Chapter 2

Interactions of Cationic Surfactants with Azo Dyes in Aqueous Solution

Aggregation of cationic surfactants and azo dyes in aqueous solution has been studied using UV-vis spectroscopy. Cationic surfactants having a single alkyl chain and a single head group as well as dicationic surfactants show interactions with MO in aqueous solution at concentrations far below the surfactants’ cmc. Unsaturation in the alkyl tail of a surfactant impedes aggregation. Aggregation is characterized by a new band in the absorption spectrum of a given dye, which is ca. 80 nm blue shifted with respect to the main absorption band of the dye in aqueous solution. An explanation for the short wavelength absorption band is advanced. The presence and position of the ionic group and the effect of structural variations of the dialkylamino substituent of an azo dye on the aggregation have been studied. In addition, interactions of cationic amphiphiles and an azo dye lacking the azobenzene unit have been investigated. The importance is discussed of both hydrophobic and electrostatic interactions in the binding process.

2.1 Introduction

Interactions of dye molecules and proteins have been extensively investigated since they mimic small molecule-macromolecule interactions, which are of major importance in biochemistry.1 Using dyes, aggregation can be followed spectrophotometrically. For example, the binding of methyl orange (MO, Scheme 2.1) to bovine serum albumin (BSA) has been investigated and the importance recognized of electrostatic interactions. Steric factors are also important in the binding process.2,3 In addition to proteins, interactions of MO with polymers and surfactants have been investigated. Charged4,5,6 as well as nonionic7,8,9 polymers bind MO, whereas cationic surfactants show interactions with the dye at concentrations below the cmc.10,11,12,13,14,15,16 Aggregation is reflected by a ca. 80 nm blue shift of the main absorption band of the dye. Interestingly, the largest spectral changes are observed at low surfactant and polymer concentrations. Upon increasing the polymer or surfactant concentration a shift is observed of the absorption band to longer wavelengths. Ultimately, the absorption spectrum of the dye resembles that of MO in organic solvents.

Dye aggregation was held responsible for the occurrence of the new band upon binding of MO to cationic polymers in aqueous solution.5,6 However, cis-trans isomerism12 of the dye

1 Our study of the interactions of C$_3$TAB and azo dyes in aqueous solution has been published in Langmuir 1999, 15, 1083, the study of the aggregation behavior of dicationic surfactants and MO has been published in Langmuir 2001, 17, 1054, and the study of the interactions of pyridinium surfactants with unsaturations in the alkyl chain and MO in aqueous solution has been accepted for publication in Langmuir.
and ion-pair formation of surfactant and dye\textsuperscript{13} have also been proposed as responsible for the spectral changes.

\begin{center}
\includegraphics[width=0.5\textwidth]{Scheme2.1.png}
\end{center}

\textbf{Scheme 2.1} Structure of Methyl Orange (MO).

This chapter reports a UV-vis spectroscopic study of the interactions of cationic surfactants and azo dyes in aqueous solutions. Effects of structural variations of both the surfactants and of the dyes have been studied. The effects of variation of surfactant tail length and structural variations of the head group of surfactants were studied using n-alkyltrimethylammonium bromides (C\textsubscript{n}TAB) and 1-methyl-4-n-alkylpyridinium iodides (C\textsubscript{mpyI}) (Scheme 2.2).

\begin{center}
\includegraphics[width=0.5\textwidth]{Scheme2.2.png}
\end{center}

\textbf{Scheme 2.2} Structures of C\textsubscript{n}TAB (left) and C\textsubscript{mpyI} (right).

The effect of pyridinium iodide surfactants with unsaturations in the alkyl tail (Scheme 2.3) on the aggregation behavior of MO was also studied. Aggregation of these surfactants in aqueous solution differs from that of the corresponding saturated compounds,\textsuperscript{17} which prompted a study the interactions of these surfactants with MO in aqueous solution.

\begin{center}
\includegraphics[width=0.5\textwidth]{Scheme2.3.png}
\end{center}

\textbf{Scheme 2.3} Structures of unsaturated pyridinium iodide amphiphiles and the saturated analogue.

In addition, the effect of non-conventional dicationic surfactants on the aggregation process was studied. Scheme 2.4 shows the structures of dicationic surfactants used in this study.
The selected dicationic surfactants are micelle forming. The morphology of aggregates formed from 12-s-12 gemini surfactants largely depends on the spacer length (s). In the case of 12-s-12 geminis, short spacers (s=2,3) induce the formation of wormlike micelles; geminis with intermediate spacers (s=4–12) form spheroidal micelles and vesicles are formed when s=16,20. The large influence of s on the aggregation behavior of gemini surfactants prompted a study of their aggregation with MO.

Bolaform surfactants are structurally related to geminis but the head groups of bolaform surfactants are connected by a long alkyl chain. The alkyl spacer of bolaform surfactants is partly stretched and partly bent in micelles.

Dicationic surfactant 12-4 is interesting because it is intermediate between a gemini surfactant and the conventional surfactant n-dodecyltrimethylammonium bromide.

In addition to the effects of different surfactants on the aggregation process the influences of structural variations have been investigated of the azo dyes on the interactions with cationic surfactants in aqueous solution. Scheme 2.5 shows the azo dyes used in this study. The effects of differences have been examined in the type and position of the ionic

### Scheme 2.4 Structures of dicationic surfactants.

### Scheme 2.5 Structures of azo dyes used in this study.
group as well as the effect of structural variations of the dialkylamino substituent on the interactions with cationic amphiphiles. In addition, the effects have been studied of dyes lacking an ionic group or the dialkylamino substituent on the aggregation process. In order to investigate the importance of the azobenzene skeleton on the aggregation process the effect was studied of n-alkyltrimethylammonium bromides on the absorption spectrum of 4-butylphenylazosulfonate (Scheme 2.6). The sulfonate group is directly attached to the azo unit in contrast to azobenzene dyes where the ionic group is attached to a second phenyl group. Longer chain analogs (C ≥ 6) have gained interest because of their photo destructible properties.20 These surfactants form micelles in aqueous solution but they lose their surface-active properties upon irradiation, which cleaves the surfactants into the corresponding phenols and hydrocarbons, which display little surface activity.

