Fundamental Properties of Amphoteric Surfactant 3-(N'-Acyl)-aminopropyl-N,N-dimethyl-N-carboxymethyl Betaine

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Abstract: A new amphoteric betaine-type surfactant of 3-(N'-acyl)-aminopropyl-N,N-dimethyl-N-carboxymethyl betaine (Cn-APNB) was prepared, whose biodegradation and hydrolysis pose no environmental problems. The physicochemical properties and surface activities such as critical micelle concentration (cmc), surface tension, foaming power, sedimentation and surface potential (adsorption) of TiO2 particles in the presence of the surfactant were measured and biodegradability and antimicrobial features were also evaluated. The mixed system of Cn-APNB with soap exhibited synergistic effect on surface tension and foaming power at a weight ratio of 1 : 9. Antimicrobial activity forward Escherichia coli and Enterococcus was weakened by the introduction of an amidopropyl group and effects on the aquatic environment were minimized.

Key words: amphoteric surfactant, betaine surfactant, antimicrobial activity, titanium dioxide

Introduction

An amphoteric surfactant containing carboxylic acid is classified into betaine type, imidazoline type, β-alanine type and α-amino type. Recently, amphoteric surfactants of various chemical structures have been widely applied to cosmetics, shampoos, detergents etc. on an industrial scale. The synthesis and interfacial phenomena for the betaine type have been reviewed and summarized1) 2). The present authors have already published works on a series of β-alanine type surfactants having hydroxyl groups3) 12). A sulfo-betaine type surfactant provides excellent lime soap dispersion properties and good detergency performances as reported by Linfield and his coworkers13) 18). An amido betaine surfactant is non-irritating for human skin. It is particularly useful for hair-shampoo, body-shampoo, textile softener etc. 3-(N'-acyl)-aminopropyl-N,N-dimethyl-N-carboxymethyl betaine surfactant (Cn-APNB) is expected to be milder than 3-(N'-acyl)-aminopropyl-N,N-dimethyl-N-sulfopropyl betaine18). Amphoteric surfactants having a peptide structure easily undergo hydrolysis or biodegradation. Therefore, a degradable surfactant would be preferred from the environmental standpoint of aquatic protection.

In this paper, sodium N'- (alkanoyl)amidopropyl-N,N-dimethyl ammonio acetate homologues (Cn-APNB) were synthesized and physicochemical properties such as cmc, surface tension, foaming power, sedimentation time, and adsorption onto a TiO2 surface were examined. Further, the mixed Cn-APNB/soap system was also evaluated concerning foaming power and surface tension. The biodegradability and antimicrobial activity were also evalu-
Experimental

Preparation

The synthesis of the betaine consists of two processes; amidolysis and quaternary ammonolysis:

\[
\text{RCOOCH}_3\text{H}_2\text{N(CH}_2\text{)}_2\text{N(CH}_3\text{)}_2 \rightarrow \text{RCONH(CH}_2\text{)}_2\text{N(CH}_3\text{)}_2\text{+CH}_3\text{OH} \\
\text{RCONH(CH}_2\text{)}_2\text{N(CH}_3\text{)}_2\text{+ClCH}_2\text{COONa} \rightarrow \text{RCONH(CH}_2\text{)}_2\text{N}^+(\text{CH}_3\text{)}_2\text{CH}_2\text{COONa}
\]

