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Novel Antimicrobial Polymer Based on Hydantoin

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Abstract Infections by microorganisms, such as gram-positive and gram-negative bacteria, virus, fungi, and protozoa are major concerns in clinical and pharmaceutical areas (drugs, medical devices, and food industry (food packaging, storage, and fresh products)). New antimicrobial polymers were prepared by the reaction of 5,5-dimethylimidazolidine-2,4-dione, 5,5-diphenylimidazolidine-2,4-dione, 1-(2,5-dioxoimidazolidin-4-yl)urea, 5,5-diphenyl-2-thioxoimidazolidin-4-one and 5-benzylidene-imidazolidine-2,4-dione with 6-O-Tosyl-N-phthaloyl chitosan and reaction of 5,5-dimethylimidazolidine-2,4-dione, 5,5-diphenyl-2-thioxoimidazolidin-4-one and 5-benzylidene-imidazolidine-2,4-dione with O-succinoyl chitosan chloride. The reaction achieved by activation of hydroxyl groups on the C-6 position of glucosamine residues of chitosan. The structure of the resulting materials was characterized with Fourier transform infrared-spectroscopy, X-ray diffraction TGA analysis and transmission electron microscope. Antimicrobial activity of the polymers was studied against Gram-negative bacteria, Gram-positive bacteria by well diffusion method and cut plug method. Polymers showed good or moderate antimicrobial activities.

Keywords Chitosan derivatives, Imidiazolidine-2,4-diones, Antimicrobial activity

1. Introduction

Infections by microorganisms, such as gram-positive and gram-negative bacteria, virus, fungi, and protozoa are major concerns in clinical and pharmaceutical areas (drugs, medical devices, and food industry (food packaging, storage, and fresh products)). The diseases caused by these microorganisms provoke serious health problems that in severe cases lead to death. Diseases related to the proliferation of microorganisms are particularly significant in hospitals where the risk of infection by microorganisms is a major concern, mainly when complicated surgical procedures are conducted. However, illnesses caused by poor personal hygiene and rotten or contaminated food should also be considered an important issue [1–3]. Therefore, the development of materials that exhibit antimicrobial activity appears to be highly relevant in health care. According to Musumeci *et al.* [4] an antimicrobial agent is a substance that kills or inhibits the development and the multiplication of microorganisms, such as bacteria, fungi, protozoa or viruses. Among numerous materials having this feature, chitosan and its derivatives can be highlighted. In what follows, some results related to the bacterial activity of chitosan and chitosan-derivatives are presented.

Chitosan is a partially deacetylated derivative of chitin, consisting of β-(1,4)-2-amino-2-deoxy-D-glucopyranose and small amounts of *N*-acetyl-D-glucosamine [5]. Chitosan-derivatives are usually obtained by chemical modification of the amino or hydroxyl (especially at C6 position in the chitosan backbone) groups of chitosan for improving the physicochemical properties [5, 6]. Chitosan and chitosan-derivatives have been extensively used to obtain polyelectrolyte complexes, due to their polycationic nature and their biological properties (biodegradability, biocompatibility, low toxicity, mucoadhesivity and antimicrobial) [7–9].

Imidazolidine-2,4-diones belong to significant heterocyclic compounds, these Imidazolidine-2,4-diones have many applications as antitumor, antiarrhythmic, anticonvulsant, herbicidal and others. Aplysinopsins isolated from marine organisms [10] are examples of imidazolidine-2,4-diones containing natural products exhibiting cytotoxicity towards cancer cells and the ability to affect neurotransmitters. In this study new microbicidal polymers were prepared by the reaction of 5,5-dimethylimidazolidine-2,4-dione **I**, 5,5-diphenylimidazolidine-2,4-dione **II**, 1-(2,5-dioxoimidazolidin-4-yl)urea **III**, 5,5-diphenyl-2-thioxoimidazolidin-4-one **VI** [11] and 5-benzylidene-imidazolidine-2,4-dione **V** [11] with 6-O-Tosyl-N-phthaloyl chitosan and reaction of 5,5-dimethylimidazolidine-2,4-dione, 5,5-diphenyl-2-thioxoimidazolidin-4-one and 5-benzylidene-imidazolidine-2,4-dione with O-succinoyl chitosan chloride. The reaction achieved by activation of hydroxyl groups on the C-6 position of glucosamine residues of chitosan. The structure of the resulting materials was characterized with Fourier transform infrared-spectroscopy, X-ray diffraction, thermo

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gravimetric analysis, and transmission electron microscope. Antimicrobial activity of the polymers was studied against Gram-negative bacteria, Gram-positive bacteria by well diffusion method and cut plug method. Polymers showed good or moderate antimicrobial activities.

2. Experimental

2.1. Materials

Chitosan (molecular weight of 300 kDa and 90% of degree of deacetylation) was purchased from Acros Organic, dimethylformamide, 1,2-diphenylethane-1,2-dione, 1,4 dioxane, benzaldehyde, imidazolidine-2,4-dione, 5,5-dimethyl imidazolidine-2,4-dione, 5,5-diphenylimidaz olidine-2,4-dione, phthalic anhydride, triethylamine, toluene-4-sulfonyl chloride, were purchased from Sigma Aldrich.

2.2. Preparation of 6-O-tosyl-N-phthaloyl chitosan (VI)

Triethylamine (6.868 g, 0.068 mol) and toluene-4-sulfonyl chloride (3.572 g, 0.0188 mol) dissolved in DMF (20 mL) were gradually added to a cooled to 4–8°C solution of N-phthaloylchitosan [12, 13] (0.54 g, 0.00188 mol) in DMF (50 mL). The mixture was stirred overnight at 8°C. The precipitate obtained by pouring the solution onto ice water, collected by filtration, washed with chloroform and dried to give 0.63 g, yield 83%. FT-IR spectrum (KBr) (cm^{-1}): 1631 cm–1 (-C=N-) 1177 cm^{-1} due to tosyl Groups and 1578 cm^{-1} aromatic group, XRD patterns show (Fig. 2) prominent peaks corresponding to the basal spacing of 2Ə= (14.43°, 17.13°).