2.2 Interactions of single-tailed cationic amphiphiles with methyl orange in aqueous solution

Aggregation of C nTAB and MO in aqueous solution was followed by UV-vis spectroscopy. Figure 2.1 shows the effects of different concentrations of C 12TAB on the absorption spectrum of MO. Successive additions of small concentrations of surfactant decrease the intensity of the absorption band at 463 nm (curves 1→4). This band is characteristic of MO in aqueous solution. Further addition of surfactant produces a new band at ca. 380 nm (curves 3-5). The absorption spectrum shows the new band at a concentration of 4 mM of C 12TAB which is more than 3 times lower than its cmc (13.3 mM).21 The intensity of the new band first increases upon increasing the surfactant concentration (curves 3→5), and then decreases (curves 5→7) until another band at ca. 430 nm appears, characteristic of MO bound to cationic micelles.

Similar experiments using different n-alkyltrimethylammonium bromides have been performed. Figure 2.2 (left) shows the effect of C nTABs (n = 10, 12, 14, 16, 18) on the position of the wavelength of maximum absorption of MO in aqueous solution at a dye concentration of 25 µM. The effect of C 18TAB, C 16TAB, and C 14TAB is similar to that of C 12TAB: at concentrations considerably below the cmc of the surfactants a strong interaction occurs, which is reflected by the appearance of the short wavelength absorption band. Again, upon increasing surfactant concentration this short wavelength absorption band gradually disappears at the expense of the micellar band. These spectral changes are absent for C 10TAB. However, upon increasing the dye concentration to 50 µM the short wavelength absorption band appears in the absorption spectrum of MO. Table 2.1 compares the cmcs of C nTABs and the aggregation concentration of C nTAB and MO at a MO concentration of 25 µM.
Interactions of Cationic Surfactants and Azo Dyes in Aqueous Solution

Figure 2.1 Effect of C_{12}TAB on the absorption spectrum of MO in aqueous solution at 30°C, [MO] = 25 μM. [C_{12}TAB]/mM: (1) 0, (2) 3, (3) 4, (4) 6, (5) 8, (6) 10, (7) 11.5, (8) 20.

Figure 2.2 Left: effect of C_{n}TAB on the position of the wavelength of maximum absorption of MO: (◊) n = 10; (●) n = 12; (○) n = 14; (♦) n = 16; (∇) n = 18; right: effect of C_{m}pyI on the absorption spectrum of MO: (▼) m = 8; (◊) n = 10; (●) n = 12; (○) n = 14. Measurements were performed at 30°C except for C_{18}TAB, which was studied at 35°C. [MO] = 25 μM.
Comparable results were obtained from experiments using 4-n-alkyl-1-methylpyridinium iodide surfactants; Figure 2.2 (right). In aqueous solutions containing low concentrations of $C_{14}$pyI, $C_{12}$pyI, and $C_{10}$pyI aggregation with MO occurs whereas interactions are absent at low concentrations of $C_8$pyI. The length of the total apolar moiety of $C_8$pyI is comparable to that of $C_{10}$TAB, and in both cases aggregation at low surfactant concentration is absent (at a MO concentration of 25 µM). The dependence of aggregation on alkyl chain length of the surfactant strongly indicates that hydrophobic interactions are important in determining intermolecular interactions.

### Table 2.1 Aggregation concentration of $C_n$TAB with MO in 25 µM MO solution and critical micelle concentrations\(^a\) of $C_n$TAB.

<table>
<thead>
<tr>
<th>$C_n$TAB</th>
<th>cac ($C_n$TAB)$^a_{MO}$/mM</th>
<th>cmc ($C_n$TAB)/mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 12</td>
<td>2.09</td>
<td>13.3</td>
</tr>
<tr>
<td>n = 14</td>
<td>0.159</td>
<td>4.41</td>
</tr>
<tr>
<td>n = 16</td>
<td>0.024</td>
<td>1.0</td>
</tr>
<tr>
<td>n = 18</td>
<td>0.014</td>
<td>2.92</td>
</tr>
</tbody>
</table>

\(^a\)In an aqueous solution of 25 µM of MO.

The short wavelength absorption band also appears in absorption spectra of aqueous solutions of MO and dodecylamine hydrochloride (DAHCl), the latter differing from $C_{12}$TAB in its head group: ammonium instead of trimethylammonium. The short wavelength absorption band was recorded for DAHCl at a concentration of 0.4 mM, whereas in aqueous solutions of $C_{12}$TAB the band appeared at 4 mM. Lowering of the aggregation concentration in the case of DAHCl can be attributed to additional hydrogen bonding between surfactant N-H moieties and the sulfonate group of MO. The cac of DAHCl is 14.7 mM.\(^12\)

### 2.2.1 Introduction of unsaturations in the surfactant alkyl chain

Surfactant self-aggregation is strongly influenced by the introduction of unsaturation in the alkyl chain: cmcs show an increase when compared to the corresponding saturated ones.\(^17\) However, within the series of surfactants with unsaturations in the alkyl chain neither the position of the unsaturation nor its configuration seems to have a large effect on the cmc. Table 2.2 compares cmcs and degrees of counter ion binding ($\beta$) of unsaturated pyridinium amphiphiles and the corresponding saturated one as determined by conductometry. Structures of the amphiphiles are shown in Scheme 2.3 (p.2). Degrees of counter ion binding are of the same order of magnitude as in the case of other pyridinium iodide surfactants (with saturated alkyl tails).\(^22\)

