Biocidal Polymers (I): Preparation and biological activity of some novel biocidal polymers based on Uramil and its Azo-dyes

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Abstract

Biocidal polymers were prepared by reacting uramil with polyacrylonitrile and polyethylacrylate, and also by co-polymerizing heterocyclic monomers based on uramil azo-dyes with tolylene-2,6-diisocyanate and toluene-2,4-diisocyanate. The prepared polymers were converted to their quaternary salts and N-halamine forms. Prepared N-halamine polymers were designed to contain multi available positions for halogenation and quaternarization and to tune the stability of halogens attached to the polymers. Antimicrobial activity of the prepared polymers against examples of Gram-positive and Gram-negative bacteria was examined.

Key words: biocidal polymers; uramil; azo-dye; antimicrobial; N-halamine.

1. Introduction

A biocidal polymer is a polymer with the ability to kill microorganisms, by acting as a source of sterilizing ions or molecules. Four types of biocidal polymers have been described; polymers containing quaternary nitrogen atoms (poly-quats) (scheme 1, a) [1, 2], polymers containing phosphonium salts (polymeric phosphonium materials) (scheme 1, b) [3-12], polymers containing halogenated sulphonamide groups [halogenated poly(styrene-co-divinylbenzene)sulphonamide polymers] (scheme 1, c) [13, 14] and N-halamine polymers (scheme 1, d) [15-25]. Some poly-quats and polymeric phosphonium materials are water-soluble. As such, they are unsuitable for drinking water sterilization since they can adversely affect human health. They can be modified to be water-insoluble by converting them to the cross-linked form or by increasing their molecular weight and used as emulsions or suspensions in water. The mode of action of the these two types of biocidal polymers depend on surface

adsorption, the polymer carries positive charges and bacteria at physiological pH carry a negative charge, resulting in cells being adsorbed. The poly-cations diffuse through the cell wall to bind to the cytoplasmic membrane resulting in disruption; releasing K^+ ions and the constituents of the cytoplasmic membrane resulting in cell death [26]. Most halogenated sulphonamide polymers are water-insoluble and their antibacterial action depends on the halogen exchange between the polymer and the bacterial cell, disturbing protein metabolism and causing cell death [13, 14].

The N-Halamine biocidal polymers described in the literature are water-insoluble; therefore they can be used safely in sterilizing drinking water and many other applications, such as disinfecting water supplies, swimming pools, hot-tubs, industrial water systems, and other applications where a sanitized water supply is required [15-25]. N-Halamine biocidal polymers have been prepared previously by introducing five or six membered heterocyclic rings containing amide or imide functional groups to be halogenated (Scheme 1, d) [15-25]. The mode of action of N-halamine biocidal polymers, it has been suggested, depends on contact between the polymer and the bacteria which results in halogen exchange, killing the bacteria. N-halamine biocidal polymers are very stable, do not decompose in water to form toxic products, or release halogen until contact with the bacteria occurs [27].

In this work, uramil (**1**) not previously exploited in biocidal polymers, was introduced on polymers (polyacrylonitrile or polyethylacrylate) (See also Scheme 7). In addition, novel monomers containing this heterocyclic system were prepared which co-polymerized with other simple monomers such as tolylene-2,6-diisocyanate and toluene-2,4-diisocyanate. These polymers were modified in two ways: first, by converting the polymer to its quaternary salt, which confers limited water solubility, resulting in effective biological activity (poly-quats) [28, 30]; the second method was halogenating the polymers to convert them to the N-halamine form which are completely water insoluble [15-25]. The prepared polymers were designed to contain more available positions for halogenation and quaternarization than described for N-halamine polymers in the literature. In addition, the uramil structure also aids stabilization of the halogens on the polymer. Prepared polymers (the modified) were tested for bactericidal activity against representative Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The unmodified polymers were insoluble in water and their activity was measured as controls. In addition, the biological activity of the unmodified polymers saturated in DMSO, as they are

completely insoluble in water and most organic solvents, was also evaluated as potential disinfectants, antibiotics and antifouling agents.

2. Experimental

2.1. Materials

Barbituric acid, granulated tin, resorcinol, *m*-phenylenediamine, fuming nitric acid, sodium nitrite, polyacrylonitrile (average M_w 150,000), polyethylacrylate (average M_w 95,000), toluene-2,4-diisocyanate, toluene-2,6-diisocyanate, bromine and iodine were supplied by Sigma Aldrich Chemicals, UK. Sodium hydroxide, hydrochloric acid, potassium permanganate, absolute ethanol, sulphuric acid, starch, sodium thiosulfate, potassium iodide, dimethylformamide (99.99%) and methanol (analytical grade reagents) were supplied by Fisher Chemicals, UK.

