Macroscopic *N*-Halamine Biocidal Polymeric Beads

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ABSTRACT: The particle size of *N*-halamine biocidal polymers was methodically modified forming beads of different sizes by blending water-insoluble *N*-halamine polyurethane with sodium alginate as the matrix and loading heterocyclic rings onto modified silica gels. The biological activity of the prepared beads and halogenated modified silica derivatives was evaluated against examples of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. The recycling possibilities and the optimum preparation conditions of the blended beads were investigated; blending prehalogenated polyurethane (5%, w/v) with sodium alginate (3%, w/v) followed by crosslinking with $CaCl_2$ (10%, w/v) at 40°C are the optimum preparation conditions for the alginate beads. J Appl Polym Sci 116: 2396–2408, 2010

Key words: *N*-halamine; beads; silica; bacteria

INTRODUCTION

N-halamine polymeric materials have been studied extensively over the last 2 decades.[1–20] Prepared by constructing heterocyclic rings containing amide or imide groups on polymeric supports,[1–20] these polymers have to be halogenated to exert biocidal action.[1–20] For such applications, *N*-halamine polymers have been prepared as powders[1,14,15] and as defined particles[4–6]; their production in definite particle size is very important for certain applications, such as their use in water filters. Suitable particle sizes can be obtained by using polymeric beads in their preparation,[4–6] by preparing monomers and polymerizing them to form the beads,[4–6] and by grafting heterocyclic monomers onto silica particles[12] [Scheme 1(a)].

In this work, *N*-halamine biocidal polymeric materials were produced in a definite particle size by preparing novel heterocyclic-modified silica gels with particle sizes in the range of 200–400 mesh, designed to increase the number of sites for halogenation, allowing a higher halogen loading for biocidal action, compared with the current modified silica gels reported in the literature [Scheme 1(a)].[12] At the same time, the design improved the stability of the halogen attached to the heterocyclic ring by incorporating stronger electron-donating groups than those suggested in the literature.[12]

In a different approach, sodium alginate was used to generate *N*-halamine polymers of different size particles, rather than the restricted size described previously in the literature.[4–6,12] Sodium alginate has been previously used to generate a matrix for the controlled release of water-soluble drugs and has been modified to carry a positive charge to act as bioactive material by adsorbing bacteria onto its surface.[21,22] In this work, sodium alginate beads were prepared by blending water-insoluble *N*-halamine biocidal polymers with sodium alginate as a matrix followed by crosslinking with calcium chloride. An *N*-halamine biocidal polymer with five available positions for halogenation was chosen to exert the biological activity of the generated beads[1,17,18] [Scheme 1(b)].

At the optimum conditions for bead preparation, the possibility of rehalogenation was investigated to improve the biological properties and prolong the life-time of the particles.

The biological activity of the prepared silica and beads was evaluated against examples of Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*).

EXPERIMENTAL

Materials

Barbituric acid, granulated tin, resorcinol, fuming nitric acid, sodium nitrite, toluene-2,6-diisocyanate,

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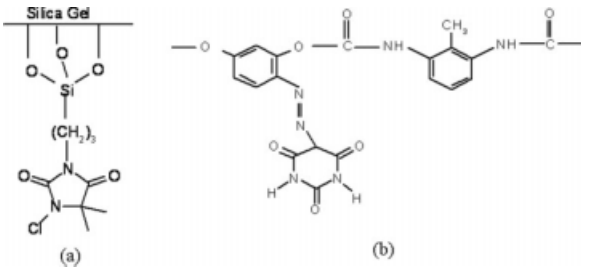


Scheme 1 (a) An example of modified silica with heterocyclic rings. (b) Heterocyclic polymer to be blended with sodium alginate.

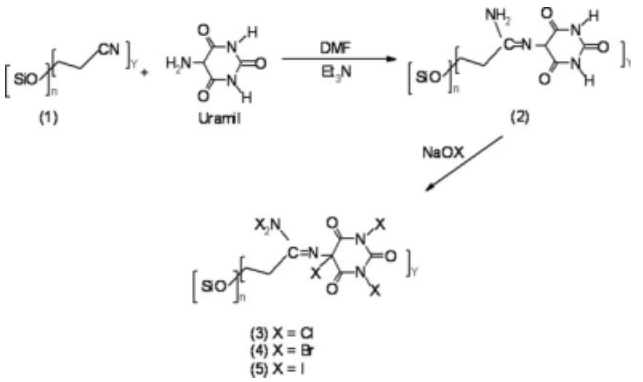
sodium alginate, bromine, iodine, 2-cyano-functionalized silica gel (200–400 mesh, extent of labeling: 1.5–2.0 mmol/g per 7% carbon loading), 3-(isocyanato)-propyl-functionalized silica gel (200–400 mesh, extent of labeling: 1.2 mmol/g loading), calcium hypochlorite, gelatin, formaldehyde, glutaraldehyde, and triethylamine were supplied by Sigma Aldrich Chemicals, UK. Sodium hydroxide, absolute ethanol, sulfuric acid, starch, sodium thiosulfate, potassium iodide, calcium chloride, *N*,*N*-dimethylformamide (DMF) (99.99%), and methanol (analytical grade reagents) were supplied by Fisher Chemicals, UK. Nutrient broth and nutrient agar were supplied by Oxoid. Cultures of *S. aureus* and *E. coli* were obtained from the faculty culture collection. Primary cultures on nutrient agar slopes and subcultures on nutrient agar plates were stored at 4°C. All chemicals were used as obtained from suppliers without extra purification.

Preparation of 2-iminouramil-functionalized (2) and 3-(*N*-barbiturourea)propyl-functionalized (6) silica gels and their halogenation

2-Cyano-functionalized silica gel (**1**) (1.14 g, 0.01 mol based on carbon load) was suspended in DMF (30 mL). Uramil (2.86 g, 0.02 mol) and triethylamine (600 μL) were added. The mixture was stirred at 120°C for 24 h. The product was filtered hot, washed with hot DMF (100 mL), and dried (Scheme 2).

Analysis: FTIR (KBr): ν_{max} (cm^{-1}), 1701 and 1668 (C=O, heterocyclic ring), 1540 (C=N), 2942 (CH), 3124 (NH), 3435 (OH), 1100 (C—O and C—N). Solid ^{13}C-NMR, 6–10 (aliphatic part carbons), 86 (CH of the ring), 151 (C=N), 152 and 162 (C=O). Elemental analysis: found (%, w/w): C, 12.3; H, 1.1; N, 6.4; calculated (%, w/w): C, 14.6; H, 1.7; N, 8.5.

The same procedure was followed to prepare 3-(*N*-barbiturourea)propyl-functionalized silica gel (**7**). 3-(Isocyanato)propyl-functionalized silica gel (**6**) (using 1.3 g, 0.01 mol based on the carbon load) and uramil (2.86 g, 0.02 mol) were refluxed together at 120°C in DMF (30 mL) in the presence of triethylamine (600 μL). The product was filtered hot, washed with hot DMF (100 mL), and dried Scheme 3.