Interactions of surfactants 1-5 with MO in aqueous solutions have been investigated using UV-vis spectroscopy. Aggregation of saturated surfactant (1) and MO is similar to that of other cationic saturated surfactants: aggregation of 1 and MO occurs at concentrations
well below the cmc of 1 and it is reflected by the appearance of the short wavelength absorption band which is ca. 80 nm blue shifted with respect to the absorption band of MO in aqueous solution. The absorption spectrum of MO shifts to that of the dye in the presence of cationic micelles when the concentration of 1 approaches its cmc. Surfactants 2 and 3, with the double bond at the end of the alkyl tail and at the 5-position (cis), respectively, largely impede the aggregation process. On the other hand, the short wavelength absorption band is observed upon addition of 4 (5-trans) to aqueous solutions of MO. The effects of surfactants 3 and 5 on absorption spectra of MO are shown as an example (Figure 2.3). The increase in absorbance around 360 nm in Figure 2.3 (left) is most likely due to absorption\textsuperscript{23} of I\textsuperscript{-} and/or due a charge-transfer absorption of the pyridinium head group.\textsuperscript{24}

<table>
<thead>
<tr>
<th>surfactant</th>
<th>cmc/M</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>9.3</td>
<td>0.77</td>
</tr>
<tr>
<td>3</td>
<td>11.6</td>
<td>0.78</td>
</tr>
<tr>
<td>4</td>
<td>9.2</td>
<td>0.80</td>
</tr>
<tr>
<td>5</td>
<td>7.9</td>
<td>0.82</td>
</tr>
</tbody>
</table>

**Table 2.2** Cmcs and degrees of counter ion binding (β) of unsaturated pyridinium amphiphiles

**Figure 2.3** Effect of surfactants with an unsaturated alkyl tail on the absorption spectrum of MO. Effect of 3 (left) and 5 (right) on the absorption spectrum of MO. [MO] = 25 μM, T = 30°C. Numbers correspond to surfactant concentrations in mM.
Introduction of an unsaturation in the alkyl tail of surfactants leads to shortening of the alkyl tail. In addition, a decrease in hydrophobicity of the unsaturated alkyl tail with respect to the saturated surfactant is usually assumed to occur. This pattern is for example reflected by the Rekker hydrophobic fragmental constants (Σf) for CH=CH and CH₂-CH₂ moieties, which are 0.63 and 1.475, respectively. The higher Σf, the more hydrophobic is the fragment. Another important difference between saturated and unsaturated alkyl tails is that unsaturation leads to a bend in the tail, which hampers aggregation of surfactants. Most likely, interplay of these three effects leads to a decrease in the packing efficiency in the hydrophobic core of dye-surfactant aggregates. Consequently, a short wavelength absorption band in the absorption spectrum of MO is absent upon addition of low concentrations of surfactant.

2.2.2 Changing the dye concentration

Figure 2.4 (left) shows the effect of changes in dye concentration on the interaction of C₁₆TAB and EO in aqueous solution. Upon increasing the EO concentration higher concentrations of surfactant are necessary to produce the short wavelength absorption band in the absorption spectrum of EO. Addition of ca. 30 µM of C₁₆TAB to an aqueous solution of 23 µM of EO is sufficient to produce this band whereas ca. 0.6 mM of C₁₆TAB is required when the dye concentration is 1.06 mM.

Figure 2.4 Left: effect of C₁₆TAB on the position of the wavelength of maximum absorption of EO in aqueous solution at 30°C, [EO]: (●) 23 µM, (○) 1.06 mM; right: vesicles formed in an aqueous solution of 0.95 mM of C₁₆TAB and 1.06 M of EO, bar represents 1 µm.

Aggregates formed at concentrations at which the absorption spectrum of EO (at a concentration of 1.06 mM) showed the short wavelength absorption band were investigated using TEM. Vesicles ranging in diameter from 20 nm to several micrometers in diameter are formed at this concentration. Figure 2.4 (right) shows that vesicles are formed in an aqueous
solution of 1.06 mM of EO and 0.95 mM of C16TAB. Vesicles at low EO concentration (25 µM) could not be detected by TEM.

2.3 Interactions of dication amphiphiles and methyl orange in aqueous solution

The effects of different dicationic surfactants (Scheme 2.4) on the absorption spectrum of MO have been investigated using UV-vis spectroscopy. Figure 2.5 shows the effect of different concentrations of eicosane-1,20-bis(trimethylammonium) dibromide (C20Me6) on the absorption spectrum of MO. Analogous to n-alkyltrimethylammonium bromide and 1-methyl-4-n-alkylpyridinium iodide surfactants,15 successive additions of C20Me6 decrease the intensity of the absorption band at 463 nm. Further addition of surfactant leads to the appearance of a new band at ca. 380 nm. Similar to the results obtained for n-alkyltrimethylammonium bromides and 1-methyl-4-n-alkylpyridinium iodides this new band first increases and then decreases in intensity upon increasing the surfactant concentration. Finally the band is replaced by the micellar band at ca. 430 nm.

