Philippine Journal of Science
140 (2): 207-219, December 2011
ISSN 0031 - 7683
Date Received: 14 Dec 2010

Regenerable Antimicrobial Polyurethane Coating Based on N-Hydroxymethylated Hydantoin

Wilfredo Bisquera Jr. and **Florentino C. Sumera***[1]

Material Science and Engineering Program, College of Science,
University of the Philippines, Diliman, Quezon City
[1]Institute of Chemistry, University of the Philippines, Diliman, Quezon City

Polyurethane coating made from castor oil and toluene diisocyanate was transformed into an antimicrobial coating by covalently attaching monomethylol hydantoin, a potential N-halamine derivative with regenerable antimicrobial properties. The coating was readily converted to N-halamine structures on exposure to a halogen source such as commercially available sodium hypochlorite. Once chlorinated, the coating became biocidal against representative organisms such as *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative), and *Candida Albicans*. The coating could be recharged repeatedly and become antimicrobial for at least five days by reapplication of the hypochlorite solution. The surface morphology, thermal and spectral properties of the coating were analyzed by SEM, contact angle, DSC, TGA, FTIR before and after chlorination. Chlorine content was determined by titration using iodometric method. The structures of the starting hydantoin derivatives were characterized by FTIR, and H^1 NMR.

Key Words: castor oil, monomethylol hydantoin, N-Halamine, polyurethane, regenerable antibacterial coating, toluenediiso-cyanate

INTRODUCTION

Due to wide spread proliferation of infectious pathogens and persistence of microorganisms on surfaces, antimicrobial polymers have attracted considerable interests. Biocidal polymers may be categorized by their mode of antimicrobial activity: biocide release or contact-kill (Grunzinger et al. 2007).

Biocide release is the most common mode for providing antimicrobial action. Biocidal agents like aldehydes, silver ions (Texter et al. 2007), and antibiotics (Piozzi et al. 2004) are incorporated into commercially important polymers during processing. However, with leaching biocides, the antimicrobial agent may broadly affect non-target organisms (Makal et al. 2006). In addition, these materials lose activity over time without the possibility of regeneration when the leaching agent is exhausted.

An alternative method, contact or non-leaching, is to covalently bond the biocide to prevent leaching. In a series of articles, Sun and Xu (1998;1999a, 1999b) attached a peroxycarboxylic acid functional group to render cellulose and other polymeric materials, antimicrobial with regenerative property by way of peroxy compounds. Also, covalently bonding monomethylol dimethylhydantoin with its amide and imide functions to some polymer preparations has been used extensively as a formaldehyde donor to prevent microbial growth (Farina et al. 1993 US Patent No. 5,252,744). Moreover, hydantoins and related cyclic amides may be grafted to polymers that reacts with hypochlorite (bleach) generating biocidal surfaces with regenerable properties (Eknoian et al.

*Corresponding author: florentino.sumera@up.edu.ph
willie_bisquera@yahoo.com

1999). For example, polyesters (Lin et al. 2002), nylon (Lin et al. 2001), and cellulosic materials (Sun et al. US Patent 6,770,287 B1) were synthesized as N-halamine derivatives for regenerable antimicrobial applications. Halamine refers to an amine, amide, imide, urea, melamine, sulfonamide, hydantoin, urethane, imidazolidinone and their derivatives in which hydrogen attached to the nitrogen atom has been replaced with a halogen atom e.g. chlorine atom (Li et al. 2007). N-halamines are stable, noncorrosive (Williams et al. 1987), regenerable, and effective oxidative contact biocide. Worley working with Sun Y. (2001, 2002) and Sun G. (2001) in a series of papers copolymerized vinyl hydantoins with acrylonitrile, methylmethacrylate, and vinyl acetate. Worley with Chen et al. (2003a, 2003b; 2004a, 2004b) grafted hydantoin to polystyrene to produce beads that can decontaminate water. In a US patent, Worley et al.(2005) showed that polystyrene), polyethylene and siloxane polymers modified with hydantoin functionality generates biocidal surfaces upon exposure with hypochlorite (bleach). Hydantoin containing diol was incorporated by Worley et al. (2003a; 2003b) into a polyurethane chain. Makal et al. (2006) developed an antimicrobial polyurethane containing 5,5-dimethylhydantoin pendant groups as surface modifier for conventional PU. A hydantoin-oxetane monomer copolymerized to polyoxetane telechelics was developed as surface modifier by Grunzinger et al. (2006,2007) for a polyurethane. Tan and Obendorf (2007) grafted dimethyl imidazolidinone to a polyurethane and applied it as an halamine antimicrobial barrier clothing.

In this paper we propose to use a monomethylol hydantoin compound not to release formaldehyde for antimicrobial action but to impart regenerable biocidal function as halamine to a substrate such as a polyurethane coating composed of castor oil and toluene diisocyanate (TDI). The optimum percent by weight of the hydantoin derivative and the reaction mixture NCO/OH working ratio for polyurethane coating will thus be investigated.

The synthesized polyurethane coating should possess antimicrobial function for at least five days against *Staphylococcus aureus*, *Escherichia coli*, and *Candida Albicans* and be regenerable upon repeated exposure to hypochlorite solution.

MATERIALS AND METHODS

Materials

The hydantoin 5,5-dimethylhydantoin (97%) and anhydrous potassium carbonate was purchased from Aldrich. Commercial castor oil was purchased from BE Instruments (local supplier) and was used without further modification. Reagent grade solvent ethyl acetate, formaldehyde (37% solution) and anhydrous sodium sulfate were obtained from J.T Baker. Potassium iodide used in the titration was purchased from Ajax Chemicals and sodium thiosulfate from Himedia. Toluene diisocyanate used in the polyurethane synthesis was donated by Resins Incorporated.

Preparation of Monomethylol hydantoins (Milkowski et al. 1979)

N-hydroxymethyl-5,5-dimethyl hydantoins or monomethylol hydantoins were synthesized according to Milkowski et al.(1979) by reacting 5.00g (0.039 mol) of 5,5-dimethylhydantoin with 2.90 ml (0.039 mol) of an aqueous 37% solution of formaldehyde (1:1 mole ratio) in a solution of 0.228g(0.00165 mol) potassium carbonate.

Synthesis of the Polyurethane Coating

Polyurethane prepolymer was first prepared by mixing 5.00g (equivalent free OH, 0.01429) Castor Oil and 3.58g (equivalent free NCO, 0.04115) Toluene diisocyanate (TDI). After a few minutes or when the prepolymer was already viscous, 1.00g (equivalent free OH 0.00632) monomethylol hydantoin dissolved in hot ethyl acetate was added to the prepolymer. The mixture was then coated on glass slides and left for 4 days inside a cabinet at ambient temperature to complete the reaction. The cured coatings were peeled off the glass substrate and then cut to desired sizes, preferably the size of a cover slip for biocidal testing.

Chlorination of hydantoin modified polyurethane coating

To convert the hydantoin moieties to biocidal halamine, the samples were immersed in diluted 20% v/v commercial bleach (Zonrox, adjusted to ≈ pH 7 with acetic acid) for two hours. After chlorination, the samples were washed with deionized water and subsequently dried for 2 hours at ambient temperature. After drying, the samples were stored inside a cabinet for 2 days then tested for biocidal activity.

