# Covalent Attachment of Quaternary Ammonium Compounds to a Polyethylene Surface via a Hydrolyzable Ester Linkage: Basis for a Controlled-Release System of Antiseptics from an Inert Surface

**P. J. McCubbin,[1]* E. Forbes,[1] M. M. Gow,[2] S. D. Gorham[1]**

[1]*Department of Chemistry and Chemical Engineering, University of Paisley, PA1 2BE, United Kingdom*
[2]*Department of Biological Sciences, University of Paisley, PA1 2BE, United Kingdom*

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**ABSTRACT:** The colonization of medical devices such as catheters, topical wound dressings, and surgical implants by micro-organisms is an ongoing problem, particularly as many strains of bacteria are becoming resistant to antibiotics. Such a problem may be addressed by a material surface that is able to provide a slow release of a disinfectant during its period of usage. To achieve this objective, a novel material was prepared in which a quaternary ammonium salt was covalently bound onto a polyethylene backbone via a hydrolyzable ester linkage, which provided a slow release of the disinfecting agent. A low-density polyethylene film was treated with glow discharge followed by the graft polymerization of acrylic acid. A tertiary amine function was introduced onto the film by the esterification of the carboxylic acid groups, via an acid chloride intermediate, with 4-hydroxy-*N*-methyl piperidine. The tertiary amine on the piperidine was then quaternized with a series of alkyl bromides of various chain lengths. The quaternary ammonium salt was released slowly by the hydrolysis of the ester bond over a 4-h period. To test the efficacy of the quaternary ammonium function itself, soluble compounds were prepared as follows. 4-Hydroxy-*N*-methyl piperidine was esterified with acetic anhydride and a corresponding series of quaternary ammonium salts prepared again by a reaction with alkyl bromides of various chain lengths. A preliminary microbiological survey of the materials included an investigation of the effect of the chain length as well as the efficacy of the soluble quaternary salts themselves. As expected, only the longer alkyl chains provided quaternary ammonium salts with bactericidal properties, chain lengths of less than 10 carbon atoms proving ineffective. Both the polymer-bound and soluble long-chain quaternary ammonium salts were effective against suspensions of *Staphylococcus aureus* and *Escherichia coli*. The results therefore indicate that such a system may well be useful in the development of biomedical materials such as surgical implants or dressings in which a slow release of a disinfectant or other physiologically active agent such as an anti-inflammatory drug may be required.
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**Key words:** biological applications of polymers; graft copolymers; polyethylene (PE); surfaces

## INTRODUCTION

The colonization of medical devices by micro-organisms is an ongoing problem.[1] Several approaches to this problem have been described in previous investigations.[1–9] For example, the bioburden at the skin infection site may be lowered by the application of povidone iodine, alcohol, or chlorohexidine or by the topical administration of antibiotics. In addition, daily flushing with heparin/vancomycin has been shown to reduce the frequency of catheter-related bacteraemia attributed to luminal colonization with Gram-positive organisms susceptible to vancomycin.[5]

Alternatively, antibiotics such as cefazolin can be adsorbed ionically onto the surface of a plastic that has been precoated with the quaternary ammonium compound tridodecylmethylammonium chloride.[6] The antibiotic is then released slowly from the surface by ion exchange. In further attempts to reduce sepsis, catheters have been coated with antiseptics such as silver sulfadiazine and chlorohexidine, and a reduction in colonization and bacteraemia has been observed.[7,8]

Another method of addressing this problem is to incorporate antiseptic substances into the plastic from which the catheter is manufactured.[8] This method allows a slow release of bacteriocide via diffusion from the polymer matrix. Clearly, any such agent that is incorporated into the plastic must be thermally stable to withstand the extrusion of the molten plastic. Alternatively, during manufacture, the polymer can be dissolved in a suitable solvent, and the antimicrobial agent can be added to the polymer solution,[9] after which the solvent is evaporated.

*Present Address: SMTL, Princess of Wales Hospital, Coity Road, Bridgend CF31 1RQ, UK.*
*Correspondence to:* S. D. Gorham (chem.ceng@paisley.ac.uk).


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Soluble polymers based on poly(glycidyl methacrylate) and containing bound quaternary ammonium and phosphonium groups have recently been reported in the literature.[10] Such polymers are effective against a range of Gram-positive and Gram-negative organisms. In addition, chemically modified poly(vinyl alcohol) has been described that contains quaternary ammonium functions bound by hydrolyzable ester groups to give a slow release of an antimicrobial agent.[11] Although these soluble materials were designed as antifouling paints and coatings, these findings suggest strongly that a similar approach may well be useful in the design of medical devices based on an insoluble plastic such as polyethylene (PE).