2.2. Preparation of polymers

2.2.1 Preparation of poly(N-iminouramil)ethylene (2) (series 1)

Polyacrylonitrile (0.50 g, 0.01 mol) and uramil (5-aminobarbituric acid) (**1**) (1.4 g, 0.01 mol) (prepared as described in the literature [29]) were heated in 30 ml of dimethylformamide/absolute ethanol (2:1, v:v) at 120°C in the presence of sodium hydroxide (0.40 g, 0.01 mol) for 48 hours. The reaction mixture was cooled and added gradually to a flask containing 50 g ice and the pH adjusted to 7 using 0.1M HCl. The reddish brown solid product was filtered, dried and weighed, which produced 1.5 g (73% yield), Scheme (7).

Analysis, FT-IR (KBr): ν_{max} (cm⁻¹) 1603 (C=O, imide), 1690 (C=O, amide), 1498 (C=N), 1218 (C-N), 3200 (N-H, imide) and 3421 (N-H amine). ¹H NMR (DMSO, 500 MHz): δ 2.04 (b.s, 2H, CH₂), 2.9 (s, 2H, NH₂), 2.1 (b.s, 1H, CH), 3.2 (s, 2H, 2NH imides), and 7.9 (s, 1H, ring CH). ¹³C NMR (DMSO, 125 MHz); ppm 28.6 (CH), 28.3 (CH₂), 33.5 (CH, ring), 36.4 (C-N), 121 (C=O, amide) and 163 (C=O, imide). Elemental analysis, found (%): C, 42.0; H, 4.1; N 27.8. Calc. for C₇H₈N₄O₃ (%): C, 42.9; H, 4.1; N, 28.6.

The same reaction was performed using polyethylacrylate (1.0 g, 0.01 mol) instead of polyacrylonitrile to prepare 5-polyacrylamidobarbituric acid (**7**) (series 2) and the final red solid product was filtered, dried and weighed, which produced 1.2 g (61% yield), Scheme (8).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm^{-1}) 1625 (C=O, no. 2), 1690 (C=O, amide, d), 1666 and 1680 (2C=O amide no. 3 and no. 1 respectively), 3113 (NH, imide) and 3447 (NH, amide) (scheme 2). ^1H NMR (DMSO, 500 MHz): δ 1.2 (b.s, 2H, CH_2), 1.5 (b.s, 1H, CH), 5.4 (s, 1H, no 1), 7.1 (s, 1H, no. 2), 10.4 (s, 1H, no. 3) and 4.1 (s, 1H, no. 4) (scheme 2). ^{13}C NMR (DMSO, 125 MHz): ppm 13.8 (CH_2), 28 (CH), 118 (ring CH), 150 (C=O, d), 163 (C=O, a), 164.9 (C=O, b) and 172 (C=O, c), (Scheme 2). Elemental analysis, found (%): C, 42.4; H, 21.4; N, 3.3. Calc. for $\text{C}_7\text{H}_7\text{N}_3\text{O}_4$ (%): C, 42.6; H, 21.5; N, 3.6.

2.2.2. Diazotization of uramil

Uramil (1.40 g, 0.01 mol) was dissolved in 5 ml concentrated sulphuric acid. The temperature was kept at 0°C using an external ice bath. A cold solution of NaNO_2 [0.69 g of NaNO_2 (0.01 mol) + 10 ml water] was added drop wise to the uramil solution with stirring to form the uramildiazonium salt (**12**), Scheme (9).

2.2.3. Preparation of 1,3-diamino-4-azo(5-barbituric acid)benzene (**13**)

m-Phenylenediamine (1.1 g, 0.01 mol) and sodium hydroxide (2.8 g, 0.07 mol) were dissolved in 10 ml water. The temperature was kept at 0°C using an external ice bath. This cold solution was added gradually to the uramildiazonium salt (**12**) with stirring. The resulting deep red solid product was filtered, dried and weighed, which produced 2.4 g (90% yield), Scheme (9).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm^{-1}) 3330 (NH, imide), 3392 (NH, NH_2 near azo group), 3429 (NH, NH_2 group away azo group), 1603 (C=O, imide), 1680 (C=O amide), 2942 (CH aliphatic) and 1411 (N=N). ^1H NMR (DMSO, 500 MHz): δ 5.4 (s, 2H, NH_2 away azo group), 1.4 (s, 2H, NH_2 near azo group), 6.1 (s, 2H, 2NH imide), 10.3 (s, 1H, ring CH) and 6.9-7.5 (m, 3H, benzene ring). ^{13}C NMR (DMSO, 125 MHz): ppm 31 (ring CH), 175.9 (C=O, amide), 220 (C=O, imide) and 96.8, 107.8, 116.9, 124.5, 145 and 150 (aromatic). Elemental analysis, found (%): C, 45.1; H, 3.6; N, 32.9. Calc. for $\text{C}_{10}\text{H}_{10}\text{N}_6\text{O}_3$ (%): C, 45.8; H, 3.8; N, 33.1.

