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SHORT COMMUNICATION

Bactericidal silver ion delivery into hydrophobic coatings with surfactants

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Abstract A much studied oil-soluble surfactant, bis[2-ethylhexyl]sulfosuccinate, sodium salt, was ion exchanged into the silver ion form and dissolved into microemulsions of immiscible polyurethane step monomers. Coating and curing of these microemulsions produced polyurethane coatings that exhibit bactericidal activity against representative Gram negative bacteria. After 24 h exposure, 0.006–0.012% weight Ag relative to coating weight (0.0013–0.0025 μmol Ag/cm^2) results in the three-log reduction in *Escherichia coli*. A slightly higher level of 0.031% weight Ag relative to coating weight (0.006 μmol Ag/cm^2) killed all of the *E. coli* after 12 h exposure. Similar results were obtained for *Pseudomonas aeruginosa*. Since the double-tail surfactant anion promotes reverse micelle formation in many different kinds of oils and solvents, it appears an excellent vector for incorporating low and effective amounts of silver ion into many industrial, hospital, and household coating formulations.

Keywords Surfactant · Antimicrobial silver · Silver ion · Bactericidal silver · Silver surfactant

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Introduction

The efficacy and importance of silver ion as an antimicrobial agent has been known for many years, and the use of silver salts to combat infection dates to ancient time [12, 22, 25]. It is known that silver ion binds to thiols in enzymes and other proteins often deactivating them [10, 16, 21, 28]. It is also thought that silver ion interferes with ion transport and respiration across the cytoplasmic membrane [2, 6, 9, 14, 23, 27]; organisms with higher turnover rates with extracellular ions appear more susceptible to silver ion poisoning.

In the past few years the incorporation of silver in various forms into clothing articles [1, 20] and coatings of diverse materials and products [4, 5, 8, 11, 13, 19] has become more and more popular, as the efficacy of silver ion in combating body odor and general bacterial infestation has become more broadly appreciated. The physical form of silver incorporation ranges from elemental metallic silver nanoparticles to salts [17] of varying sparing solubility. In all instances, however, it is the silver ion species, produced by oxidation or by dissolution that is believed to be the active agent.

Other studies have examined various coating additives and the effects of additives on the adherence of Gram-negative bacteria to coatings [7], and the efficacy of disinfectant-containing xerogel coatings [3]. In this paper we report a method for the direct incorporation of silver ion into coatings for consumer, hospital, or industrial use. The methodology with a newly reported method of formulating polyurethane coatings from immiscible monomers, called microemulsion polymerization, is used [29]. Microemulsions are thermodynamically stable solutions of two immiscible fluids (in this case propylene glycol and isophorone diisocyanate) made miscible by the action of a third chemical

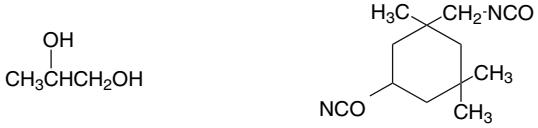
component, typically a surfactant [15, 26, 30]. The use of charged surfactant, having an ion exchangeable cation, in this case a sodium ion, can be easily transformed by ion exchange into a silver form. It is shown that such silver ion surfactants may readily be incorporated into coatings.

Challenges by two Gram-negative bacteria, *Escherichia coli* ATCC 11229, and *Pseudomonas aeruginosa* ATCC 15442, are also reported. The results illustrate that our coatings exhibit high bactericidal activity, even at quite low levels of incorporated Ag^+.

Materials and methods

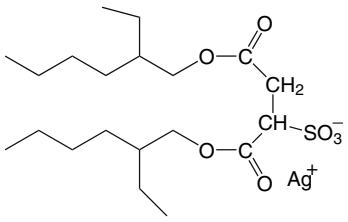
Microemulsion materials

The reactive monomers, propylene glycol (PG) and isophorone diisocyanate (IPDI) and the catalyst dibutyltin dilaurate were obtained from Aldrich (Sigma–Aldrich, Milwaukee, WI, USA). Aerosol OT (AOT, bis[2-ethylhexyl]sulfosuccinate, sodium salt), glass lantern slides (5 cm × 7.6 cm), and glass microscope slides (2.54 cm × 7.6 cm) were obtained from Fisher (Fisher Scientific, Hanover, IL, USA). The silver form of AOT, AgAOT was produced by ion exchange of AOT following the method of Pileni et al. [24].



