Chapter 7

Epilogue

This chapter summarizes and reviews the results obtained in the previous chapters, which describe aggregation processes in aqueous solutions, determined by an interplay of electrostatic and hydrophobic interactions.

7.1 Introduction

This chapter evaluates the investigations described in the previous chapters, which present studies of aggregation in aqueous solutions in which both hydrophobic and electrostatic interactions are important. For this purpose the interactions of apolar compounds modified with oppositely charged ionic moieties in aqueous solution have been studied. Intermolecular interactions have been studied as a function of the chemical architecture of both components involved in the aggregation process.

A combination of electrostatic and hydrophobic interactions favors many (bio)chemical aggregation processes. Enzyme-substrate interactions strongly rely on a combination of electrostatic and hydrophobic interactions. These interactions are also important in other fields of host-guest chemistry like the binding of substrates to cationic cyclophanes and cyclodextrines. Protein folding strongly relies on noncovalent interactions like hydrophobic, electrostatic, and hydrogen bonding interactions, which can be efficiently tuned by the α-amino acid sequence. Substantial stabilization of surfactant aggregates arises from interplay of electrostatic and hydrophobic interactions. Aggregation in aqueous mixtures of cationic and anionic surfactants occurs at concentrations, which are considerably lower than aggregation concentrations of the individual surfactants. Other examples of aggregation processes in aqueous solutions, in which both hydrophobic and electrostatic interactions play a role, include hydrolysis of a hydrophobic ester and amide by low concentrations of surfactants and interactions of surfactants and dyes.

Aggregation of apolar cationic and anionic compounds have in common the fact that aggregation takes place at concentrations far below the aggregation concentration of the individual components. However, little is known about factors governing such processes in aqueous solution. The present study was undertaken in order to gain insight into aggregation processes in aqueous solution that are influenced by both hydrophobic and electrostatic interactions. Such intermolecular interactions have been studied as a function of the molecular architecture of both components.

Chapters 2 and 3 dealt with interactions between cationic amphiphiles and anionic azo dyes in aqueous solution. In the work described in Chapter 4, the anionic component is an amphiphile as well. In addition, the anionic component contains a chromophoric unit.
Aggregation of n-alkyltrimethylammonium surfactants with a modified salicylate counter ion in aqueous solution is presented in Chapter 5. Finally, Chapter 6 reports a study of the aggregation of catanionic hydroptropes in aqueous solution.

The main observations include:

1. Aggregation in aqueous solution occurs at concentrations far below the critical aggregation concentrations of individual compounds.
2. The morphology of mixed aggregates usually differs from that of aggregates formed from the individual components.
3. A special type of dye aggregation is observed when the anionic component is a dye molecule in combination with a cationic surfactant.

Polyfunctional molecules are hydrated in a heterogeneous way because hydration of the individual polar and apolar moieties differs. Interference of the respective hydration spheres leads to mutual obstruction. Studies on the effects of solutes containing hydrophobic and hydrophilic groups on the kinetics of the hydrolysis of activated amides and esters show that the hydration spheres of ionic groups are less compatible with the hydrophobic hydration shells of neighboring methylene units than those of nonionic groups. This feature results from the stronger interactions of ionic groups with water molecules compared with nonionic groups. Consequently, the hydration sphere of ionic groups is more difficult to disrupt than that of nonionic groups. Thus, hydrophobic interactions between solute and probe molecules are hampered by the presence of polar groups.

The solubility of hydrophobic molecules is increased by polar groups. Overlap of hydrophilic and hydrophobic hydration spheres leads to a decreased “apparent” hydrophobicity of apolar groups. As a consequence, apolar compounds functionalized with polar moieties are more water-soluble than the corresponding solute lacking a polar functionality.

Aggregation of surfactants is an important example of a process in which hydrophobic interactions between the alkyl tails are hampered by the presence of polar groups. In the case of single-tailed surfactants, strong head group repulsions lead to the formation of spherical micelles and micellar growth is hampered.

Increased stabilization of hydrophobic aggregates can be achieved by turning the repulsive electrostatic head group interactions into attractive interactions. This property is demonstrated by studying interactions between apolar compounds containing oppositely charged ionic substituents in aqueous solution. The results will be discussed with respect to three different topics:

1. Aggregation of cationic surfactants and anionic azo dyes.
2. Aggregation of short-chained cationic and anionic compounds.
3. Aggregation of cationic surfactants with hydrophobic counter ions.
7.1.1  Aggregation of cationic surfactants and azo dyes