2.2.4. Preparation of poly[1,3-diamino-4(5-azobarbituric acid)benzene)-co-(tolylene-2,6-diisocyanate)] (poly-urea) (**14**) (series 3)

Monomer (**13**) (2.6 g, 0.01 mol) and tolylene-2,6-diisocyanate (1.7 g, 0.01 mol) were heated in 30 ml dimethylformamide at 90°C for 5 hours. The reaction mixture was cooled and 50 ml of methanol was added. The brown product was filtered, washed copiously with methanol, dried and weighed, which produced 4.1 g (93 % yield), Scheme (10).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm⁻¹) 1639 (C=O, imide), 1680 (C=O, amide), 3436 (N-H, broad band), 1118 (C-N) and 1472 (N=N). ¹H NMR (DMSO, 500 MHz): δ 2.2 (s, 3H, CH₃), 4.2 (s, 1H, NH, no.1), 5.9 (s, 1H, NH, no. 3), 4.8 (s, 1H, NH, no. 2), 6.7 (s, 2H, 2NH imide), 7-8.3 (m, 6H, aromatic), 6.5 (s, 1H, NH, no.4) and 9.2 (s, 1H, heterocyclic ring CH) (Scheme 3). ¹³C NMR (DMSO, 125 MHz): ppm 12.4 (CH₃), 49 (heterocyclic ring CH), 100.3, 110, 111.3, 111.7, 113, 118, 121, 123, 125, 137, 144 and 146 (aromatic), 162 (C=O, imide), 152 (C=O, a), 153 (C=O, b) and 174 (C=O, heterocyclic ring amide) (Scheme 3). Elemental analysis, found (%): C, 52.1; H, 3.5; N, 24.8. Calc. for C₁₉H₁₆N₈O₅ (%): C, 52.3; H, 3.6; N, 25.7.

The same reaction was performed using toluene-2,4-diisocyanate to prepare poly[1,3-diamino-4(5-azobarbituric acid)benzene)-*co*-(tolulene-2,4-diisocyanate)] (poly-urea) (**19**) (series 4).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm⁻¹) 1609 (C=O, imide), 1650 (C=O, amide), 3446 (N-H, broad band), 1136 (C-N) and 1547 (N=N). ¹H NMR (DMSO, 500 MHz): δ 2.1 (s, 3H, CH₃), 4.8 (s, 1H, NH, no.1), 6.7 (s, 1H, NH, no. 3), 7.0 (s, 1H, NH, no. 2), 7.3 (s, 2H, 2NH imide), 7.9-8.8 (m, 6H, aromatic), 7.7 (s, 1H, NH, no.4) and 9.1 (s, 1H, heterocyclic ring CH) (Scheme 4). Elemental analysis, found (%): C, 52.2; H, 3.4; N, 24.9. Calc. for C₁₉H₁₆N₈O₅ (%): C, 52.3; H, 3.6; N, 25.7.

2.2.5. Preparation of 1,3-dihydroxy-4(5-azobarbituric acid)-benzene (**24**)

Resorcinol (1.1 g, 0.01 mol) and NaOH (5.5 g, 0.14 mol) were dissolved in 20 ml water and added gradually to cold uramildiazonium salt (**12**). The dark purple product was filtered, washed copiously with cold water, dried and weighed, which produced 2.6 g (99% yield), Scheme (9).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm⁻¹) 1603 (C=O, imide), 1705 (C=O, amide), 1411 (N=N), 3100 (N-H), 3432 (OH) and 2942 (CH aliphatic). ¹H NMR (DMSO, 500 MHz): δ 1.3 (s, 1H, OH near azo group), 5.4 (s, 1H, OH away azo group), 6.2 (s, 2H, 2NH imides), 6.9-7.2 (s, 3H, aromatic) and 10.2 (s, 1H, heterocyclic ring CH). ¹³C NMR (DMSO, 125 MHz): ppm 49 (heterocyclic ring CH), 102.4, 103, 105, 106, 129 and 150.3 (aromatic), 158.3 (C=O, amide) and (C=O, imide). Elemental analysis, found (%): C, 45.1; H, 2.9; N, 20.9. Calc. for C₁₀H₈N₄O₅ (%): C, 45.5; H, 3; N, 21.2.

