Synthesis and Properties of Cleavable Quaternary Ammonium Compounds

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Abstract: Quaternary ammonium compounds are widely used as antiseptic and disinfectant. It is been a concern that their widespread use will lead to an increase of environmental problems, therefore the development of biodegradable surfactants is necessary. The present research is aimed at the design of novel amphiphilic molecules with similar properties to those already known but more biodegradable. Based on benzalkonium chloride (BAC), novel carbonate cleavable surfactants (CBAC) were synthesized. The breakable carbonate sites make CBAC compounds more degradable and potentially more biodegradable than their non-cleavable BAC analogues. Natural products such as fatty alcohols (C₈-C₁₆) and N,N-dimethyl-2-aminoethanol were used as reagents for the synthesis of CBAC₈-16. These amphiphilic compounds were characterized in terms of surface properties and antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and moulds. The novel surfactants showed similar surface activities in aqueous solutions when compared to BAC. Also, the surface activity/structure relationship revealed that carbonate cleavable surfactants with n-decyl group (CBAC10) showed the same behaviour as non-cleavable BAC. Furthermore, compounds containing n-octyl (CBAC8), n-decyl (CBAC10) and n-dodecyl (CBAC12) group showed strong antimicrobial activities.

Key words: quaternary ammonium, cleavable surfactants, carbonate, surface activity, antimicrobial activity

1 Introduction

The world is currently suffering the consequences of a viral pandemic, known as COVID-19, caused by the SARS-CoV-2 virus. Recently, Environmental Protection Agency (EPA) published a list of those compounds that meet criteria for disinfecting surfaces to prevent disease transmission¹. This virus has a double lipid layer into its envelope that makes it vulnerable to quaternary ammonium surfactants of the benzalkonium chloride (BAC) type. Quaternary ammonium surfactants, whose general formula contains at least one long hydrocarbon chain linked to a positively charged nitrogen atom, are the most common cationic surfactants²-⁸. These molecules interact with microbial cells causing a disorder in the bilayer membranes that produces cell lysis and death⁹. For this reason, cationic surfactants are widely used as active ingredients in disinfectant and antiseptic formulations and are employed in households, human and animal healthcare facilities, agriculture, and industry⁵. Indeed, quaternary ammonium salts are considered as high production volume chemicals due to the large quantities used⁶. Unfortunately, after utilization, these surfactants, or part of them, reach into the environment⁷.

In fact, BAC and dialkyl dimethyl ammonium chloride (DDAC) are the most predominant types of quaternary ammonium disinfectant active ingredients, Fig. 1. In particular, BAC, a mixture of n-alkyl benzyl dimethyl ammonium chlorides, is toxic to aquatic organisms, not biodegradable under anaerobic conditions, and it only degrades slowly under aerobic conditions⁶,⁹,¹⁰. Consequently, in order to facilitate its degradation, or biodegradation, after use different functional groups have been incorporated into its structure as chemically cleavable sites¹¹,¹². Nowadays, the challenge is to develop surfactants easily degradable after application. To promote this situation, different functional groups as amide, esters, and carbonates have been incorporated in surfactant molecules as a hydro-
lysis site\textsuperscript{13}. Ideally, the compounds generated after hydrolysis should be biodegradable, renewable and/or reusable and should not be toxic, acid, corrosive, etc. Several authors reported that surfactants containing a carbonate group were readily biodegradable\textsuperscript{14}. The incorporation of carbonate moieties is a viable option in the design of cleavable surfactants. Indeed, carbonate bond is slightly more stable than ester bond and afford neutral alcohols under hydrolysis\textsuperscript{5, 15}. Given the severity of the COVID-19 pandemic and the recommendations on personal care and hygiene, in addition to the disinfection of surfaces and objects of daily use, the generation of disinfectant, non-toxic and non-polluting compounds is of vital importance. In this sense, the present work deals with the design, synthesis and characterisation of compounds similar to BAC, which are easily degradable and whose final products are environmentally friendly and biodegradable. With this aim, we synthesized and characterized a series of quaternary ammonium surfactants with carbonate linkages inserted between the hydrocarbon chain and the polar head (CBAC). Their physicochemical properties, such as surface tension, critical micelle concentration, and antimicrobial activity were evaluated. The results were analysed and compared with those of BAC. It is worth mentioning that the reagents and the substances generated by hydrolysis of the CBAC compounds are: renewable, biodegradable or reusable (Scheme 1). The degradation of these new surfactants leads to the generation of fatty alcohols and readily biodegradable quaternary ammonium alcohols non-toxic to aquatic organisms\textsuperscript{14}.

