Chapter 3

Aggregation Behavior of Surfactant-Dye Salts

Aggregation of \( n \)-alkyltrimethylammonium-methyl orange, ethyl orange, and para methyl red salts (C\(_n\)TA-MO, C\(_n\)TA-EO, and C\(_n\)TA-pMR, respectively) and of dicationic surfactant-methyl orange salts was studied using differential scanning calorimetry (DSC), optical microscopy, and transmission electron microscopy (TEM). Critical aggregation concentrations (cacs) of C\(_{10}\)TA-MO and C\(_{12}\)TA-pMR were determined using conductometry and drop tensiometry. The cacs are considerably lower than those of the corresponding bromides. C\(_n\)TA—azo dye salts form myelins and vesicles above the Krafft temperature (\( T_K \)). Similarly, dicationic surfactant-MO salts formed vesicles in aqueous solution. The effect of different dye counter ions on \( T_K \) of C\(_n\)TA-azo dye salts is discussed. Myelin formation of gemini surfactant-dye salts depends on the spacer length of the gemini. Aggregation behavior of dicationic surfactant-MO salts is compared to that of aqueous mixtures of dicationic surfactants and MO containing an equivalent of NaBr.

3.1 Introduction

Surfactant molecules self-associate in aqueous solution into a variety of morphologies of supramolecular assemblies like micelles, vesicles, emulsions, fibers, and liquid crystals depending on the design of the surfactant molecule and the specific conditions under which aggregates are formed.\(^1\) For example, aggregation of \( n \)-alkyltrimethylammonium surfactants in aqueous solution is strongly dependent on the type of counter ion: spherical micelles are formed in combination with halide counter ions whereas aromatic counter ions usually induce the formation of wormlike micelles.\(^2\) Upon addition of counter ions of increased hydrophobicity to aqueous solutions of C\(_{16}\)TAB the surfactant forms vesicles.\(^3,4\) The formation of vesicles in these mixtures occurs spontaneously, i.e. without the input of mechanical energy or elaborate chemical treatment. Usually, vesicle formation from double-tailed bilayer-forming lipids needs considerable energy input, for example sonication or extrusion.\(^5\) However, spontaneous vesicle formation from bilayer forming amphiphiles can be induced by, e.g., addition of strongly hydrated counter ions,\(^6\) changes in temperature,\(^7\) or pH.\(^8\) Catanionic surfactants also spontaneously associate into vesicles.\(^9\) Such surfactants are generally more soluble in aqueous solution than common bilayer-forming surfactants and they form micelles when separately dispersed in aqueous solution.

Gemini constitute a class of surfactants that comprise two hydrophilic head groups and two alkyl chains, which are linked by a spacer at the level of the head groups.\(^10,11\) Gemini surfactants are superior to conventional single-tailed surfactants in many properties:

\(^1\) The studies of the aggregation behavior of C\(_n\)TA-azo dye salts and of dicationic surfactant-2MO salts have been published in Langmuir 1999, 15, 1083 and in Langmuir 2001, 17, 1054, respectively.
they display lower cmcs, larger surface tension reduction, lower Krafft temperatures, and better solubility in aqueous solution. Moreover, they are superior to many conventional surfactants in oil solubilization. Alkanediyl-α,ω-bis(alkyldimethylammonium) bromides have been extensively studied and their aggregation behavior strongly depends on the length of the spacer. These surfactants are usually referred to as m-s-m where m and s are the number of carbon atoms in the alkyl tail and in the spacer, respectively. In the series 12-s-12 short spacers (s=2, 3) induce the formation of threadlike micelles, spheroidal micelles are formed when s=4-12 and vesicles are formed when s=16, 20. Aqueous solutions of 12-2-12 and 12-3-12 display the formation of entangled wormlike micelles resulting in viscoelastic solutions. Dimerization via short spacers leads to reduced curvature of the aggregates formed in aqueous solution when compared to the monomeric counterparts. Aggregation numbers of micelles formed from geminis with short spacers increase rapidly upon increasing surfactant concentration. Upon increasing s to s=4-8 the distance between the head groups increases to 0.6–1.1 nm, which is close to the average distance between head groups in spheroidal micelles. Therefore, these geminis display aggregation behavior similar to conventional micelle-forming surfactants.

Bolaform amphiphiles are related to geminis since they also consist of two hydrophilic head groups at both ends (α and ω) of a spacer. However, the spacer is longer than that in the case of gemini amphiphiles. Bolas are usually membrane-spanning when incorporated into bilayers. Surfactants are called bolas when the spacer is longer than twice the alkyl tail (s>2m). Like geminis, cmcs of bolas are lower than those of the monomeric surfactants of which they consist. Moreover, they display different aggregate morphologies like vesicles and rods when dispersed in aqueous solution. Geminis have only synthetically been prepared whereas bolaform amphiphiles are naturally occurring in archaebacteria where they protect the cells against the extreme circumstances under which they live.