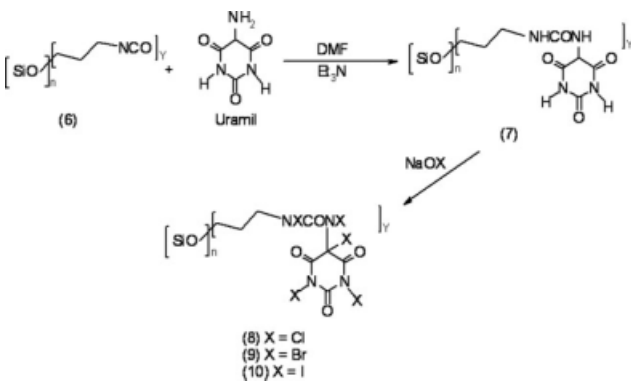
Scheme 2 Preparation of 2-iminouramil-functionalized silica gel and its halogenation.

Analysis: FTIR (KBr): ν_{max} (cm^{-1}), 1697, 1660, and 1611 (C=O), 2943 (CH), 3124 (NH), 3432 (OH), 1100 (C—O and C—N). Solid ^{13}C-NMR, 7, 23, 40 (aliphatic part carbons), 86 (CH of the ring), 153, 154, 161, 162 (C=O). Elemental analysis: found (%, w/w): C, 13.1; H, 1.4; N, 7.1; calculated (%, w/w): C, 14.3; H, 1.6; N, 8.4.

Both of these reactions were performed equally successfully using sodium hydroxide instead of triethylamine and a mixture of DMF and absolute ethanol (2 : 1) as the solvent instead of pure DMF.

Halogenation of the novel modified silica gel was performed using NaOX (X=Cl, Br, or I), and chlorination performed using commercial sodium hypochlorite (10%, w/v) by soaking the modified silica gel (1 g) in water (10 mL) and sodium hypochlorite (10 mL, 10% w/v) with stirring at ambient temperature for 1 h. Bromination and iodination were performed similarly using sodium hypobrominate, and hypoiodinate was prepared by adding bromine or iodine to a sodium hydroxide solution (10%, w/v) gradually until pH 7.

The halogenation process was followed by FTIR spectroscopy,[1] and the halogen/g content was determined using iodometric titration.[8] The values are given in Table I.



Scheme 3 Preparation of 3-(*N*-barbiturourea)propyl-functionalized silica gel and its halogenation.

TABLE I
FTIR Characterization and Halogen Content of the *N*-Halamine Biocidal-Modified Silica Gel

Modified silica	Bond	ν_{max} (cm^{-1})	Halogen content (ppm)
3 (chlorinated)	N—Cl	804	98 ± 3
4 (brominated)	N—Br	802	104 ± 10
5 (iodinated)	N—I	784	121 ± 0
8 (chlorinated)	N—Cl	799	101 ± 0
9 (brominated)	N—Br	798	113 ± 40
10 (iodinated)	N—I	804	192 ± 10

The surface of the *N*-halamine biocidal-modified silica gel was examined using scanning electron microscopy (SEM, Fig. 1).

Preparation of *N*-halamine biocidal beads

N-halamine polyurethane (**14**) was prepared according to the methodology reported earlier.[1,17,18]

Diazotization of uramil

Uramil[23] (**11**) (5-aminobarbituric acid) (1.40 g, 0.01 mol) was dissolved in concentrated sulfuric acid (5 mL). 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 + water, 10 mL) was added dropwise to the uramil solution with stirring to form the uramil diazonium salt (**12**)[1,17,18] (Scheme 4).

Preparation of 1,3-dihydroxy-4(5-azobarbituric acid)benzene (13)

Resorcinol (1.1 g, 0.01 mol) and NaOH (5.5 g, 0.14 mol) were dissolved in water (20 mL) and added gradually to cold uramil diazonium salt (**12**). The dark purple product that precipitated was filtered, washed copiously with cold water, dried, and weighed, producing 2.6 g (99% yield)[1,17,18] (Scheme 4).

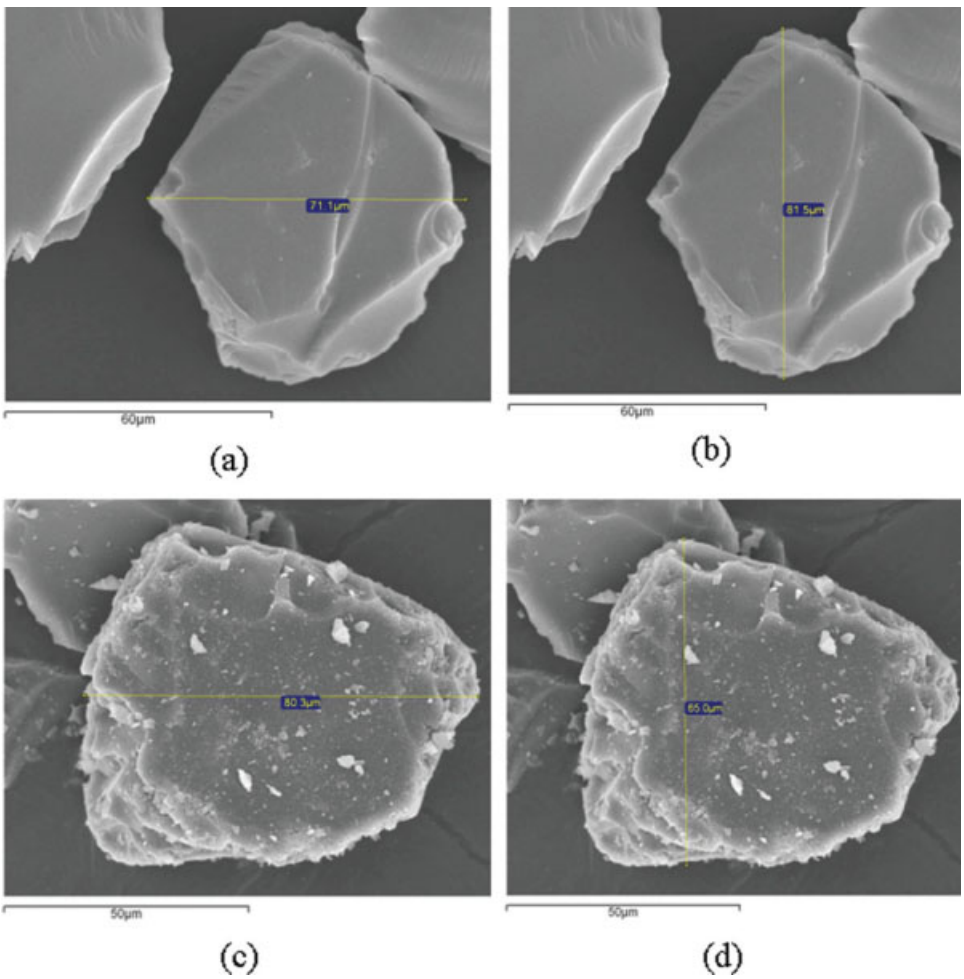
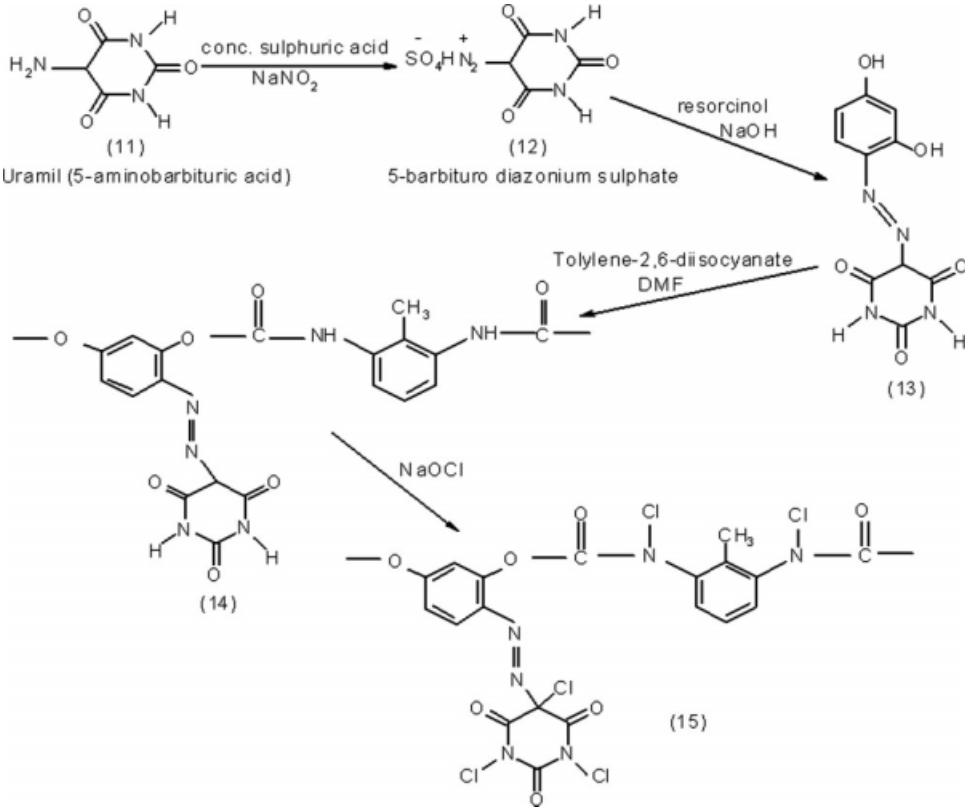