2.3. O-Succinoyl Chitosan Chloride (VII)

$SOCl_2$ (22 ml, 0.03 mol) was added drop wise to O-Succinoyl chitosan [14] (3.89 g, 0.01 mol) (3:1 molar) in anhydrous benzene (20 ml). Reaction mixture was stirred at r.t. for 24 h. The reaction mixture was heated at 60°C for 3 h. residual solid was filtered off. The precipitate formed washed several times with anhydrous benzene then dried under vacuüm to give 2.64 g, (yield 65%). [14] FT-IR spectrum (KBr) (cm^{-1}): band at 1731 cm^{-1} was attributed to carbonyl group of ester group [C=O of O (COR)] and the peak at 1662 cm^{-1} was assigned to the carbonyl group of acylamino group the signal of the phthalimide group at 1770, 1710 cm^{-1}.

2.4. Synthesis of 6-O-tosyl-N-phthaloyl chitosan / 5,5-Dimethylimidazolidine-2,4-dione (VIII)

5,5-Dimethylimidazolidine-2,4-dione I (0.128g 0.001 mol) was stirred with sodium hydroxide (0.04 g, 0.001 mol) in ethanol for 24 h at 25°C followed by addition 6-O-Tosyl-N-phthaloyl chitosan **VI** (0.457 g, 0.001 mol) portion wise. The reaction mixture was refluxed for 48 h, cooled. The residual solid was filtered off, the residue was purified by ethanol and dried under vacuum oven at 40°C for 48 h to afford 0.35g, yield 80% , FT-IR spectrum (KBr) (cm^{-1}) (Fig. 1): showed the following bands 3450 (O-H), 3280 (N-H amide, CO-NH-CPh2), 3000 (C-H aliphatic), 1771,1736 (C=O, phthalimide) 1711 (C=O amide, NH-CO-CMe), 1640, (C=O amide, NH-CO-N), 1386 (CH_2), 1159 (C-O-C), The XRD patterns show (Fig. 2) prominent peaks corresponding to the basal spacing of 2Ə= (4.94°, 14.03°, 17.40°, 18.33°, 23.85°).

2.5. Synthesis of 6-O-tosyl-N-phthaloyl chitosan / 5,5-diphenylimidazolidine-2,4-dione (IX)

5,5-diphenylimidazolidine-2,4-dione **II** (0.252 g, 0.001 mol) was stirred with sodium hydroxide (0.04 g, 0.001 mol) in ethanol for 24 h at 25°C followed by addition 6-O-Tosyl-N-phthaloyl chitosan **VI** (0.457 g, 0.001 mol) portion wise. The reaction mixture was refluxed for 48 h, cooled. The residual solid was filtered off, the purification of the residue by ethanol and dried under vacuum oven at 40°C for 48 h to afford 0.41 g, yield 75% , FT-IR spectrum (KBr) (cm^{-1}) (Fig. 1): showed the following bands 3450 (O-H), 3240 (N-H amide, CO-NH-CPh2), 3000 (C-H aromatic), 1771,1735 (C=O, phthalimide) 1723 (C=O amide, NH-CO-CPh), 1643, (C=O amide, NH-CO-N)., XRD patterns show (Fig. 2) prominent peaks corresponding to the basal spacing of 2Ə= (4.96°, 11.35°, 13.21°, 16.79°, 17.40°, 20.40°, 22.50°, 26.51°).

2.6. Synthesis of 6-O-tosyl-N-phthaloyl chitosan / 1-(2,5-dioxoimidazolidin-4-yl)urea (X)

To a suspension of 1-(2,5-dioxoimidazolidin-4-yl)urea **III** (0.158g, 0.001 mol) in DMF (10 mL), 6-O-Tosyl-N-phthaloyl chitosan **VI** (0.457 g, 0.001 mol) was added portion wise. The reaction mixture was refluxed for 48 h, cooled. The residual solid was filtered off, the residue was purified by ethanol and dried under vacuum oven at 40°C for 48 h to afford 0.36g, yield 80%, FT-IR spectrum (KBr) (cm^{-1}) (Fig. 1): showed the following bands 1777 cm^{-1} is characteristic of carbonyl in phthalimide and absorption at 1726, 1629 cm^{-1} are characteristic of carbonyl in 1-(2,5-dioxoimidazolidin-4-yl)urea, XRD patterns show (Fig. 2) prominent peaks corresponding to the basal spacing of 2Ə = (17.23°, 20.34°, 29.75°).

2.7. Synthesis of 6-O-tosyl-N-phthaloyl chitosan / 5,5-diphenyl-2-thioxoimidazolidin-4-one (XI)

Into solution of 5,5-diphenyl-2-thioxoimidazolidin-4-one **IV** (0.26g, 0.001 mol) in DMSO (10 mL), 6-O-Tosyl-N-phthaloyl chitosan **VI** (0.457 g, 0.001 mol) was added portion wise. The reaction mixture was refluxed for 48 h, cooled. The residual solid was filtered off, the residue was purified by ethanol and dried under vacuum oven at 40°C for 48 h to afford 0.45 g, yield 75%, FT-IR spectrum (KBr) (cm^{-1}) (Fig. 1): showed the following bands 3450 (O-H), 3200, (N-H), 3070 (C-H aromatic), 1752, 1725, are characteristic of carbonyl in phthalimide and absorption at 1637, (C=O) cm^{-1} is characteristic of carbonyl in

5,5-diphenyl-2-thioxoimidazolidin-4-one and at 1180 (C-S), XRD patterns show (Fig. 2) prominent peaks corresponding to the basal spacing of 2Ɵ = (11.50°, 14.74°, °, 17.70°, 21.45°, 23.96°).