Chemical and Thermal Characterizations

^{1}H spectra of hydantoin samples (10-20 mg) were obtained using a JEOL Lambda FT-NMR spectrometer at room temperature. Samples were also analyzed using IR Prestige-21 Fourier Transform Infrared Spectrophotometer. Thermal analysis of the samples was done using TA Differential Scanning Calorimeter (DSC) Q10. Thermal stability of the samples was done using Shimadzu TGA-50.

Biocidal Test (Makal et al. 2006)

A modified "Sandwich" Test for Biocidal Efficacy *(AATCC-100-1999)* was applied for the biocidal tests. *E. coli* and *S. aureus* grown in Nutrient Agar (NA) for 18-24 hours were used as bacterial test organisms. *C. albicans*, yeast used as test organism was grown in Glucose Yeast Peptone Agar (GYP) and incubated also for 18-24 hours. Bacterial and yeast suspensions were prepared with turbidity approximating that of McFarland No. 2 (7.5×10^9 average cell count/ml) and 4 (10.0×10^9 average bacterial count/ml), respectively (McFarland, 1907).

A piece of the sample polymer coated on a glass coverslip was placed on a Petri dish. Twenty microliters (20 µL) of the bacterial suspension was placed onto it after which another piece of the same sample was laid on top of the inoculum. A small sterile Erlenmeyer flask was placed on top of the two pieces of polymer samples for good contact between inoculum and sample. The contact time between the bacterial suspension and the material was 2 hours. After the contact time, the samples were placed into an Erlenmeyer flask with 100 mL of 0.02 N sodium thiosulfate and then the flask was shaken vigorously. The mixture was serially diluted to 10^{-4}. From each dilution (designated as 10^0 10^1 10^2), three (3) replicates of 25 µL were transferred onto a pre-poured Nutrient Agar plate. The plates were incubated at room temperature and the colonies were counted after 24-48 hours incubation period at room temperature. Colony counts were multiplied by 40 to get corresponding colony-forming units (CFU) per mL. Unchlorinated polyurethane without hydantoin additive was used as a control following the same procedure. Eq. (1) was used to calculate the Percent Kill, and Eq. (2) was used to calculate log reduction:

$$\text{Percent Kill} = \frac{X - Y}{X} \times 100 \qquad (1)$$

$$\text{Log Reduction} = \log\left[1 - \left(\frac{X - Y}{X}\right)\right] \qquad (2)$$

where X is the number of CFUs counted from the control plate and Y is the number of CFUs counted from the experimental plate. To estimate the percent kill, in the absence of surviving CFUs on the experimental plates, Y is assigned a value of "1", an assumption of one survivor.

Titration of Oxidative Chlorine Content (Pierce et al. 1958)

Standard iodometric/thiosulfate titration procedure was employed to determine the oxidative chlorine (Cl^+) content of the coatings. The polymer samples, 0.5 to 0.6g, were suspended overnight with constant stirring in 100 mL of 0.1 N acetic acid solutions with 0.6 g KI and starch indicator. The Cl^+ ions oxidized the I^- to I_2. The formed I_2 in solution was titrated with 0.0001 N sodium thiosulfate solution until the blue color disappeared at the end point. Unchlorinated polyurethane sample without hydantoin was also titrated with the same method and used as a control. The weight percent oxidative Cl^+ on the samples could be determined from Eq. 3.

$$\%\ Cl^+ = [\ N \times V \times 35.45/(2 \times W)] \times 100\% \qquad (3)$$

Where N and V are the normality (eqv/L) and volume (L) respectively of the $Na_2S_2O_3$ consumed in the titration, and W is the weight in grams of the sample.

Contact Angle Measurement

Contact angles of polyurethane coating and monomethylolhydantoin containing polyurethane coating were measured using a sessile drop method with FACE Contact-Anglemeter.

SEM of Polyurethane

Philips FE-SEM XL30 was used to investigate the relative concentration of chlorine on the surface. X-ray net counts were obtained at three random locations for each sample with an accelerating voltage of 25KV and collection time of 100 seconds. SEM images of polyurethane samples before and after chlorination (as is), and after washing with deionized water were recorded.

RESULTS AND DISCUSSION

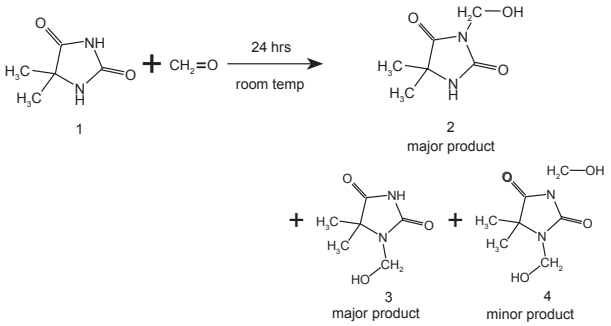
The 5,5-dimethylhydantoin was introduced into the polyurethane coating by reacting 5,5-dimethylhydantoin with formaldehyde to provide the methylene alcohol substituent subsequently reacted with the polyurethane prepolymer. The polyurethane prepolymer made of castor oil and toluene diisocyanate in a 1:2 proportion (equivalent free OH to equivalent free NCO) provided the coating that exhibited regenerable antimicrobial activity when combined with the formaldehyde derivative of 5,5-dimethylhydantoin. The synthesis of the derivatives of 5,5-dimethylhydantoin with formaldehyde to their N-monohydroxymethylated product was important in letting free the active amide or imide functional groups necessary for halamine formation and production of the antimicrobial coating.

Preparation of the Monomethylol Hydantoin Derivatives

Foelsch (U.S. Patent No. 3,987,184) disclosed the production of 45-70% 1,3-dimethylol-5,5-dimethylhydantoin by admixing 1.85-2.4 moles of formaldehyde per mole of 5,5-dimethylhydantoin 1 in

a 2:1 ratio. In this reaction, to produce monomethylol products, formaldehyde and 5,5-dimethylhydantoin 1 was reacted in an approximately one to one mole ratio. Thus, three products, 2, 3 and 4 resulted as shown in Scheme 1. Our results (from ^{1}HNMR analysis) showed that 67% of the product was monohydroxymethylated (on imide 2 and on amide 3) while the 33% was divided between the starting hydantoin and the dihydroxymethylated hydantoin (1 and 4).

Scheme 1. Reaction of 5,5-dimethylhydantoin 1 with Formaldehyde giving three products 2, 3 and 4.



This preparation provided the next reactant with a higher percentage of monomethylol than dimethylol product. The purpose was to have the hydroxy group of the monomethylol product be covalently attached to the polyurethane PU while leaving an imide or an amide nitrogen free for the reaction with the halogenating agent. A product with two hydroxymethyl units as in the dimethylol product would mask the imide and/or the amide functional groups making these groups impossible to be halogenated in principle.

FTIR of 5,5-dimethylhydantoin 1 and its methylol derivatives

As shown in Figure 1, the halamines showed the characteristic peaks of amide carbonyl group. The appearance of hydrogen bonded –OH (3417 cm-1) in formylated hydantoin mixture suggest a successful grafting of -CH2OH substituent.