Aggregation of cationic surfactants and azo dyes in aqueous solution has been studied as a function of the molecular structure of both components. A chromophore in one of the components enables aggregation to be followed by spectroscopic techniques without the use of probe molecules. Depending on the molecular architecture of the interacting species aggregation occurs at concentrations far below the aggregation concentrations of the individual compounds. Figure 7.1 schematically depicts the relevant aggregation process. Aggregation is reflected by the appearance of a new absorption band in the spectrum of the dye. This band is ca. 80 nm blue shifted with respect to the position of the absorption band of the dye in aqueous solution (route a) and is attributed to aggregation of dye molecules in a parallel fashion. Thus, within mixed dye-surfactant aggregates formed at low concentrations in aqueous solution, a special type of dye aggregation is observed. Direct information on the type of aggregates formed at low concentrations (route a) could, unfortunately, not be obtained, but, there are strong indications that the dye molecules are arranged in a parallel fashion in these aggregates, the so-called H-type of aggregation. Typical for this type of aggregation is a blue shift of the main absorption band of the dye compared to that of the monomeric dye. Further research in necessary in order to determine the exact morphology of mixed surfactant-dye aggregates. X-ray diffraction on lamellar surfactant-dye dispersions or on crystals consisting of surfactants and dyes might give the desired information.

Route b (Figure 7.1) is followed if the structural requirements for efficient aggregation are not met and, consequently, a short wavelength absorption band in the spectrum of the dyes is not observed. In such cases, a gradual shift of the absorption band occurs from that of the dye in aqueous solution to that of the dye in micellar environment.

Interactions of surfactants and dyes depend on the alkyl tail length of the surfactants and on the type and position of the substituents in the aromatic ring of the dye. Interestingly, the type of surfactant head group is not important as long as it is cationic in origin. Aggregation at low surfactant concentration is absent with anionic and nonionic surfactants.

If a tight packing of surfactants and dyes is assumed in the mixed aggregates, it is likely that surfactants and dyes have to meet certain structural requirements for efficient formation of these types of H-aggregates. The separation of methyl orange dyes arranged in a parallel way has been calculated to be 0.5-0.7 nm. Indeed, interactions of surfactants with unsaturation in the tail with azo dyes are less efficient than interactions of the corresponding saturated surfactants with azo dyes. Depending on the conformation of the unsaturation and its position in the alkyl tail, mixed dye-surfactant aggregates (in which the dyes are oriented parallel with respect to each other) are indeed formed. However, the packing of surfactants and dyes in mixed aggregates is less efficient than that in the case of saturated surfactants. This feature of unsaturated surfactants is reflected by a blue shift of the absorption band of the dyes in mixed dye-surfactant aggregates. This band is at longer wavelengths than in the case of mixed dye-surfactant aggregates composed of surfactants with saturated alkyl tails.
and dyes. Most likely, hampering of the packing of dyes and surfactants in mixed dye-surfactant aggregates is responsible for this effect. Route b is followed when steric interactions are too large; in this case aggregation is impeded by the presence of a double bond in the alkyl tail of the surfactant.

**Figure 7.1** Schematic representations of different stages in the dye-surfactant aggregation process at a fixed dye concentration. Increasing the surfactant concentration may induce dye-aggregation (route a). Further addition of surfactant leads to solubilization of the dyes in surfactant micelles. Dye-surfactant aggregation is absent in pathway b. The relevant absorption spectra are shown at each stage of the aggregation process.

Dicationic surfactants like gemini and bolaform surfactants also interact with azo dyes at low concentrations in aqueous solutions. Although the spacer connecting both head
groups of gemini surfactants has a large influence on the aggregation of geminis, it has hardly any effect on intermolecular dye-surfactant aggregation. Also the bolaform surfactant and a dicationic surfactant lacking a second alkyl tail show aggregation with azo dyes in aqueous solution similar to that for single-tailed cationic surfactants. Hence, surfactants with saturated tails interact with azo dyes at low concentrations in aqueous solution as long as the head group is cationic.

The orientation of the ionic substituent in the first aromatic ring of an azo dye plays an important role in dye-surfactant aggregation. In addition, a dialkylamino substituent is necessary for aggregation. According to the results reported in Chapter 2, efficient aggregation only occurs when the dye contains a dialkylamino and an anionic substituent on the second and first aromatic ring, respectively, which are para with respect to the azo link. The anionic substituent is necessary for efficient attractive electrostatic interactions between dyes and cationic surfactants. Apparently, the increase in hydrophobicity of the dye brought about by the dialkylamino substituent is necessary for surfactant-dye aggregation. Aggregation of surfactants and dyes containing one aromatic ring in aqueous solution at low concentrations is also observed. Apparently, the hydrophobicity of 4-n-butylphenylazosulfonate (C4PAS) is large enough to interact with cationic surfactants at low concentrations in aqueous solution. Comparison of the aggregation of azo(benzene) surfactants and conventional surfactants revealed that the azobenzene unit corresponds to 8.4 CH₂ units whereas the azophenyl moiety corresponds to 6.5 CH₂ units. This pattern indeed indicates a larger “apparent hydrophobicity” of C₄PAS compared to azobenzene dyes.