Our approach to the problem of bacterial colonization of medical devices is to provide a polymer surface to which an antimicrobial agent such as a quaternary ammonium salt is covalently bound. This linkage could be via an ester that would slowly hydrolyze, providing a release of the antiseptic component. Quaternary ammonium salts are well-known cationic molecules that are able to disrupt the bacterial cell membrane, causing the contents to leak out and resulting in the death of the cell.[12,13] Another advantage of choosing a disinfectant such as a quaternary ammonium salt is the emergence of multiantibiotic-resistant bacteria associated with catheters, vascular grafts, and prosthetic-joint-related infections.

To produce such a material, the polymer onto which the quaternary ammonium salt will be attached must either contain functional groups or be capable of being functionalized. A convenient way of introducing functional groups onto an inert polymer such as PE is the graft polymerization of a suitable monomer that itself contains functional groups, such as acrylic acid or acrylamide.[14–22]

To achieve this objective, the PE film was treated with glow discharge to produce free radicals and peroxides on the polymer surface before the treatment with acrylic acid to produce a grafted polymer of acrylic acid. The grafted poly(acrylic acid) so produced was subsequently reacted, via an acid chloride intermediate, with 4-hydroxy-*N*-methyl piperidine to form an ester. The tertiary amine function present on the piperidine was then further reacted with alkyl halides of various chain lengths to form the corresponding quaternary ammonium salts. Films so prepared were tested against both Gram-positive and Gram-negative micro-organisms for their antibacterial effect. To test the efficacy of the quaternary ammonium function itself against micro-organisms, soluble compounds were prepared from 4-hydroxy *N*-methyl piperidine, which was esterified with acetic anhydride before quaternization.

## EXPERIMENTAL

### Materials

All chemicals were purchased from Aldrich Chemicals, Ltd. (Poole, United Kingdom), and used without further purification, except for acrylic acid, which was purified by vacuum distillation before use. All solvents were analar-grade.

Low-density linear PE film was a gift from J. N. Samson (B.P. Chemicals, Grangemouth, Scotland).

IR spectra were recorded on a Nicolet (Warwick, UK) Impact Fourier transform infrared spectrometer, and the compounds were examined either as grafts on PE film or as solids in KBr pellets. UV spectra were recorded on a PerkinElmer (Beaconsfield, UK). Lamda 3 UV–vis spectrophotometer. $^1$H- and $^{13}$C-NMR were obtained in a deuterochloroform solution at 90 Mz with a JEOL (Welwyn Garden City, UK) Ex90 FT spectrometer with tetramethylsilane as a standard.

### Graft polymerization of acrylic acid onto PE

To initiate free-radical graft polymerization onto the PE surface, it was first subjected to plasma glow discharge with argon gas followed by immersion in a deaerated solution of acrylic acid monomer.[18] The PE film ($2 \times 2$ cm) was extracted for 16 h with methanol in a Soxhlet extraction apparatus to remove additives,[18] thoroughly dried, and placed in a gold-splutter apparatus with reversed polarity. The vessel was then purged with argon gas to displace the air, and a vacuum of 0.1 Torr applied. The apparatus was repurged with argon for 1 min, ionized for 60 s, and again purged with argon to release the pressure.

The graft polymerization of acrylic acid was then achieved by the immersion of the plasma-treated PE films in glass ampules containing a 20% (w/v) aqueous solutions of acrylic acid (10 mL) that had been purged with nitrogen. The ampules were sealed *in vacuo* and incubated at 60°C. After 2 h, the films were removed from the ampules, thoroughly washed with deionized water at 70°C overnight to remove all traces of the viscous homopolymer, rinsed again with deionized water at the ambient temperature, and dried after being washed with ethanol and ether.

The presence of the acid moiety on the film was confirmed by IR spectral data, which showed the appearance of the carbonyl group at 1715 cm$^{-1}$. The graft density, that is, the number of carboxyl groups grafted per centimeter squared, was determined with a dye binding assay method.[18]

### Determination of the graft density of the carboxyl groups

The method was essentially that described by Sano et al.[18] Carboxyl groups on the grafted film were complexed with a solution of toluidine blue O at pH 10 for