2.2.6. Preparation of poly[(1,3-dihydroxy-4(5-azobarbituric acid)-benzene)-*co*-(tolylene-2,6-diisocyanate)] (polyurethane) (**25**) (series 5)

Monomer (**24**) (2.6 g, 0.01 mol) and tolylene-2,6-diisocyanate (1.7 g, 0.01 mol) were heated in 30 ml dimethylformamide for 5 hours at 90°C. The reaction was

cooled and 50 ml of methanol was added. The brown product was filtered, washed copiously with methanol, dried and weighed, which produced 3.8 g (86% yield), Scheme (11).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm⁻¹) 1640 (C=O, imide), 1700 (C=O, urethane group), 1660 (C=O, heterocyclic ring amide), 3429 (N-H), 1135 (broad band for C-O & C-N bonds), 1471 (N=N) and 2920 (CH aliphatic). ¹H NMR (DMSO, 500 MHz): δ 2.2 (s, 3H, CH₃), 4.8 (s, 1H, NH no.1), 4.2 (s, 1H, NH no.2), 6.8 (s, 2H, 2NH imide), 10.5 (s, 1H, heterocyclic ring CH) and 7.0-8.4 (s, 6H, benzene rings) (Scheme 5). ¹³C NMR (DMSO, 125 MHz): ppm 11.4 (CH₃), 49 (heterocyclic ring CH), 109.9, 111.6, 113.2, 116 117.9, 118.5, 120, 121, 125, 137.3, 137.7 and 146 (aromatic), 150 (C=O, ring amide), 185 (C=O, imide), 163.0 (C=O, b) and 153.2 (C=O, a) (Scheme 5). Elemental analysis, found (%): C, 51.7; H, 3.2; N, 18.1. Calc. for C₁₉H₁₆N₈O₇ (%): C, 52.1; H, 3.2; N, 19.2.

The same reaction was performed using toluene-2,4-diisocyanate to prepare poly[1,3-dihydroxy-4(5-azobarbituric acid)benzene)-*co*-(tolulene-2,4-diisocyanate)] (polyurethane) (**30**) (series 6).

Analysis, FT-IR (KBr): $\nu_{\max}$ (cm⁻¹) 1617 (C=O, imide), 1639 (C=O, amide), 1712 (C=O, urethane), 3417 (N-H, imide), 3458 (N-H, amide), 3550 (OH), 110 (C-N & C-O) and 1457 (N=N). ¹H NMR (DMSO, 500 MHz): δ 2.0 (s, 3H, CH₃), 6.4 (s, 1H, NH, no.1), 7.1 (s, 1H, NH, no. 2), 6.9 (s, 2H, imide), 7.4-8.5 (m, 6H, aromatic), and 9.4 (s, 1H, heterocyclic ring CH) (scheme 6). ¹³C NMR (DMSO, 125 MHz): ppm 12 (CH₃), 49 (heterocyclic ring CH), 106, 110, 113, 114, 115, 116, 119, 119.6, 120, 121, 123 and 126 (aromatic), 128 (C=O, ring amide), 151 (C=O, imide), 154 (C=O, b) and 160 (C=O, a) (scheme 6). Elemental analysis, found (%): C, 51.8; H, 3.2; N, 18.5. Calc. for C₁₉H₁₆N₈O₇ (%): C, 52.1; H, 3.2; N, 19.2.

All the prepared polymers are insoluble in water and most organic solvents. The non-modified polymers dissolve partially (less than 30%) in DMSO by soaking for 3-10 days at 30-40°C. The partially dissolved polymers were filtered to carry out the analysis. However, M_w characterization was not possible due to the need to completely dissolve the material in low-viscosity solvents for GPC.

2.2.7. Halogenation process

The different types of the n-halamine polymers were halogenated by the same method: polymer was dispersed in sodium hydroxide solution and the halogen

(chlorine, bromine or iodine) was added gradually until neutralization to pH 7. The mixture was stirred for a further hour during which the temperature was kept below 5°C using an external ice bath. The resulting product was filtered, washed copiously with chlorine free water, dried and weighed [31, 32]. The resulted polymers are completely water insoluble. The amounts of polymers and sodium hydroxide used in the preparation of each N-halamine polymer and the final yield are illustrated in Table 1.

The halogenation process was followed using IR spectroscopy [30-32] and the halogen/g content was determined using iodometric titration [24]. The values are given in Table 2.

2.2.8. Quaternarization process

The polymer was dispersed in water (10 ml) and diluted HCl added. The temperature was kept below 10°C using an external ice/water bath and the mixture was stirred for 1 hour. The polymer salts were partially soluble in water (20% maximum). Methanol (50 ml) was then added to the solution to precipitate the dissolved polymer. The resulting product was filtered, washed copiously with methanol, dried and weighed [28, 30]. The amounts of polymers, and the volumes of HCl used are given in Table 3.