Figure 1 SEM of modified silica gel particles before and after halogenation. (a, b) Silica gel particles before reaction. (c, d) Silica gel particles after halogenation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]



Scheme 4 Preparation of the *N*-halamine polyurethane and its chlorination.

Polyurethane polymer preparation

Monomer (**13**) (2.6 g, 0.01 mol) and toluene-2,6-diisocyanate (0.01 mol) were heated in DMF (30 mL) 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[1,17,18] (Scheme 4).

Polyurethane chlorination

Polymer (**14**) (1 g) was stirred in NaOCl (10%, w/v) for 2 h at ambient temperature. The resulting product was filtered, washed with chlorine-free water, and dried[17,18] (Scheme 4).

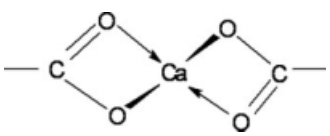
Alginate bead formation

Sodium alginate was dissolved in water (10 mL) to 2.0% w/v. A suitable polymer (see later) was added (2.5%, w/v), and the mixture was stirred for 30 min. The blend was added dropwise to a solution of calcium chloride (100 mL, 6% w/v $CaCl_2$) (Scheme 5). The beads were filtered and dried at 45°C for 24 h.

The blended polymers with alginate were as follows:

1. Prehalogenated polymer (**15**) (Scheme 4) (**AB1**) (Fig. 2).
2. Nonhalogenated polymer (**14**) (Scheme 4) (**AB2**) (Fig. 2). **AB2** halogenated after their formation by soaking the beads (1 g) in sodium hypochlorite (10% w/v, 10 mL) for 30 min.

The alginate-based beads were characterized using FTIR, SEM, and TGA analysis. **AB1**, FTIR (KBr): ν_{max} (cm^{-1}), 1593 (broad band, C=O), 2319–3634 (OH carboxylic), 3342 (OH), 3030 (CH aromatic), 2917 (CH aliphatic), 3141 (NH), 1071 (C—O), 1023 (C—N), 1549 (C=N), 658 (N—Cl), and 1412 (N=N). TGA: T_o (dm/dT_{max}), 60, 120, 210, 280, 300, 460, 700, and 1000. **AB2** (before halogenation), FTIR (KBr): ν_{max} (cm^{-1}), 1612 (broad band, C=O), 2433–3678 (OH carboxylic), 3334 (OH), 3024 (CH aromatic), 2949 (CH aliphatic), 3251 (NH), 1126 (C—O), 1112 (C—N), 1550 (C=N), and 1469 (N=N). TGA: T_o (dm/dT_{max}), 80, 140, 220, 340, 400, 450, 550, 700, and 1000. SEM images for the dried beads are shown in Figure 3.



Scheme 5 Expected complexation product between the alginate and calcium ions.

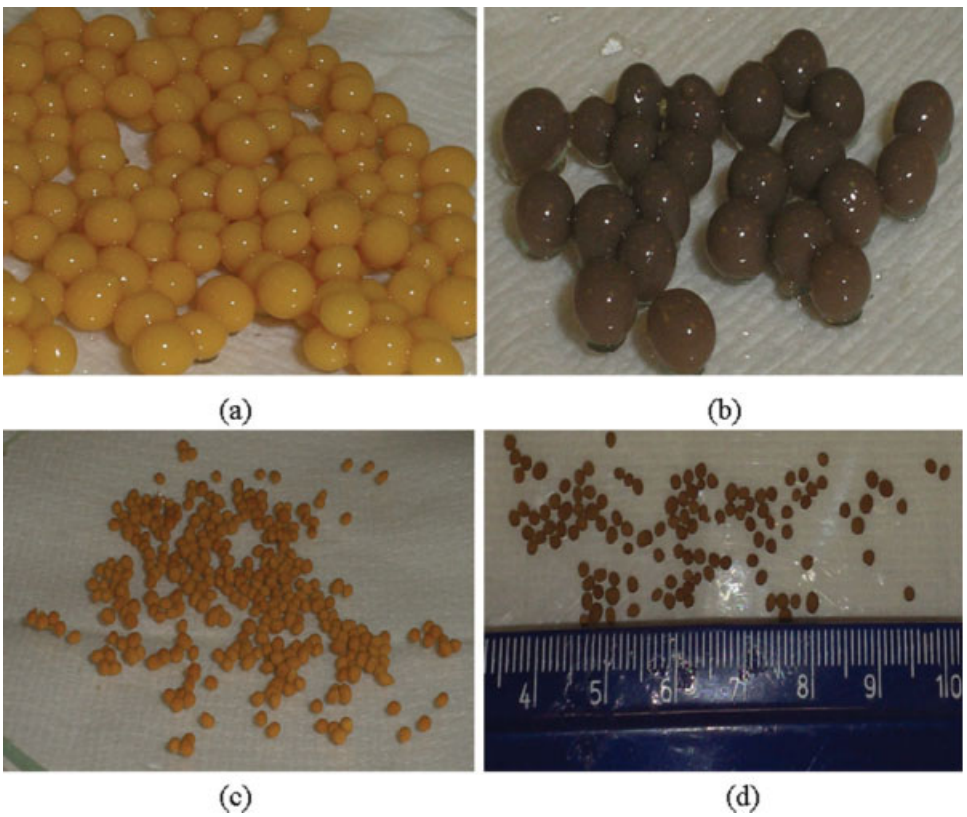


Figure 2 Photographs of the alginate beads in their hydrated and dried status. (a) **AB1** before drying, (b) **AB2** before drying and halogenation, while (c) and (d) represent **AB1** after drying (scale in mm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Determination of the biological activity of the prepared beads matrix and modified silica gels