Interestingly, the short wavelength absorption band is not observed when small amounts of C10TAB, which is “half” of the C20Me6 molecule, are added. In that case, λmax of MO gradually shifts from that in water to that of MO in the presence of cationic micelles (Figure 2.2). The cmc of C20Me646 is 7.5 mmol kg⁻¹ whereas C10TAB has a cmc of 60.2 mM.21 Similar to geminis, cacs of bolas are lower than those of the corresponding monomeric surfactants. The shifts are understood in terms of an entropy effect: upon transferring one
tail of a gemini surfactant molecule from the aqueous to the micellar phase, the second tail is already in a favorable position. This effect might both explain the lowering in cmc for bolaform surfactants compared to conventional ones and the fact that C_{20}Me_6 shows interactions with MO below the cmc whereas the interactions are absent in the case of C_{10}TAB.

Figure 2.6 shows the effect of 12-s-12, 10p-4-p10, C_{20}Me_6, and 12-4 on the wavelength of maximum absorption of MO. The surfactants all induce the short wavelength absorption band in the absorption spectrum of MO. For 12-s-12 geminis the surfactant concentration necessary for aggregation to occur decreases slightly with the spacer length: cac's of 12-s-12 and MO (at a MO concentration of 25 µM) are 7 µM, 5 µM, and 4 µM for 12-4-12, 12-8-12, and 12-12-12, respectively. The cmc decreases in the series 12-4-12 to 12-12-12 due to penetration of the spacer in between the alkyl chains. Therefore, the decrease in aggregation concentration of 12-s-12 geminis and MO upon increasing s reflects an increase in effective hydrophobicity of the surfactant in this series. Similar behavior is observed for 10p-4-p10 which shows again that a change in the type of head group has no influence on the aggregation process. Dicationic surfactant 12-4 also shows interactions with MO below its cmc (24.1 mM). Although the cmc of 12-4 is larger than that of the corresponding surfactant with a single ionic group (the cmc of C_{12}TAB is 13.3 mM), the structural variation does apparently not hamper the interactions with MO to a large extent.

![Figure 2.6](image-url) Effect of dicationic surfactants on the position of the wavelength of maximum absorption of MO: (■) 12-12-12, (▲) 12-8-12, (○) 12-4-12, (×) 10p-4-p10, (Δ) C_{20}Me_6, (♦) 12-4. Measurements were performed at 30°C except for 10p-4-p10, which was studied at 60°C. [MO] = 25 µM.
2.4 Interactions of n-alkyltrimethylammonium bromides and different azo dyes in aqueous solution

In addition to the effects of structural variations of the surfactants, the effects have been investigated of structural variations of the azo dyes on the aggregation process. The present section describes a study of the interactions of CₙTAB with different azo dyes. Results are discussed in each case in relation to MO. In Table 2.3 (p. 14) the positions are compared of absorption maxima of the dyes in different surroundings.

2.4.1 Changing the type and position of the ionic group

In order to examine the influence of variations in the type and position of the ionic group, the effects have been investigated of CₙTAB on the absorption spectrum of pMR and MR. Both dyes have a carboxylate ionic group which is positioned para with respect to the azo unit in the case of pMR and ortho in the case of MR. Aggregation of n-alkyltrimethylammonium bromides and pMR (Figure 2.7, left) is similar to that of n-alkyltrimethylammonium bromides and MO indicating that a change in the type of head group has little or no influence on the aggregation process.

![Figure 2.7](image)

**Figure 2.7** Effect of CₙTAB on the wavelength of maximum absorption of pMR (left) and MR (right) in aqueous solution at 30°C: (◊) n = 10; (●) n = 12; (〇) n = 14; (●) n = 16; (△) n = 18. [pMR] = [MR] = 25 µM. Measurements containing C₁₈TAB were performed at 35°C.

Although the MR molecule is fully conjugated its absorption maximum in aqueous solution is positioned at shorter wavelengths than in the case of pMR. Presumably, forcing the aromate system out of planarity results in a hypsochromic shift of the absorption band. The position of the absorption maximum of pMR gradually shifts from that in water to that in micellar solution upon addition of CₙTAB. Figure 2.7 (right) presents results of
experiments using MR. Geometric constraints probably prevent association of surfactant and dye. Our results are in agreement with a study on the interactions of bovine and human serum albumin with several azobenzene anions. The isomeric position rather than the nature of the anionic substituent is important for aggregation.2d

2.4.2 Effect of absence of an ionic group

The importance of the ionic group was tested using MY which lacks an ionic group. The absorption maximum of MY in aqueous solution is at 442 nm. The blue shift of the absorption maximum of MY compared to MO results from the absence of the SO₃⁻ moiety (the “pull” substituent is missing). MY does not show interactions with C₁₄TAB at low concentrations: its absorption maximum gradually shifts from that in aqueous solution to that in the presence of cationic micelles (ca. 417 nm). The band at 417 nm is characteristic for MY in a hydrocarbon-like environment since the main absorption band in ethanol is situated at 407 nm. Figure 2.8 shows the effect of C₁₄TAB and C₁₈TAB on the position of the wavelength of maximum absorption of MY.

![Figure 2.8](image)

**Figure 2.8** Effect of C₁₈TAB (▼) and C₁₄TAB (○) on the position of the wavelength of maximum absorption of MY.

2.4.3 Effect of structural variations of the dialkylamino substituent

The effect of an increase of the size of the dialkylamino substituent was studied using EO, which has a diethylamino substituent on the 4’ position whereas MO has a dimethylamino substituent. This increase leads to a shift in the wavelength of maximum absorption of the
dye in aqueous solution to longer wavelengths: 472 nm and 463 nm for EO and MO, respectively. The difference is attributed to an increase in electron donating ability of the dialkylamino substituent upon increasing the size of the alkyl groups. Figure 2.9 shows the effects of n-alkyltrimethylammonium bromides on the position of the wavelength of maximum absorption of EO. The results are similar to those obtained for MO since both dyes show the short wavelength absorption band in their absorption spectrum upon addition of small amounts of cationic surfactants. The short wavelength absorption band is positioned at longer wavelengths in the case of EO. Note that low concentrations of C_{10}TAB induce a short wavelength absorption band in the spectrum of EO whereas it is absent in the case of MO and pMR (at similar dye concentration). In the case of C_{10}TAB, the short wavelength absorption band in the spectrum of EO is at longer wavelengths than that observed with amphiphiles possessing a longer hydrocarbon tail. Again, increasing the EO concentration decreases the position of the short wavelength absorption band to 395 nm, similar to that observed for the other amphiphiles studied. These results are again fully reconcilable with the role of hydrophobic interactions in the aggregation process.

