INTRODUCTION

C31G is a unique mixture of synthetic, amphoteric surface active compounds which demonstrate broad spectrum antimicrobial properties when buffered with citric acid to a pH of approximately 5. A specific formulation of C31G contains equal molar concentrations of alkyl dimethyl glycine (alkyl N-betaine) (2) and alkyl N, N-dimethylamine oxide (1).

\[
\text{CH}_3
\begin{array}{c}
\text{R}_{12} \quad \text{N} \\
\text{CH}_3
\end{array}
\]

1 Alkyl N,N-dimethylamine oxide

\[
\text{CH}_3
\begin{array}{c}
\text{R}_{12} \quad \text{N}^+ \quad \text{CH}_2 \quad \text{C} \quad \text{O}^-
\end{array}
\]

2 Alkyl N-betaine

C31G is a mole to mole preparation of these two amphoteric surfactants. The specific gravity of this yellow transparent liquid is 1 ± 0.005 at 25°C. When C31G is diluted with distilled water, the pH of the final solution will be 4.8 ± 1 and it is stable at temperatures below 50°C. Frozen form of the mixture will thaw at room temperature to its original clear, homogeneous solution. C31G is effective on both gram positive and gram negative bacteria and its fungicidal activity is as low as 13 ppm (1-3). The mode of action of this antimicrobial agent is related to its absorption by glycolipid cell walls and its lytic effects on the cell membranes of the microorganisms(4). It promotes the healing of infected and non-infected wounds. This biological activity is attributed to its effect on fibrin formation(5). This composition also exhibits skin degreasing, cleansing and deodorizing properties(1).

Results of in vitro experiments have shown that the antimicrobial effectiveness of C31G is comparable or even superior to widely used topical dis-
infecting agents such as hexachlorophene, benzalkonium chloride and chlorhexidine gluconate\textsuperscript{(6)}. It has been established that the antimicrobial action of the surfactants is attributed to their physical properties, especially to their surface activity\textsuperscript{(7, 8)}. The purpose of the present study is to investigate the interfacial properties of C31G and also to evaluate the relationship between its antimicrobial effectiveness and surface activity.

**EXPERIMENTAL**

**Materials**

C31G (Batch No:001) is supplied by Hunterdon Pharmaceuticals, USA, and used without any further purification.

Surface tension measurements were performed using an interfacial tensiometer, Du Nuoy, Type «OS» Balance, White Elec. Co. Ltd., UK. A thermostated water bath connected to a water jacket was used to obtain constant experimental temperatures.

**Methods**

19 aqueous solutions at varying concentrations in the range of $1.91 \times 10^{-6}$ to $0.04$ mol/l were prepared by dilution using distilled water. The solutions were prepared 10-12 hours before the surface tension measurements and the fresh solution-air interface was kept undisturbed for 3-5 minutes before each measurement. Surface tensions were determined at seven different temperatures: 10°C, 20°C, 25°C, 30°C, 40°C, 50°C and 60°C.

**RESULTS AND DISCUSSION**

Figures 1-7 illustrate the plots of surface tension vs log molar concentration at seven different temperatures. The data from the linear parts of the curves is shown in Table I. Critical micelle concentrations (CMC) were obtained from the extrapolation of two segments of the surface tension vs log C plots and were listed in

![Fig. 1 - Surface tension variation of C31C as a function of log molar concentration at 10°C, Key: (●), parabolic curve; (+), linear part of the curve.](image1)

![Fig. 2 - Surface tension variation of C31C as a function of log molar concentration at 20°C, Key: (●), parabolic curve; (+), linear part of the curve.](image2)

![Fig. 3 - Surface tension variation of C31C as a function of log molar concentration at 25°C, Key: (●), parabolic curve; (+), linear part of the curve.](image3)

![Fig. 4 - Surface tension variation of C31C as a function of log molar concentration at 30°C, Key: (●), parabolic curve; (+), linear part of the curve.](image4)
Table II. Figure 8 illustrates the CMC variation as a function of temperature. The CMC decreases with temperature initially to 40°C and then it arises rapidly. This behaviour is similar to that reported for non-ionic surfactants since the amphoterics exist in solutions as zwitterionic form. According to the findings of the former workers(9), at 25°C, critical micelle concentrations of some quaternary ammonium salts varied between 20X10⁻³ and 2.3X10⁻³ mol/l. In the present study, lower CMC values of C31G were obtained. As it is known that the amphoteric surfactants possess more surface activity than tonics have, containing the same hydrophilic group since the oppositely charged ions are neutralized. Therefore, it can be considered that C31G is an advant-