Agar plate method

This experiment was performed only for the modified silica gels. Nutrient agar (Oxoid) was prepared (250 mL), held molten at 50°C, and 1.0 mL of a 24-h nutrient broth culture of either *S. aureus* or *E. coli* was added as an inoculum. The seeded agar was poured into plates; two plates for each type of bacterium, Gram-positive and Gram-negative. Wells, 5-mm diameter were cut into the agar, and small amounts of each type of modified silica gel (0.03 g) were placed in the well in the middle of the plate. The experiment was performed in triplicate, three plates for each polymer.[1] Plates were incubated for 24 h at 37°C, and the inhibition zones around the well were recorded (Fig. 4).

Stirred flask method

The biological activity of the modified silica gels and alginate beads was determined by studying their effects on bacterial viability. For the alginate beads, bacterial suspension (either *E. coli* or *S. aureus*) was prepared by inoculating 10 mL of nutrient broth in a universal bottle. The culture was incubated at 37°C for 17 h, and 0.1 mL of this suspension was used to inoculate five different universal bottles each containing 10 mL of fresh nutrient broth, which were incubated at 37°C for 17 h. Individual cultures were treated as follows: **AB1** (0.5 g), **AB2** (0.5 g), **AB2** halogenated form (0.5 g), sodium alginate beads (control, no blended polymer), and the fifth was used as a bacterial control. Viability was followed by the "Miles and Misra" method[24] at different time intervals.[17] For the modified silica gel, the same method was applied. Three universal bottles were used: the first treated with halogenated modified silica gel (0.5 g), the second treated with nonhalogenated modified silica gel (control, 0.5 g), and the third was used as a bacterial control. The viability was followed as above.

Bead regeneration

Optimum conditions were determined for "recycling" (rehalogenating) the beads.

Changing the nature of the crosslinker

Changing the ratio of calcium chloride. The most successful preparation method was obtained using calcium chloride; the beads were formed by dropping

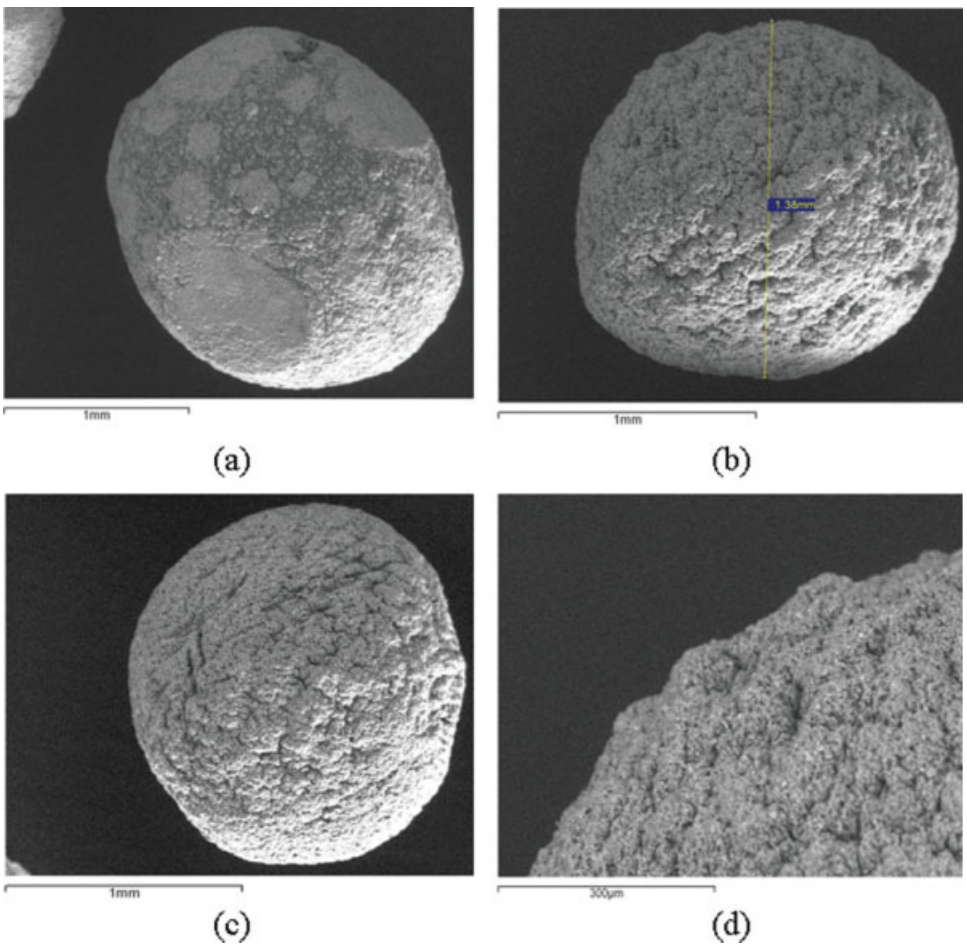


Figure 3 SEM of the beads before and after curing. (a) **AB1** noncured, (b) **AB1** cured, (c) **AB2** cured, and (d) closeup of **AB1** cured. Note: Beads were cured by heating in calcium chloride at 40°C after formation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the polymer (2.5%, w/v)/alginate (2.0%, w/v) mixture in 10 mL distilled water into a solution of calcium chloride (100 mL). The experiment was repeated using different calcium chloride concentrations, 2, 4, 6, 10, 20, and 40% (w/v). These concentrations were used both with and without curing. Curing was performed by heating the beads in the calcium chloride bath at 40°C for 12 h, whereas noncured samples were stirred in calcium chloride for 1 h at ambient temperature. The beads were filtered, washed with distilled water, and dried.

Figure 4 Inhibition zones resulting from one of the *N*-halamine biocidal-modified silica gels, in triplicate. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Using gelatin with calcium chloride. Gelatin was used in different concentrations 1–3% (w/v) with calcium chloride (10% w/v). Polymer (5%, w/v)/alginate (3%, w/v) mixture (in 10 mL distilled water) was dropped into a bath containing the mixture of calcium chloride and gelatin. The experiment was repeated using different ratios of gelatin. The beads were filtered, washed with distilled water, and dried.

Aldehydes: formaldehyde and glutaraldehyde. Aldehydes, formaldehyde and glutaraldehyde, with different ratios (1, 2, 4, 8, and 10% w/v) were added during bead preparation as crosslinkers, and the preparation method is modified as follows: sodium alginate was dissolved in distilled water (2.0% w/v, 10 mL).