2 Experimental

2.1 Materials

All chemicals used in the synthesis procedures: 1-octanol, 1-decanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, guaiacol carbonate, \(N, N\)-dimethyl-2-aminoethanol (DMAE) and benzyl chloride, were of reagent grade and used as received from Sigma-Aldrich Co., Inc. All solvents: triethylamine (Et\(_3\)N), acetonitrile (ACN) and ethyl acetate, were analytical grade and used as received from Sigma-Aldrich Co., Inc.; Nutrient Agar (NA) and Malt Extract Agar (MEA) from Merck. Microbial strains: Gram-positive (Staphylococcus aureus, Micrococcus luteus) and Gram-negative (Pseudomonas sp., Escherichia coli) bacteria, yeasts (Candida tropicalis, Saccharomyces cerevisiae) and moulds (Penicillium roqueforti, Aspergillus niger) belong to the collection of the Chair of Microbiology of the Faculty of Chemical Engineering - National University of the Littoral (FIQ-UNL).

2.2 Preparation of carbonate cleavable surfactants (CBAC8-16)

Intermediate compounds AC8-16 were prepared following a similar procedure to that reported in the literature (Scheme 2)\textsuperscript{14}. In a 25 mL bottomed flask equipped with a magnetic stir bar, guaiacol carbonate (2.742 g, 0.010 mol), 1-alkanol (0.010 mol) and Et\(_3\)N (0.726 g, 0.010 mol) were heated at 80°C for 24 h. The intermediate compound AC8-16 was obtained. DMAE (0.802 g, 0.009 mol) was then added and the reaction was maintained at 80°C for another 24 h, after which, volatile compounds were removed by
evaporation at reduced pressure. Finally, quaternary ammonium compounds CBAC8-16 were obtained by quaternization of the intermediate AC8-16 with benzyl chloride (1.392 g, 0.011 mol) in ACN, at 80°C for 8 h with stirring. Purification was carried out by evaporation of the solvent and recrystallization of the crude product (ethyl acetate) to obtain the carbonate cleavable surfactants CBAC8-16 as a white powder (see Supplementary Information).

2.3 Surface properties

The static surface tension isotherms of aqueous solutions, pH 7, were measured at 25°C using a Cole-Parmer Surface Tensiomat 21 tensiometer by the Du Noüy ring method. Each recorded surface tension value (γ) in mN/m was the mean of three consecutive measurements. The critical micelle concentration (CMC) in mM and the surface tension at CMC (γ_{CMC}) in mN/m, the molar concentration of the surfactant required to produce a drop in surface tension of 20 mN/m (C_{20}) in mM, and the negative log of C_{20} (pC_{20}) were estimated from each surface tension vs. concentration on log scale curve. The surface excess concentration (Γ) in mol/m² and the occupation area of a molecule at a surface (A_{min}) in nm², were calculated using equations 1 and 2:

\[ \Gamma = \frac{-10^{-3} \frac{d\gamma}{d\log(M)}}{2.30nRT} \]  
\[ A_{min} = \frac{10^{18}}{N_{A} \Gamma} \]  

Where n is 2 for single-type cationic surfactants, dγ/dlog(M) is the slope of the curves below the CMC, T is the absolute temperature, R is the molar gas constant (8.314 J/mol K) and N_{A} is Avogadro’s number.