Thermal Analysis of the 5,5-dimethylhydantoin and its methylol derivatives

DSC thermograms of hydantoin and hydantoin derivatives are shown in Figure 2. The hydantoin derivatives show three endotherms corresponding to melting (60°C-105°C), formaldehyde dissociation (173°C -217 °C) and decomposition onset at 274°C. This is confirmed by TGA (Figure 3) with two weight losses corresponding to formaldehyde dissociation (41.56% 184.37-231.72°C) and decomposition (49.59% 298.88-335.52°C).

DSC of 5,5-dimethylhydantoin shows two endotherms corresponding to melting (178°C) and decomposition (278-342°C). This was also confirmed by TGA with one weight loss (96.857%) corresponding to decomposition (246-274°C, Figure 3).

Polymerization and Characterization of Polyurethane-halamine

The polyurethane prepolymer was prepared by reacting castor oil and toluene diisocyanate (TDI) in a 1:2 equivalent free OH to equivalent free NCO ratio, leaving an excess of TDI for the reaction with halamine formylated 5,5-dimethylhydantoin. A working ratio was developed just well enough for coating wood or glass preventing the appearance of bubbles and avoiding the tackiness of the coated film.

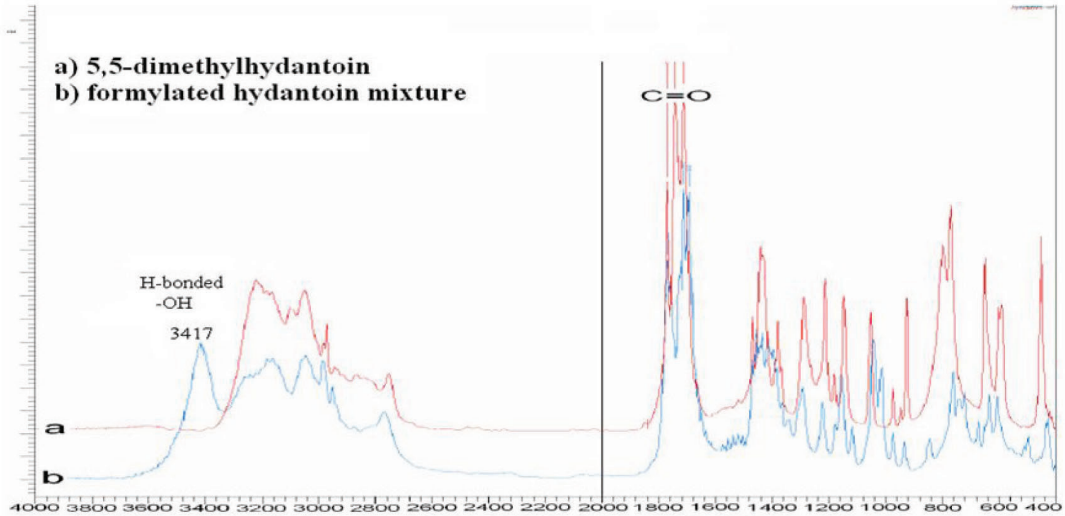


Figure 1. FTIR scan of a) 5,5-dimethylhydantoin and b) formylated hydantoin mixture showing O-H stretch (H-bonded) centered at 3417 cm^{-1} for the hydroxymethyl substituent.

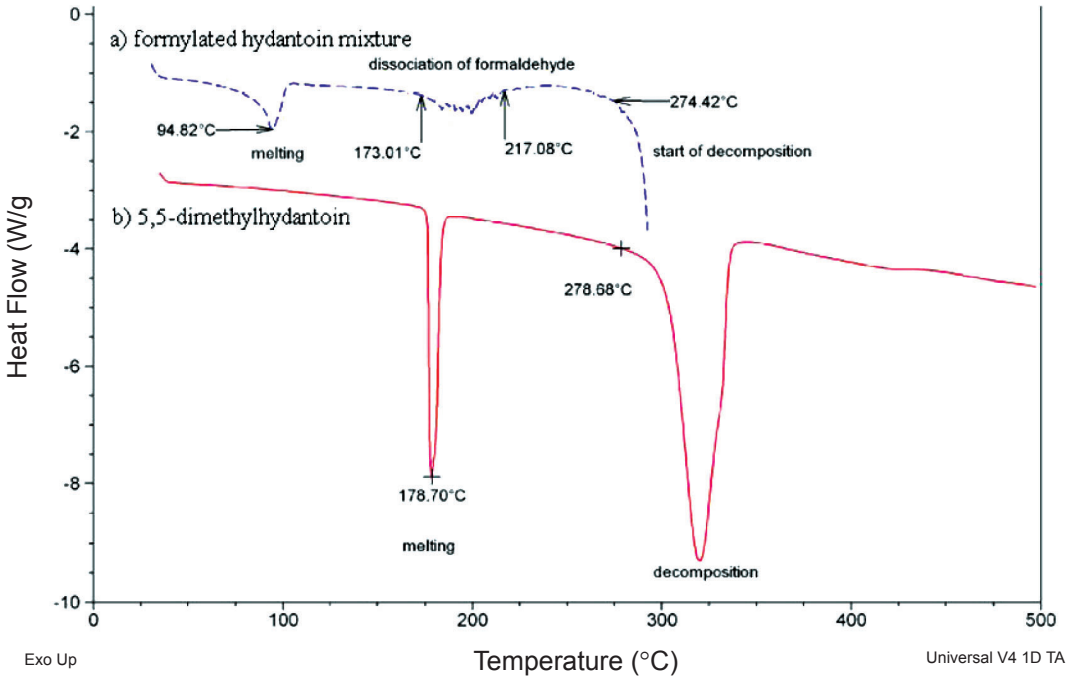


Figure 2. DSC of a) formylated hydantoin mixture and b) 5,5-dimethylhydantoin.

