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Novel antimicrobial *N*-halamine polymer coatings generated by emulsion polymerization

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Abstract

A new class of *N*-halamine polymers has been synthesized. These polymers can be emulsified in water to produce coatings which, once chlorinated, act as contact disinfectants. The surfaces inactivate bacterial organisms efficiently, requiring relatively brief contact times of several minutes. The latexes can be formed by copolymerization of a *N*-halamine precursor monomer with other monomers in water with the aid of a surfactant, or by chemically grafting the *N*-halamine precursor monomer onto an emulsified polymer backbone, followed by chlorination.

Keywords: Antimicrobial polymer coating; Emulsion polymerization

1. Introduction

There is a need for new classes of polymeric contact biocides that are stable, do not harm their surroundings, and inactivate organisms rapidly. Currently the only commercial contact biocides are polymeric quaternary ammonium compounds (quats) [1,2], polymeric phosphonium compounds [3], and halogenated sulfonamides [4]. These compounds effectively inactivate a broad range of organisms, but they have important drawbacks. The quats and phosphonium compounds are partially water soluble and hence leach into water; they need prolonged contact times (often hours) to inactivate microorganisms, are relatively expensive to produce, and once exhausted, the biocidal activity of these surfaces cannot be regenerated. The halogenated sulfonamides can be water insoluble, and they generally require lower contact times than do quats and phosphonium compounds to inactivate organisms. Their biocidal activities can be regenerated, which is desirable, but they are pH sensitive, and they release high concentrations of free halogen into their environment, which can then cause formation of toxic by-products, such as trihalomethanes [5].

In an effort to produce contact disinfectants that have sufficient pH stability to avoid release of significant levels of free halogen, and that are inexpensive to manufacture, several regenerable biocidal *N*-halamine polymers have been prepared in these laboratories. The term '*N*-halamine' herein signifies a molecule containing a nitrogen–halogen bond prepared by halogenation of an imide, amide, or amine. The first class involved modifying commercial polystyrene to incorporate the *N*-halamine moiety for use in water and air filtration systems [6,7]. These polymers are stable for over a year at room temperature, and they require short contact times (a few seconds) to inactivate a broad spectrum of organisms. Their biocidal activities are easily regenerated once exhausted by flowing an aqueous solution of free halogen over them. These polymers show considerable commercial promise for water and air filtration systems, and they are inexpensive to produce, but polystyrene is not useful for coatings, so their application as contact disinfectants is limited. In order to circumvent these limitations, members of a new class of monomers (Fig. 1) have been synthesized which can be copolymerized with numerous commercial monomers to form, upon halogenation, various *N*-halamine polymers [8]. These monomers can also be chemically grafted onto a variety of polymer backbones to form, upon halogenation, new classes of grafted *N*-halamine polymers. These polymers can then be used as contact disinfectants at surfaces, since the monomeric *N*-halamine moiety can be incorporated into any film-forming monomer or polymer. In preliminary experiments, granular *N*-halamine copolymers were made and then dissolved in an appropriate organic solvent and coated on different surfaces. The resulting coatings are clear, tough, show excellent adherence to various substrates, and are

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effective at killing bacterial organisms with a short contact time. An important drawback to these types of coatings is that they require an organic solvent, which raises toxicity and flammability concerns.

In order to make surface coatings that were more environmentally sound, dispersal of the polymers in water was considered so that organic solvents would not have to be used to dissolve the compounds. Successful water dispersal was achieved by utilizing emulsion polymerizations of the oxazolidinone monomers with various commercial monomers. In emulsion polymerizations, polymer particles are dispersed in a volume of water with the aid of a surfactant and, following evaporation of the water, the polymer particles coalesce to form a clear coating.

Emulsion polymerizations are extremely complex processes involving a minimum of four components: water, vinyl monomer, initiator, and surfactant. The process consists of dispersing a water-insoluble monomer with the aid of surfactants into the water. The polymerization is then performed, and the resulting polymer formed is then emulsified by adsorption of surfactant molecules on the surfaces of polymer particles. The product is a homogeneous emulsion of high-molecular weight polymer with a narrow molecular weight distribution. The mechanism of emulsion polymerization is still not well understood, and many viable pathways can be postulated for a particular system.