The aldehyde (formaldehyde or glutaraldehyde) was added.[22] The mixture was stirred for 1 h, and the polymer (**14** or **15**) was added to 2.5%, w/v and stirring continued for 30 min. The beads did not form; therefore, the polymer ratio was decreased from 2.5 to 1% and then to 0.05% (w/v), and the experiment was repeated but still no beads formed.

Changing the ratio of sodium alginate. Sodium alginate ratio was changed (1, 2, 3, and 4% w/v) during mixing with the polymer (**14** or **15**) (2.5% w/v), in 10 mL distilled water, followed by dropping into a calcium chloride bath (10% w/v, 100 mL). The beads were filtered, washed with distilled water, and dried.

Changing the polymer ratio

The polymer (halogenated and nonhalogenated) (**14** and **15**) ratio was changed, (2, 3, and 5% w/v) to achieve the maximum load of polymer on the beads. The blend of polymer/alginate (3%, w/v, in 10 mL distilled water) in each case was dropped into calcium chloride bath (10%, w/v, 100 mL). The beads were filtered, washed with distilled water, and dried.

The biological activity of the prepared beads under different conditions was quantified, as the effect of the beads on bacterial viability determined as described earlier (using stirred flasks method)[17] for both *E. coli* and *S. aureus*.

Swelling behavior of the beads

Beads, **AB1** and **AB2**, prepared under different conditions (0.05 g) were soaked first in tap water and then distilled water in two different universal bottles for 24 h each. The beads were filtered, the surface water absorbed with paper tissues, and the swelling ratio was calculated using eq. (1).

$$\%\ \text{Swelling} = [(\text{Polymer weight after soaking} - \textit{Polymer weight before soaking}) / \text{Polymer weight before soaking}] \times 100. \quad (1)$$

RESULTS AND DISCUSSION

To improve the particle size of *N*-halamine biocidal materials, uramil was reacted with two different types of modified silica gels: 2-cyano-functionalized silica gel (200–400 mesh) and 3-(isocyanato)propyl-functionalized silica gel (200–400 mesh). The reaction was an addition to the cyano or the isocyanate groups loaded on silica under basic conditions, which was performed successfully using triethylamine or sodium hydroxide as catalysts. The resulting products retained the stability of the halogen on the polymer.

Structures of modified silica of the same type in the literature contain heterocyclic rings with substituted methyl groups (dimethylhydantoins).[12] These methyl groups, as electron-donating groups, stabilize the halogen attached to the heterocyclic ring.[12] The novel modified silica in this study has stronger electron-donating groups that have increased the stability of the halogen attached to the heterocyclic ring, such as the amino group in modified silica[2] and the amide group in modified silica.[7]

The FTIR data of modified silica (**2**) show the disappearance of the cyano group signal (2216 cm^{-1}) of 2-cyano-functionalized silica gel and the appearance of the carbonyl group signals of the heterocyclic ring at 1701 and 1668 as well as the NH signal at 3124 cm^{-1}. The ^{13}C-NMR indicates the appearance of the carbonyl carbon signals of the heterocyclic ring at 152 and 162 ppm, whereas the CH carbon of the heterocyclic ring gives a signal at 86 ppm.

Similar results were obtained for modified silica (**7**); from the FTIR, the carbonyl peaks of the heterocyclic ring and the urea side chain appear at 1697, 1660, and 1611 cm^{-1}, whereas the NH appears at 3124 cm^{-1}, and from ^{13}C-NMR, the carbonyl peaks appear at 153, 154, 161, and 162 ppm. Therefore, FTIR and ^{13}C-NMR showed that the loading of the uramil to the modified silica gels was successful.

SEM was performed to investigate the particle size diameter and to demonstrate that the particles were not damaged during the reactions (Fig. 1). It can be seen that the silica particles still keep their average diameter but smaller particles were scratched. However, the small fragments that are separated from the silica particles will not restrict the use of these particles in some applications, such as water filters.

The biological activity of the *N*-halamine-modified silica gels was investigated using an agar plate technique (Table II).

From Table II, it can be seen that all the halogenated derivatives of both modified silica gels (**2** and **7**) have an inhibitory effect on both Gram-positive

TABLE II
Bacterial Inhibition Zone Diameters (mm) Resulting from the Halogenated Modified Silica Gels (Well Diameter 5 mm)

	E. coli	*S. aureus*
2 (control)	0.0	0.0
3 (chlorinated)	11 ± 1	11 ± 1
4 (brominated)	14 ± 1	18 ± 1
5 (iodinated)	17 ± 1	24 ± 1
7 (control)	0.0	0.0
8 (chlorinated)	11 ± 1	14 ± 1
9 (brominated)	17 ± 1	21 ± 1
10 (iodinated)	26 ± 1	28 ± 2

