Solution Properties and Emulsification Properties of Amino Acid-Based Gemini Surfactants Derived from Cysteine

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Abstract: Amino acid-based anionic gemini surfactants (2CₙdiCys, where n represents an alkyl chain with a length of 10, 12, or 14 carbons and “di” and “Cys” indicate adipoyl and cysteine, respectively) were synthesized using the amino acid cysteine. Biodegradability, equilibrium surface tension, and dynamic light scattering were used to characterize the properties of gemini surfactants. Additionally, the effects of alkyl chain length, number of chains, and structure on these properties were evaluated by comparing previously reported gemini surfactants derived from cystine (2CₙCys) and monomeric surfactants (CₙCys). 2CₙdiCys shows relatively higher biodegradability than does CₙCys and previously reported sugar-based gemini surfactants. Both critical micelle concentration (CMC) and surface tension decrease when alkyl chain length is increased from 10 to 12, while a further increase in chain length to 14 results in increased CMC and surface tension. This indicates that long-chain gemini surfactants have a decreased aggregation tendency due to the steric hindrance of the bulky spacer as well as premicelle formation at concentrations below the CMC and are poorly packed at the air/water interface. Formation of micelles (measuring 2 to 5 nm in solution) from 2CₙdiCys shows no dependence on alkyl chain length. Further, shaking the mixtures of aqueous 2CₙdiCys surfactant solutions and squalane results in the formation of oil-in-water type emulsions. The highly stable emulsions are formed using 2C₁₂diCys or 2C₁₄diCys solution and squalane in a 1:1 or 2:1 volume ratio.

Key words: gemini surfactant, amino acid-based surfactant, cysteine, surface tension, emulsification

1 INTRODUCTION

Gemini surfactants, which consist of 2 alkyl chains, 2 hydrophilic groups, and 1 spacer in a molecule, are well known to exhibit excellent surface activities. Compared to conventional monomeric surfactants, gemini surfactants exhibit better water solubility, lower critical micelle concentration (CMC), and higher efficiency in lowering water surface tension¹⁻³. Gemini surfactants also exhibit unique aggregation behaviors such as spontaneous vesicle formation at fairly low concentrations and premicelle formation at concentrations below the CMC in aqueous solution. Gemini surfactants have attracted attention as next-generation surfactants, and those with increased performance and functionality are required in the further.

The class of amino acid-based surfactants is expected to exhibit advantages such as biocompatibility and biodegradability over other surfactants. Amino acids were initially used as preservatives for medical and cosmetic applications and were found to be effective against various disease-causing bacteria, tumors, and viruses⁴. Acyl amino acid-based surfactants, which have been widely investigated, exhibit excellent properties such as good water solubility and rapid biodegradability⁵,⁶. While gemini surfactants have been thoroughly investigated, few studies have focused on the synthesis and various properties of amino acid-based gemini surfactants derived from cystine, cysteine, cystamine, and arginine⁷⁻¹⁸. Our group previously reported the synthesis, adsorption, and aggregation properties of cystine-based gemini surfactants (2CₙCys, where n represents an alkyl chain length of 8, 10, or 12 carbons, and Cys represents cystine) with 2 alkyl chains, 2 amino groups of a cystine frame and 2 hydrophilic carboxylic acid groups in alkaline solution¹⁹. There, we observed high adsorption ability at the air/water interface for alkyl chain...
length of 8 and 10, while a further increase in chain length led to decreased surface activity. Micelles formed for chain length of 8 and 10 carbons, while the coexistence of small unilamellar vesicles and rod-like micelles was observed for chain length of 12 carbons.

In this study, we synthesized new amino acid-based gemini surfactants with 2 alkyl chains, 2 cysteines, and a hexanediimide spacer derived from cysteine (2C_n diCys, where \( n \) represents an alkyl chain length of 10, 12, or 14 carbons and “di” and “Cys” indicate adipoyl and cysteine, respectively). The chemical structures of 2C_n diCys and 2C_n Cys are shown in Fig. 1. Several properties of amino acid-based gemini surfactants were evaluated, including biodegradability, surface tension, micellization, and emulsification.