2.8. Synthesis of 6-O-tosyl-N-phthaloyl chitosan / 5-benzylidene-imidazolidine-2,4-dione (XII)

5-benzylidene-imidazolidine-2,4-dione **IV** (0.188g 0.001 mol) was stirred with sodium hydroxide (0.04 g, 0.001 mol) in ethanol for 24 h at 25°C followed by addition 6-O-Tosyl-N-phthaloyl chitosan **VI** (0.457 g, 0.001 mol) was added portion wise. The reaction mixture was refluxed for 48 h, cooled. The residual solid was filtered off, the purification of the residue ethanol and dried under vacuum oven at 40°C for 48 h to afford 0.40 g, yield 75% , FT-IR spectrum (KBr) (cm^{-1}) (Fig. 1): showed the following bands 3550,(O-H), 3070 (C-H aromatic), 1715 broad band are characteristic of carbonyl in phthalimide 1658, broad band are characteristic of carbonyl in 5-benzylidene-imidazolidine-2,4-dione., XRD patterns show (Fig. 2) prominent peaks corresponding to the basal spacing of 2Ɵ = (7.79°, 15.70°, 17.74°, 26.60°).

2.9. Synthesis of O-succinoyl chitosan / 5,5-diphenylimidazolidine-2,4-dione (XIII)

To a suspension of 5,5-diphenylimidazolidine-2,4-dione **II** (0.252 0.001 mol) with O-succinoyl chitosan chloride **VII** (0.407g 0.001 mol) in 1,4 dioxane (10 ml), pyridine (0.79g, 0.001 mol) was added drop wise. The reaction mixture was stirred at r.t. for 72 h. The mixture was reflux 48 h then cooled, The residual solid was filtered off washed with 1,4 dioxane, dried under vacuüm oven at 40°C for 48 h to afford 0.40 g 65% yield. FTIR (KBr) (cm^{-1}): 3450 (O-H), 3210 is characteristic of (N-H) in 5,5-diphenylimidazolidine-2, 4-dione, 3070 (C-H aromatic), 1773 is characteristic of carbonyl in 5,5-diphenylimidazolidine-2,4-dione, 1719, 1660, broad bands are characteristic of carbonyl in phthalimide (C=O), 1400 (CH_2), 1023 (C-O-C).

2.10. Synthesis of O-succinoyl chitosan / 5,5-diphenyl-2-thioxoimidazolidin-4-one (XIV)

To a suspension of 5,5-diphenyl-2-thioxoimidazolidin-4-one **IV** (0.26g 0.001 mol) with O-succinoyl chitosan chloride **VII** (0.407g 0.001 mol) in 1,4 dioxane (10 ml), pyridine (0.79g, 0.001 mol) was added drop wise. The reaction mixture was stirred at r.t. for 72 h. The mixture was reflux 48 h then cooled, The residual solid was filtered off washed with 1,4-dioxane dried under vacuüm oven at 40°C for 48 h to afford 0.44g, 70% yield. FTIR (KBr) (cm^{-1}): 3436 (O-H), 3274 is characteristic of (N-H) in 5,5-diphenylimidazolidine-2,4-dione, 3050 (C-H aromatic), 1764 is characteristic of carbonyl in 5,5-diphenylimidazolidine-2,4-dione, 1713, 1645 broad bands are characteristic of carbonyl in phthalimide (C=O), 1390 (CH_2), 1030 (C-O-C).

2.11. Synthesis of O-succinoyl chitosan / 5-benzylidene imidazolidine-2,4-dione (XV)

To a suspension of 5-benzylideneimidazolidine-2,4-dione **V** (0.188g 0.001 mol) with O-succinoyl chitosan chloride **VII** (0.407g 0.001 mol) in 1,4-dioxane (10 ml), pyridine (0.7g, 0.001 mol) was added drop wise. The reaction mixture was stirred at r.t. for 72 h. The mixture was reflux 48 h then cooled, The residual solid was filtered off, washed with 1,4-dioxane dried under vacuüm oven at 40°C for 48 h to afford 0.42 g 76% yield. FTIR (KBr) (cm^{-1}): 3561, 3453, 3267 (O-H), (N-H), 3030 (C-H aromatic), 3150 (C-H aliphatic), 1760 band is characteristic of carbonyl in 5-benzylideneimidazolidine-2, 4-dione, 1715, 1660 broad bands are characteristic of carbonyl in phthalimide (C=O), 1380 (CH_2), 1070 (C-O-C).

2.12. Characterization

FT-IR spectra were recorded on Bruker, Tensor 27 FT-IR spectro-photomer with frequency range 4000 cm^{-1} to 400 cm^{-1} (Central Lab, Tanta University, Egypt) with KBr pellets. Scanning Electron Microscope – JEOL JSM 5200 LV (electron microscope unit, Faculty of Medicine, Tanta University, Egypt). The XRD analysis was measured by GRN, APD 2000 PRO X-Ray diffraction, using Cu Ka (1.54 Ao) at 40 Ku (Central Lab. Tanta University, Egypt), TGA data were obtained by using Shimadzu thermal analyzer system at a heating rate of 10 °C/min, sample weight of 5-6 mg, under nitrogen (20 ml/min) flow. The range investigated from 25°C- 800°C. TGA-50 furnace with a M3 microbalance, and TA72 Graphware software were employed for thermal analyses (Central Lab. Tanta University, Egypt).

3. Biological Activities of the Modified Polymers

3.1. Tested Microorganisms

The microbial strains were obtained from Bacteriological lab, Microbiology section, Botany Department, Faculty of Science, Tanta University: The Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027), Gram-positive bacteria (*Staphylococcus aureus* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228), fungi (*Candida albicans* and *Aspergillus niger*) were used to examine the antimicrobial activity of the polymers The bacterial strains were maintained on nutrient agar (3 g peptone, 5 g NaCl, 5 g beef extract, 20 g agar per liter, pH=7.4).

3.2. Well Diffusion Method for Screening of Antimicrobial Activity for Tested Complexes

The antimicrobial activity of the tested samples was determined by well diffusion method [15] Powder test samples (20 mg/ml) were dissolved in DMSO. In case of nanocomposites the samples were dispersed in DMSO.

