an adsorbed layer is unaffected both with respect to the adsorbed amount and thickness (**Fig. 13**).

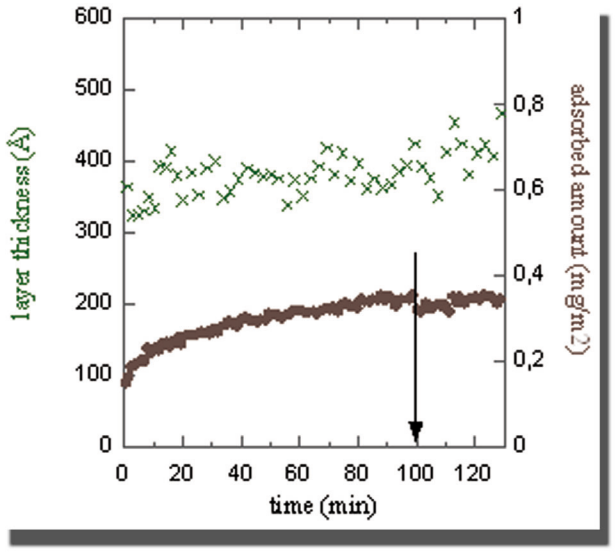

Fig 13. *Adsorbed amount and layer thickness for the adsorption of DNA on hydrophobized silica surfaces. The arrow indicates the point when rinsing with water was started*[34,35].

Addition of a cationic surfactant has a large effect on double helix DNA adsorption. For a hydrophilic surface it may or may not induce DNA adsorption depending on the concentration and the type of amphiphile used. On the other hand, effects on the adsorption of DNA upon cationic surfactant addition are quite dramatic for hydrophobic surfaces as exemplified in **Fig 14** for a short-chain cationic surfactant. The total adsorbed amount is strongly increased at the same time as the adsorbed DNA layer compacts to ca. 40-50 Å. The compaction is observed for different cationic surfactants and parallels that in bulk as observed by fluorescence microscopy and dynamic light scattering[26,34,36].

On rinsing with water, DNA invariably remains adsorbed while the surfactant may or may not be released into solution. For short-chain surfactants the diffusion-controlled desorption is fast enough for all surfactant to be removed; concomitantly, DNA decompacts and the adsorbed layer thickness essentially returns to the value obtained for DNA adsorption alone. For longer chain surfactants, desorption is much slower and an essential

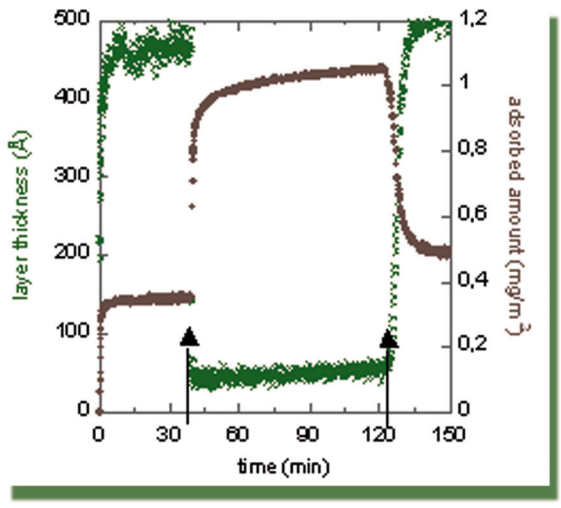

Fig. 14. *Adsorbed amount and layer thickness for the adsorption of DNA on hydrophobized silica surfaces. The arrow at t = 38 min indicates the point when the short chain cationic surfactant was added. The arrow at t = 125 min indicates the point when flushing with water was started*[34,37].

fraction remains at long times. DNA decompaction is then only partial. For a dimeric "gemini" surfactant, surfactant desorption is not observed and DNA remains in the fully compacted state[34,37].