2 EXPERIMENTAL

2.1 Materials

L-Cysteine, \( n \)-decyl bromide, \( n \)-dodecyl bromide, and adipoyl chloride were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Acetone, ethanol, ethyl acetate, hexane, methanol, tetrahydrofuran, and sodium hydroxide (NaOH) were obtained from Kanto Chemicals Co. Inc. (Tokyo, Japan). All chemicals were reagent-grade purity commercial materials and used without further purification.

2.2 Synthesis of cysteine-based gemini surfactant

\( N \)-Alkyl cysteine was synthesized in methanol containing NaOH by reacting cysteine and \( n \)-alkyl bromide (alkyl = decyl, dodecyl, or tetradecyl) according to previously described methods.\(^{15}\) Adipoyl chloride was added to a stirred solution of \( N \)-alkyl cysteine (2.2-fold molar excess for adipoyl chloride) in tetrahydrofuran at 5°C; this mixture was stirred for 24 h. After the solvent was removed by evaporation, the residue obtained was dissolved in acetone and the solution was filtered to remove nonsoluble material. After removing the solvent, the residue obtained was re-crystallized repeatedly from the mixture of ethyl acetate and ethanol, the mixture of ethyl acetate and acetone, and then the mixture of hexane and acetone, giving the cysteine-based gemini surfactant 2C_n diCys (\( n \) = 10, 12, or 14).

The structures were evaluated by using \( ^1H \) NMR (JEOL JNM-EX 500 MHz, CDCl_3) and elemental analysis (Perkin-Elmer 2400II CHNS/O). \( ^1H \) NMR (CD_3OD): 8 0.90 (6H, \( CH_2-CH_2 \)), 1.29-1.39 (12H, \( CH_2-(CH_2)_n-CH_2 \)), 1.56 (4H, -\( CH_2-(CH_2)_n-CH_2-N \)), 1.70 (4H, -N-C(=O)-CH_2-CH_2-CH_2-CH_2-C(=O)-N\)), 2.29 (4H, -N-C(=O)-CH_2-CH_2-CH_2-CH_2-C(=O)-N\)), 2.54 (4H, -\( CH_2-(CH_2)_n-CH_2-N \)), 2.83-3.03 (4H, -CH-CH_2-SH), 4.41 ppm (2H, -CH-CH_2-SH). Elemental analysis: 2C_10 diCys. Calcd for C_{30}H_{56}N_2O_6S_2: C, 59.57; H, 9.33; N, 4.63. Found: C, 60.17; H, 9.79; N, 4.47. 2C_12 diCys. Calcd for C_{38}H_{72}N_2O_6S_2: C, 63.65; H, 10.12; N, 3.91. Found: C, 63.25; H, 10.32; N, 4.12. From the values of elemental analysis, 2C_n diCys may contain very little impurities like inorganic salts. However, probably, there is no influence of these impurities on the solution properties and emulsification properties because of a small quantity.

2.3 Measurements

All the gemini surfactant solutions were adjusted to pH 13 using 0.1 mol dm\(^{-3}\) NaOH, except when used for biodegradability assays, and measurements were performed at 25°C.

2.3.1 Biodegradability

The biodegradability of the gemini surfactants was evaluated using the oxygen-consumption method based on the modified MITI (ministry of industry trade and industry) test. Activated sludge was obtained from a municipal sewage treatment plant in Osaka City (Japan). Biochemical oxygen demand (BOD) after 7, 14, 21, and 28 days was determined on the basis of the quantity of oxygen consumed. Biodegradability was estimated using the following equation:

\[
\text{Biodegradability} (\%) = \frac{\text{BOD} - \text{blank}}{\text{TOD}} \times 100
\]

where blank represents oxygen consumption (mg) in the blank dispersion and TOD represents the theoretical oxygen demand (mg).