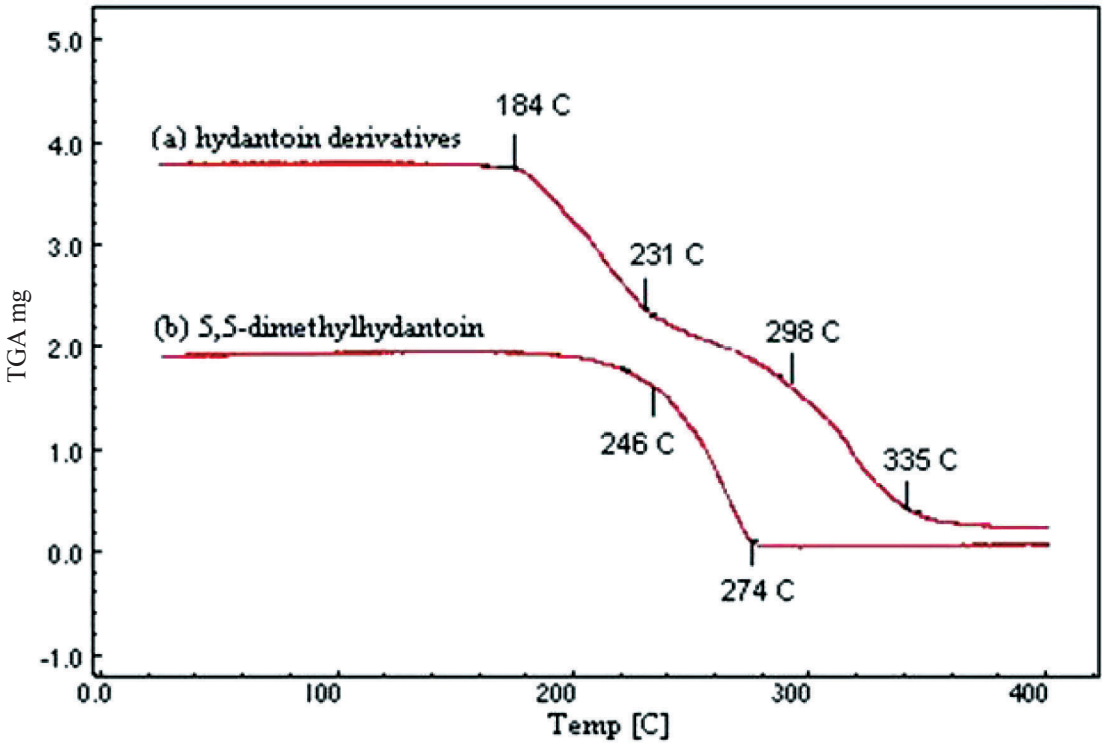


Figure 3. TGA of a) Formylated hydantoin derivatives and b) 5,5- dimethylhydantoin.

FTIR analysis of Coating

Figure 4 shows the FTIR scan of Castor Oil, Toluene diisocyanate (TDI), polyurethane and hydantoin modified polyurethane. Castor oil shows the characteristic broad intermolecular hydrogen bonded, O-H stretch at 3390 cm^{-1}. Also to be noted are the NCO band centered at 2243 cm^{-1} disappearing after four (4) days of curing and N-H stretching (hydrogen

bonded) appearing at 3319 cm^{-1}, and Amide II bands at 1531 cm^{-1} appearing in both polyurethane samples. The spectra, however, of both the PU and the modified PU with monomethylolhydantoin are very similar. This is because the amount of hydantoin incorporated into the PU was very small (<10 %) compared to the bulk of the PU.

Upon chlorination, a decrease in the N-H stretching (hydrogen bonded) (Figure 5) and N-H bending signal (not shown) was observed for both modified and unmodified samples, an indication of proton replacement with oxidative Cl at the imide, amide and urethane bonds. Shifting to lower frequency of Amide II bands was also observed with chlorinated samples.

Contact Angle of the coating

Figure 6 shows the contact angle values of polyurethane and monomethylolhydantoin modified polyurethane coating. It could be seen that there were only slight differences of θ values within the same sample even after thorough washings. The observed slight difference may be attributed to surface inhomogeneity and surface roughness

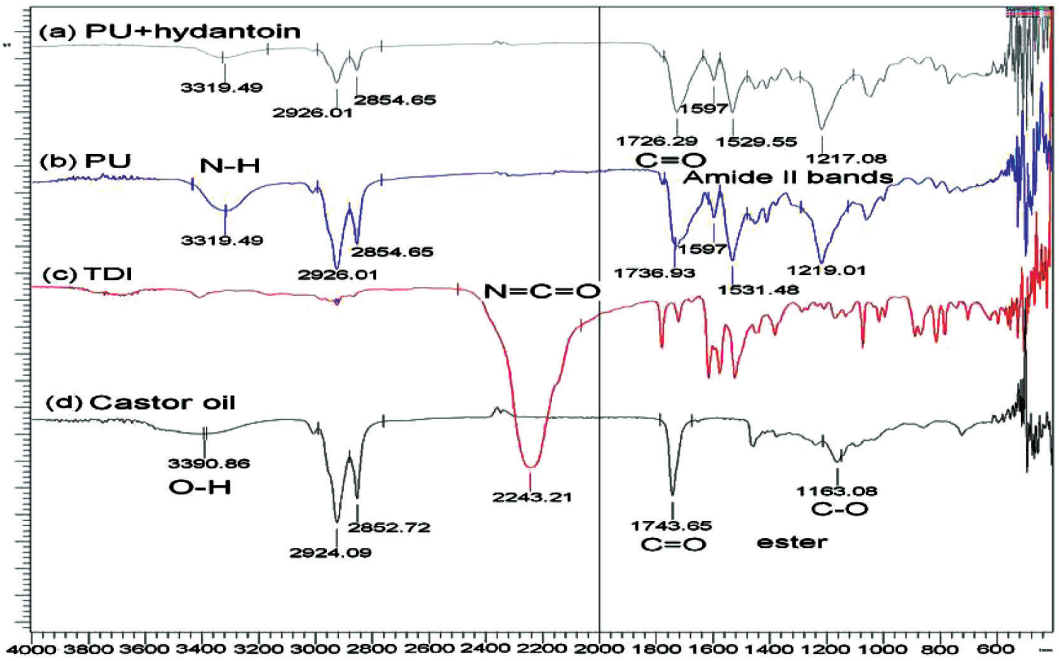


Figure 4. FTIR Scan of a) PU+10%Monomethylolhydantoin, b) PU, c) TDI, and d) Castor oil.

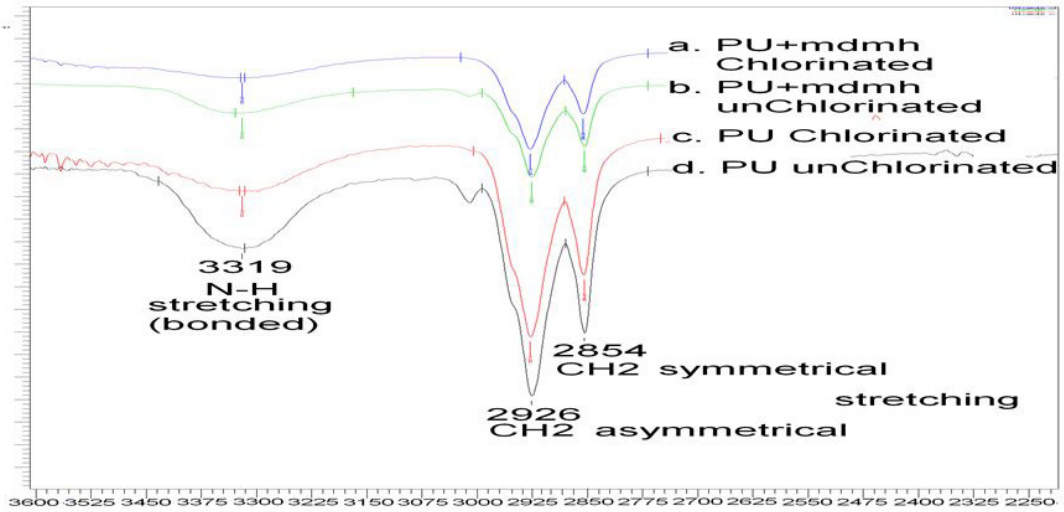


Figure 5. FTIR Scan of the N-H stretching region for the Chlorinated and Unchlorinated PU and PU + 10%Monomethylolhydantoin