Twisting the aromatic system out of planarity by substituents that are positioned ortho with respect to the azo linkage may also contribute to the reluctance of dyes to aggregate (route a, Fig. 7.1). Instead, route (b) is followed.

Various studies have shown that a wide range of azobenzene chromophores are able to aggregate in a parallel way as reflected by changes in the absorption spectrum of the chromophoric unit. Often, the chromophores are part of the same molecule and therefore they are already in close proximity. The latter is expected to facilitate chromophore interactions. Chromophore aggregation of negatively charged dye molecules can also be induced by addition of oppositely charged compounds, in agreement with other studies. The parallel orientation of chromophores was confirmed by X-ray diffraction experiments. Moreover, methyl orange (MO) molecules in Langmuir-Blodgett films composed of cationic surfactants and MO are oriented in a more or less parallel fashion and show a blue shift of the π→π* absorption band as well. A strong indication for dye aggregation was obtained by diluting an aqueous solution containing low concentrations of cationic surfactants and dyes. Upon dilution at a constant ratio of surfactant to dye the short wavelength absorption characteristic for dye (H) aggregates is replaced by the absorption band of the free dye in aqueous solution.
Clearly, efficient interaction of surfactants and dyes results from a combination of electrostatic and hydrophobic interactions. Interestingly, intermolecular aggregation occurs at concentrations where both components do not aggregate individually.

7.1.2 Aggregation of short chained cationic and anionic compounds

The compounds of choice contain a short alkyl tail connected to an ionic group. The compounds do not aggregate by themselves at the studied concentrations (< ca. 0.5 M). These short-chained compounds are hydroptropes, which aggregate in aqueous solution albeit in a less cooperative manner than surfactants. Moreover, loose aggregates with small aggregation numbers are formed.\textsuperscript{14} The hydrophobicity of methylene moieties attached to an ionic group is reduced due to destructive overlap of hydrophilic and hydrophobic hydration shells. For short-chain hydrophobic compounds (C \textless 6) with an ionic group, this leaves only 3 or less methylene moieties that have intact hydrophobic hydration shells. Hence, aggregation of the individual cationic or anionic components similar to that shown by surfactants is not observed. The apolar part is too short and (attractive) hydrophobic interactions cannot compensate the repulsive inter head group electrostatic interactions in the aggregation process. However, interaction of cationic and anionic short-chained hydrophobic compounds does occur at concentrations where the individual compounds show no sign of aggregation. Upon aggregation, the ionic hydration shells of the anionic and cationic groups destructively overlap with the release of a large number of the water molecules into the bulk. Another important factor contributing to the stability of the mixed cationic/anionic aggregates is that the smaller resulting polar hydration shell overlaps to a lesser extent with the methylene groups in both alkyl chains. In addition, the smaller (catanionic) hydration shell leads to a decrease of repulsions between hydration shells of ionic groups. Thus, whereas aggregation of apolar compounds containing ionic groups is hampered by electrostatic repulsions, aggregation of cationics is favored by attractive electrostatic interactions. The structure of the ionic group is important in determining the type of aggregate formed. If the interactions between both head groups are too strong, phase separation is observed whereas surfactant aggregates are formed (pseudo phase separation) when the inter head group interactions are weaker.

Whereas individual components are hydroptropes and do not aggregate cooperatively in aqueous solution, the cationic hydro trope combination acts like a surfactant in aqueous solution displaying cooperative aggregation behavior. Moreover, micelles are formed at concentrations where the individual hydroptropes do not aggregate.

7.1.3 Aggregation behavior of cationic surfactants with hydrophobic counter ions

Aggregation of ionic surfactants is strongly influenced by counter ions.\textsuperscript{15} Whereas n-alkyltrimethylammonium surfactants with halide counter ions aggregate into spherical micelles in aqueous solution, the aggregate morphology changes to wormlike micelles upon addition of counter ions, usually aromatics, which are more strongly bound.\textsuperscript{16} Both
electrostatic interactions between the oppositely charged ionic groups and hydrophobic interactions between the alkyl tails of surfactants and apolar portions of counter ions are responsible for the morphology change. This can also be explained using the packing parameter approach.\textsuperscript{17} The packing parameter relates the shape of the surfactant monomer to the shape of the aggregate formed by surfactants in aqueous solution. Adding oppositely charged counter ions to single-tailed ionic surfactants leads, in addition to a decrease in the effective head group area, to an increase of the volume of the surfactant alkyl tails. Consequently, the curvature of the aggregate formed in aqueous solution decreases. Upon changing the additive it should be possible to change the morphology of aggregates formed from single-tailed ionic surfactants from spherical micelles to wormlike micelles and eventually to vesicles. An increase in apolar character of the counter ion induces a further change in morphology of aggregates formed from single-tailed cationic surfactants to vesicles. This is somewhat surprising since the apolar part of the counter ion was only extended with 2 additional methylene groups with respect to salicylate, which forms wormlike micelles in combination with the same cationic surfactant.