PG

IPDI



AgAOT

Coating procedures

Coatings were made on glass slides that had been washed with acetone and water. The first set was coated on glass-lantern slides (5 cm × 7.6 cm). The second set was coated on microscope slides (2.54 cm × 7.6 cm). The polyurethane coatings were produced in situ by a recently reported microemulsion polymerization method [29]. The immiscible step-polymerization monomers propylene glycol and isophorone diisocyanate were formulated as a microemulsion using AOT. Dibutyltin dilaurate was used as a catalyst to drive the step polymerization. The microemulsions were formulated with equivalent weights of propylene glycol and isophorone diisocyanate, along with AOT at 30% (% weight AOT relative to weight of all three components). Dibutyltin dilaurate was added at 0.1% of the total composition weight. Coatings of approximately 150 μm thickness and 5 cm width were made on the glass slides using a drawdown bar. The coatings were stored for at least ten days at room temperature before being subjected to testing. These AOT coatings were used as control coatings in the subsequent challenges. AgAOT was substituted for AOT at various levels, during the microemulsion formation stage prior to coating, to prepare level series in AgAOT for the challenges. AgAOT was substituted for AOT on a weight basis from 10 to 0.01% by weight to produce test coatings. The first set of coatings on the lantern slides averaged a total coating coverage of 23 ± 3 mg/cm^2. In this first set, AgAOT was substituted at levels of 0.01, 0.1, 0.5, 1.0, 3.0, and 10.0% by weight of the AOT. These substitutions produced coatings having 0.0016–1.30 μmol Ag/cm^2. The second set of coatings on microscope slides averages a total coating coverage of 20 ± 2 mg/cm^2. In this second set of coatings AgAOT was substituted at levels of 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0% by weight of the AOT. These substitutions produced coatings having 0.0031–0.26 μmol Ag/cm^2.

Sterilization of slides

Sterilization of the slides prior to the testing was achieved by exposure of the slides to a germicidal UV lamp in a Nuaire Model NU-455-600 Class II Type A/B3 laminar flow hood (2 min on each side at room temperature). Other methods of sterilization (i.e., swabbing with 70% EtOH or autoclaving) either did not achieve complete sterilization of the slides, or compromised the integrity of the coating. Treated slides were checked for sterilization after overnight incubation at 37°C in Tryptic Soy Broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA).

Bacterial cultures

Escherichia coli ATCC 11229 and *P. aeruginosa* ATCC 15442 (Cuti-loops, Remel Europe, Dartford, Kent, UK) were selected for the testing of the antimicrobial coatings. Each bacterial strain was streaked for isolated colonies on Tryptic Soy Agar plates (TSA; Difco, Becton Dickinson) and incubated at 37°C for 12–18 h. Isolated colonies from the TSA plates were used to inoculate TSB cultures. The TSB cultures were incubated in a water bath shaker (150 rpm at 37°C), and were allowed to grow to an $A_{590\ nm}$ of 1.2–1.5 ($\sim 10^9$ CFU/ml). Bacterial cells were pelleted at 12,000×*g* (10 min at 4°C, Sorval SS34 rotor), washed once with phosphate buffer saline (PBS;

BBL, FTA Hemagglutination Buffer, Becton Dickinson), and diluted with PBS to achieve 10^5–10^6 CFU/ml. This bacterial suspension was used to inoculate the slides containing antibacterial coatings.

Application of bacteria

All inoculations of the coatings were performed in a laminar flow hood where the sterilized slides were placed into sterile Petri dishes. The prepared bacterial suspension (0.5 ml) was pipetted directly onto the slide, which spread across the entire surface of the coating, but remained on top of the slide. The initial coatings contained a 0.01–10% range of silver (AgAOT), relative to the 30% control amount of AOT in the coating composition, were tested with *E. coli* ATCC 11229 (2 runs). The second set of coatings containing a range of 0.02–2.0% of AgAOT, of the 30% AOT control amount, were challenged with *E. coli* ATCC 11229 and *P. aeruginosa* ATCC 15442. These substitution levels are expressed as % weight AgAOT relative to total weight AOT and as μmol Ag/cm^2. These coatings were tested after 0, 12, and 24 h incubation in a humidified chamber (20–25°C), with the 0% AgAOT and 0 h slides as controls.