where R stands for \(\text{C}_n\text{H}_{2n-1}\) \((n=10, 12, 14 \text{ and } 16)\). First, not less than 98% of methyl alkylcarboxylate (1.15 mol) was reacted with dimethylaminopropylamine (1.15 mol, 117 g) in the presence of sodium methoxide for 3 h at 90~100°C. After raising the temperature to 130°C, the unchanged amine is removed by topping under reduced pressure (30 mmHg) for 3~4 h. The total amine value is 197~203. During cooling, the reaction mixture was purged with nitrogen gas at 60~70°C. Subsequently, the 3-(N'-acyl)-aminopropyl-N, N-dimethyl amine obtained was quaternized using a mixture of aqueous sodium monochloroacetate and aqueous sodium hydroxide solution, the reaction temperature being maintained at 80°C for 5 h. After cooling the reaction product was neutralized to pH 7.0~7.2 with aqueous hydrochloric acid solution. The elemental analyses for C, H and N of these betaine homologues were within 1.3% of the calculated value. The structure of these betaine amphoterics was confirmed by IR analysis; 3350~3200 cm\(^{-1}\) (N-H, str. sec amide), 1640 and 1607 cm\(^{-1}\) (COO-, str.), 996 cm\(^{-1}\) (C-N, quaternary ammonium ion) and 870 cm\(^{-1}\) (N-H bending). The \(^1\)H-NMR spectra were also consistent with the structures of betaine homologues; 0.85 ppm (terminal \(-\text{CH}_3\) of long alkyl chain), 1.26 ppm \((-(\text{CH}_2)_n\text{)}, n=10, 12, 14 \text{ and } 16)\), 1.57 ppm \((-\text{CONH-C-CH}_2\text{C-N}^+)\), 1.97 ppm \((-\text{C-C-CH}_2\text{N}^+)\), 2.21 ppm \((-\text{CH}_2\text{-CONH-})\); 3.22 ppm (two methyl substituents of the ammonium ion), 3.59 ppm \((-\text{CONH-CH}_2\text{-})\), and 3.86 ppm \((\text{N}^+\text{-CH}_2\text{-COO-})\). The \(^{13}\)C-NMR spectra (500 MHz, D\(_2\)O) were also identified with the following predominant signals; 161.84 ppm \((\text{COO}-)\), 154.77 ppm \((\text{CONH-})\), 50.00 and 48.10 ppm \((-\text{N}^+\text{-}(\text{CH}_3)_2\text{)}\), 8.81 ppm \((\text{CH}_3^-)\), 11.97 ppm \((\text{CH}_3^-\text{-CH}_2^-)\). N,N-dimethyl dodecyl betaine (C\(_{12}\)-NB, C\(_{12}\)H\(_{25}\)N\(^+\)(CH\(_3\)_2CH\(_2\)COONa) purchased from Toho Chem. Ind. Co. Ltd., was employed as a reference sample.

Physicochemical and surface active properties.

The surface tension was measured by the Wilhelmy method with a Simadzu ST-1 tensiometer at 40°C. The foaming power for 0.1 wt% solution was measured at 40°C and pH 10 according to the Ross-Miles procedure. The foam height (mm) was recorded immediately after dropping the test sample and after standing for 5 min\(^3\). The sedimentation velocity power was measured by the campus disc procedure according to the Japanese Industrial Standard JIS L-1096 (1990) 6.26.3\(^\alpha\). The fabrics of wool felt (1 cm \(\times\) 1 cm, 2 mm width) which was deoiled under refluxing with diethyl ether and further with benzene/ethanol (1 : 1 vol/vol) for 8 h were employed for the sedimentation test. The lime soap dispersing requirement (LSDR), which means the number of grams required to disperse 100 g of oleic acid soap, was measured by Linfield’s procedure\(^{14}\)~\(^{16}\). Infrared spectra were obtained with a JASCO FTIR-5000 FT-IR spectrophotometer by a KBr disk method. The \(^1\)H-and \(^{13}\)C-NMR spectra were recorded with a JEOL FT-NMR (500-MHz) spectrophotometer. The measurement of \(\zeta\)-potential was carried out for a surfactant solution (50 mL) adjusted at the fixed pH value in the presence of TiO\(_2\) particles (2 mg, anatase 80%, Degussa P-25) with a Laser Zee Meter 501 (Pen. Kem Co. & Ltd.).

Biodegradability and antimicrobial activity assay

A biodegradation test of C\(_{12}\)-APNB was measured according to the orange II absorption procedure [JIS-K 3363 (1976)]. The bacterial source was an activated sludge in the wastewater treatment plant at Minamitama in the Tama river, Tokyo. The biodegradability for a ref
Reference reagent of sodium dodecyl sulfate (22 mg/L) was 98.8% after 7 to 8 days. Antimicrobial activities against *Escherichia coli* and *Enterococcus* were evaluated by a tube dilution procedure. The minimum inhibitory concentration (MIC) was judged by a plate culture method\(^{19,20}\). Reagent grade of dodecyltrimethylammonium chloride (C\(_{12}\)-TAC) and octadecyltrimethylammonium chloride (C\(_{18}\)-TAC) supplied from Wako Pure Chem. Ind. Ltd., was employed as a reference standard.

**Results and discussion**

The relationship between log cmc and carbon number of APNB is shown in Fig. 1. The cmc was determined by the break point of surface tension vs. concentration. The cmc values decreased with increasing alkyl chain length in the following order:

\[
\text{C}_{16}(0.008 \text{ mM}) < \text{C}_{14}(0.06 \text{ mM}) < \text{C}_{12}(0.45 \text{ mM}) < \text{C}_{10}(3.4 \text{ mM})
\]

A linear relationship of log cmc vs. carbon number (N) was obtained; \(\log \text{cmc} = 1.91 - 0.438\) N. As the alkyl chain length increases, the cmc values becomes smaller and generally the micelle aggregation number increases. In comparison with the cmc of C\(_{12}\)-NB, C\(_{12}\)-APNB exhibited a lower cmc than C\(_{12}\)-NB (1.58 mM) which implies that C\(_{12}\)-APNB has a larger aggregation number. The -CONH-structure in the alkyl chain of APNB is surmised to make larger bond behavior through intermolecular hydrogen bond of mutual surfactants together.