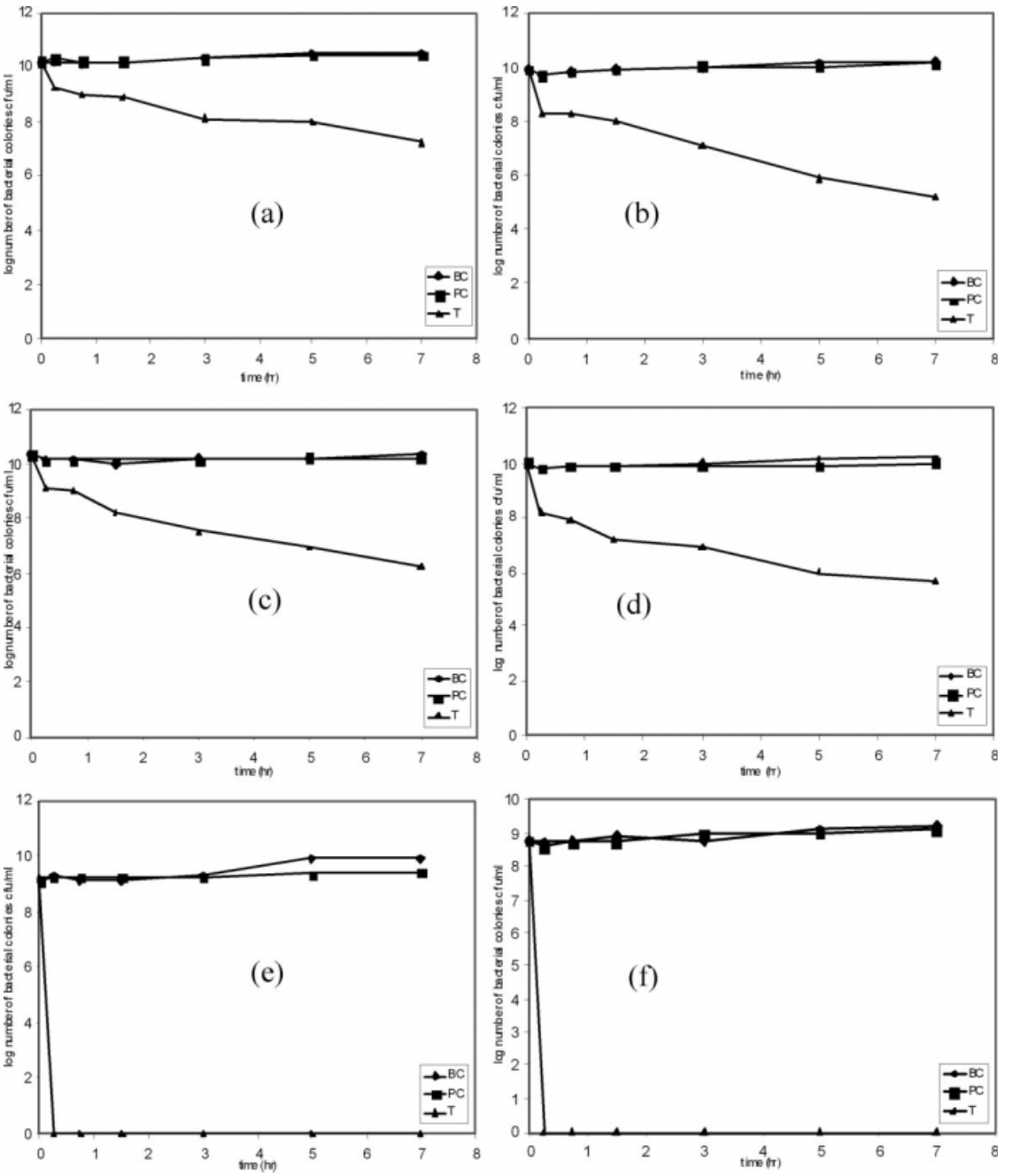


Figure 5 Effect of halogenated [chlorinated (a and b), brominated (c and d), and iodinated (e and f)] heterocyclic-modified silica gels[8–10] on *E. coli* and *S. aureus* viability, respectively. BC is the bacterial control, PC is the nonhalogenated heterocyclic-modified silica gel (control), and T is the halogenated heterocyclic-modified silica gel.

(*S. aureus*) and Gram-negative (*E. coli*) bacteria. The effect on *S. aureus* is generally greater than that on *E. coli*, and the biological activity of the *N*-halamine-modified silica gel derivatives (**3–5**) is lower than those derived from (**7**) (i.e., **8–10**).

The biological activity of the halogenated derivatives of modified silica gel (**7**) was quantified by determining their effect on bacterial viability. Chlorinated modified silica (**8**) succeeded in achieving a 3 log reduction in viability in 7 h for *E. coli* and a 4 log reduction for *S. aureus* in the same time period [Fig. 5(a,b)]. The brominated modified silica (**9**) achieved a 4 log reduction in the viability of both *E. coli* and *S. aureus* in 7 h [Fig. 5(c,d)]. The most powerful effect was achieved by iodinated modified silica. It achieved a 9 log reduction in viability in 15 min for both *E. coli* and *S. aureus* [Fig. 5(e,f)].

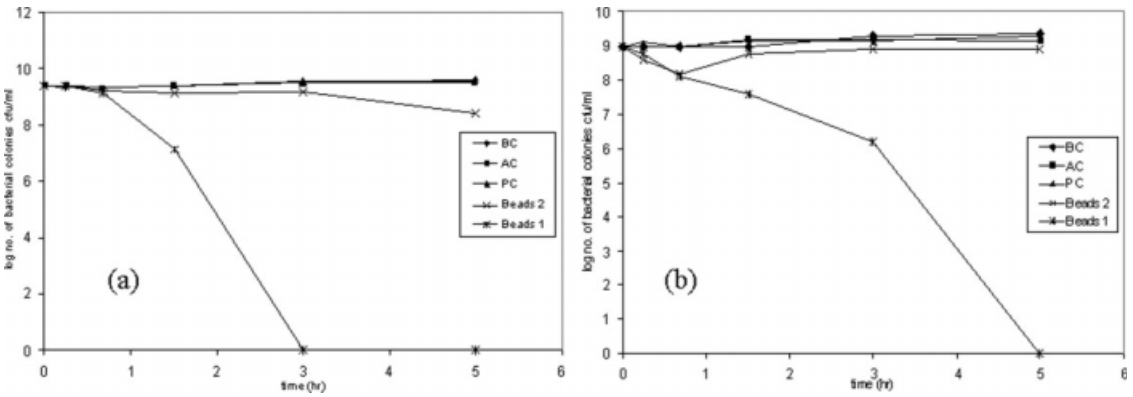


Figure 6 Biological effect of the beads matrix on (a) *E. coli* and (b) *S. aureus*. BC: bacterial control, AC: sodium alginate beads as a control, PC: **AB2** (nonhalogenation form) as a control, Beads 1: **AB1**, and Beads 2: **AB2** (halogenated form).

From the previous results, it can be seen that halogenated modified silica succeeded in reducing the viability of both Gram-positive and Gram-negative bacteria. As expected from previous results,[1,17] the iodinated modified silica has the maximum biocidal power. These results also show that it was possible to improve particle size whilst keeping moderate biological activity. Stability of the halogen attached to the heterocyclic ring of silica was improved by using a heterocyclic ring supported with stronger electron donating groups. In spite of the presence of these strong electron-donating groups attached to the heterocyclic ring, the modified silica showed moderate biological action, which maintains the balance between stability and biological activity. In comparison with similar types reported in the literature,[4–6] this stability may be reflected in reduced biological power of the modified silica. However, it may prolong the active life of the modified silica without rehalogenation as the silica will not easily loose the halogen.

Biological activity of the modified silica is lower than that of the powdered *N*-halamine biocidal polymer, prepared by our group,[1,17,18] as the number of function groups on silica is lower, which affects the number of heterocyclic rings that can be loaded onto silica. In addition, the *N*-halamine biocidal polymers in powder form present a greater surface area, which would improve contact with the bacteria.

Sodium alginate beads matrix

The final particle size of the *N*-halamine-modified silica is dependent on the particle size of the silica used as a starting material (2-cyano-functionalized and 3-(isocyanato)propyl-functionalized silica gels). For some applications, there is a need for larger particles. However, increasing the size of the starting silica may result in reducing the biological activity of the product, because the number of the functional groups on the particle surface, which are used to react with uramil, will decrease. Most of the modified silicas, and other beads reported in the literature, have the same problem.[4–6,14]

A new method has been described here, based on blending *N*-halamine polymer powders (**14** or **15**) with sodium alginate followed by crosslinking with calcium chloride, to obtain larger size insoluble particles. This method can be used to produce different sized particles because the particle size will depend on the dropper used, enabling production of a range of sizes depending on the required application. At the same time, the bioactive polymer content in the particle matrix can be increased to improve the biological

TABLE III
Swelling Ratio of the Noncured Beads Prepared with Different Ratios of Calcium Chloride

Calcium chloride ratio (%, w/v)	Swelling ratio of **AB1** without curing (%, w/v)		Swelling ratio of **AB2** without curing (%, w/v)	
	Distilled water	Tap water	Distilled water	Tap water
4	111.8	94.3	80	64
6	58	44	55.8	56
10	16	8	8	8
20	3.9	2	2	1.8
40	1.9	1.9	0	0

TABLE IV
Swelling Ratio of the Cured Beads Prepared with Different Ratios of Calcium Chloride

	Swelling ratio of **AB1** with curing (%, w/v)		Swelling ratio of **AB2** with curing (%, w/v)	
Calcium chloride ratio (%, w/v)	Distilled water	Tap water	Distilled water	Tap water
4	20	12	18	18
6	11.5	12	18	14
10	6	6	3.8	4
20	2	1.9	2	0
40	0	0	0	0

activity. Similar matrices have been used previously as control-release systems for releasing water-soluble antibiotics.[21,22] The method was modified to be used with insoluble polymers; the biocidal activity in this case depends on halogen ion release from the beads or contact with the outer surface of the beads.