2.3 Biological activity of the prepared polymers

The prepared polymers were tested against both Gram-positive and Gram-negative bacteria as follows:

2.3.1 Agar plate method

Nutrient agar (Oxoid) was prepared (250 ml), held molten at 50°C and 1.0 ml of a 24hr nutrient broth culture of either *Staphylococcus aureus* or *Escherichia coli* was added as an inoculum. The seeded agar was poured into square assay plates (24 x 24 cm); two plates for each type of bacterium, Gram-positive and Gram-negative bacteria. Thirty-six, 5 mm wells were cut into the agar according to a square pattern (chessboard). Small amounts of each polymer (0.03 g) were placed in a well in the middle of each square. The experiment was performed in replicate, three wells for each polymer. The plates were incubated for 24 hours at 37°C and the inhibition zones around the polymers were recorded. All the prepared polymers, including halogenated polymers and the quaternarized salts were examined for biocidal activity. The unmodified polymers were used as controls [28, 30-32].

The unmodified polymers (**2**, **7**, **14**, **19**, **25** and **30**), as well as monomers (**13** and **24**) were examined in their partially soluble form by dissolving them in dimethylsulphoxide (DMSO) and the solvent was used as control. For each polymer 0.01 g was dissolved in 1ml DMSO (the samples were soaked in DMSO for more than 10 days) and 0.03 ml from the prepared solution was added to the wells in the plates, the experiment was performed in replicate, three wells for each polymer.

3. Results and Discussion

N-halamine biocidal polymers have been used in sterilization technology for almost a decade [5-25]. To prepare this type of polymer a heterocyclic ring is introduced into the polymer backbone or side chain. The heterocyclic ring contains amide or imide function groups which can be halogenated [5-25]. In this work the heterocyclic polymers were prepared by reacting the ready-prepared 5-aminobarbituric acid ring (**1**) (uramil) with a polymer (Schemes 7 and 8). Uramil contains three main positions available for halogenation which gave the opportunity to prepare multi-halogen biocidal polymers (MHBP). This increases the number of available halogens per monomer unit compared with existing N-halamine polymers [5-25]. These positions can also be acidified to form poly-quats.

The positions in uramil available for halogenation are imides, rather than amides. Halogenated imide groups are considered to have potential as biocides as the imide group can easily release free halogen [35, 36]. The dissociation constant for the imide halogen in trichlorocynuric acid for example is from 1.6×10^{-12} to 8.5×10^{-4} , for amide halogen is 1.6×10^{-8} in 1,3-dichloro-2,2,5,5-tetramethylimidazolidine-4-one and for amino halogen is $<10^{-12}$ for 1-chloro-2,2,5,5-tetramethylimidazolidine-4-one [35]. This indicates that the amine halamine bond is the most stable but results in a less bioactive polymer as halogen is not easily released in aqueous media [35]. The imide halogen exhibits the lowest stability but offers the best biological action while amide halogens have intermediate stability and moderate biological activity; enough for effecting sterilization [35, 36]. The presence of the methyl groups on the heterocyclic rings in 1,3-dichloro-2,2,5,5-tetramethylimidazolidine-4-one and 3-chloro-4,4-dimethyl-2-oxazolidinone increases the stability of the halogens attached to the polymer by increasing the electron density over the ring, and this makes the hydantion based polymers the best halamine biocidal polymers currently on the market [15-25]. We took this into consideration to design a new type of halamine

polymer containing imide groups where the stability improved by increasing the electron density over the ring. In polymer (**2**) (scheme 7) prepared by reacting uramil with polyacrylonitrile; the resulting structure contains enamine groups in resonance with the ring. The resonance driven by the electron pairs of the amino group will result in increasing the electron density over the heterocyclic ring which was expected to stabilize the nitrogen-halogen bond, making the release of halogen more difficult. The same effect was expected with polymer (**7**) (scheme 8), in this case the OH group of the amidol form being the resonance driving force. The monomers (**13**, **24**) (scheme 9) were designed following a similar reasoning. In this case we expect that by coupling the heterocyclic ring over *m*-phenylenediamine or resorcinol good resonance, from the amino or hydroxyl groups through the aromatic ring and the azo group to the heterocyclic ring, increases the electron density over the heterocyclic ring. This results in increased stability of the halogen-nitrogen bond on the ring. After co-polymerization to form the poly-urea and polyurethane, the nitrogen and the oxygen lone electron pairs still have the ability to resonate with the heterocyclic ring creating stability.

Thus, by making use of uramil and the selected polymer backbones, we aimed to develop a new series of water insoluble N-halamine biocidal polymers, exhibiting a good balance of stability-biocidal activity and containing multi-available positions for halogenation (4 or more). N-halamine biocidal polymers currently available on the market depend on only one available position for halogenation based on hydantions systems [15-25]. Some previous examples of N-halamine biocidal polymers have been reported based on 2 and 3 available halogenation positions, using six membered heterocyclic rings, but the stability did not improve in these polymers over traditional halogenated hydantoins [28, 31-32].