The effect of the alkyl chain of APNB on the foaming power is shown in Fig. 2. The foam of the C\(_{10}\)-homologue was poor, whereas the C\(_{12}\)-homologue exhibited the greatest foaming power and even 5 min after dropping the APNB solution, the foam height remained the same. The C\(_{14}\)-APNB gave a relatively greater foam height (above 150 mm). The C\(_{16}\)-homologue showed a lower foam height because of hydrophobicity. The foam height of C\(_{12}\)-APNB was higher than that of C\(_{12}\)-NB.

The surface tension for each alkyl derivative solution of 0.1 wt% is also depicted in Fig. 2. The surface tension of C\(_n\)-APNB decreased with increasing alkyl chain length. The C\(_{10}\)-
APNB showed the highest surface tension, but the C₁₆-homologue was still lower. C₁₂-APNB exhibited a slightly higher value than C₁₂-NB.

**Fig. 3** shows the effect of the carbon number of APNB on sedimentation time. The C₁₀-
APNB gave longer sedimentation time. The C_{12}-APNB exhibited a minimal sedimentation time, which can be related to the water absorbing capacity. That of C_{14}- and C_{16}-homologues are higher than of C_{12}-APNB. The fact means that C_{12}-APNB has good properties of penetration or hydrous affinity into a wool felt. The referenced C_{12}-NB gave a similar value to

**Fig. 4** Lime Soap Dispersing Requirement (LSDR) on the Alkyl Chain Length of APNB in the Hard Water (CaCO_3 333 ppm) for C_n-APNB (○), C_{12}-NB (△) and C_{12}-DS (□). LSDR is the number of grams required for the dispersion of 100 g of oleic acid soap.

**Fig. 5** Effect of the Composition Ratio of C_{12}-DS/C_{12}-APNB on Foaming Power Immediately after Dipping (○) and after Standing for 5 min (●) and Surface Tension (□). The foam height for the surfactant solution (0.25 wt%) was measured at 40°C and pH=10.0.
C_{12}-APNB.

The effect of alkyl chain length on the lime soap dispersing requirement (LSDR) of APNB is depicted in Fig. 4. The C_{12}-APNB exhibited the lowest LSDR among APNB homologues. In comparison with the same dodecyl chain, the LSDR value of C_{12}-APNB is lower than that of C_{12}-NB (18 g) and sodium dodecyl sulfate (C_{12}-DS) (30 g). Irrespective of alkyl chain length of APNB, the LSDR exhibited a definite value (7-8 g). The LSDR was not affected by the alkyl chain length of C_n-APNB. The LSDR of C_n-APNB was almost steady irrespective of alkyl chain length.

The foaming power and surface tension for the C_{12}-DS/C_{12}-APNB mixed system are illustrated in Fig. 5. The foaming power was the highest at the 0.9/0.1 composition ratio. A synergistic effect was observed for the mixed system in comparison with the individual C_{12}-DS or C_{12}-APNB system. Similarly, the 0.9/0.1 mixed system of C_{12}-DS/C_{12}-APNB showed the minimum surface tension. The complex formation by the mixed micelle was estimated to yield a 0.9/0.1 composition of C_{12}-DS/C_{12}-APNB.

The ionic structure of APNB is governed by pH variation as follows:

\[
\begin{align*}
\text{C}_{n-1}\text{H}_{2n-1}\text{CONH}-(\text{CH}_2)_3\n^+\text{CH}_2\text{COOH} & \quad \text{(acidic side)} \\
\uparrow & \\
\text{C}_{n-1}\text{H}_{2n-1}\text{CONH}-(\text{CH}_2)_3\n^+\text{CH}_2\text{COO}^- & \quad \text{(neutral side)} \\
\uparrow & \\
\text{C}_{n-1}\text{H}_{2n-1}\text{CONH}-(\text{CH}_2)_3\n^+\text{CH}_2\text{COO}^- & \quad \text{(alkaline side)}
\end{align*}
\]

APNB shows a cationic form in acidic media, a zwitterionic form at the isoelectric point, and an anionic form in alkaline media. TiO_2 exhibits amphoteric properties (isoelectric point 6.8); pH variations of the dispersion cause a change in the surface changes of the TiO_2 particles^{21}.

\[
\text{(acidic side)} \quad \text{(neutral side)} \quad \text{(alkaline side)}
\]

