Conformation and dynamics of polyoxyethylene lauryl ether (Brij-35) chains in aqueous micellar solution studied by 2D NOESY and \(^1\)H NMR relaxation

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Abstract Spin-lattice relaxation time, spin-spin relaxation time and two-dimensional nuclear Overhauser enhancement spectroscopy (2D NOESY) experiments of polyoxyethylene lauryl ether (Brij-35) micelles in aqueous solutions at a concentration of 100 times the critical micellar concentration (cmc) give direct evidence that the hydrophilic polyoxyethylene chains, staying in the exterior of the micellar core, are coiled, bent and aligned around the micellar core with a certain number of water molecules included. This hydrophilic layer is in contact with the solvent, water, keeping the micellar solution stable. \(^1\)H NMR relaxation time measurements show that the first oxyethylene group next to the alkyl chain participates in the formation of the surface area of the micellar core. The motion of the hydrophilic polyoxyethylene chains is less restricted as compared with the hydrophobic alkyl chains.

Keywords: polyoxyethylene lauryl ether (Brij-35), 2D NOESY, micellar structure and dynamics, relaxation.

Polyoxyethylene lauryl ether (Brij-35) is one of the nonionic surfactants widely used both in biochemical and chemical processes for its virtues such as high stability, well-dissolved, and easily mixing with other typical surfactants for a mixed use\(^[1]\). Although it is generally accepted that the nonpolar hydrocarbon chains are packed in the interior of the micellar core, whereas the hydrophilic polyoxyethylene chains stay outside the core and move relatively freely in the solvent, keeping the micelle stable in the solution, there are controversies on the penetration of water into the hydrophobic micellar core of the surfactants\(^[2\text{-}4]\) and the mobility of the hydrocarbon chain in the interior of the micellar core. Some authors confirmed that the motion of these hydrocarbon chains is more restricted than that in a liquid hydrocarbon of the same chain length\(^[3,5]\). The ability of polyoxyethylene alkyl ethers to undergo self-aggregation in aqueous solution has been intensively studied by many methods, especially nuclear magnetic resonance (NMR), which can provide valuable information regarding molecular motion\(^[6]\). The spin-lattice relaxation time (\(T_1\)) determination of C\(_{10}\)E\(_5\) shows that the hydrophilic polyoxyethylene chains are motionally re-
stricted compared with the hydrophobic alkyl chains, and so concluded that the hydrophilic poly-

oxyethylene chains are coiled or entangled, but where no evidence of conformation’s description has
been provided$^{[4]}$. Spin-spin relaxation time ($T_2$) and self-diffusion studies of $C_{17}E_{84}$ give the simi-
lar results$^{[7]}$. Two-dimensional nuclear Overhauser enhancement spectroscopy (2D NOESY) is an
effective method to study the three-dimensional structure of large molecules$^{[8-10]}$, which can pro-
vide the information of those proton pairs with inter-nuclear distances shorter than 0.5 nm in space.
One can obtain internuclear distances from the intensity of the cross peaks$^{[11,12]}$. Kolehmainen$^{[13]}$
reported the inter- and intra-molecular distances in an aqueous solution of bile salts with aromatic
solubilizate. We have also observed these effects in an aqueous solution of TX-100$^{[14,15]}$. $^1H$ NMR
relaxation and 2D NOESY experiments were performed in this paper to determine the detailed
structure of a widely used nonionic surfactant, Brij-35 micelles.

1 Experimental

Brij-35 was the chemical pure reagent from J. T. Baker Chemicals, B. V. –Deventer of Hol-

land. D$_2$O, 99.8% deuterated, was the product of Beijing Chemical Factory of China. All NMR
experiments were performed on a Bruker ARX-500 NMR spectrometer with a proton frequency of
500.13 MHz, at 298.0 K. The $^1H$ $T_1$ (spin-lattice) and $T_2$ (spin-spin) NMR relaxation time meas-
urements were performed with inversion recovery and CPMG pulse sequences, respectively. 4.0 s
pulse delay, 32 accumulations. D$_2$O was used as solvent instead of water to weaken the water
proton signal. Meanwhile, the presaturation method was used to further suppress the proton
signal of the solvent. 2D NOESY experiments were performed with the standard three-pulse
sequence, i.e., $\pi/2$-$t_1$-$\pi/2$-$\tau_m$-$\pi/2$-$t_2$ $^{[15]}$. TPPI mode sampling, 4.0 s pulse delay, 32 accumula-
tions. Sampling data point array $t_2 \times t_1 = 1024 \times 256$; mixing times of 900, 700, 500, 400, 300,
100 and 80 ms were used in the experiment. Zero filling FT changing data point array $F_2 \times F_1 =
1024 \times 512$.