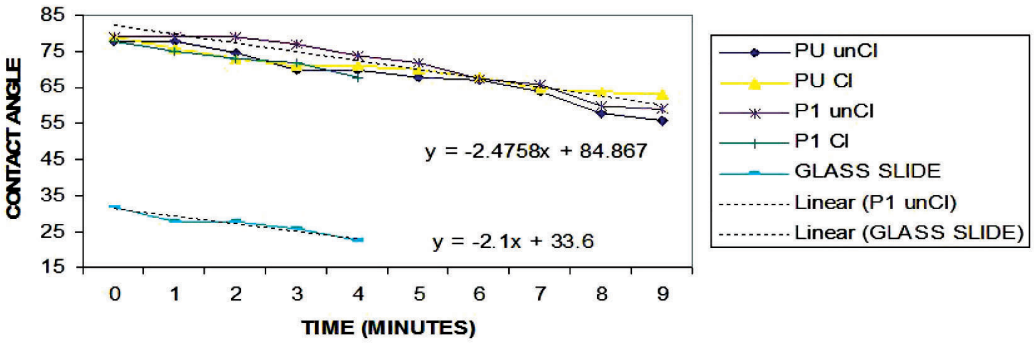


Figure 6. Change in contact angle of representative samples with respect to time note PU – polyurethane; P1 – polyu rethane+monomethylolhydantoin; Cl - chlorinated; unCl - unchlorinated

of the samples. It is interesting to note that modification with monomethylolhydantoin doesn't significantly change the contact angle of the TDI-castor oil polyurethane system, just a slight increase of 1-2°, making the modified polymer slightly hydrophobic than the unmodified PU.

All the samples including the glass slide shows a gradual and almost constant rate of decrease in contact angle with respect to time (Figure 6), suggesting that the decrease is due to water evaporation. This also demonstrates that the samples are non-leaching since there is no abrupt change in θ, an indication of migrating surface active substance from the surface.

Optimization of Monomethylolhydantoin Content

To determine the optimum percent by weight (w/v) of bioactive compound, polymer samples with 5%, 7%, and 10% w/v monomethylolhydantoin additive were soaked for 24 hours in 20% by volume (v/v) bleach to convert the hydantoin moieties to biocidal halamine. After soaking, the polyurethane coatings were washed with deionized water to remove unreacted free chlorine and then dried for two hours at ambient temperature. Two batches were prepared, the first batch designated 0 day were tested for biocidal efficacy right after drying using the modified American Association of Textile Chemists and Colorists (AATCC-100-1999) "sandwich" test, the second batch were stored inside a cabinet for a week (designated 7 day) then tested for biocidal efficacy using the same method as the first batch.

Table 1 shows the percent kill of the samples with unmodified polyurethane as control. For the first batch (0 day), unchlorinated polyurethane with 7%

Table 1. Biocidal activity of polyurethane with 0%, 5%, 7%, and 10% by weight monomethylolhydantoin after 0 and 7 days cabinet storage

Polyurethane Sample	Days of storage	CFU control*	CFU expt**	Percent Kill	Log reduction
PU unchlorinated (control)	-	1910	-	-	-
PU+7% hydantoin unchlorinated	0	1910	9470	0 %	-
PU+0% hydantoin chlorinated	0	1910	520	72.77 %	0.565
PU+5% hydantoin chlorinated	0	1910	320	83.25 %	0.776
PU+7% hydantoin chlorinated	0	1910	13.3	99.30%	2.15
PU+10% hydantoin chlorinated	0	1910	Complete kill	99.95	3.30
PU+0% hydantoin chlorinated	7	1910	5600	0 %	-
PU+5% hydantoin chlorinated	7	1910	4130	0 %	-
PU+7% hydantoin chlorinated	7	1910	4530	0 %	-
PU+10% hydantoin chlorinated	7	1910	1070	43.98 %	0.252

Note: 24 hours soaking with 20% v/v bleach solution and 2 hours challenge time with E. coli bacteria
*CFU control - colony forming unit in control plate
**CFU expt - colony forming unit in experimental plates

monomethylolhydantoin showed zero percent kill and an increased CFU (colony forming unit) compared to control. This is expected since the hydantoin moieties were not converted to biocidal halamine, and chlorination is needed to turn N-H group to N-Cl which renders the polyurethane antimicrobial by providing relatively stable form of oxidative chlorine bound to nitrogen. This observation also establishes that N-halamine moiety in the chlorinated sample is the active biocide and not the monomethylol hydantoin (MDMH) compound by itself, which is known as formaldehyde donor. Formaldehyde is an antimicrobial agent effective against a number of common microorganisms (Foelsch U.S. Patent No. 3,987,184).

Chlorinated samples for 0 day, displayed increasing log reduction with increasing monomethylolhydantoin additive as shown in Table 1. Chlorinated polyurethane without modification gave the least percent kill of 72.77% (due to the urethane groups) and a complete kill and a log reduction of 3.30 for the sample with 10% monomethylolhydantoin.

The addition of hydantoin derivative increased the amide bonds (and imide bonds) in the polyurethane (Fig 7 and 8) thus increasing biocidal halamine bonds upon chlorination of the sample. Furthermore chlorine bound to hydantoin amide bond was more stable than the amide bond in the polyurethane backbone because of the electron-donating alkyl groups substituted on the hydantoin heterocyclic ring adjacent to the oxidative N–Cl moiety which hindered the release of "free halogen" into aqueous solution (Liang et al. 2007).

Efficiency of Regeneration

To determine if the chlorine loadings of exhausted coatings could be regenerated again upon exposure to free chlorine, samples with 5% and 10% monomethylolhydantoin were prepared for biocidal efficacy after 1.5 days and 4.5 days cabinet storage. To simulate chlorine consumption, chlorinated samples were soaked in 0.02N sodium thiosulfate for 10 minutes with stirring to reduce chloramide to amide. The samples were subsequently washed with ample deionized water to remove unreacted thiosulfate. After washing, the samples were soaked in 20% v/v bleach solution for 24 hours to regenerate the halamine moieties. Table 2 shows the biocidal efficacy of the regenerated samples. From the table, all samples showed biocidal activity even after 4.5 days of cabinet storage. These demonstrated the regenerability of biocidal halamine by rechlorination with dilute bleach.

Table 2 shows the corresponding plates of the control, 5% and 10% sample. Again, the sample that showed the highest log reduction and a complete kill for both 1.5 and 4.5 days cabinet storage is the sample with 10% monomethylolhydantoin.

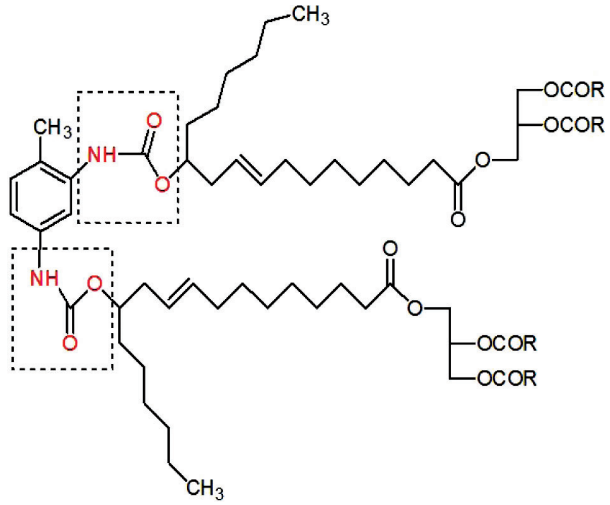


Figure 7. TDI-Castor oil polyurethane backbone showing two urethane groups.