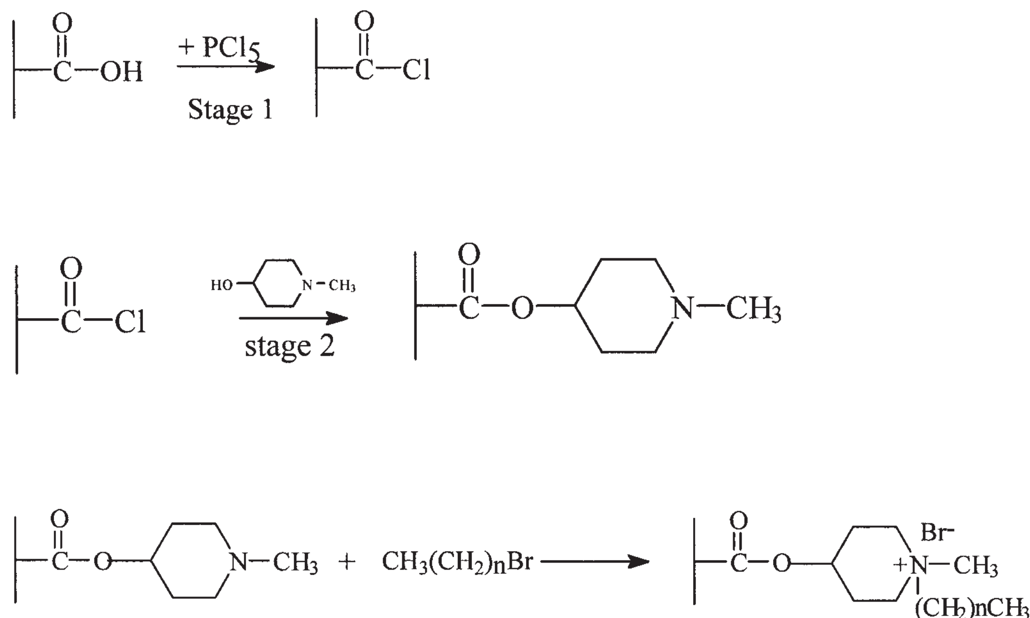


**Figure 1** Conversion of the grafted carboxylic acid to the quaternary salt ($n$ = 3, 5, 7, 9, 11, 15, or 17).

5 h at 30°C. The noncomplexed dye was removed with 0.1 m*M* sodium hydroxide, after which the desorption of dye molecules complexed to the grafted carboxyl groups was accomplished with a 50 vol % aqueous acetic acid solution. The amount of complexed toluidine blue was determined by the measurement of the absorbance at 633 nm. The total number of carboxyl groups grafted onto PE was then calculated under the assumption that each carboxyl group bound one molecule of toluidine blue.

### Acid chloride formation

Portions of pregrafted PE films (2 × 2 cm) were heated under reflux for 2 h with a solution of phosphorus pentachloride (1 g) in dry chloroform (20 mL). After reflux, the films were thoroughly washed in dry chloroform and dried. The conversion of the carboxylic acid to the acid chloride (see Fig. 1, stage 1) was confirmed by IR spectroscopy with the loss of the OH stretching vibration at 3300 $cm^{-1}$ and a shift of the carbonyl stretch from 1715 to 1780 $cm^{-1}$.

### Formation of the quaternary ammonium function

The acid chloride film was then refluxed with 4-hydroxy-*N*-methyl piperidine (1 g) in pyridine (30 mL) for 7 days. Ester formation (Fig. 1, stage 2) was confirmed by IR spectroscopy by the loss of the carbonyl stretching frequency of the acid chloride at 1780 $cm^{-1}$ and the appearance of the ester carbonyl at 1730 $cm^{-1}$.

After esterification, the tertiary amine of the piperidine ring was reacted with a series of long-chain alkyl bromides to form the corresponding quaternary ammonium salts (Fig. 1). This was achieved by the refluxing of the film (2 × 2 cm) in an ether solution (30 mL) with an excess of the appropriate alkyl bromide (1 g) for 7 days. The 1-alkyl bromides used were all straight-chain saturated compounds varying in length from 4 to 18 carbon atoms

The changes in the IR spectrum after quaternary ammonium salt formation were minimal. Therefore, to confirm that the reaction had occurred, the modified PE films were tested for the presence of bromide ions with a silver nitrate solution: a positive reaction was indicated by the formation of a precipitate of silver bromide. The presence of a quaternary ammonium salt was also confirmed by the stability studies described next.

### Stability studies: the hydrolysis of the ester linkage at pH 7

The stability of the ester linkage was investigated with a colorimetric assay in which free quaternary ammonium salts liberated into the solution were estimated with a modification of a standard literature procedure.[23] The quaternary ammonium film was incubated at the ambient temperature in a phosphate buffer of pH 7 (10 mL) for time periods of 5 min, 30 min, 60 min, 4 h, and 24 h. After incubation, the solutions were analyzed for the presence of the quaternary ammonium salt with the dye bromophenol blue as follows. The film was incubated with a mixture of 10% $Na_2CO_3$ (2 mL) and an aqueous solution of bromophenol blue (1 mL,0.4%). The mixture was then extracted with dichloromethane (10 mL), and the absorbance of the organic layer was measured at 604 nm. The method was standardized with cetylpyridinium bromide. The films were subsequently analyzed by IR spectroscopy.