2 Results

2.1 $^1H$ NMR relaxation

The concentration of Brij-35 is, expressed in terms of critical micellar concentration (cmc =
0.06 mmol/L), 100 cmc in the experiment. There are altogether seven resolvable resonance peaks
in the Brij-35 $^1H$ NMR spectrum. They represent seven kinds of chemically equivalent groups of
spins, as shown in fig. 1. Chemical shifts of the protons of the (CH$_2$CH$_2$O)$_{21}$ group (labeled as a
single group H2) are not equal to each other, but very close, and they overlap seriously, as indi-
cated by the broad signals in the 1D spectrum of fig. 2. The $T_1$, $T_2$ values, and the ratio $T_R$
($T_2 / T_1$) of all the proton signals of Brij-35 are listed in table 1.

\[
\begin{align*}
\text{CH}_3(\text{CH}_2)_9\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{21}\text{CH}_2\text{CH}_2\text{OH} \\
\text{H7} & \quad \text{H6} & \quad \text{H5} & \quad \text{H4} & \quad \text{H1} & \quad \text{H2} & \quad \text{H3}
\end{align*}
\]

Fig. 1. Formula and proton numbering of the Brij-35 molecule.
It is obvious that $T_R$ of all the proton signals of Brij-35 at a concentration of 100 cmc is about 0.2 with the exception of H2 and H3. This suggests that the motion of Brij-35 protons is not in the extreme narrowing condition ($T_1 = T_2$), and the molecules are not in the extended single molecular state. Motion of most protons is seriously restricted, especially, of the hydrophobic protons.
Table 1 The \(^1\)H \(T_1\) (ms), \(T_2\) (ms) and \(T_R\) values of the protons of Brij-35 in an aqueous solution at a concentration of 100 cmc

<table>
<thead>
<tr>
<th>Brij-35</th>
<th>(T_1)</th>
<th>(T_2)</th>
<th>(T_R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>166</td>
<td>688</td>
<td>0.24</td>
</tr>
<tr>
<td>H2</td>
<td>581</td>
<td>794</td>
<td>0.73</td>
</tr>
<tr>
<td>H3</td>
<td>858</td>
<td>1201</td>
<td>0.71</td>
</tr>
<tr>
<td>H4</td>
<td>57</td>
<td>655</td>
<td>0.09</td>
</tr>
<tr>
<td>H5</td>
<td>96</td>
<td>567</td>
<td>0.17</td>
</tr>
<tr>
<td>H6</td>
<td>124</td>
<td>666</td>
<td>0.19</td>
</tr>
<tr>
<td>H7</td>
<td>355</td>
<td>1169</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Estimated errors of \(T_1\) and \(T_2\) values are smaller than 5%.

Among the hydrophobic protons (H4—H7), H4, whose \(T_R\) values is only 0.09, is a typical proton involved in the surface layer of micellar core\[^{15}\]. \(T_R\) values of hydrophobic protons increase as their distances to the polar groups get longer, suggesting that the motion of these methylene protons gets less restricted. Because of the intrinsic internal rotation of the methyl groups, \(T_R\) values of its protons are the largest in all the hydrophobic protons. The motion of the first oxyethylene protons (H1) next to the hydrophobic chains (\(T_R = 0.24\)) is more restricted than that of other polyoxyethylene protons (H2, H3), suggesting that this oxyethylene group participates in the formation of the compact surface layer of the micellar core. Although the motion of the hydrophilic protons (H2, H3) staying in the exterior of the micellar core is less restricted than that of the hydrophobic protons (H4, H5, H6, H7) and the first polyoxyethylene protons (H1), it is still far from the extreme narrowing condition. This implies that the oxyethylene groups of the hydrophilic chain are very close to each other. The \(T_R\) values of the relaxation time measurements show that the ether bond CH\(_2\) (H4 and H1) together with the hydrophobic protons of CH\(_2\) (H5) next to the polar group participates in the formation of the surface layer of the micellar core, and the hydrophilic polyoxyethylene chains staying in the exterior of the micellar core are not extended. These results are similar to those of TX-100\[^{15}\].