**Table 1 - Statistical Data of the Linear Parts of Surface Tension vs Log C Plots of C31G**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Concentration range (10⁻⁵ mol/l)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.91 - 57.5</td>
<td>-23.67</td>
<td>-41.49</td>
<td>0.991</td>
</tr>
<tr>
<td>20</td>
<td>1.91 - 57.5</td>
<td>-22.44</td>
<td>-41.76</td>
<td>0.986</td>
</tr>
<tr>
<td>25</td>
<td>1.34 - 57.5</td>
<td>-22.24</td>
<td>-41.82</td>
<td>0.995</td>
</tr>
<tr>
<td>30</td>
<td>1.34 - 57.5</td>
<td>-21.88</td>
<td>-40.82</td>
<td>0.990</td>
</tr>
<tr>
<td>40</td>
<td>0.96 - 57.5</td>
<td>-22.51</td>
<td>-46.29</td>
<td>0.993</td>
</tr>
<tr>
<td>50</td>
<td>0.96 - 57.5</td>
<td>-19.31</td>
<td>-36.16</td>
<td>0.989</td>
</tr>
<tr>
<td>60</td>
<td>0.57 - 57.5</td>
<td>-14.08</td>
<td>-17.51</td>
<td>0.976</td>
</tr>
</tbody>
</table>

**Table 2 - Critical Micelle Concentrations (CMC) of C31G as a Function of Temperature**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>CMC (x10⁻⁴ mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6.94</td>
</tr>
<tr>
<td>20</td>
<td>5.36</td>
</tr>
<tr>
<td>25</td>
<td>5.13</td>
</tr>
<tr>
<td>30</td>
<td>4.58</td>
</tr>
<tr>
<td>40</td>
<td>3.59</td>
</tr>
<tr>
<td>50</td>
<td>3.50</td>
</tr>
<tr>
<td>60</td>
<td>4.26</td>
</tr>
</tbody>
</table>

ageous ancimicrobial agent due to its amphoteric behaviour when compared to cationic surfactants such as benzalkonium chloride. On the other hand, Rosen et al.(10) reported that the synergisms in surface tension reduction and in mixed micelle formation occurred in a binary surfactant mixtures containing betaine type zwitterionics. They found that the CMCs of or of the alkyl N-betaine and its mixture with an anionic surfactant at 25°C were 5.52 x 10⁻⁴ and 4.5 x 10⁻⁴ mol/l respectively.
The CMC of C31G mixture was found similar to the Rosen's finding for alkyl N-betaine. This suggests that the contribution of an amphoteric surfactant to the synergism in micelle formation is not significant. According to the findings of the present study, the surface tension lowering occurred by C31G at 0.005% concentration (46.17 mN/m) was found more than benzalkonium chloride did (60.38 mN/m) as given in a former study (11). The surface activity is important not only from the physicochemical point of view, but also because of the use of surfactants as the antimicrobial agents in many pharmaceutical formulations. It has been suggested that the antimicrobial action of surfactants is related to their surface activity (8). Zisman's suggestion for quaternary ammonium compounds explains that the solutions having equal antimicrobial activity possess surface tension values at the same order of magnitude (12). On the other hand, Ecanow et al. (13) defined the thermodynamic activities of the bactericidal surfactants as the ratio of the minimum bactericidal concentration (MCC) to the CMC. Afterwards this value (MCC/CMC) was calculated for quaternary ammonium salts by Weiner et al. (9). The authors, in their other work (7), also defined the thermodynamic activity as the ratio of the Gibbs surface (excess) concentration at the MCC to that at the CMC. Although the surface properties and thermodynamics of adsorption of alkyl betaines has been investigated by some workers (10,14,15), no studies have been performed concerning the interfacial properties of C31G. And also, the bacterial and antifungal concentrations (MIC, MCC) of C31G were determined by former workers (1,16).