This paper will demonstrate how emulsion polymerization in aqueous medium can be used to form contact coatings. These, once activated with free halogen, can inactivate bacterial organisms with relatively short contact times. Such compounds may then be used in paint additives, as industrial and commercial protective coatings, in-can preservatives, textile coatings, and in numerous other applications.

2. Experimental

*2.1. Poly-acrylonitrile-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2a**)*

Five g (0.025 mol) 4-(acryloxymethyl)-4-ethyl-2-oxazolidinone (**1a**) [8], 10.0 g (0.190 mol) acrylonitrile, 30.0 g (1.67 mol) water, and 0.20 g (6.94×10^{-4} mol) sodium lauryl sulfate were added to a three-necked round-bottomed flask equipped with a gas inlet and reflux condenser. The solution was stirred and heated to 60°C and purged with nitrogen for 10 min. A solution of 0.025 g (9.26×10^{-5} mol) potassium persulfate and 1.0 ml water to be used as an initiator was prepared and added to the reaction mixture after the nitrogen purge was completed. The reaction mixture was stirred for 12 h and then cooled to room temperature. Any precipitated polymer was allowed to settle, and the latex was decanted off. The product, poly-acrylonitrile-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2a**), was produced in 95% yield with prominent infrared bands in a KBr pellet at 2985, 2244, and 1750 cm^{-1}. Comparable results were obtained with compounds **1b–1d** for this co-polymerization as well as for the other co-polymerization and graft polymerization reactions (see Fig. 1) discussed below.

*2.2. Poly-vinyl chloride-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2b**)*

An emulsion copolymerization was performed with **1a** and vinyl chloride using the procedure employed above. The yield of poly-vinyl chloride-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2b**) was 90% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 2980 and 1750 cm^{-1}.

*2.3. Poly-styrene-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2c**)*

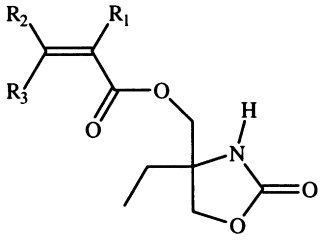
An emulsion copolymerization was performed with **1a** and styrene using the procedure employed above. The yield of poly-styrene-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2c**) was 90% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 3050, 2985 and 1750 cm^{-1}.

*2.4. Poly-vinyl acetate-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2d**)*

An emulsion copolymerization was performed with **1a** and vinyl acetate using the procedure employed above. The yield of poly-vinyl acetate-co-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**2d**) was 90% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 2985, 1772, and 1750 cm^{-1}.

*2.5. Poly-acrylonitrile-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3a**)*

Compound **1a** (2.3 g, 0.013 mol), 10.0 g poly-acrylonitrile latex which was prepared from acrylonitrile using the method previously described [9], and 20.0 g (1.1 mol) water were added to a 100 ml three-necked round-bottomed flask.



1a = $R_1 = R_2 = R_3 = H$
1b = $R_1 = R_3 = H$, $R_2 = CH_3$
1c = $R_1 = R_2 = H$, $R_3 = CH_3$
1d = $R_1 = H$, $R_2 = R_3 = CH_3$

Fig. 1. Structures of unhalogenated monomers.

The reagents were purged with nitrogen for 15 min; the flask was then sealed and heated to 60°C with stirring. Once the internal temperature reached 60°C, 0.05 g (1.9×10^{-4} mol) potassium persulfate, dissolved in 1.0 ml water, was added to the reaction mixture. The reaction mixture was stirred for 12 h, then cooled to room temperature. Any precipitated polymer was allowed to settle, and the latex was decanted off. The grafted polymer product, poly-acrylonitrile-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3a**), was produced in 90% yield with prominent infrared bands in a KBr pellet at 2985, 2240, and 1750 cm^{-1}.