With respect to C_{12}-APNB, in the acidic side below pH=4, the \(\zeta\)-potential is positive due to the effect of quaternary ammonium cation as shown in Fig. 6. As the pH value changes from pH=4 to pH=6, the potential becomes negative because of the formation of carboxyl anion (\(-\text{COO}^-\)) and the effect of \(-\text{CONH}\)-group. However, in the neighborhood of pH=7, C_{12}-APNB absorbed on the TiO_2 surface is desorbed owing to zero net charge to form immiscible aggregates in aqueous bulk solution. Therefore, the TiO_2 surface becomes naked, and the \(\zeta\)-potential shows maximum. At the alkaline side above pH=8, the \(\zeta\)-potential is shifted more negative by the formation of both \(\gamma\text{TiO}^-\) and \(\text{COO}^-\) group. On the other hand, no maximal phenomenon was observed with respect to C_{12}-NB which exhibits a positive potential below pH=7, zero potential at about pH=7 and negative potential in the range above pH=7.
Biodegradability and antimicrobial properties

The biodegradability of C12-APNB is not less than 97% for the concentration of 29 mg/L after culturing of test organism solution for 7 to 8 days. This amidobetaine is very easily biodegradable because of cleavage of the peptide bond. The antibacterial activity of C12-APNB was evaluated in comparison with that of C12-NB, C12-TAC and C18-TAC as summarized in Table 1.

<table>
<thead>
<tr>
<th>Microorganism concentration</th>
<th>Escherichia coli $10^6$ cells/mL</th>
<th>Enterococcus $10^6$ cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12-APNB</td>
<td>$&gt;10000$</td>
<td>500</td>
</tr>
<tr>
<td>C12-NB</td>
<td>2000</td>
<td>250</td>
</tr>
<tr>
<td>C12-TAC</td>
<td>250</td>
<td>20</td>
</tr>
<tr>
<td>C18-TAC</td>
<td>1000</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Biodegradability and antimicrobial properties

Generally, an antimicrobial property is profoundly affected by a hydrophobic chain length\(^{22,23}\). Since C12-APNB is corresponding to sixteen molecular length of a hydrophobic chain containing a peptide bond, the antimicrobial activity of C12-APNB is further weak. In comparison with the same alkyl chain, C12-TAC showed lower minimum inhibitory concentration (MIC) than C12-NB (8 times lower for *Escherichia coli* and 12.5 times lower for *Enterococcus*), which is attributable to the effect of –COOH group. Generally, the relationship be-
tween antimicrobial activity and biodegradability is reciprocal. However, C12-APNB has mildly ecological characteristics, probably due to both properties.

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References

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[総説] コレステロール分子系の集合形態と認識機能
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コレステロールは分子間相互作用による自己合成分を有していることから、環境を反映した集合体を容易に形成する。本総説では、クラウンエーテル、ポリオキシメチルアミノプロピルのコレステロール誘導体を液滴、ゲル、水面単分子膜として用いたときの会合挙動と認識機能について述べた。

[報文] 両性界面活性剤 3-(N'-アシル)-アミノプロピル-N,N-ジメチル-N-カルボキシメチルペタインの基礎的性質
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ペプチド構造を有する 3-(N'-アシル)-アミノプロピル-N,N-ジメチル-N-カルボキシメチルペタイン型両性界面活性剤は容易に加水分解して生分解されるため、水質保全の観点から有用な界面活性剤である。単系体での emc、表面張力、起泡性、浸透性、および TiO2 表面への吸着など物理化学的性質や生分解性及び抗菌性などの測定を行い、混合系では起泡性および表面張力を測定した。3-(N'-アシル)-アミノプロピル-N,N-ジメチル-N-カルボキシメチルペタインはセッケンと混合ミセルを形成して、特に前者と後者の重量比 1/9 においては相乗効果により表面張力は低下し、起泡力が増大した。TiO2 共存下における電位は pH 6 で極小、pH 8 付近で極大を示し複雑な挙動を示す。大腸菌および腸球菌の平板法による最少発育抑制濃度 (MIC) は、アミドプロピル基の長さによりさらに高められ、環境への影響が少ない。

[報文] 狭い付加モル分布をもつポリオキシエチレン/ポリオキシプロピレン型非イオン界面活性剤の性質
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複合金属触媒固体触媒を用いて高級アルコールにエチレンオキシド (EO) およびプロピレンオキシド (PO) をブロック重合させたアルコール EO/PO 付加体（NREP）は、塩基触媒を用いた同型の EO/PO 付加体（BREP）に比べ、素早い泡切れを示す。これは EO/PO 付加モル分布が狭いため未反応アルコール等の砕水性成分が減少したこと、あるいは末端の PO 付加構造が変化したため気-液界面における NREP の分子占有面積が増加することで起因するものと考察される。また、原料に亜硫酸アルコールを用いた場合、この泡切れの良さは更に促進される。このように特異な泡挙動をもつ NREP は、非イオン界面活性剤としても優れた特徴をもつ、その生分解性も BREP と比べ優れている。