Although the effect of counter ions on the aggregate morphology of n-alkyltrimethylammonium surfactants has been widely studied, morphology changes of aggregates formed from gemini and bolaform surfactants as induced by different counter ions have not been previously addressed in any detail. Moreover, the effect of large hydrophobic counter ions like azo dyes on the aggregation behavior of either conventional or non-conventional cationic surfactants has not been assessed. This chapter describes a study of the aggregation behavior of n-alkyltrimethylammonium-azo dye salts (CnTA-MO, CnTA-pMR, CnTA-EO where n is the number of carbon atoms in the alkyl chain). In addition, the aggregation behavior of dicationic surfactant-methyl orange salts was investigated. The surfactants are didodecyl-α,ω-bis(dimethylalkyl)ammonium 2MO (12-s-12 2MO, spacer length s=4, 8, 12), N,N’-(1,4-butanediyl)-bis-(4-decyl)-pyridinium 2MO (10p-4-p10 2MO), eicosane-1,20-bis(trimethylammonium) 2MO (C20Me6 2MO), and N-dodecyl-N,N,N’,N’,N’,-pentamethyl-N,N’-butanediylammonium 2MO (12-4 2MO) salts. Structures of surfactants and dyes are shown in Scheme 3.1.

Thus, whereas the previous chapter reported on interactions of surfactants and dyes at low concentrations (µM range) in aqueous solution, the present chapter describes a study of
the aggregation behavior of surfactants and dyes at high concentrations (mM range) in aqueous solution. Moreover, aggregation was studied at equimolar ratios of surfactants and dyes whereas previously interactions were investigated as a function of surfactant concentration at a constant dye concentration. Dye-surfactant salts were studied using differential scanning calorimetry (DSC) for determining Krafft temperatures ($T_K$). The formation of myelins was studied in so-called phase penetration experiments using optical microscopy.\textsuperscript{29} Cacs were determined using both surface tension experiments and conductometry. The morphology of aggregates formed from surfactant-dye salts in aqueous solution was investigated by negative staining transmission electron microscopy (TEM).

<table>
<thead>
<tr>
<th>surfactants</th>
<th>dyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_n H_{2n+1} N^+ (CH_3)_3$</td>
<td></td>
</tr>
<tr>
<td>$C_nTA^+, n = 10, 12, 14, 16, 18$</td>
<td></td>
</tr>
<tr>
<td>$nC_{12}H_{25}N' - (CH_3)_2 - (CH_2)<em>9 - (CH_2)<em>2 N^+' - nC</em>{12}H</em>{25}$</td>
<td></td>
</tr>
<tr>
<td>$12-s-12, s = 4, 8, 12$</td>
<td></td>
</tr>
<tr>
<td>$nC_{10}H_{21} - N^+ - (CH_2)<em>4 - N^+' - nC</em>{10}H_{21}$</td>
<td></td>
</tr>
<tr>
<td>$10p-4-p10$</td>
<td></td>
</tr>
<tr>
<td>$(CH_3)<em>2 N' (CH_2)</em>{10} N' (CH_3)_3$</td>
<td></td>
</tr>
<tr>
<td>$C_{20}Me_6$</td>
<td></td>
</tr>
<tr>
<td>$nC_{12}H_{25}N' (CH_2)_2 (CH_2)_4 - N' (CH_3)_3$</td>
<td></td>
</tr>
<tr>
<td>$12-4$</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Only in combination with MO.

$\Rightarrow C_nTA-MO$, $C_nTA-pMR$, $C_nTA-EO$

$12-s-12$ $2MO$, $10p-4-p10$ $2MO$, $C_{20}Me_6$ $2MO$, $12-4$ $2MO$

**Scheme 3.1** Structures of surfactant–dye salts
3.2 Chemical identification of surfactant-dye salts

Precipitation occurs in aqueous solutions of surfactants and dyes when mixed at surfactant concentrations below the surfactants’ cmc. Isolation of the precipitates and identification by $^1$H NMR spectroscopy revealed a 1:1 ratio of surfactant-to-dye for singly charged surfactant-dye salts and a 2:1 ratio of surfactant to dye for dicationic surfactant-dye salts. A 1:1 ratio of surfactant to dye for the C$_{16}$TA-MO salt was confirmed by elemental analysis (see experimental section). Precipitation of dye-surfactant salts in a 1:1 molar ratio has been observed before for aqueous solutions of cationic surfactants and anionic dyes as well as for solutions of anionic surfactants and cationic dyes.$^{24}$

Table 3.1 shows melting points of n-alkyltrimethylammonium–azo dye salts. Melting points decrease upon increasing the number of carbon atoms in the surfactant alkyl tail (n) although differences are not extremely large, particularly for the different C$_n$TA-MO salts. Depending on the type of surfactant, melting points of ionic surfactants either increase or decrease upon increasing the tail length.$^{31}$

<table>
<thead>
<tr>
<th>C$_n$TA-MO</th>
<th>mp/°C</th>
<th>C$_n$TA-pMR</th>
<th>mp/°C</th>
<th>C$_n$TA-EO</th>
<th>mp/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 10</td>
<td>237-239</td>
<td>n = 10</td>
<td>196-199</td>
<td>n = 10</td>
<td>215-217</td>
</tr>
<tr>
<td>n = 12</td>
<td>236-239</td>
<td>n = 12</td>
<td>188-195</td>
<td>n = 12</td>
<td>203-205</td>
</tr>
<tr>
<td>n = 14</td>
<td>230-232</td>
<td>n = 14</td>
<td>184-187</td>
<td>n = 14</td>
<td>188-191</td>
</tr>
<tr>
<td>n = 16</td>
<td>227-229</td>
<td>n = 16</td>
<td>165-169</td>
<td>n = 16</td>
<td>177-180</td>
</tr>
<tr>
<td>n = 18</td>
<td>220-222</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Precipitation of surfactant-dye salts also occurs in aqueous solutions of dicationic surfactants and dyes. In this case the crystals consist of a surfactant to dye ratio of 1:2. Table 3.2 presents melting points of dicationic surfactant-2MO salts.