2.3.2 Surface tension

The surface tension measurements were performed with a Krüss K100 tensiometer using the Wilhelmy plate technique. To obtain equilibrium surface tension, sets of measurements were performed until the change in the surface tension was less than 0.01 mN m\(^{-1}\) every 60 s. The CMC and the surface tension at the CMC (\( \gamma_{\text{ac}} \)) were determined from the breakpoint of the surface tension versus the logarithm of the concentration curve. In most cases, the surface excess concentration (\( \Gamma \)) in mol m\(^{-2}\) and the area occupied by molecule (\( \Lambda \)) of the gemini surfactants at the air/water interface were calculated using the following
Gibbs adsorption isotherm equations \(^{(19)}\): 
\[
\Gamma = \frac{-1}{iRT} \frac{\mathrm{d}y}{\mathrm{d}nC}
\tag{2}
\]
\[
\Lambda = \frac{1}{N_A}\Gamma
\tag{3}
\]
Here, \(\gamma\) represents the surface tension in mN m\(^{-1}\), \(R\) is the gas constant (8.31 J mol\(^{-1}\) K\(^{-1}\)), \(T\) is the absolute temperature, \(C\) is the surfactant concentration, \(\langle \mathrm{d}y/\mathrm{d}nC \rangle\) is the slope below the cmc in the surface tension plots, and \(N_A\) is Avogadro’s number. We use \(i = 1\) in this study, because the solution contains excess sodium hydroxide.

The Gibbs free energy of micellization and adsorption \(\langle \Delta G_{\text{mic}} \rangle\) and \(\langle \Delta G_{\text{ads}} \rangle\), respectively, of the gemini surfactants can be obtained from the following equations \(^{(19)}\):
\[
\Delta G_{\text{mic}}^{\text{ads}} = RT\left(\frac{1}{2} + \beta\right)\ln\left(\frac{\text{CMC}}{55.3}\right) - \left(\frac{RT}{2}\right)\ln 2
\tag{4}
\]
\[
\Delta G_{\text{ads}}^{\text{ads}} = \Delta G_{\text{ads}}^{\text{mic}} - \frac{\pi_{\text{CMC}}}{\Gamma}
\tag{5}
\]
Here, \(\pi_{\text{CMC}}\) denotes the surface pressure at the CMC (\(\pi_{\text{CMC}} = \gamma_0 - \gamma_{\text{CMC}}\)), where \(\gamma_0\) and \(\gamma_{\text{CMC}}\) are the surface tensions of water and the gemini surfactant solution at the CMC, respectively. \(\beta\) is the apparent degree of counterion binding at the micelle/solution interface, calculated from \(\beta = 1 - \alpha\) (\(\alpha\) is the degree of ionization for micelle). Here, the \(\alpha\) value is taken as 1 in all cases because the variation in conductivity with the concentration does not show a clear breakpoint around the CMC in the solution at pH 13.

2.3.3 Dynamic light scattering (DLS)

The DLS measurements were performed with the DLS-7000 (Otsuka Electronics Co., Ltd., Japan) using an argon laser (\(\lambda = 488\) nm) at a scattering angle of 90°. All gemini surfactant solution was filtered with a 0.2 μm membrane filter of mixed cellulose acetate before the measurements. For spherical particles, the diffusion coefficient extrapolated to zero concentration \(D_0\) is converted to the apparent hydrodynamic diameter \(\langle D_n \rangle\) using the Stokes-Einstein relation \(^{(21)}\):
\[
D_n = \frac{kT}{3\pi\eta D_0}
\tag{6}
\]
where \(k\) is the Boltzmann constant, \(T\) is the absolute temperature, and \(\eta\) is the viscosity of the solution.

2.3.4 Emulsion stability

The stability of the emulsion prepared was estimated using the 2-point conductivity method with a DualCon stability tester (ITEC-IFAC Technology GmbH & Co. KG, Duisburg, Germany). Two pairs of electrodes were used to determine conductivity at the top \(\left(\kappa_t\right)\) and bottom \(\left(\kappa_b\right)\) of the sample placed in a cylindrical container. The difference between these conductivity values \(\Delta \kappa = \kappa_b - \kappa_t\) was used to determine the colloidal stability of the emulsions prepared.

2.3.5 Droplet size of emulsion

Droplet size in the emulsion was measured using a fiber-optics particle analyzer FPAR-1000 (Otsuka Electronics Co., Ltd., Osaka, Japan) using a laser diode (\(\lambda = 660\) nm) at a scattering angle of 160°. The size of particles in the emulsion was obtained using the Stokes-Einstein relation equation \((6)\).