*2.6. Poly-vinyl chloride-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3b**)*

A grafting reaction was performed with **1a** and poly-vinyl chloride using the procedure employed above. The yield of poly-vinyl chloride-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3b**) was 70% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 2980 and 1750 cm^{-1}.

*2.7. Poly-styrene-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3c**)*

A grafting reaction was performed with **1a** and poly-styrene using the procedure employed above. The yield of poly-styrene-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3c**) was 89% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 3050, 2985 and 1750 cm^{-1}.

*2.8. Poly-vinyl acetate-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3d**)*

A grafting reaction was performed with **1a** and poly-vinyl acetate using the procedure employed above. The yield of poly-vinyl acetate-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3d**) was 92% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 2985, 1772, and 1750 cm^{-1}.

*2.9. Poly-vinyl alcohol-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3e**)*

A grafting reaction was performed with **1a** and poly-vinyl alcohol using the procedure employed above. The yield of poly-vinyl alcohol-g-4-(acryloxymethyl)-4-ethyl-2-oxazolidinone latex (**3e**) was 95% of that theoretically expected. The product exhibited prominent infrared bands in a KBr pellet at 3400, 2980, and 1747 cm^{-1}.

2.10. Preparation of surface films and coatings

Unchlorinated polymer latex compounds were cast into thin films on various surfaces and then chlorinated so that the biocidal efficacy of these surfaces could be determined. The general method for coating granular polymeric materials on to various substrates was as follows. The substrate to be coated was washed, autoclaved, and dried at 100°C before the polymer solution was added. Sufficient polymeric solution containing the oxazolidinone monomeric unit within the co-polymer in the concentration range 10–50 wt% was added to coat the surface of the substrate without running over the sides. Then the material with the polymer solution coating it was heated in an oven at 80–100°C until the water was removed. In all cases, the coating was clear, tough, resistant to abrasion, and had good adherence to the substrate. Once coated, the surface was chlorinated with a dilute solution of sodium hypochlorite (3000 ppm free chlorine) by soaking the surface in the aqueous solution for 20 min. The surface was then removed, washed with synthetic chlorine-demand-free water, and dried at room temperature overnight to ensure all residual sodium hypochlorite was removed.

2.11. Surface bacterial testing

Each surface to be tested for biocidal activity against *Staphylococcus aureus* (ATCC 6538) was applied to a circular glass coverslip measuring 12 mm in diameter. The surfaces were chlorinated in the same manner as mentioned above. Fifty μl of a suspension containing 10^6 CFU *S. aureus* were placed on the surface, and a 25 μl aliquot was removed at a predetermined time, and the active halogen was quenched by sterile 0.02 N sodium thiosulfate. The aliquot was then applied to the dried surface of a Petri dish containing nutrient agar. After incubation at 37°C for 48 h, the bacterial colonies were counted. Control samples containing unchlorinated precursor surfaces were handled in the same manner.

2.12. Zone of inhibition studies for fabrics

Zone of inhibition studies were performed on fabric materials coated with various poly *N*-halamines. The coated fabric samples were cut into 1–1.5 cm squares prior to chlorination and dried thoroughly after chlorination, and they were placed on a Tryptic Soy agar plate which was inoculated with *S. aureus* (ATCC 6538). The plates were incubated for 24 h at 37°C, and the zones of inhibition were then measured.

2.13. Efficacies of poly N-halamines against Salmonella enteritidis

Fabric materials coated with various poly *N*-halamines were challenged with the bacterium *Salmonella enteritidis* according to protocol #100 of the American Association of Textile Chemists and Colorants (AATCC), slightly modified to accommodate small sample size. In this test procedure, each fabric sample was prepared as a disc of diameter

1.0 cm and challenged with 100 μl of a suspension containing 10^6 CFU of the bacteria for a contact time of 10 min at ambient temperature. Control samples coated with unchlorinated polymers were similarly challenged. At the end of the incubation period each sample was immersed in 10 ml of 0.02 N sodium thiosulfate solution in a 50 ml test tube to quench chlorine biocidal action and was agitated vigorously for 60 s. Aliquots of 100 μl were removed from the tubes, serially diluted in sterile water, and plated in duplicate on 10 cm diameter Trypticase-Soy agar plates for 24 h at 37°C. The numbers of colony forming units of surviving bacteria present in the eluates were determined, and compared to the total numbers detected in the eluates from the corresponding challenged control samples, to establish the percent reduction brought about by each of the chlorinated polymers.