<table>
<thead>
<tr>
<th>Surfactant-2MO</th>
<th>mp/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-4-12 2MO</td>
<td>254-257</td>
</tr>
<tr>
<td>12-8-12 2MO</td>
<td>188-193</td>
</tr>
<tr>
<td>12-12-12 2MO</td>
<td>208-210</td>
</tr>
<tr>
<td>10p-4-p10 2MO</td>
<td>189-194</td>
</tr>
<tr>
<td>C$_{20}$Me$_6$ 2MO</td>
<td>249-251</td>
</tr>
<tr>
<td>12-4 2MO</td>
<td>259-265</td>
</tr>
</tbody>
</table>
3.3 Optical microscopy and differential scanning calorimetry

A change of crystalline surfactant material to closed bilayer structures can be followed under an optical (polarizing) microscope using the phase penetration technique. In such an experiment, water is brought into contact with solid surfactant material and, upon heating, the formation of myelins at the boundary of the crystal/water interphase can be observed. Myelins are wormlike structures and have been proposed to consist of surfactant layers alternating with water layers that are wrapped around a core axis of water. Myelin formation can also be detected using DSC. The temperature of myelin formation \( T_{\text{myelin}} \) corresponds to an endothermic transition in a DSC enthalpogram. The solubility of surfactant-dye salts in aqueous solution shows a sudden increase when heated above \( T_{\text{myelin}} \). Therefore, myelin formation can be compared to the Krafft temperature phenomenon for micellar systems when \( T_{\text{myelin}} \) is defined as the temperature above which the solubility of micelle-forming surfactants shows a drastic increase. At \( T_{K} \), the solubility of the surfactant equals the cmc. In other words, the myelin temperature of bilayer-forming surfactants corresponds to the Krafft temperature \( (T_{K}) \) of micelle-forming surfactants.

3.3.1 \( n \)-Alkyltrimethylammonium–azo dye salts

Figure 3.1 shows myelins formed from \( C_{10} \)TA-MO as observed by optical microscopy. Temperatures at which myelins are formed can also be determined in DSC experiments since the transition corresponds to an endothermic heat effect that can be detected calorimetrically.

![Figure 3.1 Myelins formed from \( C_{10} \)TA-MO upon water penetration. \( T = 44^\circ C \), the bar represents 50 µm.](image-url)
Figure 3.2 shows an example of a DSC plot for C_{10}TA-MO. The endothermic transition upon heating corresponds to the Krafft temperature whereas the exothermic transition upon cooling corresponds to crystallization of the material. The transition corresponding to crystallization of the material always occurred at a temperature lower than the Krafft temperature and sometimes a transition in the cooling scan was not even observed. This pattern has been often found and depends on the crystallization behavior of the material. Table 3.3 shows the critical temperatures as determined by DSC (T_K) and optical microscopy (T_{myelin}) for surfactant-MO salts.

![Diagram of DSC plot](image)

**Figure 3.2** DSC plot of C_{10}TA-MO in aqueous solution.

Changes in Krafft temperature can often be explained by either a change in the solubility of the surfactant monomer or by a change in the stability of the solid state. Differences in monomer solubility have been suggested to be reflected by differences in enthalpy of melting or solvation whereas changes in stability of the solid state are reflected by differences in melting points. Unfortunately, the low solubility of surfactant-dye salts prevented the determination of enthalpies of solvation. Krafft temperatures of n-alkyltrimethylammonium-dye salts did not show a clear trend upon changing n. Apparently, both the surfactant solubility and the stability of the solid state contribute to the Krafft temperature and it is not a matter of one effect dominating the other. Figure 3.3 schematically shows the relation of the Krafft temperature and n of C_nTA-MO, C_nTA-pMR, and C_nTA-EO salts. With the exception of C_{10}TA-MO, Krafft temperatures of C_nTA-EO salts are lower than those of the corresponding MO salts. Since melting points of C_nTA-pMR salts...
are lower than those of the corresponding MO salts the differences can most likely be explained by a difference in the stability of the solid state of the surfactant-dye salts. Krafft temperatures of \( \text{C}_n \text{TA-EO} \) salts are lower than both those of \( \text{C}_n \text{TA-MO} \) and \( \text{C}_n \text{TA-pMR} \) salts. This effect cannot be attributed to differences in the stability of the solid state since melting points of \( \text{C}_n \text{TA-EO} \) are between those of \( \text{C}_n \text{TA-MO} \) and \( \text{C}_n \text{TA-pMR} \). Most likely, the differences in Krafft temperatures can be explained by increased solubility of \( \text{C}_n \text{TA-EO} \). This idea is supported by the fact that precipitation of \( \text{C}_n \text{TA-EO} \) in aqueous solutions of \( \text{C}_n \text{TAB} \) and \( \text{EO} \) as studied by UV-vis spectroscopy (see Chapter 2) did not occur whereas precipitation of \( \text{C}_n \text{TA-MO} \) and \( \text{C}_n \text{TA-pMR} \) under similar conditions occurred within several minutes.