3 RESULTS AND DISCUSSION

3.1 Biodegradability

Biodegradability of cysteine amino acid-based gemini surfactants \((2\text{C}_n\text{diCys} \text{ and } 2\text{C}_n\text{diCys})\) after 7, 14, 21, and 28 days was investigated using the BOD method for activated sludge. The results are shown in Table 1, along with data for the corresponding monomeric surfactant \((\text{C}_n\text{Cys})\) and 2 types of sugar-based gemini surfactants with lactobionamide headgroups \((2\text{C}_{12}\text{Lac} \text{ and } 2\text{C}_{12}\text{peLac})\), which have been reported previously \(^{(21)}\). The biodegradability of \(2\text{C}_n\text{diCys}\) and \(2\text{C}_n\text{diCys}\) increased with time; the biodegradability of \(2\text{C}_{10}\text{dCys}\) remained nearly constant after 14-28 days, whereas that of \(2\text{C}_{14}\text{dCys}\) continued to increase. Although the biodegradability of \(2\text{C}_{12}\text{diCys}\) was not measured, probably, the results will be almost as near as those of \(2\text{C}_{10}\text{dCys}\) or \(2\text{C}_{14}\text{dCys}\). The amino acid-based gemini surfactants used in the present study were more rapidly biodegraded than the corresponding monomeric and sugar-based gemini surfactants as well as the conventional surfactant sodium dodecyl sulfate (50.0%) \(^{(22)}\). Chemical tests showed that for more than 60% BOD, the chemical should be regarded as readily biodegradable. Notably, amino acid-based gemini surfactants, particularly those with a short chain length of \(n = 10\), are highly biodegradable. One reason may be the existence of 2 amide bonds on both edges in a spacer connected by N-alkyl cysteine. Therefore, the present amino acid-based gemini surfactants are environmentally friendly surfactants.

3.2 Surface tension

Surface tension of the cysteine-based gemini surfactant \(2\text{C}_n\text{diCys}\) determined at 25°C as a function of concentra-

<table>
<thead>
<tr>
<th>Chemical</th>
<th>BOD/TOD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
</tr>
<tr>
<td>(2\text{C}_{10}\text{diCys})</td>
<td>28.5</td>
</tr>
<tr>
<td>(2\text{C}_{12}\text{diCys})</td>
<td>48.8</td>
</tr>
<tr>
<td>(2\text{C}_{14}\text{Cys})</td>
<td>4.5</td>
</tr>
<tr>
<td>(2\text{C}_{12}\text{Lac})</td>
<td>11.9</td>
</tr>
<tr>
<td>(2\text{C}_{12}\text{peLac})</td>
<td>3.5</td>
</tr>
</tbody>
</table>