Two methods of preparing beads were compared: mixing chlorinated polymer (**15**) directly with sodium alginate (**AB1**) and mixing nonhalogenated polymer (**14**) with sodium alginate followed by chlorination (**AB2**).

The resulting beads were characterized using FTIR, TGA, and SEM. FTIR and TGA confirmed the presence of both sodium alginate and *N*-halamine polymers in the blend. Characteristic signals for the heterocyclic polymers appeared in the FTIR, such as the azo group (1425 cm^{-1}), NH (3230 cm^{-1}), and carbonyl group (1601 cm^{-1}). The N—Cl signal appeared in the FTIR spectrum at 658 cm^{-1}. TGA peaks for **AB1** showed a shift to lower values compared with **AB2** (nonhalogenated), because the burning rate of the halogenated polymer is faster than that of the nonhalogenated polymer due to the conversion of NH to N—Cl.[25] For example, the water signal, which appears from 80 to 100°C, has moved down to 75–85°C. Sodium alginate and the blended polymer showed peaks of decomposition of their main chain from 200 to 300°C.[26] These peaks appear for both of them when the TGA is performed for each one separately. The beads were examined by SEM (Fig. 3) and photoimaging (Fig. 2).

The effect of **AB1** and **AB2** on bacterial (*E. coli* and *S. aureus*) viability was quantified. During the biological activity, three controls were used: **AB2** (nonhalogenated), sodium alginate beads (without blended polymers), and bacterial control (no beads).

AB1 achieved a 9 log reduction in 3 h for *E. coli*, whereas the halogenated form of **AB2** achieved 1 log reduction in 5 h [Fig. 6(a)]. For *S. aureus*, **AB1** achieved a 9 log reduction in 5 h, whereas **AB2** (halogenated form) achieved only a 1 log reduction in 5 h [Fig. 6(b)].

The results indicated that **AB2** (halogenated form) has low biological activity because of the longer halogenation time required to enable halogen penetration to the polymer particles. These data suggest that the best way to prepare the beads is that used for preparing **AB1**, that is, mixing chlorinated polymer directly with sodium alginate.

AB1 showed a better biological action than the modified silica but lower than the polymer powder itself[17] perhaps because the ions take longer to diffuse out of the beads. The beads release the same quantity of ions as calculated but over a longer time

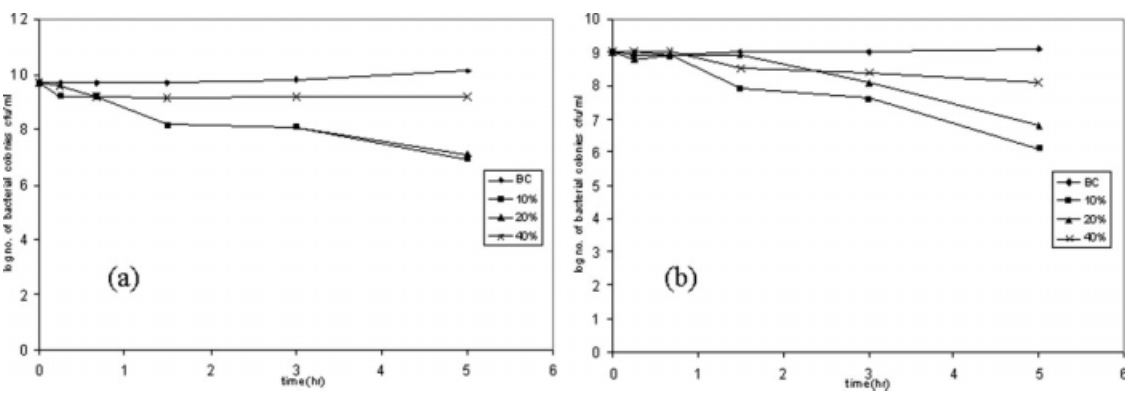


Figure 7 Effect of changing the crosslinking agent (calcium chloride) (with curing) on the biological activity of the beads (**AB1**) against (a) *E. coli* and (b) *S. aureus*. BC is the bacterial control.

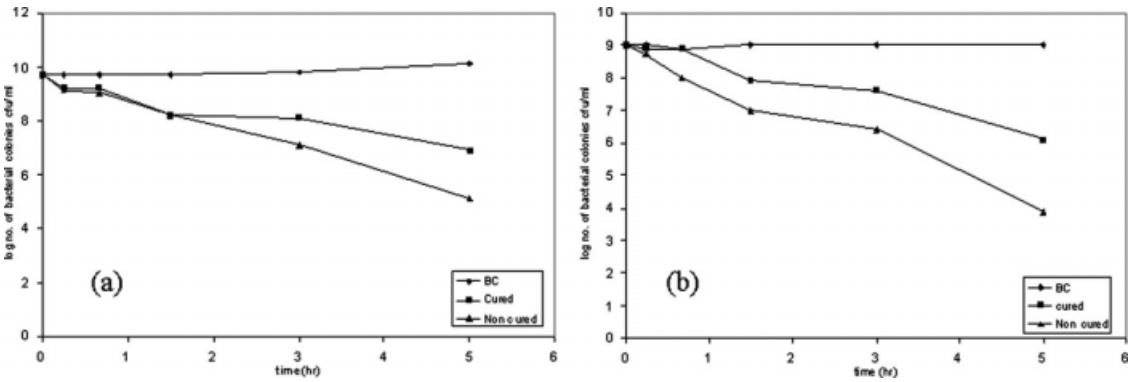


Figure 8 Comparing the biological activity of the cured and noncured forms of **AB1** against (a) *E. coli* and (b) *S. aureus*. BC is the bacterial control.

period—6 h to release the same amount of halogen as released by the powder.[17]

At the same time, the contact effect between the polymer and the cells will be low as the cells can only contact the outer surface of the beads. Moreover, the effective amount of polymer in the beads is lower than that used directly in the case of polymer powder evaluation.

Rehalogenation

To enable bead rehalogenation, the optimum conditions for bead preparation were identified. Polymer was blended with sodium alginate and added dropwise to baths containing different concentrations of calcium chloride to yield beads with different ratios of calcium chloride content 2, 4, 6, 10, 20, and 40% (w/v), cured and noncured. Curing was performed at 40°C for 12 h, whereas noncured samples were stirred in calcium chloride for 1 h after drop formation at ambient temperature.