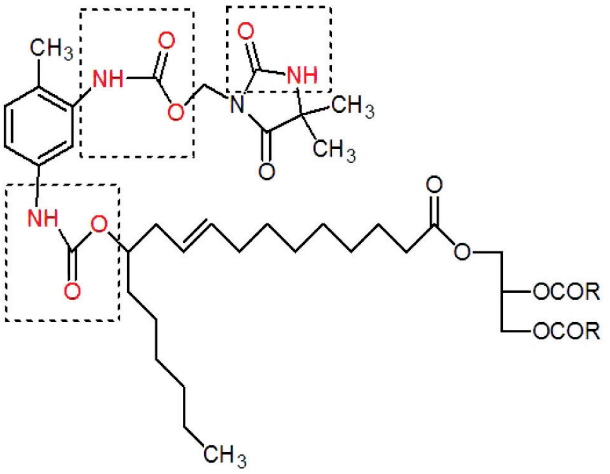


Figure 8. Hydantoin terminated TDI-Castor oil polyurethane with two urethane and one amide group.

Soaking Optimization (pH of solution and soaking time)

After determining the optimum weight percent monomethylolhydantoin additive (which was 10%) and regenerability of the samples, the next step was to determine the optimum soaking parameter. Polyurethane with 10% monomethylolhydantoin were soaked in varying time of 1, 2, 3, and 5 hours in acidified and basic 20% v/v bleach solution. Table 3 shows the biocidal efficacy of PU+10% monomethylolhydantoin with *E. coli* by different soaking parameters. From the table, it could be observed that samples soaked in acidified bleach solution gave a greater log reduction and percent kill than the corresponding samples soaked in basic bleach solution. This is better understood by looking at the available free chlorine species at varying pH. At low pH, the predominant form of free chlorine was from hypochlorous acid, and

Table 2. Biocidal efficacy of regenerated coatings with 2 hours challenge time by E. coli bacteria after 1.5 and 4.5 days of cabinet storage.

Polyurethane Sample	Days of storage	CFU control*	CFU expt**	Percent Kill	Log reduction
PU unchlorinated (control)	-	13200	-	-	-
5% monomethylolhydantoin, chlorine regenerated	1.5	13200	1730	86.87 %	0.882
10% monomethylolhydantoin, chlorine,regenerated	1.5	13200	Complete kill	99.99 %	4
5% monomethylolhydantoin, chlorine,regenerated	4.5	13200	5470	58.56 %	0.383
10% monomethylolhydantoin, chlorine,regenerated	4.5	13200	Complete kill	99.99 %	4

*CFU control - colony forming unit in control plate
**CFU expt - colony forming unit in experimental plates

Table 3. Biocidal efficacy of polyurethane with 10% w/v monomethylolhydantoin with different soaking parameter after 0 and 4.5 days of cabinet storage.

Polyurethane sample		Soaking Time (hours)	CFU control*	CFU expt**	Percent Kill	Log reduction
10 % monomethylol hydantoin 4.5 days storage	Acidified	1	15300	6130	59.93 %	0.397
		2	15300	533	96.52 %	1.46
		3	15300	1640	89.28 %	0.970
		5	15300	1090	92.88 %	1.15
	Basic	1	15300	7200	52.94 %	0.327
		2	15300	9330	39.02 %	0.215
		3	15300	11200	26.80 %	0.135
		5	15300	10800	29.41 %	0.151
10 % monomethylol hydantoin 0 days storage	Acidified	24	10700	Complete kill	99.99 %	4
	Basic	24	10700	Complete kill	99.99 %	4

Note: 2 hours challenge time with *E. coli* bacteria
*CFU control - colony forming unit in control plate
**CFU expt - colony forming unit in experimental plates

at high pH (pH 11) it was entirely from hypochlorite ion. Note (Table 3) that 2 hours soaking in acidified bleach solution was already adequate to give the sample sufficient chlorine loading and a log reduction of 1.46.

Activity in two different environment (room cabinet, toilet)

To simulate the biocidal efficacy of the samples in a natural environment, two batches of polyurethane with 10% monomethylolhydantoin were prepared. Both batches were soaked for two hours (2 hrs) in acidified 20% v/v bleach solution for chlorination. The first batch was stored inside a room cabinet and the second batch was left inside a cubicle of a toilet. Table 4 shows the antimicrobial activity of the samples. From the table, samples left inside the cabinet for 0, 1, and 5 days showed the highest log reduction of 4.00 (complete kill). On the contrary, samples left inside the toilet exhibited a log reduction of 1.50 for the first day and a log reduction of 0.551 for the 5 days exposure. It could be seen that samples left inside the cabinet have a greater antimicrobial activity or chlorine loading compared to corresponding samples left inside the toilet. This experiment showed that microbial activity(or the presence of organic matter that has considerable chlorine demand) in the toilet is much greater than inside the room cabinet, which is expected.

Activity Against Three Representative Organisms

The last phase of the experiment was to challenge the modified polyurethane samples against three representative organisms: *Staphylococcus aureus* for gram positive bacteria, *Escherichia coli* for gram negative bacteria, *Candida Albicans* for yeast. Table 5 shows the antimicrobial activity of the modified polyurethane

Table 4. Comparison of antimicrobial activity of PU with 10% by weight monomethylolhydantoin in two different environments (cabinet and toilet)

Polyurethane	Days of exposure	CFU control*	CFU expt**	Percent Kill	Log reduction
PU unchlorinated (control)	-	8400	-	-	-
PU with10%monomethylol hydantoin chlorinated (Stored inside a cabinet)	0	8400	Complete kill	99.99 %	4
	1	8400	Complete kill	99.99 %	4
	5	8400	Complete kill	99.99 %	4
	8	8400	5600	33.33 %	0.176
	11	8400	7330	12.74 %	0.059
PU with10%monomethylol hydantoin chlorinated (Left inside a toilet)	1	8400	267	96.82 %	1.50
	5	8400	2360	71.90 %	0.551
	8	8400	7730	7.98 %	0.036
	11	8400	9330	-	-

Note: 2hr soaking in acidified 20% bleach solution
*CFU control - colony forming unit in control plate
**CFU expt - colony forming unit in experimental plates

Table 5. Antimicrobial activity of 10% by weight of monomethylolhydantoin with 2hr soaking in acidified 20% bleach solution for 3 test organism (*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*) 2 days cabinet storage.

Polyurethane		Test organism	Challenge time (hrs)	CFU control*	CFU expt**	% Kill	Log reduction
PU unchlorinated control		*S. aureus*	2	2150	-	-	-
PU + 10 %	Trial 1	*S. aureus*	2	2150	Complete kill	99.95 %	3.30
	Trial 2	*S. aureus*	2	2150	Complete kill	99.95 %	3.30
PU unchlorinated control		*E. coli*	2	6530	-	-	-
PU + 10 %	Trial 1	*E. coli*	2	6530	Complete kill	99.98 %	3.70
	Trial 2	*E. coli*	2	6530	Complete kill	99.98 %	3.70
PU unchlorinated control		*C. albicans*	2	960	-	-	-
PU + 10 %	Trial 1	*C. albicans*	2	960	227	76.35 %	0.626
	Trial 2	*C. albicans*	2	960	320	66.67 %	0.477

*CFU control - colony forming unit in control plate
**CFU expt - colony forming unit in experimental plates

samples against the three representative organisms. A complete kill and a log reduction of 3.30 and 3.70 for *S. aureus* and *E. coli* respectively were observed while a 0.626 log reduction of for *C. albicans*.