![Figure 2.9](image.png)

**Figure 2.9** Effect of C_{n}TAB on the wavelength of maximum absorption of EO: (◊) n = 10; (●) n = 12; (○) n = 14; (♦) n = 16; (∇) n = 18. Measurements containing C_{18}TAB were performed at 35°C. [EO] = 23 µM.

### 2.4.4 Effect of the absence of the dialkylamino substituent

The effect of the absence of the dialkylamino substituent in the dye on aggregation is addressed by studying the interactions between C_{n}TAB and ABS (Scheme 2.5, p. 3). The absorption maximum of ABS in aqueous solution is at 320 nm. The substantial
hypochoromatic shift compared to MO is attributed to the absence of the “pull” substituent. When dissolved in water the dye does not show a blue shift of the absorption maximum upon addition of small amounts of cationic surfactants. Rather, the main absorption band shows a ca. 5-nm red shift upon going from aqueous solution to a micellar environment. Thus, like an ionic group, the dialkylamino substituent is a prerequisite for efficient binding at low surfactant concentrations.

Table 2.3 Wavelengths of maximum absorption of the dyes in different media.

<table>
<thead>
<tr>
<th>dye</th>
<th>water $\lambda_{\text{max}}$/nm</th>
<th>micelles $\lambda_{\text{max}}$/nm</th>
<th>short wavelength absorption band/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO</td>
<td>463</td>
<td>431</td>
<td>ca. 380</td>
</tr>
<tr>
<td>EO</td>
<td>472</td>
<td>452</td>
<td>ca. 395</td>
</tr>
<tr>
<td>pMR</td>
<td>463</td>
<td>428</td>
<td>ca. 375</td>
</tr>
<tr>
<td>MR</td>
<td>429</td>
<td>416</td>
<td>---b</td>
</tr>
<tr>
<td>MY</td>
<td>440</td>
<td>420</td>
<td>---b</td>
</tr>
<tr>
<td>ABS</td>
<td>318</td>
<td>325</td>
<td>---b</td>
</tr>
</tbody>
</table>

*aStructures of azo dyes are shown in Scheme 2.5 (p.31). bNot observed.

2.5 Interactions of n-alkyltrimethylammonium bromides and 4-butyphenylazosulfonate in aqueous solution

4-Butylphenylazosulfonate (C4PAS) does not contain the azobenzene skeleton, rather the sulfonate ionic group is directly connected to the azo unit (Scheme 2.6). This results in a maximum in absorption of the dye at 307 nm in aqueous solution; its extinction coefficient is 1.2x10³ m² mol⁻¹ in aqueous solution. For comparison, the maximum absorption of (phenylazo)methane (in ethanol) is at 259 nm whereas for azobenzene it is at 319 nm in the same solvent. Figure 2.10 (left) shows the effect of C16TAB on the absorption spectrum of C4PAS. The absorption maximum of C4PAS in aqueous solution undergoes a blue shift of ca. 20 nm to 287 nm upon addition of small amounts of C16TAB and it shifts to 295 nm upon increasing the surfactant concentration above the cmc. The trend in the shifts of $\lambda_{\text{max}}$ upon addition of cationic surfactants is similar to that of MO, EO, and pMR: low concentrations of surfactants induce the largest spectral shifts and the wavelength of maximum absorption of the dye in micellar solution is between that of the absorption band in aqueous solution and in aqueous solution containing low surfactant concentrations. Figure 2.10 (right) shows the effect of n-alkyltrimethylammonium bromides (n = 12, 14, 16, 18) on the position of the main absorption band of C4PAS in aqueous solution. The studied surfactants all induce the absorption band at 287 nm at low surfactant concentrations. The position of this band shifts to longer wavelengths upon decreasing n: $\lambda_{\text{max}}$ of the short wavelength absorption band is 288 nm in the presence of C18TAB whereas it shifts to 292 nm in the presence of small
amounts of C$_{12}$TAB. This effect is attributed to a decrease in hydrophobic interactions in the mixed dye-surfactant aggregates upon shortening of the alkyl tail of the surfactant.

Figure 2.10 Left: effect of C$_{16}$TAB on the absorption spectrum of C$_{4}$PAS. [C$_{16}$TAB]: (1) 0, (2) 20 µM, (3) 60 µM, (4) 200 µM, (5) 9.5 mM. Right: effect of C$_{n}$TAB on the position of the absorption maximum of C$_{4}$PAS: (◊) n = 10; (●) n = 12; (○) n = 14; (▲) n = 16; (∇) n = 18.

Thus, the lack of a phenyl group in C$_{4}$PAS does not impede the aggregation of surfactants and dyes at low concentrations in aqueous solution. However, the shift of the wavelength of maximum absorption of the dye upon aggregation with small amounts of cationic surfactants is much smaller than that of MO, EO, and pMR.