An anionic surfactant added to a DNA-cationic surfactant layer may adsorb as a monolayer on top of that and or may associate to the cationic surfactant with subsequent partial DNA decompaction. After rinsing of the DNA-cationic surfactant layer, added anionic surfactant does not adsorb demonstrating that under these conditions the layer is negatively charged. By using cationic and anionic surfactant in combination, it is thus, as illustrated in **Fig. 15**, possible to fine-tune DNA compaction at surfaces.

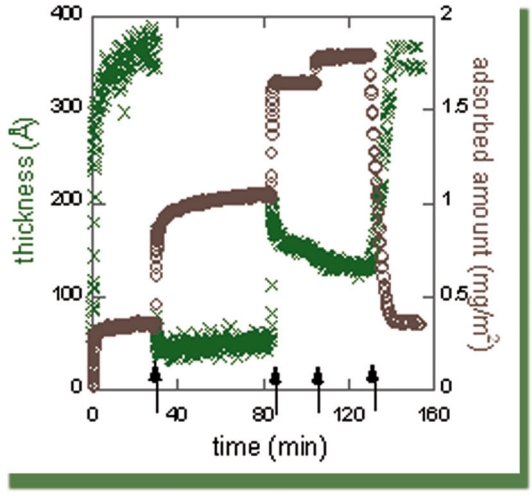

Fig 15. *Adsorbed amount and layer thickness for the adsorption of DNA on hydrophobized silica surfaces. The arrow at t = 30 min marks when the short chain cationic surfactant was added. The arrow at t = 85 min and 100 min indicates the point when anionic surfactant was added. The arrow at t =130 min marks the point when flushing with water was started*[34,37].

CONCLUSIONS

Mixed colloid systems receive a strongly increased interest in technical applications and, due to advances in theoretical and experimental possibilities, a renewed interest in life sciences; the area of colloidal biology is predicted to gain strongly significance[38].

After pointing out some basic aspects of surfactant and amphiphilic polymer self-assembly, we examined recent developments that emphasize similarities in association in bulk and at interfaces by reviewing some examples of the interfacial behaviour of mixed polymer-surfactant systems.

The manipulation of DNA has a wide range of applications as well as biological implications. It is well established that, in bulk, DNA can be compacted by many cationic co-solutes, polycations, polyamines, multivalent metal ions, and cationic surfactants and lipids. Here, we emphasize the behaviour of mixed systems of DNA and a cationic surfactant on solid surfaces. While DNA adsorbs itself on hydrophobic substrates in a very extended conformation, a cationic surfactant can strongly compact the adsorbed layer. In addition, we find that the adsorbed state of DNA can be fine-tuned by desorbing the cationic surfactant and by adding an anionic surfactant.

REFERENCES

1. Holmberg, K.; Jönsson, B.; Lindman, B. Surfactants and *Polymers in Aqueous Solution, 2nd Ed.*; John Wiley & Sons Ltd: West Sussex, 2003.

2. Evans, D.F.; Wennerström, H. The Colloidal Domain: *Where Physics, Chemistry, Biology and Technology Meet*; Wile-VCH: New Tork, 1999.

3. Atkin, R.; Craig, V.S..J.; Wanless, E.J.; Biggs, S. *Adv. Colloid Interf. Sci*. **2003**, *103*, 219-304.

4. Ingram, B.T.; Ottewill, R.H. In *Cationic Surfactants*; M.J. Schick, F. M. F., Ed.; Marcel Dekker, Inc: New York, 1991, pp 88-140.

5. Tiberg, F. PhD Thesis. *Physical Chemistry 1*; Lund University: Lund, 1994.

6. Alexandridis, P.; Lindman, B. Eds. *Amphiphilic Block Co-Polymers*, Elsevier, **2000**.

7. Alexandridis, P. *Curr. Opin. Colloid Interface Sci.* **1997**, 2, 478-498.

8. Alexandridis, P.; Olsson, U.; Lindman, B. *Polym. Prepr*. (*Am. Chem. Soc., Div. Polym. Chem.*) **1997**, *38*, 591-592.