2.4 Antimicrobial activity

The antimicrobial activity and the minimum inhibitory concentration (MIC) of the novel cleavable carbonate surfactants CBAC8-16 against microbial strains were estimated using a protocol adapted from the well diffusion test.**

Accordingly, petri dishes, 90 mm in diameter, were filled with 15 mL of inoculated agar. NA for bacteria and MEA for yeasts and moulds were used. Holes, 8 mm in diameter, were cut out of the agar and 70 µL of CBAC8-16 aqueous solutions were introduced into each hole. Non-cleavable BAC was used as a reference drug and sterile water was used as a negative control. All procedures were performed with sterile instruments under sterile condition to avoid contamination. The petri dishes were incubated for 24 h under suitable conditions depending on the test microorganism: 37°C for bacteria or 30°C for yeast and moulds. A clear area around a test hole after the incubation period indicates that the compound was active against the evaluated microorganism. All measurements were performed in duplicate tests.

2.5 Hydrolytic degradation

Hydrolytic degradation tests were carried out by dissolving 20 mg of CBAC16 in 1 mL of: a) acetic acid (75%) ; b) hydrochloric acid solution (0.41 M) ; and c) sodium hydroxide solution (0.41 M) ; at 80°C for 20 h. The organic phases were analyzed by ^1H NMR in chloroform.

3 Results

To promote sustainable processes, the reagents and the substances generated by biodegradation of the surfactants, such as fatty alcohols and quaternary ammonium alcohols, are renewable, biodegradable or reusable. Also, the synthesis of CBAC was carried out at moderate reaction temperatures without isolation of the intermediate products (AC8-16 and C8-16) minimizing the amount of waste. CBAC8-16 compounds were obtained as a white powder in a suitable global yield of around 44-55%, Table 1. Moreover, guaiacol carbonate could be considered a renewable starting material because is a by-product obtained from lignin pyrolysis. In addition, guaiacol carbonate is considered a reusable material because can be regenerated. Because of commercial BAC is a mixture of n-alkyl benzyl...
dimethyl ammonium chlorides, the use of this kind of compounds or published data may not be related to our experimental results and lead to erroneous interpretations. Owing to this, benzyl dimethyl dodecyl ammonium chloride, BAC12, was synthesized and used as a reference drug for comparison purposes. Note that the molecules compared, CBAC8-16 and BAC12, have similar structure and the same counterion.

### 3.1 Surface properties

Figure 2 shows the plots and the breakpoint of the surface tension vs. molar concentration, log scale, curves for CBAC8-16 compounds and BAC12 in aqueous solution. Graphs illustrate a well-defined CMC and surface tension at the CMC for each pure compound. C₂₀, pC₂₀, CMC/C₂₀, Γ, and Amin are determined and listed in Table 1. A good linear relationship was found between the log(CMC) and the number of atoms in the linear chain (R² = 0.9958).

### 3.2 Antimicrobial activity

Regarding antimicrobial activity, it was evaluated against the bacterial and fungal strains listed in Table 2. For comparison purposes, BAC12 was also included as a reference drug while water was used as a negative control. First, a preliminary screening test was carried out. Thus, aqueous solutions (1500 ppm) of the synthesized molecules were assessed against the different microorganisms. The presence

![Fig. 2](image-url)  
**Fig. 2** Surface tension vs. concentration of CBAC8-16 and BAC12 in aqueous solution at 25°C.

### Table 1  Yield and surface properties.

| Compound | Yield% (mM) | CMC (mM) | γ<sub>CMC</sub> (mN/m) | C₂₀ (mM) | pC₂₀ | CMC/C₂₀ | Γ × 10⁶ (mol/m²) | Amin × 10¹³ (nm²) |
|----------|------------|----------|----------------|---------|-----|---------|----------------|-----------------|
| CBAC8    | 44         | 16.94    | 33.2           | 3.68    | 2.4 | 4.6     | 2.2            | 74.9            |
| CBAC10   | 46         | 3.89     | 32.6           | 0.58    | 3.2 | 6.7     | 1.9            | 89.8            |
| CBAC12   | 48         | 1.20     | 32.7           | 0.22    | 3.7 | 5.5     | 2.1            | 81.1            |
| CBAC14   | 45         | 1.04     | 34.0           | 0.08    | 4.1 | 12.4    | 1.3            | 129.4           |
| CBAC16   | 55         | 0.38     | 34.5           | 0.03    | 4.6 | 16.6    | 1.1            | 148.7           |
| BAC12<sup>b</sup> | 4.33 | 35.4 | 0.93 | 3.0 | 4.7 | 1.9 | 86.8 |

Experimental uncertainties are estimated to be ± 0.03 mM on CMC and ± 1 mN/m on γ<sub>CMC</sub> values.