In order to obtain accurate interfacial and thermodynamic data, the surface pressure values (surface tension of solvent minus surface tension of solution) were plotted against log C. Because of the parabolic nature of the plots, the polynomial equations of the curves were computed to determine the slopes \( d\pi/d\log C \). Then, the values of surface (excess) concentration (T), area per molecule (A) and standard free energy of adsorption (\( \Delta G^\circ \)) were calculated from the slopes of the curves extrapolated at zero concentration. Table III shows the thermodynamic and interfacial data of C31G obtained by the application of the Gibbs adsorption equation to the finding of the present study.

As it is pointed out in a recent study performed with the zwitterionic surfactant (7), the negative values of Gibb's free energy suggested a strong interaction between surfactant and water, those interactions would tend to hinder the self-association process of the surfactant. The data of the present study in good agreement with the above-mentioned conclusion. Figure 10 also illustrates the free energy change of adsorption of C31G at the air-water interface as a function of temperature. In addition, thermodynamic activities based on the critical micelle concentrations and surface concentrations are shown in Table IV. The thermodynamic activity necessary for killing the microorganisms by C31G was found 0.99. This value was greater than that Weiner's findings for quaternary ammonium salts (max 0.93) (7). Thus, such a comparison between C31G and quaternary ammonium salts based on their surface active properties will contribute to evaluate the antimicrobial effectiveness of this new amphoteric surfactant mixture.

### Table III - Interfacial and Thermodynamic Parameters of C31G at Various Temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Slope* ( (\pi / \log C) )</th>
<th>Surface (Excess) Concentration ( (x 10^{-6} \text{ mole/m}^2) )</th>
<th>Area per Mole. ( (x 10^{-20} \text{ m}^2/\text{mole}) )</th>
<th>Free Energy ( (\Delta G^\circ) ) (K.Cal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>283</td>
<td>72.30 x 10^{-3}</td>
<td>11.24</td>
<td>14.77</td>
<td>-6.18</td>
</tr>
<tr>
<td>293</td>
<td>53.03 x 10^{-3}</td>
<td>9.14</td>
<td>18.16</td>
<td>-7.15</td>
</tr>
<tr>
<td>298</td>
<td>58.37 x 10^{-3}</td>
<td>11.00</td>
<td>15.09</td>
<td>-7.04</td>
</tr>
<tr>
<td>303</td>
<td>58.84 x 10^{-3}</td>
<td>11.01</td>
<td>15.08</td>
<td>-7.14</td>
</tr>
<tr>
<td>313</td>
<td>51.02 x 10^{-3}</td>
<td>8.79</td>
<td>18.89</td>
<td>-7.74</td>
</tr>
<tr>
<td>323</td>
<td>38.34 x 10^{-3}</td>
<td>6.61</td>
<td>25.12</td>
<td>-8.76</td>
</tr>
<tr>
<td>333</td>
<td>31.76 x 10^{-3}</td>
<td>5.47</td>
<td>30.35</td>
<td>-9.56</td>
</tr>
</tbody>
</table>

(*) The slopes, \( (\pi / \log C) \) → 0, were estimated from the polynomial equations of the surface pressure vs logC curves.
TABLE IV – THERMODYNAMIC ACTIVITY OF C31G BASED ON CMC AND SURFACE (EXCESS) CONCENTRATION AT 25°C

<table>
<thead>
<tr>
<th>MCC/CMC*</th>
<th>PCMC (mol/m²)</th>
<th>PMCC/PCMC</th>
<th>∆G° a/w (K.Cal/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.048</td>
<td>10.10 x 10⁻⁶</td>
<td>0.99</td>
<td>-7.18</td>
</tr>
</tbody>
</table>

(*) Minimum bactericidal concentration against Pseudomonas aeruginosa (2.46 x 10⁻⁵ mol/l)

In conclusion, it can be considered that the results represented in this work are valuable for further evaluation of C31G as an additive or an active substance in pharmaceutical preparations such as contact lens wetting, cleansing soaking solutions.

Acknowledgement
The authors wish to thank the Pharmacetical Technology Department of Faculty of Pharmacy, Ankara University.

Fig. 9 – Standard free energy change of adsorption as a function of temperature

References