Recovery of surviving bacteria

At each time point, 25 ml of sterile PBS was pipetted into the Petri dish completely submerging the slide, and a sterile plastic inoculating loop was used to release any viable bacteria remaining on the surface of the coating. Samples were plated onto TSA plates, and incubated at 37°C for at least 24 h. Plates containing between 30 and 300 isolated colonies were counted.

Results and discussion

Results of the first replicate set of tests are shown in Table 1 for *E. coli* applied to the coatings spread onto lantern slides (first set of coatings). The data taken from the samples at 0 h were set up to confirm the concentration of the inoculum used. The overall weight percent of silver in the coating composition is listed in column 2, and column 1 lists the Ag per unit area in units of μmol Ag/cm^2. The overall Ag weight percents of column 2 were obtained from the % substitution of AgAOT for AOT by multiplying by the factor 0.3 (control weight fraction of AOT), and subsequently by the factor 0.204 (the weight fraction of Ag in AgAOT). The μmol Ag/cm^2 results were obtained by analyzing the actual weight of coating per unit area, applying the column 2 results, and converting from g per unit area to μmol//cm^2 by dividing with the atomic weight of Ag.

Table 1 Initial test results for AgAOT doped polyurethane coatings exposed to *E. coli* ATCC 11229 for 24 h on lantern slide coatings

[a]μmol Ag/cm^2	% weight Ag in Coating	CFU/ml of *E. coli* at 24 h[b]
0	0	4.2×10^6; 2.8×10^6
0.0016	0.00061	8.4×10^3; 5.9×10^6
0.013	0.0061	1.2×10^3; 0
0.060	0.031	0; 0
0.12	0.061	0; 0
0.39	0.18	0; 0
1.30	0.61	0; 0

[a] The total coating coverage averaged 23 ± 3 mg/cm^2

[b] The bacterial concentration of the initial inoculum (0 hr) of bacteria used to inoculate the slides was calculated to be $4.9 \times 10^5 \pm 1.1 \times 10^5$ CFU/ml ($n = 14$). CFU/ml of survivors from duplicate plates

This first set of tests indicates that after 24 h exposure, a reduction in bacterial count by over three orders was obtained with 0.0061% weight Ag in the overall coating (0.013 μmol Ag/cm^2). Levels at or above 0.031% weight Ag (0.060 μmol Ag/cm^2) resulted in complete killing of the *E. coli*.

A set of three challenges was applied to the second set of coatings, made on microscope slides. The results, shown in Table 2, after 24 h essentially nearly confirm the results of the first set of challenges on lantern slides. At and above 0.012% weight Ag relative to overall coating weight (at and above 0.0025 μmol Ag/cm^2), the number of viable bacteria was reduced by 3-logs.

The results after 12 h exposure (Table 2) show that complete killing of the *E. coli* appears to be obtained with levels above 0.031% weight Ag relative to overall coating weight (at and above 0.00597 μmol Ag/cm^2). These more muted results, in comparison with 24 h exposure results, appear consistent with an exposure effect.

The coatings made on microscope slides were also tested against *P. aeruginosa* (Table 3). Similarly to *E. coli*, the smallest substitutional level examined at 24 h exposure, 0.0012% weight Ag relative to overall coating weight (less than 0.0031 μmol Ag/cm^2) appears very effective at killing the *P. aeruginosa*, as do all the higher challenge levels. However, the levels of 0.0031 and 0.012% (% weight Ag relative to coating weight) did not result in complete eradication in each challenge, although the assayed levels of CFU/ml in these cases are not statistically significantly different than zero. Resolution of the quantitative efficacy of this silver delivery approach for *P. aeruginosa* must await more protracted testing, but the level of 0.031% weight Ag relative to coating weight (0.006 μmol Ag/cm^2) and above appear to kill all of the *P. aeruginosa* at both 12 and 24 h exposure.