2.6 Effect of ionic strength on the aggregation process

Aggregation of surfactants and dyes is strongly influenced by the ionic strength. Figure 2.11 shows the effect C$_{16}$TAB on the absorption maximum of MO in the presence of different concentrations of sodium chloride. The short wavelength absorption band appears at lower surfactant concentrations upon increasing the NaCl concentration, but it is almost diminished upon further increasing the ionic strength. The results can be interpreted in terms of increased counter ion binding to the mixed surfactant-dye aggregates responsible for the short wavelength absorption band. Assuming that dye aggregation is responsible for the short wavelength absorption band (this feature will be discussed in the next section), the paired dye interactions are largely eliminated at higher NaCl concentrations. The sensitivity to NaCl reflects the importance of electrostatic interactions in the aggregation process.\textsuperscript{31,32} This is corroborated by the fact that interactions between anionic surfactants (e.g. SDS) and MO are absent\textsuperscript{11} whereas cationic surfactants with comparable alkyl chain length (e.g. C$_{12}$TAB) induce changes in the absorption spectrum of MO.
Figure 2.11 Effect of NaCl on the interactions of C_{16}TAB and MO in aqueous solution, [NaCl]: (□) 0.01 M, (▲) 0.1 M, (●) 0.7 M.

2.7 Origin of the short wavelength absorption band

Although a short wavelength absorption band in the absorption spectrum of MO has been observed in numerous studies its origin is still debated.\textsuperscript{5,6,10,11,12,13,14,15} Explanations that account for the blue shifted absorption band include cis-trans isomerism\textsuperscript{12} of the azo dye, surfactant-dye ion-pair formation,\textsuperscript{13} and dye aggregation.\textsuperscript{5,6} A conformational change of the dye could be ruled out on the basis of resonance Raman spectroscopy which showed that the dye retains its trans configuration upon interaction with surfactants and proteins.\textsuperscript{33} An explanation of the short wavelength absorption band in terms of ion-pair formation seems unlikely since it is not clear why ion-pairs would only form at low surfactant concentration. Most likely, the short wavelength absorption band is caused by dye aggregation although there is no direct information on the structure of surfactant-dye aggregates formed at low surfactant concentration. Dye aggregation has been proposed to be responsible for the short wavelength absorption band of MO in the presence of surfactants\textsuperscript{10,15} and polymers.\textsuperscript{4,5,6} A hypsochromic shift in the absorption spectrum of dendrimers derivatized with azobenzene moieties has also been ascribed to interactions between the chromophores.\textsuperscript{34} Similarly, the hypsochromic shift of $\lambda_{\text{max}}$ upon aggregation of azobenzene-based surfactants has been attributed to aggregation of the chromophoric units.\textsuperscript{35} Moreover, parallel orientation of azobenzene units in cast films of azobenzene-containing amphiphiles has been verified by X-ray diffraction experiments.\textsuperscript{36} Cast films have a pseudo-crystalline nature of molecular
ordering and mechanical flexibility like polymer films. The spectral properties of immobilized bilayer films are similar to those of the surfactants in aggregates in aqueous solution. 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 $\pi \rightarrow \pi^*$ absorption band as well.37

Figure 2.12 shows possible orientations of surfactants and dyes in mixed surfactant-dye aggregates. In both cases charge neutralization occurs. Orientation A implies that the core of the mixed aggregates consists of surfactant alkyl tails whereas the dyes are considered as the counter ions. Although the dyes are in close proximity, dye aggregation in the sense of exciton formation is unlikely since formation requires close packing of dyes, which are present in the diffuse Stern layer. Moreover, the size of the molecules in the Stern layer is similar to that of those in the core of the aggregate. Usually, apolar molecules are directed inward the aggregate. This pattern is represented in orientation B. The dyes are also in close proximity and oriented parallel with respect to each other. Dye aggregates are unlikely to consist of more than two or three dye monomers because of the creation of regions of negative and positive charge which is unfavorable. Measurements on monolayer behavior of C$_{16}$TAB and MO at the water CCl$_4$ interface suggest that both components equally populate the interface.38

![Figure 2.12](image)

**Figure 2.12** Possible orientations of surfactants and dyes in surfactant-dye aggregates.

In a study on the interactions of MO and cationic poly(3-methyl-1-vinylimidazolium) methosulfate (PMVI) the interaction energy ($\Delta E = E_m - E' = E'' - E_m$; Scheme 1.8) for dyes in parallel orientation was estimated from the spectral shift of the 370 nm band relative to the monomer band.10 The main absorption band of MO in aqueous solution consists of two unresolved bands centered at 476 nm (21000 cm$^{-1}$ which corresponds to 251 kJ mol$^{-1}$) and 417 nm (24000 cm$^{-1}$, corresponds to 287 kJ mol$^{-1}$). Interaction energies of 6000 cm$^{-1}$ (72 kJ mol$^{-1}$) and 3000 cm$^{-1}$ (36 kJ mol$^{-1}$) are calculated depending on which monomer band is taken as the
These energies correspond to separations of 0.5–0.7 nm for dyes of unit oscillator strength in parallel orientation as calculated from the molecular exciton model.\textsuperscript{39} It was argued that a structure in which MO are aggregated onto PMVI was unlikely to exceed dimerization.\textsuperscript{10} Molecular models indicate that higher-order dye aggregates would introduce too much strain. The distance between next-neighbor imidazolium groups is approximately 0.5 nm, which would agree perfectly with the separation calculated from spectral shifts using the molecular exciton theory.\textsuperscript{10}

The idea of dye aggregation is supported by the fact that the short wavelength absorption band disappears upon dilution of an aqueous solution of 0.31 mM of C\textsubscript{12}TAB and 25 µM of MO. Figure 2.13 shows normalized absorption spectra of EO in an aqueous solution of C\textsubscript{12}TAB. Upon decreasing both the dye and surfactant concentration at a constant ratio the short wavelength absorption band is being replaced by the absorption band of EO in aqueous solution (curves 1\textendash}4) indicating that aggregation of dye molecules indeed occurs.