<sup>a</sup> Isolated global yield.

<sup>b</sup> Non-cleavable benzyl dimethyl dodecyl ammonium chloride (BAC12).

| Compound | S. aureus | M. luteus | E. coli | P. sp | C. tropicalis | S. cerevisiae | P. roqueforti | A. niger |
|----------|-----------|-----------|---------|-------|---------------|---------------|---------------|---------|
| CBAC8    | +         | +         | +       | +     | +             | +             | +             | +       |
| CBAC10   | +         | +         | +       | +     | +             | +             | +             | +       |
| CBAC12   | +         | +         | +       | -     | +             | +             | +             | +       |
| CBAC14   | +         | +         | -       | -     | -             | -             | +             | -       |
| CBAC16   | -         | -         | -       | -     | -             | -             | +             | -       |
| BAC12    | +         | +         | +       | +     | +             | +             | +             | +       |

<sup>a</sup> Development was observed in the negative control 24 h.

(+) presence of an inhibition zone around the hole; (−) absence of inhibition zone.
Table 3 Quantitative antimicrobial activity of carbonate surfactants.

| Strain                  | CBAC8 (ppm) | CBAC8 (mM) | CBAC10 (ppm) | CBAC10 (mM) | CBAC12 (ppm) | CBAC12 (mM) | BAC12 (ppm) | BAC12 (mM) |
|-------------------------|-------------|-------------|--------------|-------------|--------------|-------------|-------------|------------|
| Gram-positive bacteria   |             |             |              |             |              |             |             |            |
| *S. aureus*             | 150         | 0.40        | 30           | 0.08        | 50           | 0.12        | 30          | 0.09       |
| *M. luteus*             | 400         | 1.08        | 30           | 0.08        | 40           | 0.09        | 10          | 0.03       |
| Gram-negative bacteria   |             |             |              |             |              |             |             |            |
| *E. coli*               | 950         | 2.55        | 90           | 0.23        | 100          | 0.23        | 30          | 0.09       |
| *P. sp*                 | 1250        | 3.36        | 950          | 2.38        | >1500        | >3.50       | 300         | 0.88       |
| Fungi: Yeasts            |             |             |              |             |              |             |             |            |
| *C. tropicalis*         | 400         | 1.08        | 20           | 0.05        | 350          | 0.82        | 30          | 0.09       |
| *S. cerevisiae*         | 1300        | 3.50        | 1000         | 2.50        | 550          | 1.28        | 30          | 0.09       |
| Fungi: Moulds           |             |             |              |             |              |             |             |            |
| *P. roqueforti*         | 1100        | 2.96        | 1000         | 2.50        | 1300         | 3.04        | 50          | 0.15       |
| *A. niger*              | 650         | 1.75        | 350          | 0.88        | 90           | 0.21        | 20          | 0.06       |

* Development was observed in the negative control 24 h.

of an inhibition zone around the hole was used as a criterion for the definition of an active compound. The results are shown in Table 2. Those compounds that showed strong antimicrobial activity, CBAC8, CBAC10, and CBAC12, were selected and evaluated quantitatively. MICs values expressed in ppm and mM are shown in Table 3 and Fig. 3.

3.3 Hydrolytic degradation
Hydrolytic degradation was analyzed by $^1$H NMR in chloroform using the aromatic signals and the methylene protons adjacent to the carbonate group. It was found that CBAC compounds were stable in acetic acid; partially hydrolysable in hydrochloric acid solution; and readily hydrolysable in sodium hydroxide solution (see Supplementary Information).

4 Discussion
4.1 Surface properties
Based on the calculated parameters, the efficacy in lowering the surface tension for the synthesized carbonate surfactants, in decreasing order, is: CBAC16 > CBAC14 > CBAC12 > CBAC10 > CBAC8 (Table 1 and Fig. 2). This behaviour is consistent with most of the results reported in the literature for cationic surfactants.