It was noticed that increasing calcium chloride content, with or without curing, decreases the swelling behavior of the beads (Tables III and IV), which in turn affects the biological activity; however, it increases the possibilities of beads rehalogenation. Raising calcium chloride ratio over 10% (w/v), with or without curing, reduces the biological activity of the beads (**AB1** and **AB2**), while using this ratio (10% calcium chloride) with curing maintains a good balance between biological activity and rehalogenation [Fig. 7(a,b)]. This ratio (10% w/v) with curing enables rehalogenation up to three times without any damage to the beads, which had been noticed with rehalogenation if the beads had been prepared using a low calcium chloride ratio and without curing. From Figure 7(a), the beads prepared with 10–20% (w/v) calcium chloride (with curing) achieved a 3 log reduction in 5 h for *E. coli*, whereas beads formed with 40% (w/v) calcium chloride with curing did not show good biological activity. Similar behavior was noticed with *S. aureus*, and with 40% (w/v) calcium chloride the beads achieved 1 log reduction [Fig. 7(b)].

Similar results were obtained when comparing the biological activity of cured and noncured beads prepared using 10% (w/v) calcium chloride [Fig. 8(a,b)]. The noncured beads show more biological activity perhaps because without curing, the beads swell more enabling ion release.

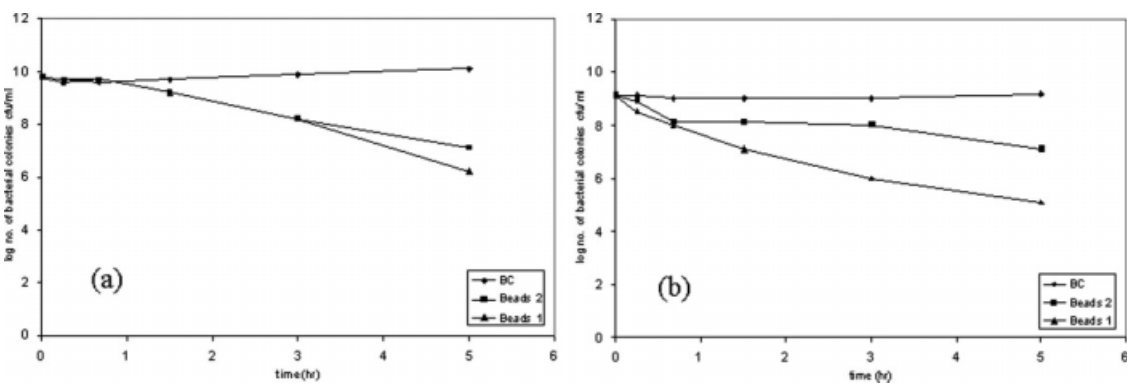


Figure 9 Effect of increasing the polymer ratio up to 5% (w/w) on the biological activity of the beads against (a) *E. coli* and (b) *S. aureus*. BC is the bacterial control, Beads 1 is **AB1**, and Beads 2 is **AB2** (halogenated form).

TABLE V
Swelling Ratio of the Beads with Curing with 20% (w/w) Calcium Chloride with Different Ratios of Polymer

	Swelling ratio of **AB1** with curing (%, w/v)		Swelling ratio of **AB2** with curing (%, w/v)	
Polymer ratio (%, w/v)	Distilled water	Tap water	Distilled water	Tap water
2	18	12	16	3.8
3	21.6	16.6	16	14
5	26	22	30	26

Other types of crosslinkers, such as aldehydes,[22] were investigated instead of salts. Unfortunately, beads did not form. Several trials were carried out using different ratios of aldehydes (1, 2, 4, 8, and 10% w/v) but no insoluble material was formed.

Sodium alginate concentration was varied (1, 2, 3, and 4% w/v) to investigate the effect on the formation of beads. Increasing the ratio of sodium alginate increases the viscosity, making the formation of "drops" difficult, but enhances the rehalogenation character of the beads by increasing the crosslinking possibilities. These different concentrations of alginate were mixed with the *N*-halamine polymer (2.5% w/v) in water (10 mL) and dropped into a calcium chloride bath (10% w/v) with and without curing.

Increasing the ratio of the polymers (2, 3, and 5% w/v) increases the biological activity of the beads (both **AB1** and the halogenated form of **AB2**). The beads were prepared by dropping into a bath containing 40% (w/v) calcium chloride as a crosslinker with curing overnight at 40°C, and the sodium alginate ratio was also increased to 3%. It was seen that **AB1** achieved a 3 log reduction in 5 h against *E. coli*, whereas **AB2** (halogenated form) achieved a 2 log reduction against the same bacterium [Fig. 9(a)]. For *S. aureus*, it was noticed that **AB1** achieved a 4 log reduction in 5 h, whereas **AB2** (halogenated form) achieved a 2 log reduction in the same time period [Fig. 9(b)]. Increasing the polymer ratio increased the biological activity of the beads, despite crosslinking at high concentration of calcium chloride (40%) compared with the results reported using the same calcium chloride ratio with low polymer concentration (2.5%) [Fig. 9(a,b)].

Gelatin was also investigated at concentrations of 1–3% (w/v) with calcium chloride (10% w/v). Using gelatin enabled a more spherical-shaped bead to develop and supported rehalogenation process as it can share in the complexation with the calcium ion. However, using gelatin with **AB1** may result in losing halogen through exchange between the halogenated polymer and gelatin during bead formation, which may reduce the biological activity of the beads.

From the previous data, the best blend for bead formation is as follows: 3% (w/v) sodium alginate and 5% (w/v) *N*-halamine biocidal polymer (**15**), crosslinked with calcium chloride 10% (w/v), followed by curing at 40°C overnight.

The swelling behavior of the beads

The swelling behavior of the beads was determined in tap and distilled water for cured and noncured beads (Tables III and IV). From Tables III and IV, noncured beads swell more than cured and increasing the concentration of calcium chloride decreases swelling. At the same time, increasing the polymer ratio increases swelling behavior even with curing (Table V).

The previous data indicate the possibility of producing blended beads of different sizes and good biological activity, which can support many applications requiring flexible flow rate. Although the beads are larger than the prepared modified silica gel, they showed better biological action because of the presence of the blended *N*-halamine polymer powder. Optimizing bead preparation supports bead recycling. Producing beads as a blend with water-insoluble polymers provides a new method of bead production containing water-insoluble polymers and of different sizes that can release bioactive ions (halogen). Additional effects may be explained on the basis of contact between the bacterial cells and the outer surface of the beads. Bioactive beads of large size and good swelling behavior may encourage using this type of bead in water filters in the future without restricting the water flow rate.

CONCLUSIONS

Particle size was improved by loading bioactive heterocyclic rings onto modified silica gels and preparing beads containing bioactive polymer powder. Bead formation generated bioactive beads of different sizes that release bioactive ions rather than water-soluble molecules. The best conditions for bead formation were mixing 3% (w/v) sodium alginate with 5% (w/v) *N*-halamine biocidal polymer (**15**) and crosslinking them by dropping into calcium chloride solution (10% w/v) followed by curing at 40°C overnight. Blending prehalogenated polymer with alginate (**AB1**) is better than blending the beads

with nonhalogenated polymer followed by halogenation (**AB2**).

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