Wells were then created and 50 µl of the samples were pipetted into each well to detect the most sensitive microorganisms against investigated polymers. DMSO was used as negative control. The plates were incubated at 37°C for bacteria were examined for inhibition zones development. A caliper was used to measure the inhibition zones. Three replicates were carried out at least. The inoculum's concentrations were 6.5 x 105 CFU for bacteria. After incubation for 24 h for bacteria, yeasts and at 48 h for fungi, the agar plates were checked for the diameter of inhibition zone. The reaction of the microorganisms with antimicrobial polymers was determined by the size of the inhibitory zone.

3.3. Cut Plug Method for Screening of Antimicrobial Activity for Tested Complexes

Cut plug method was employed to determine the antimicrobial activity of the chosen products as follows: Freshly prepared spore suspension of different test microorganisms (0.5 ml of about 10^6 cells/ml) was mixed with 9.5 ml of melting sterile Sabouraud's dextrose medium (for fungi) or nutrient agar medium (for bacteria) at 45°C, poured on sterile Petri dishes, and left to solidify at r.t. Regular wells were made in the inoculated agar plates by a sterile cork borer of 0.70 mm diameter. Each well was filled with 20 mg of each tested powder. Three replicas were made for each test, and all plates were incubated at 27°C for 3 days for fungi, and at 32°C for 24 h for bacteria. Then the average diameters of inhibition zones were recorded in centimeters, and compared for all plates. [16]

3.4. Minimal Inhibitory Concentrations (MICs)

The most antimicrobial active compounds were chosen to determine their MIC values against the most sensitive microorganism by tube dilution assay.

3.5. Tube Dilution Assay

Nutrient broth was used for the tube dilution to determine MICs of tested bacteria. The MIC value of tested compounds was determined using two-fold broth micro-dilution. One millimeter of sterile media was added to 1 ml of different compound concentrations (100, 50, 25, 12.5, 6.3, 3.2 and 1.6, 0.8, 0.4 and 0.2 mg/1 ml). The tubes were then inoculated with a drop of microbial suspension (25 µl) and incubated at 37°C for 24 h. Tetracycline (100-0.0031 mg/ml) was used as standards antimicrobial drug (positive control). DMSO was used as the negative control. After incubation, certain amount of each tube was spreeded on agar plates and then incubated for 24 h, and then the numbers of colonies were counted. The MIC endpoints were detected as the lowest concentration of tested compounds at which the growth of the tested microorganisms was inhibited. The minimum bactericidal concentration (MBC) endpoints are defined as the lowest concentration of antimicrobial agent that kills > 99.9% of the initialbacterial population and detected when no visible growth of bacteria was observed on the agar plates. [17]

3.6. Statistical Analysis

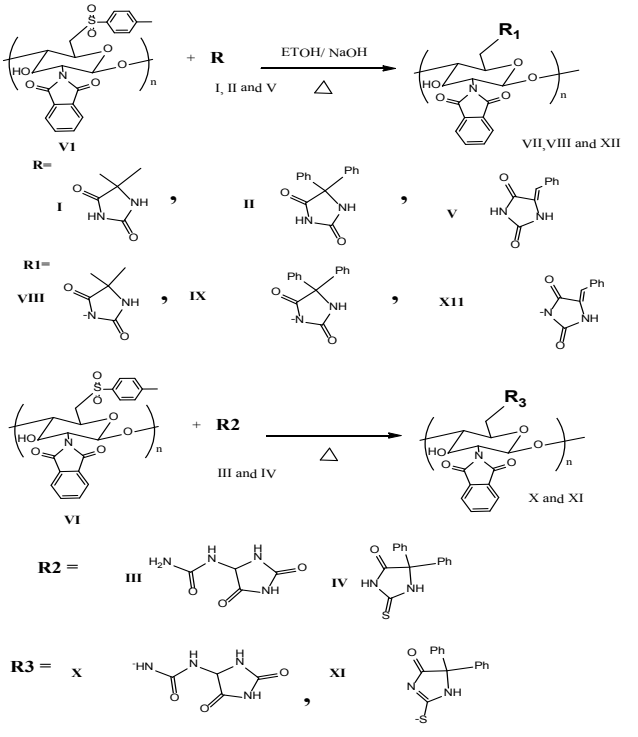
All experiments and analytical determinations were replicated at least three times and mean value was calculated.

3.7. Uses of the most Efficient Antimicrobial Polymers Water Treatment

Fixed quantity of the complexes (50 mg) were placed into different column for purifications certain amount of previously prepared infected water (5 ml of about 106 cells/ml) with some microorganism ((Escherichia coli ATCC 8739, Staphylococcus aureus ATCC 6633, Staphylococcus epidermidis ATCC 12228)). The inhibition of the complexes were taken throw different times (30 min, 90 min and 180 min) in order to establish the best result and Percentage of surviving cells was recorded for each tested material.

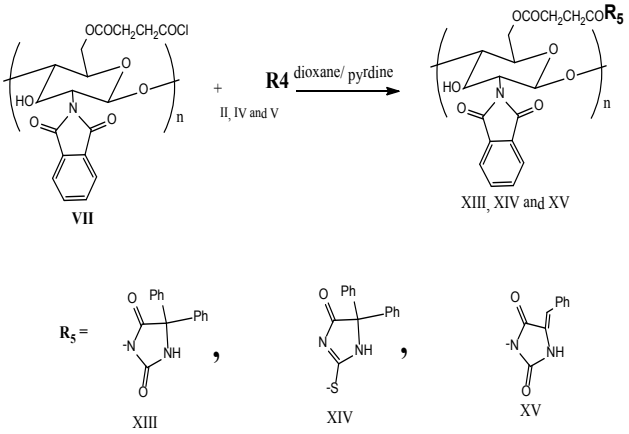
4. Results and Discussion

In this work, A series of polymers containing chemically bound imidazolidine-2,4-dione have been prepared by reaction of 5,5-dimethylimidazolidine-2,4-dione **I**, 5,5-diphenylimidazolidine-2,4-dione **II**, 1-(2,5-dioxoimidazolidin-4-yl)urea **III**, 5,5-diphenyl-2-thioxoimidazolidin-4-one **VI** and 5-benzylidene-imidazolidine-2,4-dione **V** with 6-O-Tosyl-N-phthaloyl chitosan. Modification on the C6 of chitosan was carried out by the nucleophilic replacement of the (tosyl) group with N in imidazolidine-2,4-dione or S 2-thioxoimidazolidin-4-one.