### Table 3.3

<table>
<thead>
<tr>
<th>C(_n)TA-MO</th>
<th>C(_n)TA-pMR</th>
<th>C(_n)TA-EO</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>( T_{\text{myelin}} / ^\circ \text{C} )</td>
<td>( T_K / ^\circ \text{C} )</td>
</tr>
<tr>
<td>10</td>
<td>43</td>
<td>41.9</td>
</tr>
<tr>
<td>12</td>
<td>72</td>
<td>72.3</td>
</tr>
<tr>
<td>14</td>
<td>68</td>
<td>65.2</td>
</tr>
<tr>
<td>16</td>
<td>79</td>
<td>81.0</td>
</tr>
<tr>
<td>18</td>
<td>74</td>
<td>81.7</td>
</tr>
</tbody>
</table>

\( \text{a} \)No transition in the DSC enthalpogram.

Micelle-forming surfactants often display Krafft temperatures which are dependent on e.g. the type of counter ion\(^{29,30,31}\), the degree of unsaturation\(^{32}\), or branching\(^{30}\) of the alkyl chain. Also the size of the head group significantly influences the Krafft temperature.\(^{31,33}\) Krafft temperatures of ionic surfactants usually increase upon increasing alkyl chain length. This trend can be explained by differences in energy necessary to break down the crystal lattice when melting points increase with increasing surfactant alkyl tail length. On the other hand, when melting points decrease upon increasing \( n \) (which is the case with the studied surfactants), the effect of the change in solubility of ionic surfactants upon increasing \( n \) is apparently larger than that of the change in stability of the solid state.

The behavior of \( \text{C}_n \text{TA-EO} \) salts in phase penetration experiments is different from that of the corresponding MO and pMR salts. \( \text{C}_n \text{TA-EO} \) salts form myelins upon addition of water at room temperature but they start swelling at temperatures close to the Krafft temperature as detected by DSC. Figure 3.4 illustrates this behavior for \( \text{C}_{16} \text{TA-EO} \). Myelin figures are formed at 23\(^\circ \text{C} \) (Figure 3.4, left) and they undergo swelling at temperatures above \( T_K \) (Figure 3.4, right). A lamellar structure is present below and above the Krafft temperature. Similar effects have not been previously reported.
Figure 3.3 Schematic representation of Krafft temperatures of n-alkyltrimethylammonium-dye salts: (Δ) C_nTA-MO, (o) C_nTA-pMR, (×) C_nTA-EO. The Krafft temperature of C_{14}TA-EO is taken as the temperature at which myelins start to swell.

Figure 3.4 Myelins formed from C_{16}TA-EO at room temperature (left) and swollen myelins at 60°C. The bar represents 80 µm.
3.3.2 Dicationic surfactant 2MO salts

The formation of myelins formed from dicationic surfactant 2MO salts was also studied in phase penetration experiments using optical polarization spectroscopy. In addition, Krafft temperatures were determined in DSC experiments. Table 3.4 shows critical temperatures of dicationic surfactant 2MO salts as determined by DSC and optical microscopy. Critical temperatures obtained by both methods are in good agreement. The solution behavior of 12-s-12 2MO surfactants is dependent on the spacer length: the Krafft temperature increases upon decreasing s from 12 to 8, whereas 12-4-12 2MO does not dissolve at all. Moreover, 12-8-12 2MO forms myelins at $T_{\text{myelin}}$, whereas 12-12-12 2MO dissolves at $T_{\text{myelin}}$. Although the difference in $T_K$ for 12-8-12 2MO and 12-12-12 2MO is not large, it can be reproduced in DSC measurements. Most likely, the effect of differences in solubility of the surfactant dominates the effect of the stability of the solid state of 12-s-12 2MO salts. The absence of myelin formation of 12-4-12 2MO might be partly due to its high melting point and consequently a result of the stability of the solid state. Both the degree of counter ion binding and the nature of the counter ion have been shown to affect $T_{\text{myelin}}$. Krafft temperatures of di-n-alkylphosphates increase upon increasing the counter ion binding: $T_K$ for sodium di-n-alkylphosphates are higher than those of their corresponding potassium analogues reflecting a smaller counter ion binding for the latter. An increase in counter ion binding results in less hydrated head groups and this hampers the penetration of water into the crystal. Moreover, Ca$^{2+}$ binds so strongly that calcium di-n-dodecylphosphate does not form myelins upon water penetration. The degree of bromide counter ion binding to 12-s-12 surfactants decreases in the series $s=4, 8, 12$. Most likely, the MO counter ion binding will also follow this trend. This pattern explains the difference in $T_K$ for the 12-s-12 2MO surfactants: 12-12-12 2MO will show the lowest degree of counter ion binding and therefore it displays a lower $T_K$ than 12-8-12. Apparently the binding affinity of MO for 12-4-12 is so high that hydration does not take place and myelin formation is inhibited. This effect is probably enhanced by the relatively stable solid state formed by 12-4-12 2MO. Thus, the spacer length has a profound influence on the solution behavior of 12-s-12 2MO surfactants in an aqueous environment as observed by DSC and optical microscopy. Most likely, the absence of myelin formation for 10p-4-p10 2MO can also be explained by a high binding constant between MO and 10p-4-p10. No water can penetrate into the head group region and lyotropic mesophases are not formed. The formation of myelins was also observed for C$_{20}$Me$_8$ 2MO and for 12-4 2MO although $T_K$ was accompanied by only a weak transition in the DSC enthalpogram due to its low solubility in water.
Table 3.4 Krafft temperatures as determined by optical microscopy ($T_{\text{myelin}}$) and DSC ($T_K$) of dicationic surfactant 2MO salts.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$T_{\text{myelin}}$ / °C</th>
<th>$T_K$ / °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-4-12 2MO</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>12-8-12 2MO</td>
<td>86</td>
<td>87.6</td>
</tr>
<tr>
<td>12-12-12 2MO</td>
<td>86 c</td>
<td>85.6</td>
</tr>
<tr>
<td>10P-4-P10 2MO</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>C$_{20}$Me$_6$ 2MO</td>
<td>78</td>
<td>80.5</td>
</tr>
<tr>
<td>12-4 2MO</td>
<td>&lt;23</td>
<td>22.1 d</td>
</tr>
</tbody>
</table>

*aNo myelin formation at $T<100$°C. *bNo transition in the DSC enthalpogram from 0 – 100°C. *cCompound dissolves without the formation of myelins. *dWeak transition.