Reacting uramil with polyacrylonitrile gave polymer (**2**) (scheme 7). IR characterisation indicated that the reaction had reached completion (disappearance of the cyano peak at 2240 cm⁻¹). Using polyethylacrylate instead of polyacrylonitrile gave polymer (**7**) (scheme 8) also quantitatively, IR characterisation showed complete disappearance of ester carbonyl group peak at 1735 cm⁻¹.

The halogenation of polymer (**7**) was a highly exothermic reaction, unlike the halogenation of (**2**). In spite of careful temperature control and slow addition of halogen to polymer (**7**) the halogenation yield was very low. A plausible explanation is that some amide bonds connecting the heterocyclic ring to the polymer were

hydrolysed during halogenation. This is supported by IR data which shows the presence of carboxylic acid OH group in polymer (8) ($2423\text{--}3510\text{ cm}^{-1}$) (Scheme 8) and the low halogen content measured, compared with similar polymers (polymers 8-10, Table 2).

Monomers (13, 24) (scheme 9) were prepared by coupling uramildiazonium salt with resorcinol and *m*-phenylenediamine. These monomers were considered as novel azo-dyes. These two monomers were co-polymerized with tolylene-2,6-diisocyanate and toluene-2,4-diisocyanate to form polyurethanes (25, 30) and poly-ureas (14, 19). In polyurethanes (25, 30) there are 5 available positions for halogenation and in poly-ureas (14, 19) there are 7 positions for halogenation. From the IR data the azo group peak appeared around 1411 cm^{-1} [33, 34] in both of the two monomers (13 & 24) (and in the resulting polymers) indicating that the coupling was successful. This method of preparation resulted in the development of MHBP with an even greater number of available halogens than polymers (2) and (7).

Tested against bacteria, including Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), the polymers were divided into three categories: The first group is the polymer without any modification (water insoluble polymers) and this category includes polymers (2), (7), (14), (19), (25) and (30) that acted as controls. The second category contains the water insoluble N-halamine biocidal polymers resulting from chlorination, bromination and iodination of the first category and this includes polymers (3), (4), (5), (8), (9), (10), (15), (16), (17), (26), (27), (28), (31), (32) and (33). The third category contains the quaternarized forms (has a limited water soluble amount), which resulted from acidification of the first group and this includes polymers (6), (11), (18), (23), (29) and (34). The polymers results from modifications to polymer (2), (7), (14), (19), (25) and (30) were considered as series 1, 2, 3, 4, 5 and 6 respectively (scheme 7-8, 10-11). Biological activity was tested by the agar plate method described earlier. Zones of inhibition of bacterial growth around each polymer were recorded after incubation at 37°C for 24 hours (Tables 4 and 5).

From tables 4 and 5, the controls (the original polymers without modification) did not show any biological activity; these polymers are not soluble in water and do not release any effective ion that can diffuse into the medium. The second category, N-halamine biocidal polymers, showed significant inhibition zones. These polymers are also water-insoluble; implying their mode of action is the release into the medium of

soluble halogen-containing ions that are lethal to the bacterial cells. The action of the third category (Poly-quats) depends on the solubility of the polymer itself. The conversion of the polymer to its quaternary salt increases its solubility (to a limited extent) so it can diffuse through the medium and exert its biocidal effect. Polymer (7) and its modified polymer (8) (chlorinated) did not show any bactericidal activity while the other modified forms of it showed low biological activity. This may be due to the halogenation of this polymer being a highly exothermic reaction which resulted in hydrolysis of some heterocyclic rings leading to a decrease in its biological power. From tables 4 and 5 it was clear that the N-halamine polymers in series 3-6 show greater biological activity than series 1 and 2; perhaps because the number of available positions for the halogen in series 3-6 is greater than that in series 1 and 2, which is reflected in the amount of halogen loading on the polymer in table 2. The biological activity of the quaternarized forms is similar; especially series 1 which becomes very similar to series 3-6, and may be because the repeating unit molecular weight of the poly-quats in series 1 is very low compared to the available positions for acidification on its surface.

The control polymers (2), (7), (14), (19), (25) and (30) as well as the monomers (13 and 24), were also tested as bioactive materials by dissolving them in an organic solvent (they are dissolving partially in DMSO) and using this solvent as a control. The results are in table 6.

DMSO was used as the control and its inhibition zone diameter was 5 mm for both *E. coli* and *S. aureus*. The only exception is monomer (24) which was dissolved in water. From table 6 it was clear that all the prepared polymers and monomers show biological activity. This indicates a potential use for these polymers as antifouling agents and as antiseptics.