Scheme 1. Modification of (VI) with (I- V)

While the reaction of 5,5-dimethylimidazolidine-2,4-dione, 5,5-diphenyl-2-thioxoimidazolidin-4-one and 5-benzylidene-imidazolidine-2,4-dione with O-succinoylation of chitosan chloride was carried out by the nucleophilic replacement of the chloride group with NH in imidazolidine-2,4-dione or = S 2-thioxoimidazolidin-4-one.



Scheme 2. Modification of (VII) with (II, IV and V)

In FT-IR, compound **(VIII)** showed the following bands 3450 (O-H), 3280 (N-H amide, CO-NH-CPh2), 3000 (C-H aliphatic), 1771,1736 (C=O, phthalimide) 1711 (C=O amide, NH-CO-CMe), 1640, (C=O amide, NH-CO-N), 1386 (CH_2), 1159 (C-O-C), compound **(IX)** showed the following bands 3450 (O-H), 3240 (N-H amide, CO-NH-CPh2), 3000 (C-H aromatic), 1771,1735 (C=O, phthalimide) 1723 (C=O amide, NH-CO-CPh), 1643, (C=O amide, NH-CO-N)., compound **(X)** showed the following bands 1777 cm^{-1} is characteristic of carbonyl in phthalimide and absorption at 1726, 1629 cm^{-1} are characteristic of carbonyl in 1-(2,5-dioxoimidazolidin-4-yl)urea, compound **(XI)** showed the following bands 3450 (O-H), 3200, (N-H), 3070 (C-H aromatic), 1752, 1725, are characteristic of carbonyl in phthalimide and absorption at 1637, (C=O) cm^{-1} is characteristic of carbonyl in 5,5-diphenyl-2-thioxoimidazolidin-4-one and at 1180 (C-S), compound **(XII)** showed the following bands 3550, (O-H), 3070 (C-H aromatic), 1715 broad band are characteristic of carbonyl in phthalimide 1658, broad band are characteristic of carbonyl in 5-benzylidene-imidazolidine-2,4-dione , compound **(XIII)** showed the following bands 3450 (O-H), 3210 is characteristic of (N-H) in 5,5-diphenylimidazolidine-2,4-dione, 3070 (C-H aromatic), 1773 is characteristic of carbonyl in 5,5-diphenylimidazolidine-2,4-dione, 1719, 1660, are characteristic of carbonyl in phthalimide (C=O), 1400 (CH_2), 1023 (C-O-C)., compound **(XIV)** showed the following bands 3436 (O-H), 3274 band is characteristic of (N-H) in 5,5-diphenylimidazolidine-2,4-dione, 3050 (C-H aromatic), 1764 is characteristic of carbonyl in 5,5-diphenylimidazolidine-2,4-dione, 1713, 1645 are characteristic of carbonyl in phthalimide (C=O), 1390 (CH_2), 1030 (C-O-C)., Compound **(XV)** showed the following bands 3561, 3453, 3267 (O-H), (N-H), 3030 (C-H aromatic), 3150 (C-H aliphatic), 1760 band is characteristic of carbonyl in 5-benzylideneimidazolidine-2, 4-dione, 1715, 1660 are characteristic of carbonyl in phthalimide (C=O), 1380 (CH_2), 1070 (C-O-C). [18].

Scanning electron microscopy was used for the characterization of materials from the initial one to the complexes. The surface morphology of **VI** and its modifications were obtained via usage of – JEOL JSM 5200 LV SEM and are shown in (Fig. 2). Compound **VI** surfaces morphology are seemed to be smooth (A) but compound **VIII** has different shape like fractions of sheets while compound **VII** surfaces morphology are seemed to be little rough but compound **XIII** has different crystalline surfaces morphology.