2.14. Efficacies of poly N-halamine coatings against Pseudomonas aeruginosa in flowing water

N-Halamine polymers **2d** and **3d** were coated onto the surfaces of pieces of polyurethane medical catheters as substrates. Analogous unchlorinated samples were also prepared to be used as controls. The samples, which were approximately 2–3 mm^2 in surface area and 150 μm thick, were placed in coarse mesh histological specimen bags in a 15 ml chamber through which a suspension of *Pseudomonas aeruginosa* (10^5 CFU/ml) was flowed constantly at a rate of approximately 200 ml/day for a total of 3 days. Samples were removed at 24 h intervals, fixed in 4% glutaraldehyde for 2 h, dehydrated by exposure to ethanol rinses, coated with gold (20 nm) using a Sputter Coater Model SC500, and subjected to analysis using a JEOL Scanning Electron Microscope for comparison of the extent of adherence of the *Pseudomonas* organisms to the test and control surfaces.

3. Results and discussion

The synthesis of the emulsion polymers was straightforward. The best results were obtained by the addition of the initiator after all of the components were added. This provided the highest yield with a minimal amount of precipitated polymer formation. Also, it was found that the initiator level should be below 5% of the total concentration, since higher concentrations reduce the molecular weight, enhance polymer branching, and increase the amount of precipitate formed.

The synthesis of graft copolymers is a very complex process since many undesirable reactions can occur. Chemical grafting is the method of choice since γ-radiation grafting is expensive and difficult to control. The only limitation to chemical grafting is that the targeted polymer backbone must be in a surfactant-stabilized latex form. The reason for this is, that it is hypothesized [10] that a radical is transferred on to the polymer backbone via the surfactant, which then promotes grafting. The desired reaction is that the radical on the polymer will attack a monomer molecule in the grafting process. To insure high grafting efficiency, it is necessary to have a large excess of monomer present, or have a highly reactive monomer. The latter is preferable, since a large excess of monomer will also form homopolymer which can be difficult to separate from the grafted polymer. An undesirable reaction that can occur is that the radical on the polymer backbone can fold back and attack another site on the polymer. This causes crosslinking and is undesirable because the crosslinked polymer will precipitate out of solution and not give grafted product. To reduce crosslinking, it is best to have a monomer present that is much more reactive than the polymer. The grafting procedure in this work was optimized to produce the highest grafting efficiency and the lowest amount of crosslinking. This was achieved by using monomers (**1a–d**) which are very reactive and adding the initiator in low concentrations at higher temperatures. This procedure allowed the polymers to remain in solution while providing the highest amount of grafted product. Also, any homopolymer formed precipitated out of solution and was removed by decanting off the latex.

The films formed by the various latexes, containing oxazolidinone monomer within the co-polymer in the concentration range 10–50 wt%, were clear, showed good abrasion resistance, and had good adherence to various substrates. Poly-vinyl chloride films tended to discolour slightly during processing because they are heat sensitive. The chlorination time differed with the different types of coatings, since some were more hydrophillic than others and required less time for chlorination. For example, compound **2a** was chlorinated for 20 min; however, the more hydrophillic polymer **2d** only needed a 10 min chlorination time. In all cases the two types of control experiments yielded plates which contained confluent growth too numerous to count indicating that the bacterial samples were viable. Inactivation of the organism was considered to be at least 99.9999% when no colonies were detected in the thiosulfate-quenched aliquots. The results are tabulated in Table 1. The data in Table 1 demonstrate that all of the *N*-halamine biocidal compounds tested were effective at inactivating *S. aureus* on a glass surface.