\(^*\) taken from Ref. 21.
tion is shown in Fig. 2. Surface tension decreased as concentration increased and reached a clear breakpoint, which was defined as the CMC. Table 2 summarizes the values for CMC, surface tension at the CMC (γ_{CMC}), surface excess concentration (Γ), and area occupied per molecule (A) for 2C_{12}Cys, which were obtained based on surface tension plots as well as data reported for the gemini surfactant \( N\)-dialkyl cystine (2C_{12}Cys) and the corresponding conventional monomeric surfactant \( N\)-dodecyl cysteine (C_{12}Cys)\(^{15}\). 2C_{12}Cys with \( n = 10 \) and 12 exhibited excellent surfactant active properties when compared to monomeric C_{12}Cys, as follows: (i) the CMC values of 2C_{12}Cys were lower than that of C_{12}Cys; (ii) the \( \gamma_{\text{CMC}} \) values of 2C_{12}Cys were lower than that of C_{12}Cys; (iii) A values of 2C_{12}Cys were significantly lower than twice the area of C_{12}Cys. These results were observed for previously reported various gemini surfactants\(^{3-5}\). Similar to the trend exhibited by conventional surfactants, the CMC of 2C_{12}diCys decreased with an increase in alkyl chain length from 10 to 12; however, an increase in chain length from 12 to 14 resulted in an increased CMC. This is often observed for gemini surfactants because of a lower aggregation tendency due to the steric hindrance of the bulky spacer as well as premicelle formation at concentrations below the CMC\(^{23,24}\). The slope for the linear relation between the logarithm of the CMC and alkyl chain length for 2C_{12}diCys (\( n = 10 \) and 12) was 0.35, which is slightly higher than values for conventional ionic monomeric surfactants (0.3)\(^{19}\). The CMC of 2C_{12}diCys was also higher than that of the similar cystine gemini surfactant 2C_{12}Cys. This can be explained by the difference in spacer structure between 2 gemini surfactants, i.e., the 2C_{12}diCys possesses 2 amide groups in the spacer, while the 2C_{12}Cys does not. The 2C_{n}diCys with \( n = 10 \) and 12 showed lower surface tensions of 30–32 mN m\(^{-1}\). This indicates that the gemini surfactants with amide groups adsorb and orient themselves efficiently at the air/water interface, as has been shown for conventional surfactants. An increase in alkyl chain length from 12 to 14 resulted in decreased efficiency in reducing water surface tension. This indicates that the gemini surfactant with 2 tetradecyl chains was poorly packed at the air/water interface due to the presence of 2 long chains. A values for 2C_{12}diCys decreased significantly with an increase in alkyl chain length from 12 to 14 carbons, indicating that interactions between the chains were much stronger when chain length increased. The results for the A values were not consistent with those for the \( \gamma_{\text{CMC}} \) values. 2C_{12}diCys may exhibit a small area because of the formation of a film by relatively long alkyl chains at the air/water interface, resulting in less effective interfacial adsorption. 2C_{12}diCys also showed a larger occupied area than 2C_{12}Cys due to the bulky molecular structure of the spacer.

These results are also supported by the Gibbs free energies of micellization and adsorption (\( AG_{\text{mic}} \) and \( AG_{\text{ads}} \), respectively), which can be obtained using eqs. (4) and (5), and parameters such as \( pC_{20} \) and the CMC/C\(_{20}\) ratio.

**Table 2** Critical micelle concentration, surface tension, surface excess concentration, and area occupied per molecule at the air/water interface for amino acid-based gemini surfactants and the corresponding monomeric surfactants.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>CMC (mmol dm(^{-3}))</th>
<th>( \gamma_{\text{CMC}} ) (mN m(^{-1}))</th>
<th>( 10^4T^* ) (mol m(^{-2}))</th>
<th>( A ) (nm(^2)/molecule)</th>
<th>( pC_{20} )</th>
<th>CMC/C(_{20})</th>
<th>( -\Delta G_{\text{mic}} ) (kJ mol(^{-1}))</th>
<th>( -\Delta G_{\text{ads}} ) (kJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C(_{10})diCys</td>
<td>0.106</td>
<td>32.3</td>
<td>1.81</td>
<td>0.919</td>
<td>5.91</td>
<td>85.6</td>
<td>34.4</td>
<td>78.4</td>
</tr>
<tr>
<td>2C(_{12})diCys</td>
<td>0.0211</td>
<td>31.2</td>
<td>1.88</td>
<td>0.883</td>
<td>6.64</td>
<td>91.6</td>
<td>41.6</td>
<td>90.4</td>
</tr>
<tr>
<td>2C(_{14})diCys</td>
<td>0.0528</td>
<td>41.5</td>
<td>3.72</td>
<td>0.447</td>
<td>4.84</td>
<td>3.62</td>
<td>40.2</td>
<td>81.4</td>
</tr>
<tr>
<td>2C(_{12})Cys(^a)</td>
<td>0.00116</td>
<td>40.9</td>
<td>3.12</td>
<td>0.532</td>
<td>5.40</td>
<td>29.2</td>
<td>22.8</td>
<td>32.8</td>
</tr>
<tr>
<td>C(_{12})Cys(^a)</td>
<td>0.0903</td>
<td>35.4</td>
<td>2.53</td>
<td>0.657</td>
<td>5.09</td>
<td>11.0</td>
<td>17.4</td>
<td>31.9</td>
</tr>
</tbody>
</table>