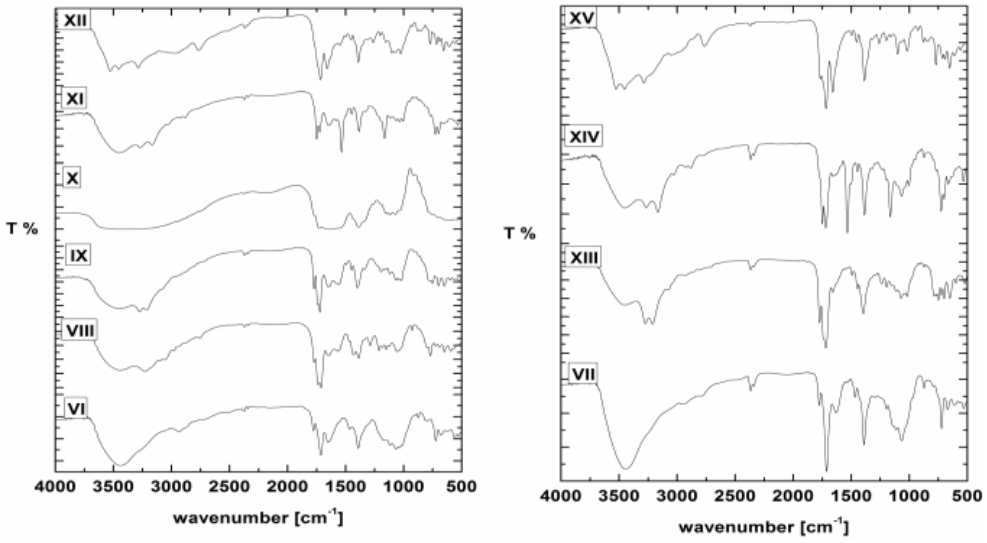


Figure 1. FT-IR spectra of VI-XV

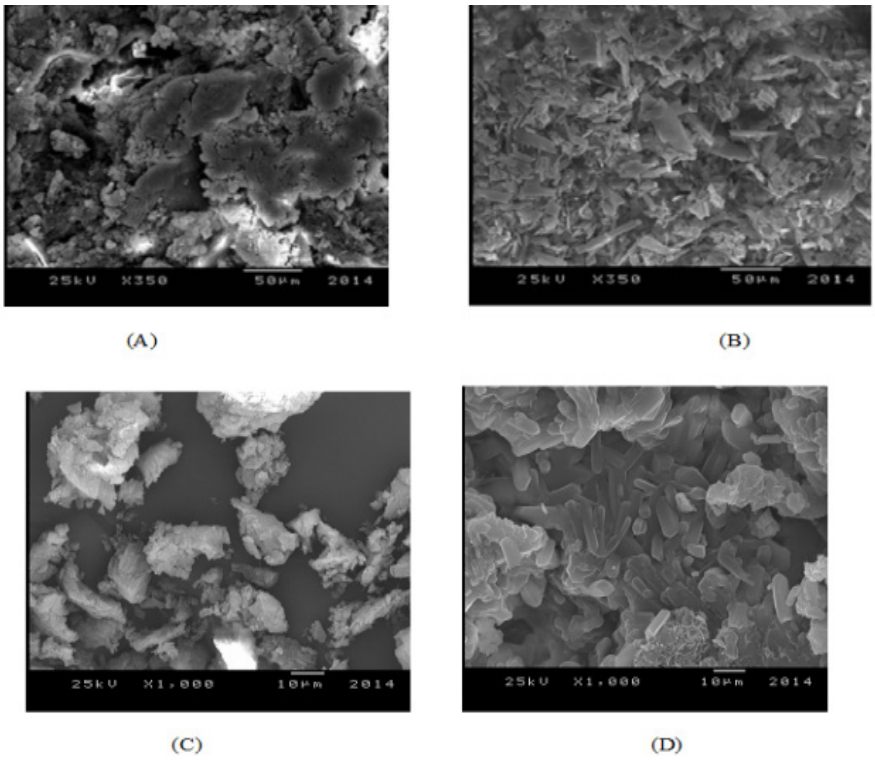


Figure 2. SEM images of (A) VI, (B) VIII, (C) VII and (D) XIII

In XRD patterns of 6-O-tosyl-N-phthaloyl chitosan (Fig 3) show prominent peaks corresponding to the basal spacing of 2Θ = 17.22° is observed. [19] This peak was shifted to different d-spacing after modification with compounds I-V cause changing in the crystalline structure. The basal spacing (d001) of compounds VIII-XII was increased to 2Θ = (4.94°, 14.03°, 17.40°, 18.33°, 23.85°), 2Θ = (4.96°, 11.35°, 13.21°, 16.79°, 17.40°, 20.40°, 22.50°, 26.51°), 2Θ = (17.23°, 20.34°, 29.75°), 2Θ = (11.50°, 14.74°, °, 17.70°, 21.45°, 23.96°), 2Θ = (7.79°, 15.70°, 17.74°, 26.60°) respectively confirming the modification of compounds I-V with compound VI while O-succinoyl chitosan show prominent peaks corresponding to the basal spacing of 2Θ = 17.13° is observed. This peak was shifted to different d-spacing after modification with compounds II, IV and V cause changing in the crystalline structure. The basal spacing (d001) of compounds 99-101 was increased to 2Θ = (11.55°, 16.55°, 18.30°, 20.49°, 26.19°), 2Θ = (11.64°, 14.46°, °, 17.94°, 21.63°, 23.70°), 2Θ = (8.10°, 13.47°, °, 15.94°, 24.63°, 26.52°) respectively confirming the modification of compounds VII with compound (II, IV and V).

In the TGA Analysis [20] (Fig 4) for compound **VI** it was found that two major weight loss patterns were observed in the temperature range of 80-100°C and 250- 815°C for tosyl chitosan. The first weight loss corresponds to evaporation of absorbed water. The second prominent weight loss was observed at 250- 815°C due to the loss of structural tosyl group. Compound **IX** show a sharp weight loss at around 200-400°C. It is explained that decomposition of intercalated polymer took place in the temperature range of 300-400°C. A weight loss at 100°C and 650°C is due to loss of water and 5,5-diphenyl-imidazolidin-2,4-dione group, respectively. In addition, the thermal degradation patterns from TGA have evidenced the presence of the 5,5-diphenyl-imidazolidin-2,4-dione in the polymer backbone confinement. Considering the above results, it is consistently believable that the introduction of organic components into organic polymers can improve their thermal stability. In case of compounds **VII**, **XIII** and **XIV**, and. It was found that two major weight loss patterns were observed in the temperature range of 80-100°C and 250- 806°C for O-succinoyl chitosan. The first weight loss corresponds to evaporation of absorbed water. The second prominent weight loss was observed at 250- 815°C due to the loss of structural succinoyl group. **XIII** and **XIV** show a sharp weight loss at around 200-500°C. It is explained that decomposition of intercalated polymer took place in the temperature range of 300-400°C. A weight loss at 100°C and 650°C is due to loss of water and 5,5-diphenyl-imidazolidin-2,4-dione group for compound **XIII**. A weight loss at 100°C and 800°C is due to loss of water and 5,5-diphenyl-2 thioxo-imidazolidin-4-one group for compound **XIV** In addition, the thermal degradation patterns from TGA have evidenced the presence of the 5,5-diphenyl-imidazolidin-2,4-dione and 5,5-diphenyl-2-thioxo-imidazolidin-4-one in the polymer backbone confinement. Considering the above results, it is consistently believable that the introduction of organic components into organic polymers can improve their thermal stability.