Titration of oxidative Chlorine

Figure 9 shows the graph of active chlorine loadings of the samples with time. The graph also compares the average percent chlorine of chlorinated modified (PU+10%monomethylolhydantoin) and chlorinated unmodified polyurethane(PU) samples. Here, modified polyurethane (PU+10%mono-methylolhydantoin) shows a higher active chlorine loading versus the unmodified polyurethane.

Scanning Electron Microscopy

Figure 10c and 10f shows the SEM photomicrographs of the unchlorinated polyurethane and polyurethane with monomethylolhydantoin additive. It could be seen that both surface are fairly smooth, there was no phase separation, and the monomethylolhydantoin additive mixed well with the TDI-castor oil polyurethane system. Deposition of unreacted chlorine compounds as salt grains on the surface was however observed (Figure 10a and 10d) more apparent in the coating with monomethylolhydantoin but these readily dissolved upon washing with deionized water (Figure 10b and 10e).

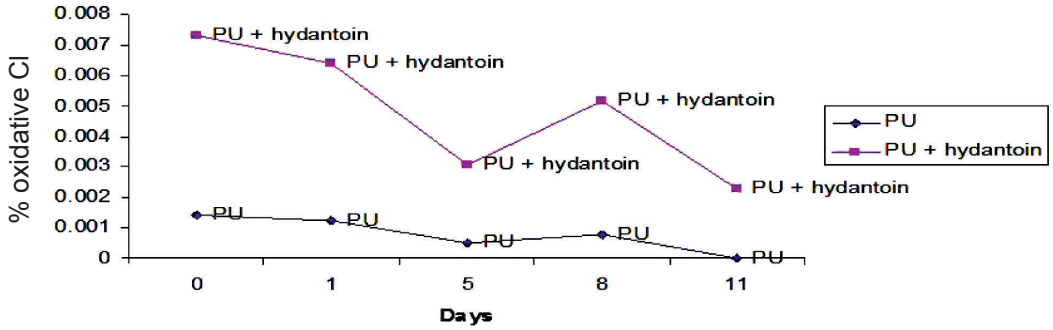


Figure 9. Average percent oxidative chlorine of polyurethane samples (PU) and PU + monomethylolhydantoin (10%) determined by titration.

PU with Monomethylolhydantoin (10%) PU only

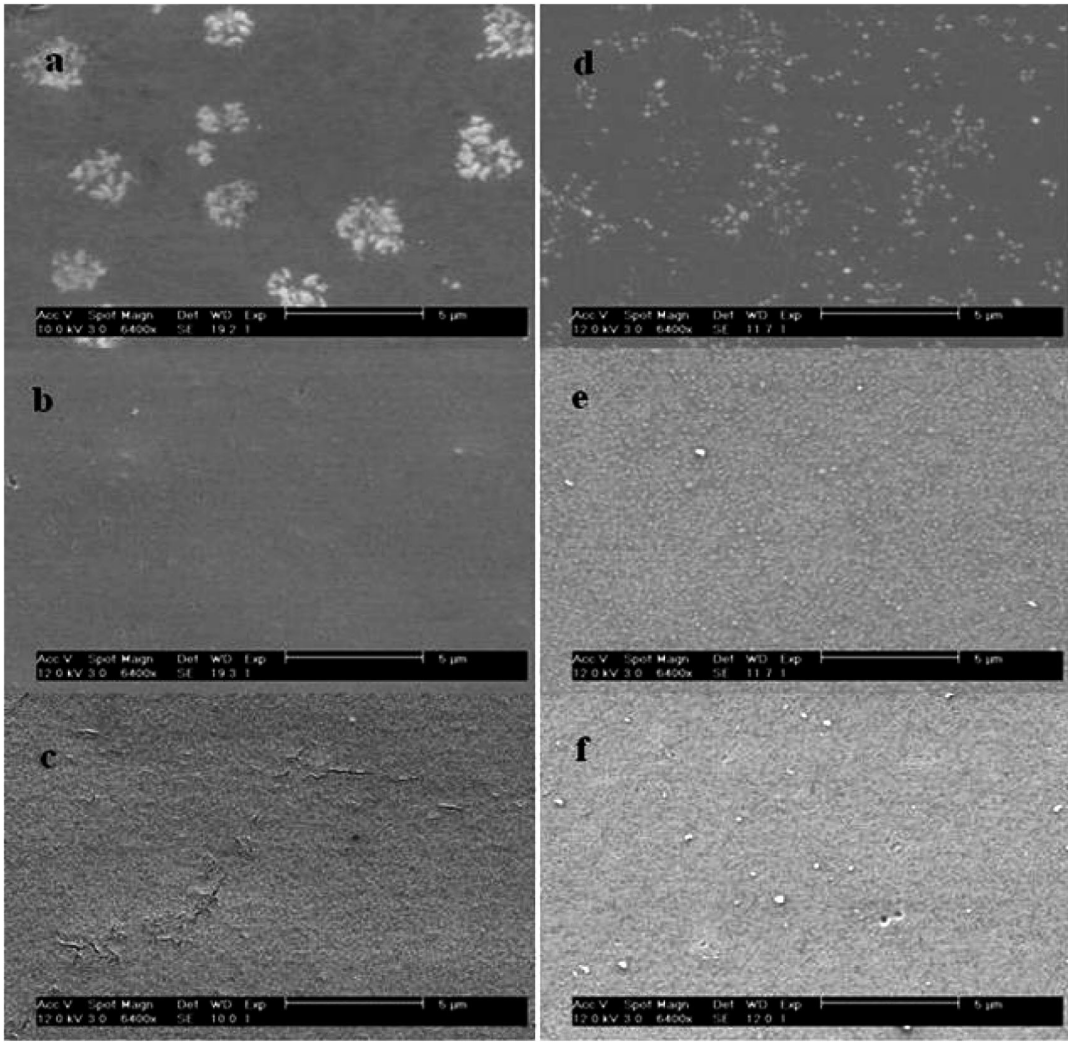


Figure 10. SEM a) PU+ monomethylolhydantoin(10% w/v) showing deposited chlorine compounds; b) PU+monomethylolhydantoin(10% w/v) after washing with deionized water; c) PU+monomethylolhydantoin(10% w/v) before chlorination; d) PU with deposited chlorine compounds after chlorination; e) PU after washing with deionized water; f) PU before chlorination.

CONCLUSION

In this research, derivatives, monoformylated 5,5-dimethylhydantoins or monomethylol hydantoins were successfully synthesized and grafted to polyurethane prepolymer composed of castor oil and toluene diisocyanate. The synthesized polyurethane coatings were readily converted to regenerable bio-cidal N-halamine structures on exposure to chlorine bleach. Coatings with 10% by weight hydantoin derivative and two hours soaking time in acidified 20% by volume chlorine bleach showed sufficient oxidative chlorine loading that remains biocidal for at least 5 days. The chlorinated coatings exhibited antimicrobial effects against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans.*

The formylated derivatives of 5,5-dimethylhydantoin dissolved in ethyl acetate solvent, mixed well and were compatible with the TDI-castor oil polyurethane system as shown in the SEM images with fairly smooth surface. Addition of 10% by weight of hydantoin derivative to the polyurethane system did not significantly alter its wetting property.