\(^a\) taken from Ref. 15.
posed by Rosen (Table 2). The absolute values of $\Delta G_{\text{ads}}^\circ$ were more than twice as high as those of $\Delta G_{\text{mic}}^\circ$ for all alkyl chain lengths, suggesting that the adsorption tendency of gemini surfactants at the air/water interface is higher than their tendency for micellization. Absolute values of $\Delta G_{\text{mic}}^\circ$ and $\Delta G_{\text{ads}}^\circ$ increased with an increase in alkyl chain length from 10 to 12, while an increase in the chain length to 14 reduced these values. However, CMC/C$_{50}$ ratio values of 2C$_n$diCys with $n = 10$ and 12 were quite high. This indicates that adsorption tendency of gemini surfactants at the air/water interface was significantly higher than their tendency for micellization in an aqueous solution, leading to decreased surface tension as described above. The CMC/C$_{50}$ ratio value became extremely low when alkyl chain length was increased to 14. The difference between 2C$_{12}$diCys and 2C$_{14}$diCys was much larger for absolute values of $\Delta G_{\text{mic}}^\circ$ than $\Delta G_{\text{ads}}^\circ$. These results indicate that the gemini surfactant with long alkyl chains prefers aggregate formation in solution than adsorption at the interface. This is also related to the increase in surface tension, i.e., inefficient interfacial adsorption. These results were also supported by the pC$_{50}$ parameter, i.e., the value decreased when alkyl chain length was increased from 12 to 14, indicating the tendency of the surfactant to form aggregates relative to its tendency to adsorb at the interface. Thus, it was found 2C$_n$diCys gemini surfactants with $n = 10$ and 12 were adsorbed strongly at the air/water interface. They oriented themselves efficiently due to the strong hydrophobic interactions between the alkyl chains, despite the bulky structure of their spacer, thus resulting in decreased surface tension. A further increase in alkyl chain length resulted in micelle formation prior to interfacial adsorption.

### 3.3 Micelle formation in solution

To confirm the formation of aggregates for the cysteine-based gemini surfactants in solution, dynamic light scattering (DLS) measurements were performed and apparent hydrodynamic diameter ($D_h$) was obtained using eq. (6). The concentrations used for 2C$_n$diCys differed with alkyl chain length because of the problem of scattering intensity or solubility in water. The values of $D_h$ were 4.9 ± 1.9 nm for 2C$_{12}$diCys (4.15 mmol dm$^{-3}$, CMC × 39), 2.4 ± 0.4 nm for 2C$_{12}$diCys (1.05 mmol dm$^{-3}$, CMC × 50), and 2.5 ± 1.2 nm for 2C$_{14}$diCys (24.9 mmol dm$^{-3}$, CMC × 472). Based on their sizes, the aggregates formed were micelles. Small micelles are thought to be closely packed due to strong interactions between 2 alkyl chains. We attempted cryogenic transmission electron microscopy (cryo-TEM) observation for the micelles of 2C$_n$diCys; however, a clear image was not observed because of the formation of small micelles. The aggregation behavior of 2C$_n$diCys differed from that of the 2C$_n$Cs cystine-based gemini surfactants, which have been reported previously (16). For $n = 12$, DLS, cryo-TEM, and SANS measurements showed that 2C$_{12}$diCys simultaneously formed 2 aggregates of small rod-like micelles that were 15 nm in length and small unilamellar vesicles with a diameter of 20 nm at a concentration of 0.832 mmol dm$^{-3}$ in solution. This may have resulted from the difference in the structure of the spacer group and the near, i.e., 2C$_{12}$Cys possesses an alkylene spacer containing disulfide bond, while 2C$_{12}$diCys possesses a comparatively rigid hexamidine spacer group. Therefore, 2C$_n$diCys surfactants could not easily form large aggregates because of the large curvature; in contrast, 2C$_n$Cs surfactants easily formed relatively large aggregates such as rod-like micelles and unilamellar vesicles because of their small curvature compared to 2C$_n$diCys.