3.4 Critical aggregation concentrations

Cac’s of some of the surfactant-dye salts have been determined by conductivity and surface tension experiments. Figure 3.5 (left) shows a conductivity plot for C$_{10}$TA-MO. Experiments were performed at temperatures above the Krafft temperature of the surfactant, in the case of C$_{10}$TA-MO experiments were performed at 50° C. The cac of C$_{10}$TA-MO is 0.83 mM as indicated by the break in the conductivity plot. The aggregation concentration is dramatically lowered compared to that of C$_{10}$TAB, which forms micelles at a concentration of 60.2 mM. The degree of counter ion binding ($\beta$) as calculated from the slopes before and after the cvc in the plot of conductivity versus surfactant concentration is 91%. This high degree of counter ion binding implies the formation of aggregates in which both components are present in (almost) equal quantities. This formation is confirmed by analysis of bilayer material. A vesicular solution of C$_{10}$TA-MO was centrifuged in an ultra centrifuge at 50°C at 25000 rpm for 10 min. The bilayer material was collected and analyzed by $^1$H NMR spectroscopy that showed that the material consists of a 1:1 molar ratio of surfactant and dye. Efforts have been made in order to determine cac’s of other C$_n$TA-azo dye salts but these experiments unfortunately failed most likely due to solubility problems. The cac of C$_{12}$TA-pMR has been determined by conductometry and amounts to 1.55 mM. The counter ion binding $\beta$ equals 0.72. Again, the cac of C$_{12}$TA-pMR is much lower than that of the corresponding Br$^-$ salt, which has a cmc of 13.3 mM.34
The cac of C_{10}TA-MO in aqueous solution was also determined by drop tensiometry. Figure 3.5 (right) shows the surface tension plot of C_{10}TA-MO in aqueous solution at 50°C. The break in the plot indicates the cac and occurs at 0.83 mM, which is in perfect agreement with the result obtained from conductometry. The area occupied by the surfactant at the air-water interface as determined from the slope of the plot before the cac using the Gibbs isotherm for a 1:1 electrolyte is 79.9 Å^{2}.\textsuperscript{35} This corresponds to 39.9 Å^{2} per chain if both the surfactant and counter ion contribute to an equal amount to the area. Which is somewhat trivial of course. In a study on mixed monolayers of C_{16}TAB and MO at the CCl_{4}/water interface it was observed that MO occupies an area of 46 Å^{2} whereas the interaction product occupies an area of 89 Å^{2}.\textsuperscript{36} The areas of n-alkyltrimethylammonium surfactants with halides as the counter ions are 60–75 Å^{2},\textsuperscript{37} indicating a decrease in the area per chain when the counter ion of C_{10}TA^{+} is MO. The surface tension value at the cac (\gamma_{cac}) equals 41.0 mNm^{-1}.

Aggregation of surfactant-dye salts was also followed by UV-vis spectroscopy. Figure 3.6 shows UV-vis spectra of C_{10}TA-MO in aqueous solution at concentrations below and above the cac. Clearly, the short wavelength absorption band appears as a shoulder in the absorption spectrum of MO at concentrations above the cac as indicated by the arrow. Thus, the short wavelength absorption band is associated with the formation of vesicles. Higher surfactant concentrations could not be measured because of the high absorption of the solutions, even when using 1 mm cuvettes.
3.5 Electron microscopy

The morphology of aggregates formed from surfactant-dye salts was investigated using TEM. Surfactant solutions were prepared by addition of 0.5 mL of water to 2-3 mg of the surfactant-dye salts. The mixtures were vortexed and subsequently heated while stirring to above the respective Krafft temperatures.

3.5.1 n-Alkyltrimethylammonium-dye salts

Formation of myelins by surfactant-dye salts in phase penetration experiments is a strong indication that the studied surfactants will display vesicle formation in aqueous solution. To test this hypothesis, aggregates formed from these surfactants in aqueous solution were studied using TEM. Electron micrographs were obtained of a 5.2 mM aqueous solution of C\textsubscript{10}TA-MO (Figure 3.7 left) and of a 2.8 mM aqueous solution of C\textsubscript{12}TA-pMR (Figure 3.7 right). Vesicles with diameters of 400-1000 nm were observed. Vesicles formed from C\textsubscript{12}TA-MO, C\textsubscript{14}TA-MO, and C\textsubscript{16}TA-EO in aqueous solutions are of similar size. Vesicle solutions were stable for more than one week when kept above T\textsubscript{K} but readily flocculated upon cooling.