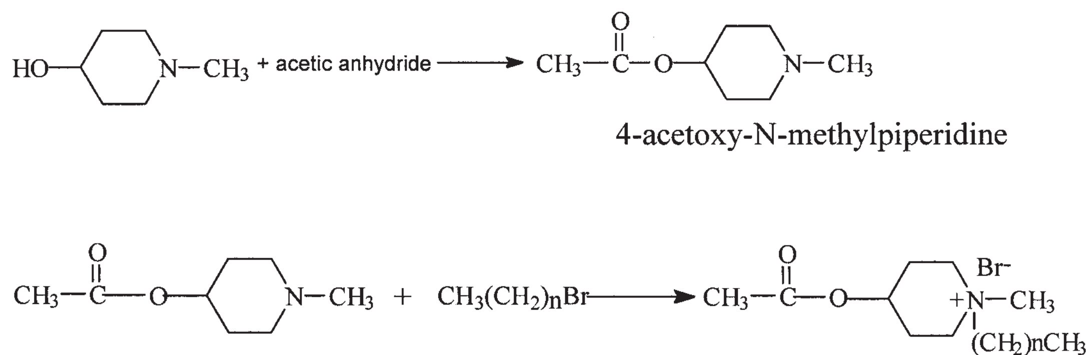


**Figure 2** Preparation of the soluble quaternary ammonium salts [$n$ = 3 (e.g., 4-acetoxy-*N*-butyl-*N*-methylpiperidinium bromide), 5, 7, 9, 11, 15, or 17].

### Preparation of the soluble ester compounds

The acetate ester of 4-hydroxy-*N*-methyl piperidine was prepared as follows. 4-Hydroxy-*N*-methyl piperidine (5 g) was added to acetic anhydride (10 mL) containing one drop of concentrated sulfuric acid ($H_2SO_4$). The resulting solution was heated in a boiling water bath for 10 min, and the reaction mixture was poured into ice-cold water (5 mL). A saturated $NaHCO_3$ solution was then added to give a final pH of 10–11. Extraction with diethyl ether followed by rotary evaporation gave a residue that was subsequently analyzed by IR and NMR spectroscopy, and this confirmed that the acetate had been formed. The reaction is shown in Figure 2.

The following assignments are proposed for the $^1$H- and $^{13}$C-NMR spectra.

$^1$H-NMR ($CDCl_3$, δ, ppm): 1.6–1.8 (m, $CH_2$CH$_2$N), 2.0 (s, 3H, $CH_3$N), 2.3 (s, 3H, $CH_3$COO), 2.6 (m, CH$_2$$CH_2$N), 4.7–4.9 (m, 1H, O$CH$CH$_2$). $^{13}$C-NMR ($CDCl_3$, ppm): 20 ($CH_3$COO), 30 (CH$CH_2$), 45 ($CH_3$N), 52 ($CH_2$N), 69 (O$CH$CH$_2$), 170 (CH$_3$$CO_2$).

The IR spectrum showed the characteristic ester carbonyl peak at 1740 cm$^{-1}$ (C═O).

### Preparation of the soluble quaternary ammonium salts

A series of quaternary ammonium salts was then prepared from the 4-acetoxy-*N*-methylpiperidine as follows. A solution of the tertiary amine (0.025 mol) in ether (50 mL) containing an *n*-bromoalkane (0.1 mol) was heated under reflux for 7 days. The chain length of the *n*-bromoalkanes ranged from 4 to 18 carbon atoms (Fig. 2).

The product precipitated and was recrystallized from ethyl acetate.

Quaternization of the tertiary amine was confirmed by NMR spectroscopy. One striking difference in the spectrum following quaternization was a downfield shift in the $^{13}$C- and $^1$H-NMR signals for the methyl group attached to the nitrogen (N$CH_3$). There was also a corresponding increase in the number of signals due to the addition of the alkyl chain, which varied in length from 4 to 18 carbons. The following assignments are proposed as an example of the data obtained for the $^1$H- and $^{13}$C-NMR spectra for the compound for which $n$ was 5.