#### 3.4 Emulsification properties with squalane

The oil-in-water (O/W) type emulsion was prepared by using ultrasonic homogenizer for mixtures of the gemini surfactants 2C$_n$diCys solution and squalane (1 : 1, 2 : 1 v/v). Figure 3 shows an example of the change in conductivity at the top and bottom phases ($\kappa_1$ and $\kappa_2$, respectively), and their difference ($\Delta \kappa = \kappa_2 - \kappa_1$) in emulsion, determined at 25°C as a function of time for 2C$_{12}$diCys solution (0.528 mmol dm$^{-3}$): squalane = 1 : 1 v/v. Conductivity $\kappa_1$ increased slightly with time, while $\kappa_2$ decreased. The $\Delta \kappa$ increased over time. Generally, stable emulsion systems do not exhibit conductivity changes with time. The shape of the conductivity curves can indicate instability such as creaming, sedimentation, coalescence, and flocculation. For example, for coalescence of emulsion, particle size and the

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Fig. 3 Variation in conductivity of the top and bottom phases, and their difference with time for the O/W type emulsion by 2C$_{12}$diCys solution (0.528 mmol dm$^{-3}$) and squalane (1 : 1 in volume ratio) at 25°C.
number of particles decreases, leading to a decreased viscosity and increased conductivity. For sedimentation and flocculation, viscosity is increased and ion mobility is decreased due to aggregation, thereby decreasing conductivity. For creaming, due to gravitational forces, inhomogeneity increases, leading to a conductivity difference between the top and bottom of the sample container.

The 2C12diCys surfactant exhibits typical behavior for emulsion creaming. The change in the $\Delta \kappa$ of the emulsion for 2C$_{n}$diCys solution (CMC $\times$ 25 for 2C$_{10}$diCys and 2C$_{12}$diCys, CMC $\times$ 70 for 2C$_{10}$diCys and squalane (1 : 1, 2:1 v/v) determined at 25°C as a function of time is shown in Fig. 4. Emulsion creaming was also observed for 2C$_{10}$diCys and 2C$_{14}$diCys in a 1 : 1 volume ratio (Fig. 4(a)). The increase in the $\Delta \kappa$ of 2C$_{10}$diCys in an initial short time was extremely higher than that of 2C$_{14}$diCys. This suggests that the emulsion formed by 2C$_{10}$diCys is unstable compared with 2C$_{14}$diCys. The $\kappa_2$ values for 2C$_{10}$diCys were close to 0 after 400 min, indicating that the emulsion completely separated into the aqueous phase. The $\Delta \kappa$ of 2C$_{12}$diCys increased gradually, and the $\kappa_2$ decreased slowly with time a little unlike 2C$_{14}$diCys, although the $\kappa_2$ was nearly the same behaviors, indicating that very slow creaming. Emulsion stability for 2C$_{10}$diCys was not high, even when the concentration was increased to 70 times the CMC (Fig. 4(b)). For the 1:1 volume ratio, the emulsion stability of 2C$_{12}$diCys with $n = 12$ and 14 was higher than that with $n = 10$, indicating that the stability of emulsion droplets improved when chain length increased due to formation of a strong film by the strong hydrophobic interaction between alkyl chains placed in the droplet. For the 2 : 1 volume ratio, the emulsion formed by 2C$_{10}$diCys increased stability by increasing water volume, while for 2C$_{12}$diCys and 2C$_{14}$diCys, no difference was observed in emulsion stability (Fig. 4(c)).

Droplet size measurements for the emulsion formed by the 2C$_{n}$diCys solution and squalane were performed using a fiber-optics particle analyzer with the Marquardt analysis.

![Graph](image-url)

**Fig. 4** Variation in conductivity difference between the top and bottom phases of the emulsion with time at 25°C: (a) 2C$_{n}$diCys solution (CMC $\times$ 25): squalane = 1:1 (in volume ratio); (b) 1: 1 (CMC $\times$ 70 for 2C$_{10}$diCys, and CMC $\times$ 5 for 2C$_{12}$diCys and 2C$_{14}$diCys); (c) 2: 1 (CMC $\times$ 25).

**Fig. 5** Size distribution of emulsion droplets in 2C$_{12}$diCys solution (0.528 mmol dm$^{-3}$) and squalane (1 : 1 in v/v ratio).