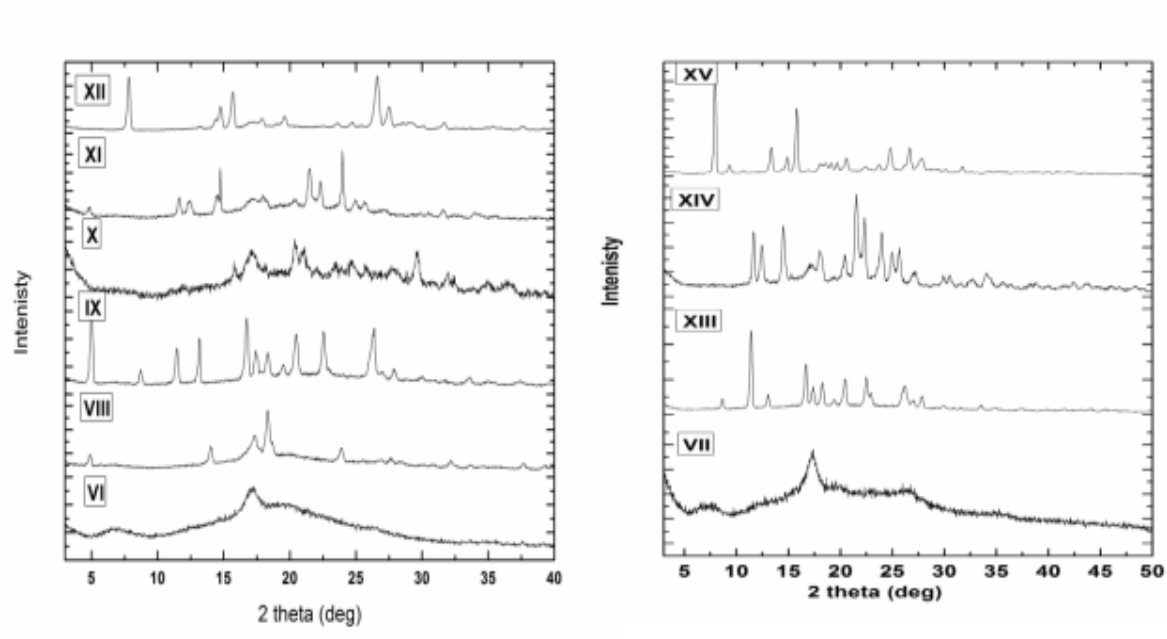


Figure 3. X-Ray spectra of VI -XV

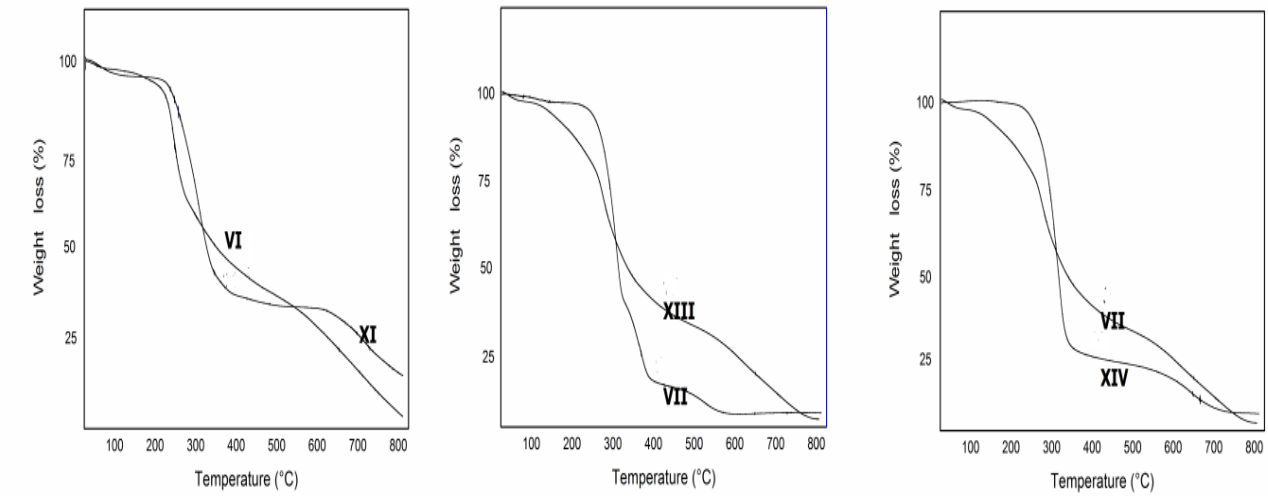


Figure 4. TGA analysis of VI, VII, IX, XIII and XIV

The results of the biocidal activity of tested polymer are presented in Table 1-4. The tested material shows a strong antibacterial activity against Staphylococcus aureus and Escherichia coli, the inhibition indices of compound **X** and **XIV** against *S. aureus* were 32 and 30 respectively. Compared to the other chitosan-derivatives, compound **XI** presented higher inhibition indices on *E. coli* [21, 22], however this property depends on pH, the insertion of alkyl and/or aryl groups increased the hydrophobic property of chitosan-derivatives.

These characteristics enhanced the interaction with the cell membrane of microorganisms and improved the antimicrobial activity of the chitosan compounds. A previous work reported by Zhong et al. [21] showed the preparation of acetyl and phenyl-thiosemicarbazone chitosan-derivatives and further evaluation of antimicrobial activities. The results also indicated that the antimicrobial action of the derivatives has a relationship with the grafted groups with different inductivity. In another study, Zhong et al. [22] obtained chloracetyl phenyl-thiosemicarbazone chitosan (CAPTCCHT) derivatives containing different R-substituent groups, the correlation between the grafted group structure and antimicrobial activities was further evaluated.

The results showed the antimicrobial activities of some derivatives were higher than unmodified chitosan. The bactericidal actions of the synthesized compounds were related to the positive polarity of the N atom and to the distribution of the electron atmosphere in the C-S groups (**XI** and **XIV**).