Figure 3.6 Absorption spectra of C\textsubscript{10}TA-MO in aqueous solution below and above the cac. [C\textsubscript{10}TA-MO]/mM: (1) 0.63, (2) 0.97; T = 50\degree C.
3.5.2 Dicationic surfactant-dye salts

Attempts were made to prepare aqueous solutions of dicationic surfactant-dye salts but suspensions were formed in each case due to their low solubility. When studied by TEM, the surfactant-dye salts were found to form vesicular structures in aqueous solution. Vesicles showed a rather large size distribution with diameters ranging from 25 nm – 1 µm. In addition, crystals of 200 nm – 1.5 µm in width and several microns in length were often observed. Crystals were not observed for 12-4 2MO by TEM but on visual inspection the solution contained crystals. Probably, the crystals were too large and flowed off the grid during the blotting process. Figures 3.8 shows types of aggregates as observed by TEM in aqueous solutions of C_{20}Me_{6} 2MO, 12-4-12 2MO, and 12-12-12 2MO, respectively. In a sample taken from an aqueous solution of C_{20}Me_{6} 2MO different types of aggregates were observed: spherical vesicles of 30 – 500 nm in diameter, long wormlike vesicular structures of 30 – 500 nm in diameter and several microns in length, and sheets of several microns in width and length (Figure 3.8, top left). Sometimes, the tubular vesicular structures were wrapped around spherical vesicles. Surprisingly, vesicles were also formed in an aqueous solution of 12-4-12 2MO (Figure 3.8, top right) and in an aqueous solution of 10p-4-p10 2MO. In addition, crystals were observed. Both compounds failed to show myelin formation. Also a transition in the DSC enthalpogram was absent for both compounds. Interestingly, vesicles were observed in an aqueous solution of 12-12-12 2MO although the electron micrographs were dominated by sheets and crystals (Figure 3.8, bottom left). Formation of myelins for 12-12-12 2MO was not observed in phase penetration experiments. Indeed, myelin formation is not an absolute prerequisite for vesicle formation. The dependence of the solution behavior of 12-s-12 2MO surfactants on s as observed in phase penetration experiments was not found.
for the aggregation behavior of these surfactants in dilute aqueous solution. Vesicle formation was observed for each of the 12-s-12 2MO salts in aqueous solution.

![Image of electron micrographs](image)

**Figure 3.8** Negatively stained electron micrographs of C$_{20}$Me$_6$ 2MO (top left), 12-4-12 2MO (top right), and 12-12-12 2MO (bottom left) in aqueous solution. The bar represents 500 nm.

### 3.5.3 Aqueous mixtures of dicationic surfactants and dyes

The morphology of aggregates formed in aqueous mixtures of dicationic surfactants and MO was also studied by TEM. In this case, the solutions were prepared by mixing aqueous solutions of surfactants and dyes in a 1:2 molar ratio. Note that the solutions contained an equimolar amount of NaBr. The surfactant concentration was 0.6 mM whereas the MO concentration was 1.2 mM. Aqueous solutions of surfactants and dyes were only stable for several hours after which precipitation occurred. However, aqueous solutions containing 12-4 and MO were stable for ca. 5 days. Samples taken from aqueous mixtures of 12-s-12 geminis and MO showed the formation of spherical vesicles and crystals. Both types of aggregates showed a rather large size distribution similar to that observed for surfactant-dye salts. An aqueous solution of 10p-4-p10 and MO contained vesicles ranging in diameter
from 300 nm – 1.5 µm. Aqueous mixtures of 12-4 and MO show spherical vesicles of 30 nm – 1 µm in diameter and wormlike vesicular structures of 50 – 100 nm in diameter and several microns in length. Figure 3.9 (left) shows a negatively stained electron micrograph of aggregates formed in an aqueous solution of 12-4 and MO in a mixing ratio of 1:2. Whereas an aqueous solution of C_{20}Me_{6} 2MO displayed different types of vesicular structures, spherical vesicles of 100 nm – 1 µm in diameter were observed in an aqueous mixture of C_{20}Me_{6} and MO (1:2). In addition, crystals of 500 nm – 1 µm in width and of several microns in length were identified in aqueous mixtures of the bolaform amphiphile and MO. Types of aggregates formed in aqueous solutions of surfactant-dye salts and their dimensions are similar to those formed in aqueous mixtures of surfactants and MO for 12-4-12 2MO and 12-8-12 2MO. Figure 3.9 (right) shows vesicles and crystals formed in an aqueous mixture of 12-8-12 and MO in a mixing ratio of 2:1. Vesicles and crystals were formed in aqueous solutions of 10p-4-p10 2MO and 12-4 2MO whereas only vesicular structures were present in aqueous mixtures of 10p-4-p10 and 12-4 and MO. However, 10p-4-p10 2MO crystallized a few hours after mixing whereas precipitation took several days in the case of an aqueous solution of 12-4 and MO.

**Figure 3.9** Negatively stained electron micrographs of aqueous mixtures of 12-4 and MO (left) and 12-8-12 and MO (right). The bar represents 1 µm. Surfactants and dyes were mixed in a 1:2 ratio.

### 3.6 Counter ion-induced morphology changes

The preferred morphology of the aggregate of the studied surfactants changed from micelles to vesicular structures in the presence of hydrophobic MO counter ions. Apparently, the shape of the surfactant molecules changed from conical for n-alkyltrimethylammonium bromides and the dicationic surfactants with bromide counter ions to cylindrical when the counter ion of n-alkyltrimethylammonium and the studied dicationic surfactants is MO. The
effective head group area decreased due to electrostatic interactions of cationic surfactant head groups and the anionic counter ions whereas the volume of the apolar part increased. This corresponds to an increase in $P$, which relates the shape of the surfactant to the morphology that the aggregate will adopt in aqueous solution. $P$ can be calculated using eq. 1.

$$P = \frac{V}{a_0 l}$$

$V$ is the volume of the hydrocarbon part of the surfactant, $l$ its alkyl chain length, and $a_0$ the mean cross-sectional head group surface area. Surfactants with $0<P<1/3$ form micelles in aqueous solution, $1/3<P<1/2$ indicates the formation of wormlike micelles whereas surfactants with $1/2<P<1$ display vesicle formation.