$^1$H-NMR ($CDCl_3$, δ, ppm): 0.9 [t, 3H, $CH_3$(CH$_2$)$_n$], 1.5 [broad, s, ($CH_2$)$_4$], 2.15 (m, 4H, CH$CH_2$CH$_2$), 2.3 (s, 3H, $CH_3$COO), 3.5 (s, 3H, N$CH_3$), 3.84.1 [br m, N$CH_2$(CH$_2$)$_4$, $CH_2$N], 5.2 (br t, 1H, O$CH$CH$_2$). $^{13}$CNMR ($CDCl_3$, ppm): 13.1 [$CH_3$(CH$_2$)$_n$], 21.2 ($CH_3$COO), 22 [CH$_3$$CH_2$(CH$_2$)$_4$], 24 [CH$_3$CH$_2$$CH_2$(CH$_2$)$_3$], 25 (CH$_3$-CH$_2$CH$_2$$CH_2$), 32 [NCH$_2$$CH_2$(CH$_2$)$_n$], 50 (OCH$CH_2$), 57 [N$CH_2$(CH$_2$)$_n$], 58 (N$CH_3$), 62 (OCHCH$_2$$CH_2$), 64.5 (O$CH$), 170.1 ($CO_2$CH$_3$).

The IR spectrum showed the ester carbonyl peak at 1740 cm$^{-1}$ (C═O).

### Microbiological analysis

The antibacterial effects of the modified films as well as those of the soluble quaternary ammonium compounds were evaluated with Gram-negative (*Escherichia coli*, strain NTCC 9001) and Gram-positive (*Staphylococcus aureus*, strain NCIMB 6571) organisms.

According to the literature,[24–29] longer chain lengths of 10 carbon atoms and greater are most active against micro-organisms. The effect of the alkyl chain length on the antibacterial activity of our own soluble quaternary ammonium compounds was investigated with a serial dilution method[25] as follows.

An overnight culture of either *E. coli* or *S. aureus* was prepared in an oxoid nutrient broth (NB) at 37°C to give an initial cell count of $10^8$ colony forming units per milliliter (cfu/mL) to ensure the presence of sufficient inoculum [NB: pH 7.4 ± 0.2, Lab Lemco powder (1.0 g/L), yeast extract (2.0 g/L), peptone (5.0 g/L), and NaCl (5.0 g/L)].

For both bacteria, 1 mL of this overnight culture was incubated with 9 mL of a $10^{-5}M$ solution of a quaternary ammonium salt. In this initial microbiological survey, we chose two incubation periods: 24 h or 6 days. A shortage of material did not permit the num-

**TABLE I**
**Amount of Quaternary Ammonium Salt Released from a Film Over a 24-h Period**

| Time | Detected quaternary ammonium salt (mol $\times 10^6$) |
|---|---|
| 5 min | ND |
| 30 min | ND |
| 1 h | 0.009 |
| 4 h | 3.35 |
| 24 h | 3.12 |

ND = not detected.

ber of time periods to be extended to include shorter and intermediate time periods. A control experiment was carried out simultaneously in which no disinfectant was added. After incubation, each incubation was serially diluted by a factor of 10 five times more.

Next, aliquots (0.1 mL) of each dilution were plated onto oxoid place count agar and incubated at 37°C for 24 h. After incubation, plates containing between 30 and 300 colonies were counted, and the colony forming units were recorded.

In the next series of experiments, the effect of the quaternary ammonium salt concentration ($1 \times 10^{-5}$ to $1 \times 10^{-8}M$) on the antibacterial efficacy of the compounds was investigated. The initial bacterial count was again $10^8$ cfu/mL.

A cell count of $10^8$ cfu/mL was higher than that expected normally. For this reason, further experiments were carried out with a lower cell count of $10^5$ or $10^6$ cfu/mL, values consistent with those used by other workers.[28,30,31] In addition, the immobilized quaternary ammonium salt samples (films) were investigated only with long alkyl chain lengths of 12 and 18 carbon atoms (as discussed later). The serial dilution method was employed as described previously, and the film samples were incubated with 1 mL of a bacterial suspension containing $10^5$ or $10^6$ cfu/mL in 9 mL of $H_2O$. Serial dilutions were subsequently prepared, plated, and incubated at 37°C for 24 h, and the colonies were counted. The colony forming units represent the approximate numbers of viable organisms.

## RESULTS

### Stability of the ester linkage

Table I shows the amount of quaternary ammonium salt released from the polymer over a 24-h period. The results show that only a trace of the quaternary ammonium salt was detected after 1 h under the assay method used, but after 4 h, the entire antibacterial agent had been released.

The IR spectrum of the film following hydrolysis showed a shift in the carbonyl C═O stretching frequency from 1730 to 1715 cm$^{-1}$ together with the appearance of a strong band at 3500 cm$^{-1}$, which was expected for carboxylic acid formation. Because of the time-consuming nature of preparing sufficient quantities of film, a full kinetic analysis of the antiseptic release was not carried out at this stage.

