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# Biocidal polymers (II): Determination of biological activity of novel *N*-halamine biocidal polymers and evaluation for use in water filters

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ABSTRACT

Novel *N*-halamine biocidal polymers were prepared by co-polymerizing a heterocyclic ring-based monomer with tolylene-2,6-diisocyanate and toluene-2,4-diisocyanate. The resulting polyurethanes were halogenated; chlorinated, brominated or iodinated. The rate of bacterial killing by the halogenated derivatives was determined both with and without halogen quenching and one of them was evaluated for use in water filters. The effect of these polymers on bacterial growth-rates was also determined.

## 1. Introduction

*N*-halamine polymers are an important class of biocidal polymers [1–16]. This type of polymer is prepared by introducing a heterocyclic ring containing amino, amide or imide groups into the polymer structure followed by halogenation to the corresponding *N*-halamines, which confers on the polymer its biological activity [1–16]. Biocidal activity is modulated by halogen stability on the polymer [1–16]; halogenated amines are more stable than amides and imides [8]. In comparison, halogenated imides exhibit the lowest stability but show the most powerful biocidal activity [8,16]. In this work the prepared heterocyclic ring contains imide groups. The N-halogen bond has been stabilized by introducing electron donating groups on the ring; however, a high level of biocidal activity is still apparent [16]. Halogen loading of these polymers was increased with respect to other examples in the literature by choosing a heterocyclic ring (uramil) (**1**), Scheme 2, [16] that can be charged with a maximum of three halogens per unit. After polymerization, the number of the available positions for halogenation was increased to five (Scheme 2) [16] in comparison with the two or three available positions in those similar polymers currently available [1–9,13,14] (examples in Scheme 1d and e). We previously reported polymers prepared using uramil that showed good biological activity and good stability [16]. These polymers were prepared by reacting polyacrylonitrile and polyethylacrylate with uramil (Scheme 1a and b) but the number of available positions for halogens was lower than for those polymers now described. In addition, uramil-derived poly-urea was also prepared (Scheme 1c) [16], the number of positions available for halogens is 7 per repeating unit, and this will be evaluated in future work.

In this work we focus on a uramil-derived polyurethane (**4**) which was halogenated with Cl, Br or I and the most appropriate selected for different applications (e.g., drinking

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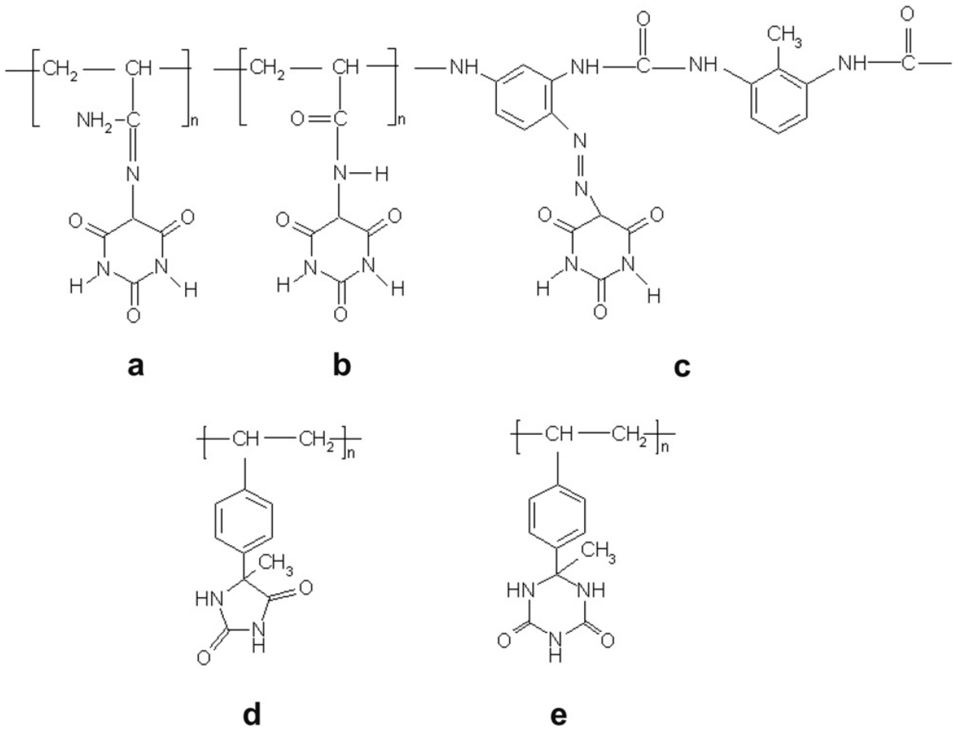

**Scheme 1.** Different types of *N*-halamine biocidal polymer.

water filters and sterilization). An analogous uramil-derived polyurethane (**8**) was chlorinated to compare its biological activity with the chlorinated form of (**4**). The bacterial killing power of each halogenated derivative, both with and without halogen quenching, was evaluated for cultures of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The effect of the non-halogenated polymer (**4**) on these bacteria was also examined.

## 2. Experimental

### 2.1. Materials

Barbituric acid, granulated tin, resorcinol, fuming nitric acid, sodium nitrite, toluene-2,4-diisocyanate, tolylene-2,6-diisocyanate, bromine and iodine were supplied by Sigma Aldrich Chemicals, UK. Sodium hydroxide, hydrochloric acid, potassium permanganate, sulphuric acid, sodium thiosulfate and dimethylformamide were supplied by Fisher Chemicals, UK. Nutrient broth and Nutrient agar (Oxoid).

### 2.2. Preparation of polymers

The polymers under investigation were prepared according to the methodology reported earlier [16], as follows:

#### 2.2.1. Diazotization of uramil

Uramil [17] (**1**) (5-aminobarbituric acid) (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 uramil diazonium salt (**2**) [16], Scheme 2.

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

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 uramil diazonium salt (**2**). The dark purple product that precipitated was filtered, washed copiously with cold water, dried and weighed, producing 2.6 g (99% yield), Scheme 2 [16].

Analysis, FTIR (KBr): $\nu$ ($cm^{-1}$) 1603, 1705, 1411, 3100, 3432 and 2942. $^1H$ NMR (DMSO, 500 MHz): $\delta$ 1.3 (s, 1H), 5.4 (s, 1H), 6.2 (s, 2H), 6.9–7.2 (s, 3H) and 10.2 (s, 1H). $^{13}C$ NMR (DMSO, 125 MHz): ppm 49, 102.4, 103, 105, 106, 129, 150.3 and 158.3. Elemental analysis, found (%): C, 45.1; H, 2.9; N, 20.9. Calculated for $C_{10}H_8N_4O_5$ (%): C, 45.5; H, 3; N, 21.2 [16].

#### 2.2.3. General procedure for the polyurethane polymers (*4*) and (*8*)

Monomer (**3**) (2.6 g, 0.01 mol) and a suitable diisocyanate (0.01 mol) were heated in 30 ml dimethylformamide for 5 h at 90 °C. The reaction was cooled and 50 ml of methanol added. The brown product was filtered, washed copiously with methanol, dried and weighed, Scheme 2 [16].

Poly[(1,3-dihydroxy-4(5-azobarbituric acid)-benzene)-co-(tolylene-2,6-diisocyanate)] (**4**) was prepared using