This study provided for potential application of monomethylolhydantoin modified PU coating in medical clinics and hospitals or even domiciles against the spread of periodic and dangerous microbial contaminations.

ACKNOWLEDGMENTS

The authors would like to thank the National Chemistry Instrumentation Center (NCIC) at the Ateneo de Manila University for the proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectrometric analyses; the Institute of Chemistry of the University of the Philippines for the use of the following instruments, FT-IR, TGA, and DSC ; the National Institute of Physics for the use of their SEM; the DOST-ITDI for the use of the FACE Contact-Anglemeter; and the Microbiology Laboratory of the NSRI of the University of the Philippines; the Materials Science and Engineering Program of the College of Science, University of the Philippines at Diliman for the research assistantship of one of the authors.

REFERENCES

[AATC] American Association of Textile Chemists and Colorists, 1999. “Sandwich” test“ (AATCC-100-1999):Antibacterial finishes on textile materials: Assessment of AATC technical manual (149-151) 21003.

CHEN YJ, WORLEY SD, KIM J, WEI CI CHEN TY, SANTIAGO JI. Biocidal poly(styrenehydantoin) beads for disinfection of water. 2003. Ind. Eng Chem Res 42:280-284.

CHEN YJ, WORLEY SD, KIM J, WEI CI CHEN TY, SANTIAGO JI . 2003. Biocidal polystyrene beads. 2. Control of chlorine loading. Ind Eng Chem Res 42:5715-5720.

CHEN YJ, WORLEY SD, HUANG TS, WEESE J, KIM J, WEI CI. 2004. Biocidal beads III. Comparison of N-halamine and quat functional groups. J Appl Polym Sci 92:363-367.

CHEN YJ, WORLEY SD, HUANG TS, WEESE J, KIM J, WEI CI. 2004. Biocidal beads. IV. Functionalized methylated polystyrene. J Appl Polym Sci 92:368-372.

EKNOIAN MW, WORLEY SD, BICKERT J, WILLIAMS JF. 1999. Novel antimicrobial N-halamine polymer coatings generated by emulsion polymerization. Polymer 40(6):1367-1371.

FARINATE, BURG DA. 1993. Process for preparing methylolhydantoins. US Patent No. 5,252,744 October 12 1993.

FOELSCH DH. 1976. Dimethylol Dimethylhydantoin Solution. US Patent No. 3,987,184 October 19, 1976.

GRUNZINGER SJ, WYNNE KJ. 2006. Polyurethanes from novel 1,3-propylene co-telechelics having pendant hydantoin and methoxymethyl groups, Polymer 47(11)423-4237.

GRUNZINGER SJ, KURT P, BRUNSON KM, WOOD L, OHMAN DE, WYNE KJ .2007. Biocidal activity of hydantoin-containing polyurethane polymeric surface modifiers. Polymer 48:4653-4662.

LIANG J, WU R, WANG J-W, BAMES K, WORLEY SD, CHO U, LEE J, BROUGHTON RM, HUANG T-S. 2007. N-halamine biocidal coatings. J Ind Microbiol Biotechnol 34:157-163.

LI S, LI L, SPOON W. 2007. Color indicator for halamine treated fabric. US Patent Application Publication No. 2007/0218562 A1 Publication Date September 20, 2007.

LIN J, CAMMARATA V, WORLEY SD. 2001. Infrared characterization of biocidal nylon. Polymer 42:7903-7906.

LIN J, WINKELMANN C, WORLEY SD, KIM JH, WEI CI AND CHO UC. 2002. Biocidal polyester. J Appl Polym Sci 85:177-182.

MAKAL U, WOOD L, OHMAN DE, WYNE KJ. 2006. Polyurethane biocidal polymeric surface modifiers. Biomaterials 27:1316-1326.

MCFARLAND J. 1907. The nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J.Am. Med. Assoc. 49:1176-1178.

MILKOWSKI J D, VEBER, DF, HIRSHCHMANN, R. 1979. Thiol Protection with Acetamidomethyl Group: S-Acetamidomethyl-L-Cysteine Hydrochloride. Organic Synthesis, Coll. 6:5 (1988), 59:190 (1970).

PIERCE WC, HAENISCH EL AND SAWYER DT. 1958. Quantitative Analysis: Chapter 15 Iodimetry and Iodometry, John Wiley & Sons Inc. New York and London, 4th edition.

PIOZZI A, FRANCOLINI I, OCCHIAPETI L, VENDITTI M, MARCONI W. 2004. Antimicrobial activity of polyurethanes coated with antibiotics: A new approach to the realization of medical devices exempt from microbial colonization. International Journal of Pharmaceutics 280:173–183.

SUN G, XU X. 1998. Durable and regenerable antibacterial finishing of fabrics: biocidal properties. Textile Chemist and Colorist 30;6:26-30.

SUN G, XU X. 1999. Durable and regenerable antibacterial finishing of fabrics: fabric properties. Textile Chemist and Colorist 31;1:21-24.

SUN G, XU X. 1999. Durable and regenerable antibacterial finishing of fabrics: chemical structures. Textile Chemist and Colorist 31;5:31-35.

SUN G, XU X, CLIVER, D. 2004. Biocidal Cellulosic Material. US Patent No. 6,770,287 B1, Date: August 3, 2004.

SUN G, SUN Y, CHEN T, WORLEY SD. 2001. Novel refreshable N-halamine polymeric biocides containing imidazolidin-4-one derivatives. J Polym Sci Pol Chem 39:3073-3084.

SUN Y, SUN G. 2002. Synthesis, characterization, and antibacterial activities of novel N-halamine polymer beads prepared by suspension copolymerization. Macromolecules 35:8909-8912.

SUN Y, SUN G. 2001. Novel regenerable N-halamine polymeric biocides. I. Synthesis, characterization, and antibacterial activity of hydantoin-containing polymers. J Appl Polym Sci 80:2460-2467.

TEXTER J, ZIEMER P, RHOADES S, CLEMENS D. 2007. Bactericidal silver ion delivery into hydrophobic coatings with surfactants. J Ind Microbiol Biotechnol 34:571–575.

WILLIAMS DE, WORLEY SD, BAMELA SB, SWANGO LJ.1987. Bactericidal activities of selected organic N-halamines. Applied Environmental Microbiology 53:2082-2089.

WORLEY SD, CHEN Y, WANG J-W, WU R, LI Y. 2005. N-halamine siloxanes for use in biocidal coatings and materials. US Patent No. 6,969,769 B2

WORLEY SD, LI Y. 2003. Heterocyclic amine diol compounds and their biocidal derivatives. US Patent Application Publication No. US 0220415 A1

WORLEY SD, LI F, WU R, KIM J, WEI CI, WILLIAMS JF. 2003;A novel N-halamine monomer for preparing biocidal polyurethane coatings. Surf Coat Int Pt B-C 86:273-277.

WORLEY SD, SUN G, SUN W, CHEN T-Y. 1996, Polymeric cyclic N-halamine biocidal compounds US Patent No. 5,490,983.