The importance of hydrophobic interactions is supported by the fact that interactions between MO and C\textsubscript{12}TAB are absent in ethanolic solutions. Solvophobic interactions between solute molecules are weak in nonaqueous solvents like dimethylformamide, toluene and ethanol,\textsuperscript{40} although recently vesicle formation has been reported for ethanol-water solutions and even for pure ethanol.\textsuperscript{41} On the other hand, electrostatic interactions in ethanol are stronger than those in water on the basis of the relative permittivities of both solvents (24.3 for ethanol and 78.5 for water),\textsuperscript{42} but apparently no aggregation results from this effect. Moreover, the wavelength of maximum absorption of MO in water only slightly increases upon addition of tetramethylammonium bromide (TMAB). A red shift of 4 nm was observed upon addition of 3.1 M of TMAB. This again confirms that hydrophobic interactions are important for aggregation.

2.8 Conclusions

Interactions of cationic amphiphiles and azo dyes as studied by UV-vis spectroscopy occur at low concentrations in aqueous solution. Aggregation is reflected by a ca. 80 nm hypsochromic shift of the main absorption band. Dye aggregation is responsible for the short wavelength absorption band. Upon increasing the surfactant concentration dilution of

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2_13.png}
\caption{Effect of dilution on the short wavelength absorption band. [C\textsubscript{12}TAB] (mM) and [EO] (M): (1) 5 mM, 25 µM, (2) 2.4 mM, 12 µM (3) 1.7 mM, 8.6 µM (4) 0.26 mM, 1.3 µM.}
\end{figure}
Interactions of Cationic Surfactants and Azo Dyes in Aqueous Solution

the dye molecules occurs and the absorption spectrum shifts to that of the dye in cationic micelles. The importance of hydrophobic interactions is revealed by the dependence of aggregation on the surfactant alkyl tail length and on the size of the alkyl substituents on the dye nitrogen. Moreover, interactions are absent in ethanolic solutions. The importance of electrostatic interactions was revealed by the effect of NaCl on the aggregation process.

2.9 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.

2.10 Experimental section

General remarks. C₈TAB (Acros), C₁₀TAB (Lancaster), C₁₂TAB (Sigma), C₁₄TAB (Aldrich), C₁₆TAB (Merck), and C₁₈TAB (Fluka) were dried in vacuo before use. Tetramethylammonium bromide (Acros) was recrystallized from 80% methanol. Dodecylamine hydrochloride (DAHCl) was prepared by dissolving dodecylamine (Aldrich) in water and adding one equivalent of HCl. The pH of DAHCl solutions was adjusted to 6. The purity of all surfactants was checked by ¹H-NMR spectroscopy. Column chromatography was performed using neutral Al₂O₃ (activity III), which was prepared by addition of 4.9 mL of water to 100 g of neutral Al₂O₃. C₈pyI, C₁₀pyI, C₁₂pyI, and C₁₄pyI were synthesized according to a literature procedure. Methyl orange (MO), ethyl orange (EO), methyl yellow (MY), and para-methyl red (pMR) were obtained from Acros Organics. Azobenzene was obtained from Aldrich. MO was crystallized from doubly distilled water. EO was dried in vacuo during one night in order to remove traces of ethanol present in the commercial product. Water was demineralized and distilled twice in an all-quartz distillation unit. ¹H NMR spectra were measured at 200 or 300 MHz on a Varian Gemini-200 or a Varian VXR-300 spectrophotometer, respectively. Melting points were determined on a Kofler hot-stage or a Mettler FP 2 melting point apparatus equipped with a Mettler FP 21 microscope. Elemental analyses were performed in the analytical department of our laboratory by Mr. Jan Ebels, Mr. Harm Draaijer, and Mr. Jannes Hommes.

Alkanediyl-α,ω-bis (dimethyl dodecylammonium bromide) surfactants (12-s-12, s = 4, 8, 12). These surfactants were synthesized by a modified literature procedure. N-dodecyl-N,N-dimethy lamine and the appropriate α,ω-dibromoalkane were refluxed in ethanol in a 4:1 ratio for 2 days (n = 4) or 5 days (n = 8, 12). The concentration in α,ω-dibromoalkane was ca. 0.3 M. The reaction was followed by ¹H NMR spectroscopy and was stopped when complete conversion of α,ω-dibromoalkane had occurred. After evaporation of the solvent, the product was purified by column chromatography. Excess N-dodecyl-N,N-dimethy lamine was eluted with CH₂Cl₂. Subsequent elution with CH₂Cl₂/10% MeOH gave
both monoalkylated product and the desired gemini amphiphile. Surfactant fractions, which contained monoalkylated product, were recrystallized from CH₃CN. Gemini surfactants 12-s-12 were obtained as white solids in 70–75 % yield. The surfactants were pure as determined by ¹H NMR spectroscopy. 12-4-12, mp 225-228°C (dec.), lit. 215°C (dec); 12-8-12, mp 192-195°C, lit. 206-208°C; 12-12-12, mp 129-134°C, lit. 137-138°C. Melting points differ from literature values although those values show mutual differences themselves. Most likely, the differences can be attributed to the presence of traces of water in the gemini surfactants. The purity of gemini 12-12-12 was checked by elemental analysis. Anal. Calcd. C₄₀H₈₆N₂Br₂ (754.94): C 63.64; H 11.48; N 3.71; Br 21.17. Found: C 63.50; H 11.55; N 3.85; Br 21.11.