9. Alexandridis, P.; Holmqvist, P.; Lindman, B. *Colloids and Surfaces A* **1997**, *129-130*, 3-21.

10. Karlson, L.; Nilsson, S.; Thuresson, K. *Colloid Polym. Sci*. **1999**, *277*, 798-804.

11. Karlson, L.; Joabsson, F.; Thuresson, K. *Carbohydrate Polymers* **2000**, *41*, 25-35.

12. Karlson, L. PhD Thesis *Physical Chemistry 1*; Lund University: Lund, 2002.

13. Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; W.H. Freeman and company: San Francisco, U.S.A., 1980.

14. Khan, M. O.; Mel'nikov, S. M.; Jönsson, B. *Macromolecules* **1999**, *32*, 8836-8840.

15. Gelbart, W.M.; Bruisma, R.F.; Pincus, P.A.; Parsegian, B.A. *Physics Today* **2000**, *53*, 38-45.

16. Tinland, B.; Pluen, A.; Sturm, J.; Weill, G. *Macromolecules* **1997**, *30*, 5763-5765.

17. Liu, H.; Gapinski, J.: Skibinska, L; Patkowski, A.; Pecora, R. J. *Chem. Phys*. **2000**, *113*, 6001-6010.

18. Dunlap, D.D.; Maggi, A.; Soria, M.R.; Monaco, L. *Nucleic Acids Research* **1997**, *25*, 3095-3101.

19. Lasic, D.D.; Templeton, N.S. Adv. *Drug Delivery Rev*. **1996**, *20*, 221-266.

20. Templeton, N. S.; Lasic, D.D. *Mol. Biotechn*. **1999**, *11*, 175-180.

21. Verma, I. M.; Somia, *N. Nature* **1997**, *389*, 239-242.

22. Khan, M. O.; Jönsson, B. *Biopolymers* **1999**, *49*, 121-125.

23. Khan, M. O. PhD Thesis. *Theoretical chemistry*; Lund Universiy: Lund, 2001.

24. Joabsson, F. PhD Thesis. *Physical Chemistry 1*; Lund University: Lund, 2001.

25. Joabsson, F.; L.indman, B. *Progr. Colloid Polym. Sci*. **2000**, *116*, 74-78.

26. Joabsson, F.; Thuresson, K.; Lindman, B. *Langmuir* **2001**, *17*, 1499-1505.

27. Mel'nikov, S. M.; Segeryev, V. G.; Yoshikawa, K. *J. Am. Chem. Soc*. **1995**, *117*, 2401-2408.

28. Dias, R.S.; Lindman, B.; Miguel, M. G. *J. Phys. Chem*. B **2002**, *106*, 12608-12612.

29. Dias, R.S. PhD Thesis. Universidade de Coimbra: Coimbra, 2003.

30. Rädler, J. O.; Koltover, I.; Salditt, T.; Safinya, C. R. *Science* **1997**, *275*, 810-814.

31. Zantl, R.; Baicu, L.; Artzner, F.; Sprenger, I.; Rapp, G.; Rädler, J.O. *J Phys. Chem. B* **1999**, *103*, 10300-10310.

32. Koltover, I.; Salditt, T.; Rädler, J. O.; Safinya, C. R. *Science* **1998**, *281*, 78-81.

33. Leal, C.; Wadsö, L.; Olofsson, G.; Miguel, M.; Wennerström, H. J *Phys. Chem. B* **2004**, *108*, 3044-3050.

34. Cárdenas, M. PhD Thesis. *Physical Chemistry 1*; Lund University: Lund, 2004.

35. Cárdenas, M.; Braem, A.; Nylander, T.; Lindman, B. *Langmuir* **2001**, *19*, 7712-7718.

36. Cárdenas, M.; Schillén, K.; Nylander, T.; Jansson, J.; Lindman, B. *Phys. Chem. Chem. Phys*. **2004**, *6*, 1603-1607.

37. Cárdenas, M.; Nylander, T.; Thomas, R.K.; Lindman, B., 2004. Manuscript.

38. Annual Report 2003, Physical Chemistry 1, Lund University. (www.fkem1.lu.se)