Pervious work suggest that N-halamine biocidal polymers act by contact.²⁰⁻²³ However, the results of our inhibition zone assays on water insoluble polymers indicates another mechanism, based on the release of active species. Further investigations into these polymers are being conducted to quantify the biological activity and determine the mode of action the results will be communicated in due course. Optimisation of conditions to achieve maximum halogenation of these novel biocidal polymers is also under investigation together with using uramil (1) to produce other novel monomers and polymers for use in different applications.

4. Conclusions

Insoluble N-halamine polymers and partially soluble poly-quats biocidal polymers were prepared and their structures were determined using different spectroscopic techniques. These polymers have multi-available positions for halogenation and the halogen-nitrogen bond was stabilized by introducing electron donating groups on the heterocyclic ring to balance the stability and enhance the biological activity. Most of these novel polymers show high disinfecting power against different types of bacteria (Gram-positive and Gram-negative) especially the N-halamine polymers. The partially soluble forms of the control polymers and the soluble monomers also have potential as disinfecting agents.

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$-(\text{CH}_2-\text{CH})_n-$ <p> $\text{A} = \text{Cl}^-, \text{Br}^-$ $\text{R} = \text{alkyl group}$ (a) </p>	$-(\text{CH}_2-\text{CH})_n-$ <p> $\text{A} = \text{Cl}^-, \text{Br}^-$ $\text{R} = \text{alkyl group}$ (b) </p>
$-(\text{CH}_2-\text{CH})_n-$ <p> $\text{X} = \text{Cl}, \text{Br}$ $\text{R} = \text{H}, \text{Na}, \text{Cl}, \text{alkyl group}$ (c) </p>	$-(\text{CH}_2-\text{C})_n-$ <p> $\text{X} = \text{Cl}, \text{Br}$ $\text{R} = \text{H}, \text{CH}_3, \text{R}' = \text{alkyl group}$ (d) </p>

Scheme 1. Structures of the different biocidal polymers.

Scheme 2. Numbering the hydrogen and carbon atoms in 5-polyacrylamidobarbituric acid.

Scheme 3. Numbering the hydrogen and carbon atoms in poly-urea (**14**).

Scheme 4. Numbering the hydrogen and carbon atoms in poly-urea (**19**).

Scheme 5. Numbering the hydrogen and carbon atoms in polyurethane (**25**).

Scheme 6. Numbering the hydrogen and carbon atoms in polyurethane (**30**).

Scheme 7. Preparation of poly(N-iminouramil)ethylene and its halogenation and acidification, series 1.

Scheme 8. Preparation of 5-polyacrylamidobarbituric acid and its halogenation and acidification, series 2.

Scheme 9. Diazotization of uramil and its coupling with *m*-phenylenediamine and resorcinol.

Scheme 10. Preparation of poly[1,3-diamino-4(5-azobarbituric acid)-benzene)-*co*-(tolylene-2, 6-diisocyanate)] (poly-urea) and its halogenation and acidification, series 3.

Scheme 11. Preparation of poly[(1,3-dihydroxy-4(5-azobarbituric acid)-benzene)-*co*-(tolylene-2,6-diisocyanate)] (polyurethane) and its halogenation and acidification, series 5.

Table 1

Amounts of polymers and sodium hydroxide used in the preparation of the N-halamine polymers and the final yields

Product.	Reactant (weight, quantity).	Sodium hydroxide (g)	Final yield
3 (chlorinated)	2 (1.96 g, 0.01 mol)	1.6 (0.04 mol)	2.0 g (67%)
4 (brominated)	2 (1.96 g, 0.01 mol)	1.6 (0.04 mol)	2.7 g (63%)
5 (iodinated)	2 (1.96 g, 0.01 mol)	1.6 (0.04 mol)	3.1 g (54%)
8 (chlorinated)	7 (1.97 g, 0.01 mol)	1.6 (0.04 mol)	1.5 g (44%)
9 (brominated)	7 (1.97 g, 0.01 mol)	1.6 (0.04 mol)	2.0 g (39%)
10 (iodinated)	7 (1.97 g, 0.01 mol)	1.6 (0.04 mol)	2.5 g (36%)
15 (chlorinated)	14 (0.44 g, 0.001 mol)	0.28 (0.007 mol)	0.5 g (74%)
16 (brominated)	14 (0.44 g, 0.001 mol)	0.28 (0.007 mol)	0.6 g (61%)
17 (iodinated)	14 (0.44 g, 0.001 mol)	0.28 (0.007 mol)	0.8 g (62%)
20 (chlorinated)	19 (0.44 g, 0.001 mol)	0.28 (0.007 mol)	0.6 g (89%)
21 (brominated)	19 (0.44 g, 0.001 mol)	0.28 (0.007 mol)	0.7 g (71%)
22 (iodinated)	19 (0.44 g, 0.001 mol)	0.28 (0.007 mol)	0.9 g (70%)
26 (chlorinated)	25 (0.44 g, 0.001 mol)	0.20 (0.005 mol)	0.4 g (66%)
27 (brominated)	25 (0.44 g, 0.001 mol)	0.20 (0.005 mol)	0.6 g (67%)
28 (iodinated)	25 (0.44 g, 0.001 mol)	0.20 (0.005 mol)	0.7 g (64%)
31 (chlorinated)	30 (0.44 g, 0.001 mol)	0.20 (0.005 mol)	0.5 g (82%)
32 (brominated)	30 (0.44 g, 0.001 mol)	0.20 (0.005 mol)	0.7 g (78%)
33 (iodinated)	30 (0.44 g, 0.001 mol)	0.20 (0.005 mol)	0.8 g (73%)