### Microbiological testing

The effect of the alkyl chain length on the efficacy of the soluble quaternary ammonium salts as disinfectants is shown in Table II. The results highlighted two features of the quaternary ammonium compounds tested: a general increase in the activity with increasing alkyl chain length and a difference in the efficacy of the compounds against the Gram-positive and Gram-negative organisms. This was particularly apparent for the longer alkyl C18 chain length, the shorter chains, apart from C10, appearing less effective. With both organisms, the results obtained with C10 would suggest it to be more effective than chain lengths other than C18. This is at variance with the general and expected trend and may well be an artifact, particularly as there is an increase in the antimicrobial activity from C12 to C18, the maximum activity being observed with the longest chain length. It must also be emphasized that these results represent a preliminary survey only and that it would be unwise to draw any firm conclusions at this stage, particularly as no full statistical analysis could be carried out. The longest chain length quaternary ammonium compound was considerably more active against the. *aureus* than *E. coli*, as would be expected from the results obtained from other workers.[26–29] However, the difference in activity was not as apparent with the C16 chain length and lower chain lengths. Further experiments on the soluble ester compounds were, therefore, limited to chain lengths of 10 and 18 carbon atoms. All subsequent experiments on the polymer-bound quaternary ammonium salts were carried out with chain lengths of 12 and 18 carbon atoms.

**TABLE II**
**Effect of the Chain Length of the Quaternary Ammonium Soluble Ester Compounds Against *E. coli* and *S. aureus***

| Salt chain length | *S. aureus* (cfu/mL) | | *E. coli* (cfu/mL) | |
|---|---|---|---|---|
| | 24 h | 6 days | 24 h | 6 days |
| Control | $6.25 \times 10^7$ | $3.0 \times 10^7$ | $3.7 \times 10^5$ | $1.06 \times 10^8$ |
| C4 | $1.95 \times 10^7$ | $6.5 \times 10^6$ | $2.65 \times 10^6$ | $3.0 \times 10^8$ |
| C6 | $1.10 \times 10^7$ | $1.75 \times 10^7$ | $3.6 \times 10^6$ | $4.95 \times 10^7$ |
| C10 | $3.4 \times 10^3$ | $1.0 \times 10^3$ | $5.45 \times 10^3$ | $6.2 \times 10^3$ |
| C12 | $5.0 \times 10^6$ | $5.7 \times 10^5$ | $1.78 \times 10^5$ | $9.05 \times 10^7$ |
| C16 | $3.24 \times 10^4$ | $3.7 \times 10^4$ | $1.33 \times 10^4$ | $4.85 \times 10^4$ |
| C18 | $5.0 \times 10^1$ | 0 | $2.74 \times 10^4$ | $6.45 \times 10^4$ |

The initial cell count of *S. aureus* was $1.14 \times 10^8$ cfu/mL, and that of *E. coli* was $1.04 \times 10^8$ cfu/mL.

**TABLE III**
**Effect of the Concentration of Soluble Quaternary Ammonium Salts on Bactericidal Activity**

| | *S. aureus* | | *E. coli* | |
|---|---|---|---|---|
| | C10 | C18 | C10 | C18 |
| Control | $3.05 \times 10^7$ | $3.05 \times 10^7$ | $6.30 \times 10^7$ | $6.30 \times 10^7$ |
| $1 \times 10^{-5}$ *M* | 0 | 0 | 0 | 0 |
| $1 \times 10^{-6}$ *M* | $8.36 \times 10^6$ | $2.00 \times 10^2$ | $4.45 \times 10^7$ | $2.24 \times 10^7$ |
| $1 \times 10^{-8}$ *M* | $2.18 \times 10^7$ | $1.19 \times 10^7$ | $2.65 \times 10^7$ | $3.45 \times 10^7$ |
| $1 \times 10^{-10}$ *M* | $1.65 \times 10^6$ | $2.35 \times 10^7$ | $4.35 \times 10^7$ | $6.05 \times 10^7$ |

The initial concentration of *S. aureus* was $2.9 \times 10^7$ cfu/mL, and that of *E. coli* was $1.38 \times 10^8$ cfu/mL.

The effect of varying the concentration of the soluble quaternary ammonium salts on the antibacterial activity is shown in Table III. From the results presented in Table III, it can be seen that both the C18 and C10 materials were effective at a concentration of $1 \times 10^{-5}M$ against *S. aureus* and *E. coli*. However, at the lower concentration of $1 \times 10^{-6}$, only the C18 material appeared to show any activity and only against *S. aureus*. More dilute samples did not appear to be effective against either organism. The reason for this sharp cutoff in activity is not clear at this stage. However, as mentioned previously, the initial cell count in these experiments was greater than that used by some researchers,[28,30,31] and to investigate fully the activity of the quaternary ammonium compounds, further experiments were carried out with a lower initial cell concentration. Furthermore, the concentration of the quaternary ammonium salt, which had been immobilized, was expected to be lower than $1 \times 10^{-5}$; in all further experiments, it was reduced to $1 \times 10^{-6}M$.