Counter ion-induced morphology changes for conventional surfactants from spherical to wormlike micelles are well known. For example, aromatics induce the formation of wormlike micelles from spherical micelles in aqueous solutions of n-alkyltrimethylammonium bromides. Morphology changes from spherical micelles to vesicles as induced by counter ions have also been studied, although less extensively. Addition of oppositely charged surfactants usually leads to the formation of bilayer structures. Therefore, it is tempting to describe C$_n$TA-azo dye and dicationic surfactant-2MO salts as catanionic surfactants. A characteristic of catanionic surfactants is the formation of vesicles in binary mixtures whereas micelles are formed in aqueous solutions of the separate surfactants. However, azo dyes are hydrotropes rather than surfactants since they lack surfactant properties like effective lowering of the surface tension and a well-defined critical aggregation concentration. Nevertheless, azo dyes like MO induce the formation of vesicles when mixed with oppositely charged surfactants in aqueous solution analogous to catanionic surfactants. However, we contend that the dye molecules should be seen as hydrophobic counter ions.

Aggregation of gemini and bolaform surfactants with hydrophobic counter ions has not been reported before. Moreover, this aggregation is one of the few examples of vesicle formation in aqueous solutions of micelle-forming gemini and bolaform surfactants. Formation of vesicles has been reported in aqueous solutions of an anionic gemini surfactant and hexadecyltrimethylammonium bromide (C$_{16}$TAB) and upon addition of hexanol to an aqueous solution of gemini surfactant 12-2-12. On the other hand, mixed micelles of gemini and monomeric surfactants have been extensively studied. Similar experiments on bolaform and non-bolaform amphiphiles have been reported.

3.7 Conclusions

Upon changing the counter ion from bromide to MO, EO, or pMR the morphology of aggregates formed by n-alkyltrimethylammonium surfactants and dicationic surfactants changes from micelles to vesicles. This change was indicated by phase penetration experiments showing myelin formation and by TEM, which revealed the formation of vesicles from surfactant-azo dye salts. Cacs of surfactant-dye salts as determined by
conductometry and surface tension measurements show a dramatic decrease when compared to the surfactants with halide counter ions. Krafft temperatures are sensitive to small changes in the surfactant tail length as well as to structural changes in the dyes although no clear relation between $T_K$ and surfactant alkyl tail length or dye structure was observed. Krafft temperatures of gemini-2MO salts decrease upon increasing the spacer length as a result of decreased counter ion binding upon increasing $s$.

3.8 Acknowledgment

Mr. Jan van Breemen is gratefully acknowledged for his help with performing electron microscopy experiments and for his help with the interpretation of the electron micrographs.

3.9 Experimental section

**General remarks.** $^1$H NMR spectra were measured at 200 or 300 MHz on a Varian Gemini-200 or a Varian VXR-300 spectrophotometer, respectively. Elemental analyses were performed by Jan Ebels, Harm Draaijer, and Jannes Hommes.

**UV-vis spectroscopy.** UV-vis absorption spectra were recorded as described in section 2.10.

**Optical microscopy.** Melting points were determined using either a Kofler hot-stage or a Mettler FP 2 melting point apparatus equipped with a Mettler FP 21 microscope. Phase penetration experiments were performed using an Olympus BX 60 polarization microscope equipped with a Linkam THMS 600 hot stage.

**Transmission Electron microscopy (TEM).** Transmission electron micrographs were obtained using a JEM 1200 EX electron microscope operating at 80 kV. Samples were prepared on carbon-coated collodion grids and stained with uranyl acetate (UAc) or phosphotungstic acid (PTA).

**Differential Scanning Calorimetry (DSC).** DSC measurements were performed on a Perkin Elmer DSC-7 apparatus using stainless steel pans. The reference cell contained an empty pan. Heating and cooling scans were run with scan rates of 3 Celsius min$^{-1}$.

**Surface tension experiments.** Critical aggregation concentrations (cacs) were determined by drop tensiometry using a Lauda TVT1 drop tensiometer. Details are given in Section 2.10.

**Conductivity experiments.** Critical aggregation concentrations as determined by conductivity were obtained using a Wayne-Kerr Autobalance Bridge B642 fitted with a Philips electrode PW 9512101 having a cell constant of 0.71 cm$^{-1}$. Solutions in the conductivity cell were stirred magnetically and thermostatted at the desired temperature. Concentrations were corrected for volume changes.
Surfactant-dye salts. Surfactant-dye salts were obtained by isolating precipitates formed in aqueous solutions of surfactants and dyes. C₅TA-azo dye salts were prepared by adding an equimolar amount of the aqueous dye solution to an aqueous surfactant solution. The final concentration of the surfactant was lower than the surfactants’ cmc. Solutions were left to stand for a few days after which the suspensions were centrifuged. The major part of the supernatant was pipetted off the fine precipitate. Crystalline surfactant-dye salts were obtained by suction. To prepare dicationic surfactant-2MO salts, typically 40 mL of a 1.2 mM aqueous solution of MO was added to 40 mL of an aqueous solution containing 0.6 mM of surfactant at 60°C. Solutions were cooled to room temperature and crystals were isolated by suction. Purities of surfactant-MO salts were checked using ¹H NMR spectroscopy. As indicated by this technique, the ratio of surfactant to dye was 1:1 for C₅TA-azo dye salts and 1:2 in the case of dicationic surfactants in the presence of MO, which was to be expected on the basis of the charges of surfactants and dyes.