Table 2

IR characterization and halogen content of N-halamine polymers.

Polymer	Bond	$\nu_{\max}$ (cm ⁻¹)	Halogen content (ppm)
3 (chlorinated)	N-Cl	593	101 ±12
4 (brominated)	N-Br	688	114 ±18
5 (iodinated)	N-I	802	116 ±22
8 (chlorinated)	N-Cl	784	40 ±16
9 (brominated)	N-Br	680	68 ±15
10 (iodinated)	N-I	583	93 ±32
15 (chlorinated)	N-Cl	763	190 ±11
16 (brominated)	N-Br	618	198 ±10
17 (iodinated)	N-I	777	201 ±26
20 (chlorinated)	N-Cl	619	187 ±14
21 (brominated)	N-Br	618	191 ±12
22 (iodinated)	N-I	689	206 ±20
26 (chlorinated)	N-Cl	600	160 ±17
27 (brominated)	N-Br	712	178 ±22
28 (iodinated)	N-I	789	193 ±30
31 (chlorinated)	N-Cl	634	176 ±11
32 (brominated)	N-Br	683	183 ±25
33 (iodinated)	N-I	722	197 ±23

Table 3

Amounts of polymers and HCl used in the preparation of poly-quats and the final yields

Product.	reactant (weight, quantity)	1M HCl quantity	Final yield
6	2 (1.96 g, 0.01 mol)	30.1 ml	2.1 g (70%)
11	7 (1.97 g, 0.01 mol)	30.1 ml	2.0 g (70%)
18	14 (0.44 g, 0.001 mol)	6.30 ml	0.5 g (83%)
23	19 (0.44 g, 0.001 mol)	6.30 ml	0.4 g (67%)
29	25 (0.44 g, 0.001 mol)	4.90 ml	0.5 g (74%)
34	30 (0.44 g, 0.001 mol)	4.90 ml	0.5 g (74%)

Table 4

Inhibition zones diameters (mm) around different polymers on agar plates containing Gram-negative bacteria

	series 1	series 2	series 3	series 4	series 5	series 6
Control	0	0	0	0	0	0
Chlorinated	7 ±1	0	18 ±1	29 ±0	24 ±1	28 ±1
Brominated	18 ±1	8 ±1	22 ±0	27 ±1	22 ±0	29 ±0
Iodinated	9 ±1	6 ±1	15 ±1	15 ±0	18 ±1	10 ±1
Poly-quats	15 ±2	8 ±0	6 ±1	13 ±1	16 ±1	13 ±1

Table 5

Inhibition zones diameters (mm) around different polymers on agar plates containing Gram-positive bacteria

	series 1	series 2	series 3	series 4	series 5	series 6
Control	0	0	0	0	0	0
Chlorinated	6 ±1	0	19 ±1	23 ±1	28 ±1	28±0
Brominated	14 ±1	10 ±0	20 ±1	21 ±1	24 ±1	25 ±1
Iodinated	9 ±0	8 ±1	18 ±1	31 ±2	19 ±1	20 ±1
Poly-quats	15 ±1	8 ±1	7 ±0	11 ±1	21 ±1	19 ±1

Table 6

The inhibition zones (mm) resulted from the dissolved control polymers and monomers

Compound no.	<i>E. coli</i>	<i>S. aureus</i>
DMSO (control)	5 ±0	5 ±0
2 (series 1 control)	9 ±1	7 ±1
7(series 2 control)	8 ±1	9 ±1
13 (monomer)	8 ±1	6 ±1
14 (series 3 control)	9 ±0	9 ±1
19 (series 4 control)	10 ±1	9 ±1
24 (monomer)	9 ±1	8 ±1
25 (series 5 control)	10 ±1	8 ±1
30 (series 6 control)	10 ±1	7 ±1