Table 2 Challenge results for AgAOT doped polyurethane coatings exposed to *E. coli* for 12 and 24 h on microscope slide coatings (second coating set)

[a]μmol Ag/cm^2	% weight Ag in Coating	CFU/ml of *E. coli* at 12 h	CFU/ml of *E. coli* at 24 h
0	0	5.8×10^5, 2.9×10^5, 4.1×10^5	6.8×10^6, 3.4×10^5, 1.2×10^5
0.00310	0.0012	6.5×10^4, 4.2×10^4, 1.21×10^5	1.1×10^5, 1.0×10^3, 3.0×10^3
0.00737	0.0031	1.4×10^5, 1.3×10^5, 5.8×10^4	1.5×10^3, 0, 3.0×10^3
0.00131	0.0061	5.0×10^2, 1.1×10^4, 3.0×10^4	0, 1.7×10^4, 0
0.00250	0.012	5.5×10^4, 9.5×10^3, 3.5×10^4	0, 0, 0
0.00597	0.031	0, 0, 0	0, 0, 0
0.0122	0.061	0, 0, 0	0, 0, 0
0.0261	0.122	0, 0, 0	0, 0, 0

[a] The total coating coverage averaged 20 ± 2 mg/cm^2

[b] The bacterial concentration of the initial inoculum (0 h) of bacteria used to inoculate the slides was calculated to be 4.9×10^5 ± 1.1×10^5 CFU/ml (n = 14). CFU/ml of survivors from duplicate plates

Table 3 Challenge results for AgAOT doped polyurethane coatings exposed to *P. aeruginosa* for 12 and 24 h on microscope slide coatings (second coating set)

[a]μmol Ag/cm^2	% weight Ag in Coating	CFU/ml of *P. aeruginosa* at 12 h	CFU/ml of *P. aeruginosa* at 24 h
0	0	5.5×10^4, 3.3×10^5, 1.0×10^5	5.0×10^4, 3.2×10^5, 2.2×10^5
0.00310	0.0012	3.0×10^4, 9.5×10^5, 2.6×10^4	0, 0, 0
0.00737	0.0031	1.1×10^4, 1.2×10^5, 3.3×10^4	0, 0, 7.5×10^2
0.00131	0.0061	4.8×10^3, 2.5×10^2, 6.3×10^3	0, 0, 0
0.00250	0.012	1.5×10^3, 1.5×10^3, 3.8×10^3	6.3×10^3, 0, 5.0×10^2
0.00597	0.031	0, 0, 0	0, 0, 0
0.0122	0.061	0, 0, 0	0, 0, 0
0.0261	0.122	0, 0, 0	0, 0, 0

[a] The total coating coverage averaged 20 ± 2 mg/cm^2

[b] The bacterial concentration of the initial inoculum (0 h) of bacteria used to inoculate the slides was calculated to be 4.9×10^5 ± 1.1×10^5 CFU/ml (n = 14). CFU/ml of survivors from duplicate plates

These results show that delivery of silver ion by AgAOT is effective as a Gram-negative bactericide at very low levels of exposure, 0.01–0.03% weight Ag relative to coating weight (0.003–0.006 μmol Ag/cm^2) with 24 h exposure and at levels of 0.003% weight Ag relative to coating weight (0.006 μmol Ag/cm^2) with 12 h exposure.

It is difficult to compare these results with those reported by Galeano et al. [11], because details of the AgION antimicrobial coatings on steel coupons are uncertain. Galeano et al. found that their silver-zeolite based coatings inactivated vegetative *Bacillus anthracis*, *B. cereus*, and *B. subtilis* cells by a factor of 10^3 on exposures of 5–24 h, but not the respective spores. It was implied that the overall silver ion concentration in theses active test coatings was 2.5% (w Ag/w coating). The substantial activity differences of silver in our respective studies might be due to the more homogeneous distribution of silver in our coatings, dramatic differences in silver ion mobility in the polyurethane of our study versus the silver ion mobility in whatever binder (unspecified) was used in the Galeano et al. [11] coatings, or to some other, as yet unassigned, reason.

The sodium form of AOT is perhaps the most studied oil soluble surfactant and tends to form reverse micelles in most nonaqueous solvents. Since its shape is reverse conical, and it has a negative packing parameter [18], it is expected to be an excellent vector for incorporating low and effective amounts of silver ion into many industrial, hospital, and household coating formulations. The inclusion of AgAOT into almost any nonaqueous coating formulation is straightforward, therefore, and thermodynamically favored. Further experiments with Gram-positive bacteria and other organisms will be reported subsequently. They are not discussed here because of confounding mechanistic features.

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