Concerning the effectiveness of the cationic surfactants, the lowest CMC, $\gamma_{\text{CMC}}$, and $C_{20}$ values distinguish surfactant CBAC16 as the most powerful of all compounds synthesized in this work. In addition, it was found that CBAC12, CBAC14 and CBAC16 exhibit better surface activity than BAC12. The $C_{20}$, $pC_{20}$ and $\text{CMC}/C_{20}$ values reveal a suitable tendency of the carbonate surfactants to adsorb at the surface and to reduce the surface tension. Moreover, $\Gamma$ and $\Gamma_{\text{mic}}$ values for CBAC8, CBAC10, and CBAC12 indicate that these surfactants may have been able to accommodate more efficiently their saturated chains at the air/water interface. Based on these observations, it can be concluded that the carbonate group is an appropriate linker between the hydrocarbon chain and the polar head.

It can be noted that CBAC10 is similar to BAC12 when the alkyl chain and ethylene carbons (-OCH2CH2-) were considered. Indeed, CBAC10 is comparable to BAC12 in all the surface parameters measured. Based on these observa-
main conclusions were drawn:

4.3 Hydrolytic degradation

Results show that the degradation of CBAC compounds under hydrolysis in hydrochloric acid solution and sodium hydroxide solution lead to the generation of non-toxic neutral alcohols and benzyl-choline. These results are in agreement with those reported in literature\(^1\). This behaviour could be attributed to differences in the cell envelope of Gram-positive and Gram-negative bacteria\(^2\). Results also have shown that Escherichia coli strain seems to be more sensitive than Saccharomyces cerevisiae, and that Aspergillus niger strain seems to be more sensitive than Penicillium roqueforti. It is noteworthy that there is no activity difference between CBAC10 and CBAC12, and their activities are milder than non-cleavable BAC. Therefore, in basis of environmental factors, the use of the new compound CBAC10 is preferred.

4.2 Antimicrobial activity

Results show that Staphylococcus aureus and Micrococcus luteus were more sensitive to CBAC8, CBAC10, and CBAC12 than Pseudomonas sp. and Escherichia coli (Table 2). These results are in agreement with those reported in literature\(^7\). This behaviour could be attributed to differences in the cell envelope of Gram-positive and Gram-negative bacteria\(^2\).

Results also have shown that Escherichia coli strain seems to be more sensitive than Pseudomonas sp. CBAC10 exhibits similar antimicrobial activity than BAC12 and it seems to be more active than CBAC8 and CBAC12 (Fig. 3). These results suggest that the inserted group could act as two extra methylene groups\(^14\), \(^23\). In addition, the synthesized compounds with the alkyl chain lengths from C\(_{6}\) to C\(_{12}\) exhibited moderate activity against yeasts and moulds. Results also have illustrated that Candida tropicalis strain was more sensitive than Penicillium roqueforti. It is noteworthy that there is no activity difference between CBAC10 and CBAC12, and their activities are milder than non-cleavable BAC. Therefore, in basis of environmental factors, the use of the new compound CBAC10 is preferred.

4 Conclusions

The influence of carbon chain size and the incorporation of carbonate cleavage sites on the surface properties and antimicrobial activity of benzalkonium chloride-like compounds was studied in aqueous solutions. The following main conclusions were drawn:

\- CBAC8, CBAC10, and CBAC12 compounds showed surface properties and antimicrobial activity comparable to the commercial BAC.

\- These novel cleavable compounds would be more degradable than the commercial BAC used as a reference, and their decomposition under hydrolysis lead to the generation of non-toxic readily biodegradable neutral alcohols and benzyl-choline.

\- The inserted carbonate group acts as an integral part of the non-polar tail, in the same way as two extra methylene groups.

\- Based on the structural similarity of these new hydrolysable surfactants with respect to BAC, and on comparable values from surface and biological tests, it is proposed that these new compounds would be effective in inactivating SARS-CoV-2 virus.

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