Zone of inhibition studies were performed for various fabric materials coated with poly *N*-halamines. The zones

Table 1
Efficacies of *N*-halamine coatings against *S. aureus* on glass[a]

Compound	Chlorination time (min)	Age (days)	Contact time for 6-log inactivation of *S. aureus* (min)
2a	20	5	10–20
2d	10	10	5–10
3a	20	30	5–10
3d	10	15	5–10
3e	30	3	30–60

[a]All of the coatings contain a concentration of 10 wt% oxazolidinone within the co-polymers

Table 2
Efficacies of poly *N*-halamine coatings against *S. aureus* on fabric[a]

Polymer	Fabric material[b]	% weight increase	Zone (mm)[c]
2a	Printcloth	16.5	0.5
2a	Cotton	23.2	1.0
2d	Printcloth	21.6	0.1
2d	Cotton	30.5	0.2
3b	Printcloth	29.6	0.5
3b	Cotton	22.1	0.5
3d	Printcloth	13.5	0.8
3d	Cotton	26.8	1.2
3	Printcloth	29.6	0.3
3	Cotton	32.3	0.5

[a]All of the coatings contain a concentration of 10 wt% oxazolidinone within the co-polymers
[b]Printcloth consists of 54/46 cotton/polyester blend
[c]Length in mm from the edge of the fabric to the viable bacteria

of inhibition were measured, and the results are tabulated in Table 2. The bacteria were not able to colonize on the fabric samples, and small zones of inhibition were produced around them. These cloth samples were actively bactericidal for several days, and the coatings did not change the overall textile appearance or properties drastically until the weight increase exceeded 30%.

For the coated fabric samples tested against *Salmonella enteritidis*, the numbers of colony forming units of surviving bacteria present in the eluates were determined, and compared to the total numbers detected in the eluates from the corresponding challenged control samples, to establish the percent reduction brought about by each of the chlorinated polymers. The data are tabulated in Table 3. The three polymer coatings tested were clearly effective at significantly reducing the numbers of CFU of *Salmonella enteritidis* over a 10 min contact period.

Finally the results of the biocidal test in which water contaminated with *Pseudomonas* was flowed over the surfaces showed good resistance to bacterial adherence. At each test period the bacteria were observed to be adherent in increasing numbers to the control sample surfaces, so that by 72 h there was extensive slime formation over the entire surface as a homogeneous layer. In contrast, no biofilm formation occurred on the chlorinated test sample at any observation point. A few colonies of *Pseudomonas* were adherent on the **3d** surfaces after 72 h, but the **2d** surface was almost entirely free of bacteria at this sampling time. It may be concluded from these data that surfaces treated with these *N*-halamine polymers can effectively inhibit biofilm formation by *Pseudomonas aeruginosa* for up to 72 h.

The biocidal activities for the granular, coated glass, and textile surfaces could be replenished by soaking the coated material in a solution of free chlorine, and comparable results were obtained once the *N*-halamine was reactivated (data not shown). This technique should also be successful for the bacteria flow experiments discussed above, although the experiments have not yet been performed.

Table 3
Efficacies of poly *N*-halamine coatings against *S. enteritidis* on fabric[a]

Polymer	Fabric material[b]	% dry weight gain on fabric	% reduction of bacteria in 10 min
2a	Printcloth	20.3	97.0
2d	Printcloth	18.5	99.99
3	Printcloth	22.3	99.9

[a]All of the coatings contain a concentration of 10 wt% oxazolidinone within the co-polymers
[b]Printcloth consists of 54/46 cotton/polyester blend

4. Conclusion

It can be concluded from this work that members of a new class of *N*-halamine biocidal polymer coatings have been synthesized. These coatings, once chlorinated, are effective at inactivating both Gram-negative and -positive bacterial organisms in relatively short contact times. The emulsified polymers can be prepared by copolymerization of the *N*-halamine precursor monomer with another monomer, or by grafting the *N*-halamine precursor monomer onto an emulsified polymer, followed by chlorination. These emulsions show commercial promise and should be useful in a broad range of applications.

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