2.2 2D NOESY experiment

2D NOESY spectra for Brij-35 aqueous solution at a concentration of 100 cmc were obtained with mixing times of 900, 700, 500, 400, 300, 100 and 80 ms. Contour plots of the 2D spectra obtained with mixing times of (a) 80, (b) 300, (c) 500 and (d) 700 ms are shown in fig. 2. Cross-peaks between protons on adjacent carbon atoms (H6-H7, H6-H5, H6-H4, H5-H4, H5-H1, H5-H2, H6-H1 and H6-H2, etc.) are observed. The presence of cross-peaks between water protons (HW) and the hydrophilic protons H2 is the evidence of interactions of the hydrophilic polyoxyethylene chain with water molecules. The presence of cross-peaks between water protons(HW) and the hydrophobic protons H6 suggests that the micellar core is penetrated to some extent by water molecules.

The evolution of the intensities of the cross-peaks in the 2D NOESY spectra of Brij-35 aqueous solution with the mixing times is shown in fig. 3. The intensities of cross-peaks between
the hydrophobic proton pairs (H6-H4, H6-H5 and H6-H7) reach their maximum already at a mixing time of 400 ms. However, those of H6-H1, especially H6-H2, still increase gradually. This indicates that the diffusion of magnetization along the hydrophobic chain is possible in this system, although spin-diffusion does not completely couple all the nuclei in the micelle. It is reasonable to ensure that the intensities of the cross-peaks are not destroyed by spin-diffusion at the mixing time of 300 ms.

So the distances between proton pairs in Brij-35 micelles were calculated with the measured intensities of the cross-peaks at the mixing time of 300 ms\cite{12}. The following formula was used with the aid of the known distance H5-H4 ($r_{54} = 0.282$ nm) in the molecule:

$$r_{mn} / r_{54} = (I_{54} / I_{mn})^{1/6},$$

where $I_{54}$ and $I_{mn}$ are the intensities of the cross-peaks between H5 and H4 and between the two nuclei of interest, respectively, $r_{54}$ and $r_{mn}$ are the corresponding internuclear distances. It should be mentioned that the proton signal of the hydrophobic alkyl chain (H6) and the polyoxyethylene chain (H2) are seriously overlapped resonances of 9 and 42 methylene groups, respectively. The intensities of the cross-peaks of H6 and H2 with other protons are the contribution of the sum of many protons. The internuclear distances between H2 and other protons listed in table 2 are the average values of the twenty-one oxyethylene groups. Eighteen methylene protons of the hydrophobic alkyl chain (H6), and four methylene protons of the oxyethylene group (H1) next to the nonpolar hydrophobic alkyl chain are also taken into account in calculation of the internuclear distances from the intensities of the corresponding cross-peaks. Therefore, the inter-proton distances calculated according to the above formula is actually the geometrically average distances between groups having chemically equivalent protons. Although these internuclear distances calculated are not precise, they can characterize the conformation of the system that we are studying. Inter-proton distances calculated from the 2D NOESY spectra are listed in table 2 as $r_{mn}$ and the inter-proton distances calculated from HYPERCHEM are listed as $r_{cal}$. Here the internuclear distances between H2 and other protons are the average distances from protons on each oxyethylene group to the proton of interest (i.e., the distance between the center of gravity of the
protons in the polyoxyethylene chain and the protons of interest). It should be mentioned that the NOESY effect can be either intra- or inter-molecular in origin, and it is impossible to discriminate between them when one deals with interaction between molecules of the same kind. Therefore, we can only assume that the inter-proton distances obtained by the 2D NOESY experiment are the weighted averages of these two interactions. These values being averaged cannot give the exact conformation of the molecule; however, they can give valuable information about the change of conformation qualitatively.

<table>
<thead>
<tr>
<th>Proton pairs</th>
<th>( r_{cal} )</th>
<th>( r_{mn} )</th>
<th>Proton pairs</th>
<th>( r_{cal} )</th>
<th>( r_{mn} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H6-H1</td>
<td>0.585</td>
<td>0.456</td>
<td>H5-H4</td>
<td>0.282</td>
<td>0.282</td>
</tr>
<tr>
<td>H6-H2</td>
<td>4.52</td>
<td>1.61</td>
<td>H5-H1</td>
<td>0.505</td>
<td>0.302</td>
</tr>
<tr>
<td>H6-H4</td>
<td>0.282</td>
<td>0.262</td>
<td>H5-H2</td>
<td>4.16</td>
<td>1.39</td>
</tr>
<tr>
<td>H6(1)-H1(1)</td>
<td>0.511</td>
<td>–</td>
<td>H5-H2(1)</td>
<td>0.722</td>
<td>–</td>
</tr>
<tr>
<td>H6(1)-H1(2)</td>
<td>0.659</td>
<td>–</td>
<td>H5-H2(2)</td>
<td>0.866</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2  Internuclear distances, \( r \) (nm), between protons of Brij-35 in an aqueous solution at a concentration of 100 cmc calculated from the intensities of the cross peaks of proton pairs in the 2D NOESY map with mixing time of 300 ms, and by HYPERCHEM calculation\(^a\)

\( a \) Inter-nuclear distances are averaged values of different configurations due to the existence of flexible chains in the molecule. \( r_{cal} \), calculated by HYPERCHEM; \( r_{mn} \), calculated from the intensities of the cross peaks in the 2D NOESY map with mixing time of 300 ms. H6(1), the methylene protons nearest to H5; H1(1), protons of the first oxyethylene group nearest to the alkyl chain of Brij-35. Estimated error is 0.03 nm.