Similarly, micelle-forming surfactants become vesicle-forming upon addition of dye counter ions. This observation has also been explained using the packing parameter approach.

Connecting an alkyl tail to the azobenzene unit of azo dyes leads to the formation of micelles in aqueous solution. Vesicles are formed in combination with cationic surfactants if the alkyl tails are longer than 6 carbons. The average vesicle size increases on decreasing the mismatch between the lengths of the alkyl tails of cationic and anionic surfactant. The results are in agreement with experimental and theoretical studies, which emphasize the importance of nonideal mixing of both components over the inner and outer bilayer leaflets. Micelles are formed in mixtures with short-chain surfactants ($C \leq 6$). Again, a subtle change in the structure of one of the components leads to significant morphology changes.

### 7.2 Hydrophobic and electrostatic interactions

In Chapter 1 different processes in aqueous solution were distinguished in which hydrophobic interactions play a major role. At concentrations far below the aggregation concentrations of the individual components, encounter complexes can be formed. Although the particles are individually hydrated under these conditions, they sometimes meet each other in aqueous solution. Short-lived clusters are then formed that have a longer lifetime than those in organic solvents. This clustering results from the more favorable partial desolvation that occurs upon cluster formation in aqueous solution than in an organic environment. The formation of encounter complexes is initially driven by pairwise hydrophobic interactions. Kinetic medium effects of inert solutes on hydrolysis reactions have been explained by encounter complex formation.\textsuperscript{18}

Bulk hydrophobic interactions are important in molecular assembly processes like surfactant aggregation. In these processes aggregation occurs when the mixing entropy is insufficient to overcome the unfavorable Gibbs energy of hydration of the apolar solute.
molecules. Host-guest complexation in water involves the binding of substrates to hosts like enzymes or macrocycles like cyclodextrines or cyclophanes. Although the complexes usually consist of a 1:1 ratio of substrate to host, many apolar residues are involved in the complexation. Therefore, host-guest complexation is thought to involve both bulk and pairwise hydrophobic interactions. In addition to hydrophobic interactions other noncovalent interactions like electrostatic and hydrogen bonding interactions are important in the aggregation processes. Often, an interplay of different interactions occurs. The stability of the folded state of proteins strongly relies on a combination of noncovalent interactions. Particularly the combination of different types of noncovalent interactions efficiently tunes the folding of proteins in aqueous solution. The studies described in this thesis address aggregation of cationic and anionic compounds in aqueous solution, which strongly rely on a combination of bulk hydrophobic interactions and attractive electrostatic interactions. The aggregation of amphiphilic molecules in aqueous solution can be viewed as pseudo-phase separation, which occurs above the solubility limit of the apolar solutes and only takes place when the mixing entropy of the solute is not sufficient anymore to compensate for the loss of entropy of the water molecules entering the hydration shells of the apolar solute molecules. A final phase-separated state is not reached due to the favorable surfactant head group-water interactions that prevent the formation of large aggregates, which would ultimately lead to phase separation. However, phase separation is observed when interactions between ionic groups are too strong. For example, precipitation occurs in aqueous mixtures of oppositely charged dyes and surfactants and also in mixtures of cationic and anionic hydrotropes. Thus, although electrostatic interactions strongly favor aggregation of amphiphilic molecules, phase separation is also facilitated by combined electrostatic and hydrophobic interactions.

Figure 7.2 schematically shows the destructive overlap of hydrophobic and hydrophilic hydration shells. This overlap results, for example, in deviations in additivity of the contributions of the first three methylene moieties in the alkyl tail of hydrophilic functionalized apolar solutes to the interactions with other apolar solutes. However, introducing oppositely charged ionic groups stabilizes hydrophobic aggregates. Electrostatic interactions strongly favor aggregation and destructive overlap of the ionic hydration shells leads to a release of hydration water from the hydrophilic hydration shell. The effect of stabilization of amphiphilic aggregates by hydrophobic and electrostatic interactions is schematically shown in Figure 7.2. The hydrophilic hydration shell of the catanionic complex is expected to be smaller than that of the individual components although its exact size in unknown as well as the extent to which it overlaps with the hydrophobic hydration shell of the complex.
Figure 7.2 Aggregation of amphiphilic molecules leads to hydration shell overlap, a schematic representation.

7.3 References