Table 1. Survey results of well diffusion method

Tested material	Diameter of inhibition zone (mm) against:			
	Escherichia coli	Staphylococcus epidermidis	Staphylococcus aureus	Pseudomonas aeruginosa
chitosan	00	00	00	00
DMSO	00	00	00	00
I	15	12	18	11
II	13	08	09	13
III	16	07	11	06
IV	22	11	15	08
V	13	06	13	13
VI	00	09	11	06
VII	00	00	00	00
VIII	17	12	24	11
IX	20	09	27	00
X	26	19	32	08
XI	37	24	27	12
XII	17	00	22	00
XIII	13	10	22	12
XIV	00	10	30	15
XV	22	30	27	09

Table 2. Survey results of cut plug method

Tested material	Diameter of inhibition zone (mm) against:			
	Candida albicans	*Aspergillus niger*	*Escherichia coli*	*Staphylococcus aureus*
Chitosan	0**0**	**00**	**00**	**00**
DMSO	00	00	00	00
I	12	14	13	09
II	08	14	07	11
III	11	11	10	08
IV	14	12	06	13
V	07	05	14	12
VI	16	15	15	17
VII	00	00	00	00
VIII	13	13	13	12
IX	12	11	13	13
X	13	12	14	13
XI	14	12	14	13
XII	12	11	12	11
XIII	14	12	13	12
XIV	15	15	15	16
XV	14	13	12	13

Table 3. MIC results

Complex	Microorganism	Percentage of surviving cells (% Optical density)					
		Concentration (mg/ml)					
		0.0	6.25	12.5	25	50	100
VIII	*Escherichia coli*	100	58	24	16	9	10
VIII	*Staphylococcus aureus*	100	55	26	12	13	12
IX	*Staphylococcus aureus*	100	69	32	30	31	32
X	*Staphylococcus aureus*	100	74	55	22	21	22
XI	*Escherichia coli*	100	48	18	7	7	8
XI	*Staphylococcus epidermidis*	100	64	37	22	22	22
XI	*Staphylococcus aureus*	100	76	58	32	21	22
XII	*Staphylococcus epidermidis*	100	79	42	29	30	29
XIII	*Staphylococcus epidermidis*	100	73	35	19	19	19
XIV	*Staphylococcus aureus*	100	61	36	20	17	8
XV	*Escherichia coli*	100	70	38	24	22	22
XV	*Staphylococcus epidermidis*	100	66	20	21	21	21
XV	*Staphylococcus aureus*	100	72	40	23	22	22

Table 4. The most efficient antimicrobial polymers water treatment

Complex	Microorganism	Percentage of surviving cells (% Optical density)		
		Concentration (mg/ml)		
		30 min	90 min	180 min
VIII	*Escherichia coli*	52	34	25
VIII	*Staphylococcus aureus*	26	19	9
X	*Staphylococcus aureus*	47	27	15
XI	*Escherichia coli*	18	11	12
XI	*Staphylococcus aureus*	40	32	30
XIV	*Staphylococcus aureus*	39	38	39
XIV	*Staphylococcus epidermidis*	48	34	20
XV	*Staphylococcus aureus*	56	42	35

Antimicrobial activity was decreased when the strong electron-donating group (–CH3) was present at the C5-position of the hydantoin derivatives and that activity enhanced if a strong electron-withdrawing group (–2ph) (**IX** and **XIII**) was present at the position. These results demonstrate that the bioactivity of (**VIII -XV**) derivatives is affected by the positive polarity of the N atom and the distribution of the electron atmosphere in the C-S group [18, 19]. The adhesion of (**VIII–XV**) to the surface of a bacterium alters its membrane properties ultimately causing death [22].

An increase in the inhibition of Gram-positive more than Gram- negative bacteria was observed. This is may be attributed to the physiological difference in the structure of the cell wall of the two strains. In fact, Gram negative bacteria have thick layer of phospholipids rather than the peptidoglycan comparing to the gram positive which has thin layer of peptidoglycan. The negative charges of the phospholipids enhance the adhesion power of poly cationic polymer on the cell wall. This result confirmed the rupture of the cell wall rather than the nuclear protein interaction mechanism. The interaction of the amine groups of modified chitosan with the cell wall decreases their selective permeability, which leads to leakage of the intracellular substances, such as electrolytes, UV absorbing material, protein, amino acids, glucose, and lactate dehydrogenase. As a result, chitosan and modified chitosan inhibit the normal metabolism of microorganisms and finally lead to the death of this cell [23]. The antimicrobial activity of modified chitosan shows small-moderate inhibitory effect against all fungi species table 2. The well diffusion method is found to be better result than cut plug method that is due to increase the ability of the modified chitosan to spread through Petri dishes allowing the interaction between the tested material and the microorganism. Modified chitosan show interesting result when it used as column for removal of microorganism from water table 4. Mechanism of antibacterial activity of chitosan and its derivatives is still not resolved. However, it

is proposed that the charge density of modified chitosan absorbed onto the negatively charged cell surface of bacteria leads to the leakage of proteinaceous and other intracellular constituents [24]. The additional effect derived from the hydrophobic–hydrophobic interactions between the aryl substituent and the hydrophobic interior of the bacterial cell wall is proposed from our results. Similar results were reported by Kim et al. [24].

5. Conclusions

Modification of some imidiazoldin-2,4-dione derivatives on the C-6 position of glucosamine residues of chitosan was achieved by protecting amino functionality by phthaloylation and "activating" primary hydroxyl groups of chitosan. Modification of chitosan with different imidiazolidine-2,4-dione derivatives **VIII–XV** show large improvement of the biological activity against the selected bacteria while compound **VI** and **VII** show pour effect. Anti-microbial activity of compound **VIII–XV** are more effective against Gram-positive than Gram-negative because the more complex architecture of the cell envelope in Gram-negative bacteria, which includes an outer and inner membrane with the periplasmic space in between.

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