The results of the effect of the initial cell count on the bactericidal activity of the soluble quaternary ammonium salts are presented in Table IV. In this experiment, only the quaternary ammonium salt with the longest alkyl chain of 18 carbon atoms was used because of its greater efficacy, as demonstrated previously. The results demonstrate that at initial cell counts of $2.05 \times 107$ and lower, the C18 material was particularly effective against the *S. aureus* organism. In comparison, under the conditions employed, only a small reduction in *E. coli* was found.

Table IV shows that the quaternary ammonium salt had a much greater efficacy against the Gram-positive species than the Gram-negative organism, as previously observed. This was especially true when the initial cell count was of the order of $10^6$ cfu/mL. Reducing the initial cell count had little, if any, effect when the test organism was *E. coli*, although some reduction in the colony forming units could be observed. These results are in contrast to *S. aureus*, for which a complete reduction to 0 cfu/mL viable organisms could be seen at initial values of $2.05 \times 10^4$, $2.05 \times 10^5$, and $2.05 \times 10^6$ cfu/mL.

From these experiments, it can be seen that an initial cell count of $10^5$ or $10^6$ cfu/mL is optimal for demonstrating the antibacterial activity of soluble quaternary ammonium compounds. For this reason, these concentrations of viable organisms were employed to test the activity of the immobilized, covalently bound quaternary ammonium salts. The results of these investigations are presented in Tables V and VI.

The results presented in Table V show that, with an initial cell count of approximately $10^6$, the covalently bound quaternary ammonium compounds were more effective against *S. aureus*, more bactericidal activity being observed with the C18 material. In contrast, both the C12 and C18 materials showed no effect against *E. coli* under the conditions of the experiment (Table V). However, as shown in Table VI, with an approximately 10-fold reduction in the initial cell count, the activity of the C18 film was observed against both organisms tested. Little effect was observed with the C12 film. The covalently bound materials were by no means as effective as the soluble salts, with which a total eradication of *S. aureus* was observed at this lower initial cell concentration (Table III). However, this difference may be a concentration effect of the antimicrobial compound. In other words, although the unstable ester bond does hydrolyze to release the quaternary ammonium salt, the actual concentration of the disinfectant obtained in the incubation mixture may be insufficient to deal with an initial organism concentration of $10^6$ cfu/mL.

The aforementioned observations concerning the possible concentration effect of the quaternary ammonium salt are in part borne out by the results obtained

**TABLE IV**
**Effect of Lowering the Initial Cell Concentration on the Bactericidal Activity of the Soluble Quaternary Ammonium Salts**

| *S. aureus* | | | *E. coli* | | |
|---|---|---|---|---|---|
| Initial (cfu/mL) | Control | C18 | Initial (cfu/mL) | Blank | C18 |
| $2.05 \times 10^8$ | $5.30 \times 10^7$ | $1.33 \times 10^7$ | $9.75 \times 10^7$ | $4.50 \times 10^7$ | $4.15 \times 10^7$ |
| $2.05 \times 10^7$ | $1.29 \times 10^7$ | $7.65 \times 10^3$ | $9.75 \times 10^6$ | $1.29 \times 10^7$ | $6.36 \times 10^6$ |
| $2.05 \times 10^6$ | $1.4 \times 10^5$ | 0 | $9.75 \times 10^5$ | $2.67 \times 10^6$ | $1.54 \times 10^5$ |
| $2.05 \times 10^5$ | $8.85 \times 10^3$ | 0 | $9.75 \times 10^4$ | $2.75 \times 10^5$ | $4.85 \times 10^4$ |
| $2.05 \times 10^4$ | $7.85 \times 10^2$ | 0 | $9.75 \times 10^3$ | $1.95 \times 10^4$ | $1.2 \times 10^4$ |

**TABLE V**
**Antibacterial Activity of the Covalently Bound Quaternary Ammonium Salts with an Initial Cell Count of $10^6$ cfu/mL**

| | *S. aureus* initial | | *E. coli* initial | |
|---|---|---|---|---|
| | $1.95 \times 10^6$ cfu/mL | $7.0 \times 10^6$ cfu/mL | $6.0 \times 10^6$ cfu/mL | $3.0 \times 10^6$ cfu/mL |
| Control | $7.6 \times 10^4$ | $1.69 \times 10^6$ | $1.95 \times 10^6$ | $6.5 \times 10^6$ |
| C12 Film | $3.4 \times 10^4$ | $1.9 \times 10^5$ | $6.5 \times 10^6$ | $6.3 \times 10^6$ |
| C18 film | $4.15 \times 10^3$ | $1.55 \times 10^5$ | $2.35 \times 10^6$ | $7.4 \times 10^6$ |