Eicosane-1,20-bis(trimethylammonium) dibromide (C₂₀Me₆). Bola amphiphile C₂₀Me₆ was synthesized according to a literature procedure. In short, 300 mg (0.68 mmol) of 1,20-dibromoeicosane was added to 6 mL of a 33% ethanolic solution of dimethylamine. The solution was refluxed for 48 h after which the reaction was complete as indicated by ¹H NMR spectroscopy. The reaction mixture was cooled to room temperature and after addition of 15 mL of diethyl ether, precipitation of C₂₀Me₆ occurred. The product was isolated by suction as white crystals in 93% yield (350 mg; 0.63 mmol). Bola amphiphile C₂₀Me₆ was pure as determined by ¹H NMR spectroscopy. Mp >230°C.

N,N'-(1,4-butanediyl)-bis-(4-decyl)-pyridinium dibromide (10p-4-p₁₀). A solution of 1.99 g (9.1 mmol) of 4-decylpyridine and 0.66 g (3.1 mmol) of 1,4-dibromobutane in 7 mL of abs. ethanol was refluxed for 24 h under a nitrogen atmosphere. After evaporation of the solvent, the reaction mixture was purified by column chromatography. Excess pyridine was removed by CH₂Cl₂, the product was eluted with CH₂Cl₂/10% MeOH. The product was recrystallized from CH₃CN in order to remove monoalkylated compound. White crystalline 10p-4-p₁₀ was obtained after ion exchange using a Dowex 1×8 200-400 mesh column in 66% yield (1.34 g, 2.04 mmol). Mp 206-208°C (dec). ¹H NMR (200 MHz, CDCl₃) δ 0.85 (t, 6H, CH₃), 1.24-1.28 (m, 28H CH₂ alkyl tails), 1.65 (m, 4H, β-CH₂), 2.42 (m, 4H, CH₂ spacer), 2.82 (t, 4H, α-CH₂), 5.09 (m, 4H, N+-CH₂), 7.74 (d, 4H, CH ar), 9.67 (d, 4H, CH ar). ¹³C NMR (200 MHz, CDCl₃) δ 12.59 (p), 21.13, 26.44, 27.57, 27.68, 27.72, 27.85, 27.97, 28.03, 30.31, 34.41 (s), 57.86 (s), 126.34 (t), 143.20 (t), 161.61 (q). Anal. Calcd. C₃₄H₅₈N₂Br₂ (654.66): C 62.38; H 8.93; N 4.28. Found C 62.30; H 8.82; N 4.43; Br 24.24.

N-dodecyl-N,N,N',N',N',-pentamethyl-N,N'-butanediylammonium dibromide (12-4). A solution of 2.1 g (10.8 mmol) of N-(4-bromobutyl)-N,N,N-trimethylammonium bromide and 2.74 g (12.9 mmol) of N-dodecyl-N,N-dimethylamine in 40 mL of abs. ethanol was heated under reflux. After 72 h the ratio of starting compounds to product was constant and the reaction was stopped. Separation of the products was achieved by column chromatography. Excess N-dodecyl-N,N-dimethylamine was eluted with CH₂Cl₂. Subsequently, CH₂Cl₂/15% MeOH was used for elution of the product. After removal of the
solvents, 12-4 was obtained as a hygroscopic white solid (mp 215-217°C) in 62% yield (3.27 g, 6.7 mmol). 1H NMR (200 MHz, CD3OD) δ 0.87 (t, 3H, CH3), 1.25-1.35 (m, 18H, CH2 alkyl tail), 1.75 (m, 2H, β-CH2), 2.08 (m, 4H, CH2), 3.31 (s, 6H, N+(CH3)2), 3.43 (m, 11H, N+(CH3)3 and α-CH2). 13C NMR (200 MHz, CD3OD) δ 11.54 (p), 17.76, 18.17, 20.75, 20.82, 24.54, 27.38, 27.55, 27.70, 27.74, 27.83, 30.15 (s), 48.41 (p), 50.78, 50.85, 50.93 (p), 61.41, 62.92, 63.80 (s). Anal. Calcd. C21H48N2Br2 (488.43): C 51.64; H 9.91; N 5.74; Br 32.72. Found: C 51.13; H 9.77; N 5.73; Br 32.67.

**Azobenzene sulfonate.** ABS was synthesized by sulphonation of azobenzene according to a literature procedure.50 Mp 120-122°C (lit. 127°C).50

**4-Butylphenylazosulfonate.** C4PAS was synthesized by diazotation of 4-butylaniline according to a literature procedure for the preparation of 4-alkylphenylazosulfonates.20 Mp>230°C. 1H NMR (300 MHz, D2O) δ (t, 3H, CH3), 1.31 (m, 2H, CH2), 1.80 (m, 2H, CH2) 2.64 (t, 2H, CH2), 7.35 (d, 2H, CH ar), 7.78 (d, 2H, CH ar).

**UV-vis spectroscopy.** UV-vis absorption spectra were recorded using a Perkin-Elmer λ5 or λ12 spectrophotometer equipped with a thermostated cell compartment. MO, pMR, MR, C4PAS, and ABS concentrations were 25 µM, the EO concentration was 23 µM, and that of MY was 12 µM. Solutions except for those containing C4PAS were prepared in 0.02 M sodium borate buffers adjusted to pH 9.4.

**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).

**Differential scanning calorimetry (DSC).** DSC measurements were made using 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 degrees min⁻¹.

**Surface tension experiments.** Critical aggregation concentrations (cacs) were determined by drop tensiometry using a Lauda TVT1 drop tensiometer equipped with a LAUDA RM6 thermostat bath. Cacs were determined from ca. 15 data points ranging from 0.1 times the cac to 10 times the cmc.

2.11 References

Chapter 2


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Interactions of Cationic Surfactants and Azo Dyes in Aqueous Solution

27 The Krafft temperature of 10p-4-p10 is 51.7°C as determined by differential scanning calorimetry.
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