It is obvious that inter-proton distances of adjacent carbon atoms in the micelles agree with those of the single molecule calculated by HYPERCHEM. Inter-proton distances of the proton pairs which have cross-peaks presented in the 2D spectra, especially those between the hydrophilic protons (H1 and H2) and the hydrophobic protons (H6 and H5), respectively, are much shorter than those of the single molecule calculated by HYPERCHEM, where the hydrophilic polyoxyethylene chain is in the extended form, so only when the hydrophilic chain is coiled, such close vicinity would be possible between the hydrophilic and the hydrophobic chains. This agrees quite well with the results of relaxation experiments.

3 Discussion

3.1 Structure of micellar core and penetration of water

Although controversies of different opinions on the penetration of water into the micellar core exist, the 2D NOESY spectrum of Brij-35 in aqueous solution at a concentration of 100 cmc does not give evidence about interaction between water protons and any protons of a Brij-35 molecule other than H2 and H6. The presence of cross-peaks between water protons and the hydrophilic protons H2 suggests that water molecules are involved in the coiled polyoxyethylene chains. The presence of cross-peaks between water protons and the hydrophobic protons (H6) suggests that the micellar core is penetrated to some extent by water molecules. This agrees quite well with the previous work\(^{12-4}\). It is also likely that such penetration would occur in a coiled or entangled chain configuration\(^4\).
3.2 Conformation of the hydrophilic chain

We can conclude that the hydrophilic polyoxyethylene chain of Brij-35 micelles in aqueous solution at a concentration of 100 cmc is coiled or bent in certain degrees both from the spin-lattice and spin-spin relaxation times (table 1) and the 2D NOESY spectrum (fig. 2). The reason is as follows: All the $T_R$ values of protons of the Brij-35 in the aqueous solution are much smaller than unit, suggesting that the motion of these protons is far from the extreme narrowing condition.

It is well known that protons situated 0.5 nm and farther apart in space do not show cross-peaks in the 2D NOESY map. Molecular simulation by HYPERCHEM tells us that the distance between H6(1), the methylene protons nearest to H5, and the first oxyethylene protons H1(1) is very much larger than 0.5 nm. But cross-peaks between proton pairs of the oxyethylene chain and of the alkyl chain (H6-H1, H6-H2, H5-H1 and H5-H2) appeared in the 2D NOESY spectrum, besides the fact that internuclear distances between protons obtained from the 2D NOESY spectrum, are very much shorter than those calculated by HYPERCHEM, further indicating that the hydrophilic polyoxyethylene chains are coiled and bent.

In forming micelles, the polyoxyethylene chains might become coiled or bent and aligned along the surface of the Brij-35 micellar core. A layer thick in dimension and loose in structure surrounding the micellar core is formed. The loose layer allows a certain amount of water molecules to be included, forming a thick hydrophilic layer, which keeps in contact with the solvent water, rendering the micellar solution stable. This conformation provides the possibility of strong intermolecular interaction between the hydrophobic protons in the micellar core and those of the polyoxyethylene chains. It supports the somewhat restricted motion of the polyoxyethylene chain of Brij-35 micelles mentioned above. The motion of the first oxyethylene protons (H1) next to the hydrophobic chain ($T_R = 0.24$) is more restricted than that of the other polyoxyethylene protons (H2, H3), suggesting that this oxyethylene group participates in the formation of the compact surface layer of the micellar core.

4 Conclusion

The micellar conformation of nonionic surfactants, TX-100 and Brij-35, shows that their hydrophilic polyoxyethylene chains, staying in the exterior of the micellar core, are coiled, bent and aligned around the micellar core with a certain number of water molecules included. This hydrophilic layer is in contact with the solvent, water, keeping the micellar solution stable. Especially the first oxyethylene group next to the hydrophobic chain participates in the formation of the surface layer of the micellar core. The motion of the hydrophilic polyoxyethylene chains is less restricted compared with the hydrophobic alkyl chains.

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