**Table 3** Droplet size ($\mu$m) of emulsion formed by cysteine-based gemini surfactant solution and squalane.

<table>
<thead>
<tr>
<th>W:O</th>
<th>CMC $\times$ 25</th>
<th>CMC $\times$ 25</th>
<th>CMC $\times$ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2C$_{10}$diCys</td>
<td>5.7 ± 2.9</td>
<td>1.3 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>2C$_{12}$diCys</td>
<td>1.6 ± 0.8</td>
<td>1.6 ± 0.7</td>
<td>2.5 ± 2.0</td>
</tr>
<tr>
<td>2C$_{14}$diCys</td>
<td>1.8 ± 0.6</td>
<td>1.5 ± 0.7</td>
<td>4.5 ± 1.2</td>
</tr>
</tbody>
</table>

* not measured.
method. An example of the size distribution of droplets in the emulsion formed by the 2C_{14}diCys solution (0.528 mmol dm$^{-3}$) and squalane in a volume ratio of 1:1 has been shown in Fig. 5. The droplet size of the emulsion was found to be 1600 ± 800 nm. The droplet sizes in the emulsion formed by the 2C_{diCys} solution and squalane (1:1 and 2:1 v/v ratio) are shown in Table 3. A slight correlation between droplet size and emulsion stability was observed. Droplet size for the emulsion formed by 2C_{10}diCys was significantly large when compared to that observed for other emulsion systems, resulting in unstable emulsion formation. However, the emulsion containing a large amount of water exhibited a decreased droplet size. This is consistent with emulsion stability values obtained using the conductivity method. For the emulsion of 2C_{12}diCys and 2C_{14}diCys, droplet size was nearly the same, even when water volume was increased from 1:1 to 2:1 for the water to squalane ratio. This indicates that the emulsion formed by these surfactants was stable at volume ratios of 1:1 to 2:1. When the concentration of 2C_{diCys} was decreased to 5 times the CMC, the droplet size in the emulsion increased. The results of the conductivity test also showed that the emulsion formed by 2C_{10}diCys at 5 times the CMC had nearly the same stability as that at 25 times the CMC, while the emulsion formed by 2C_{12}diCys completely separated into the aqueous phase, indicating unstable emulsion formation. Emulsion stability was influenced not only by the volume ratio of water and oil but also by surfactant concentration.

4 CONCLUSIONS

In this study, we designed and synthesized novel amino acid-based anionic gemini surfactants derived from cysteine; these surfactants had 2 alkyl chains (lengths of 10, 12, and 14), 2 carboxylate headgroups, and a hexanediamide spacer. We investigated the properties of gemini surfactants for biodegradability, surface tensiometry, and DLS measurements as well as the emulsification properties of their aqueous solutions and squalane. Gemini surfactants with alkyl chain lengths of 10 and 12 efficiently adsorbed and oriented at the air/water interface despite the bulky spacer structure, which is analogous to conventional surfactants. When the chain length was increased to 14 carbons, the surfactants showed poor adsorption and packing at the air/water interface. The CMC values decreased with an increase in alkyl chain length from 10 to 12, while the increase in chain length to 14 resulted in an increased CMC, indicating a low aggregation tendency such as premicelle formation at concentrations below the CMC. This has been reported for various gemini surfactants with alkyl chains of length above 14 or 16. Gemini surfactants formed compact small micelles in solution and showed no dependence on alkyl chain length due to the large curvature of the molecule. Furthermore, aqueous solutions of gemini surfactants exhibited stable oil-in-water type emulsions with droplet sizes of 1.5 – 1.8 μm on shaking with squalane in the same volume fraction or twice the volume of water to squalane.

A large number of gemini surfactants have been designed and synthesized in many studies and their properties have been investigated. However, there has been little research regarding amino acid-based gemini surfactants that are environmentally friendly. The results of this study may facilitate future studies regarding the structure-property relationships of new gemini surfactants.

References

10) Pérez, L.; Pinazo, A.; Rosen, M. J.; Infante, M. R. Surface active properties at equilibrium of novel gemini