C₅TA-dye salts. Melting points of C₅TA-dye salts are shown in Table 3.1. ¹H signals in NMR spectra of C₅TA-azo dye salts are similar within each series of surfactants with similar dyes as the counter ions but the integrated signal from the CH₂ groups of the surfactant alkyl chain differ. ¹H NMR data of one of the salts are given as an example for each series.

C₁₂TA-MO. ¹H NMR (300MHz, CDCl₃) δ 0.85 (t, 3H, CH₃), 1.22 (m, 18H, CH₂ alkyl chain), 1.64 (m, 2H, CH₂), 3.09 (s, 6H, N(CH₃)₂), 3.33 (s, 9H, N+(CH₃)₃), 3.40 (m, 2H, CH₂), 7.83 (d, 2H, CH ar), 7.85 (m, 4H, CH ar), 7.99 (d, 2H, CH ar).

C₁₈TA-pMR. ¹H NMR (300MHz, CDCl₃) δ 0.87 (t, 3H, CH₃), 1.22 (m, 30H, CH₂ alkyl chain), 1.64 (m, 2H, CH₂), 3.08 (s, 6H, N(CH₃)₂), 3.29 (m, 11H, N+(CH₃)₃ and N+CH₂), 6.74 (d, 2H, CH ar), 7.83 (m, 4H, CH ar), 8.17 (d, 2H, CH ar).

C₁⁴TA-EO. ¹H NMR (300MHz, CDCl₃) δ 0.87 (t, 3H, CH₃), 1.23 (m, 28H, CH₂ alkyl chain and CH₃ (EO)), 1.65 (m, 2H, CH₂), 3.32-3.48 (m, 15H, N(CH₂CH₃)₂, N+(CH₃)₃, N+CH₂), 6.71 (d, 2H, CH ar), 7.83 (m, 4H, CH ar), 7.97 (d, 2H, CH ar).

C₁₆TA-MO. The precipitate formed from a solution of C₁₆TAB and MO was analyzed. For the 1:1 adduct, C₃₃H₆₆N₄SO₃ (588.89), we find: calcd. C 67.31, H 9.58, N 9.51, S 5.44; found C 66.91, H 9.44, N 9.42, S 5.11.

12-s-12 2MO salts. Melting points of dicationic surfactant-2MO salts are shown in Table 3.2.

12-4-12 2MO. ¹H NMR (300MHz, DMSO, 50°C) δ 0.89 (t, 6H, CH₃), 1.31 (m, 36H, CH₂ alkyl chain), 1.72 (m, 4H, CH₂), 2.71 (s, 12H, N(CH₃)₂), 3.04 (s, 18H, N+(CH₃)₃), 3.28 (m, 4H, CH₂), 6.86 (d, 4H, CH ar), 7.68-7.84 (m, 12H, CH ar).
\(^1\)H NMR data for 12-8-12 2MO and 12-12-12 2MO are similar but differ in integration of the signal at 1.31 ppm which is larger due to the presence of additional CH\(_2\) groups in the spacer.

**10p-4-p10 2MO.** \(^1\)H NMR (300MHz, CD\(_3\)OD) \(\delta\) 0.77 (t, 6H, CH\(_3\)); 1.18 (m, 14H, CH\(_2\) alkyl tails); 1.57 (m, 4H, CH\(_2\)); 1.96 (m, 4H, CH\(_2\) spacer); 2.76 (t, 4H, CH\(_2\)); 2.99 (s, 12H, N(CH\(_3\))\(_2\)); 4.48 (m, 4H, N\(^{+}\)(CH\(_2\)))\(_n\)); 6.76 (d, 4H, CH ar); 7.68-7.81 (m, 14H, CH ar); 8.66 (d, 4H, CH ar).

**C\(_{20}\)Me\(_6\) 2MO.** \(^1\)H NMR (300MHz, DMSO, 50°C) \(\delta\) 1.31 (m, 32H, (CH\(_2\))\(_{16}\)); 1.73 (m, 4H, CH\(_2\)); 3.08 (s, 12H, N(CH\(_3\))\(_2\)); 3.24 (m, 22H, N\(^{+}\)(CH\(_3\))\(_3\) and N\(^{+}\)CH\(_2\)); 6.86 (d, 4H, ar); 7.74 (d, 8H, CH ar); 7.81 (d, 4H, CH ar).

**12-4 2MO.** \(^1\)H NMR (300MHz, CD\(_3\)OD) \(\delta\) 0.87 (3H, t, CH\(_3\)); 1.27 (m, 18H, CH\(_2\) alkyl chain); 1.72 (m, 2H, CH\(_2\)); 1.83 (m, 4H, CH\(_2\)); 3.22-3.46 (m, 4H, CH\(_2\)N\(^{+}\)); 3.06 (s, 6H, N(CH\(_3\))\(_2\)); 6.83 (d, 2H, CH ar); 7.84 (m, 4H, CH ar); 7.93 (d, 2H, CH ar).

### 3.10 References


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