in this experiment. In other words, when the initial cell count was reduced from $10^6$ to $10^5$ cfu/mL, the films appeared to be much more effective disinfecting agents (Table VI). Indeed, with an initial cell count of $1.95 \times 10^5$ cfu/mL, a complete reduction to zero viable cells was observed with *S. aureus*. However, in the slightly higher concentration of $7 \times 10^5$ cfu/mL, only a reduction to $4 \times 10^2$ cfu/mL was observed. At the initial cell concentrations of $6 \times 10^5$ and $3 \times 10^5$, the C12 material appeared to have little effect on *E. coli*, whereas the C18 immobilized quaternary ammonium salt was far more effective, resulting in reductions to $5 \times 10$ and $6.5 \times 10^2$, respectively. However, further work in which sufficient experiments are carried out to allow a full statistical analysis needs to be carried out before any conclusive evidence can be obtained.

## DISCUSSION

The results presented in this study demonstrate how a quaternary ammonium salt bound to a PE backbone by a hydrolyzable ester linkage can be prepared that may form the basis of a device providing a release of a disinfectant at a contaminated or infected site. Such a system may well have applications in surgical prostheses and wound dressings. A preliminary microbiological survey has shown that films prepared in this way show antibacterial properties, being particularly effective against Gram-positive organisms at an initial cell concentration of $10^5$ cfu/mL. The efficacy of the quaternary ammonium functions themselves has been confirmed by the preparation of soluble quaternary ammonium salts containing the same moieties as the covalently bound materials. As expected from published results,[26–29] those quaternary ammonium salts formed from the longer alkyl chains proved to be the most effective.

The chemical and spectroscopic analysis of the obtained products confirmed the proposed structures and that the intended series of reactions had been carried out. Again, an initial microbiological survey of the materials so produced demonstrated their efficacy as antimicrobial agents. Extended microbiological studies were beyond the scope of this investigation.

The preliminary microbiological data taken from these initial experiments could not be analyzed for any statistical variance because of a lack of sufficient material. The reason for this is the time-consuming nature of the experimental procedure, which did not allow sufficient samples of covalently bound, ester-linked quaternary ammonium salts to be produced in a reasonable time. As with the results of the soluble quaternary ammonium compounds, the results of these initial experiments do, however, demonstrate along with those of other workers that compounds with the longer alkyl chain length are more effective as antibacterial agents, showing greater activity toward the Gram-positive species than the Gram-negative species. This is also seen when the cell count is reduced. The immobilized ester samples did not appear to be as effective as the corresponding quaternary ammonium salts under the experimental conditions employed, although this may well be attributable to the amount of quaternary ammonium salt bound to the film.

The quaternary ammonium compound was completely released from the polymer after 4 h, a trace being detectable after 1 h. Because of the time-consuming nature of the experimental work involved in producing the antibacterial surfaces, a full kinetic analysis of the release of the disinfectant was not carried out at this stage, although it is intended that this will form the basis of a future study. In addition, future work on these surfaces will include full release profiles as well

**TABLE VI**
**Antibacterial Activity of the Immobilized Quaternary Ammonium Salts with an Initial Cell Count of $10^5$ cfu/mL**

| | *S. aureus* initial | | *E. coli* initial | |
|---|---|---|---|---|
| | $1.95 \times 10^5$ cfu/mL | $7.0 \times 10^5$ cfu/mL | $6 \times 10^5$ cfu/mL | $3.0 \times 10^5$ cfu/mL |
| Control | $5.3 \times 10^3$ | $1.11 \times 10^4$ | $5.65 \times 10^5$ | $6.2 \times 10^5$ |
| C12 film | $1.0 \times 10^2$ | $1.67 \times 10^4$ | $2.0 \times 10^5$ | $1.42 \times 10^6$ |
| C18 film | 0 | $4.0 \times 10^2$ | $5.0 \times 10^1$ | $6.5 \times 10^2$ |

as the possibility of using other ester linkages that may provide different rates of release.

Further microbiological investigations, such as growth tests, would also be necessary to determine whether the action of the soluble and immobilized quaternary ammonium salts is bactericidal or bacteriostatic.

In conclusion, preliminary experimental screening has shown that a self-disinfecting surface has been produced that could form the basis of a controlled-release system that may well find application in the manufacture of novel surgical prostheses and topical wound dressings. Future experiments that could be conducted would include using a variety of bacterial concentrations, including European standard test procedures. In addition, a statistical evaluation would also be carried out in each case.

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