Synthesis, characterization and antimicrobial activity of Schiff bases modified chitosan-graft-poly(acrylonitrile)

Magdy W. Sabaa a, Ali M. Elzanaty b, Omayma F. Abdel-Gawad b,∗, Esraa G. Araf a b

a Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt
b Department of Chemistry, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

A R T I C L E   I N F O
Article history:
Received 29 January 2017
Received in revised form 16 September 2017
Accepted 19 November 2017
Available online 21 November 2017

Keywords:
Chitosan
Grafted chitosan
Schiff’s base
Antibacterial
Antifungal activity

A B S T R A C T
Graft copolymerization of chitosan (Ch) with acrylonitrile (AN) prepared by free radical polymerization using potassium persulfate (PS) as initiator. Optimization of Graft copolymerization of acrylonitrile on to chitosan was performed by studying the main parameters that affecting the grafting process such as initiator and monomer concentrations, reaction time and temperature of the polymerization process to study their influence on percent grafting (%G), grafting efficiency (GE%) and percent homopolymer (%H). Modified grafting chitosan was done by Schiff’s base derivatives using different aldehydes. They are characterized by FT-IR, X-ray diffraction, thermal analysis and scanning electron microscope. Their antibacterial activities against Streptococcus pneumonia (RCMB 010010), Staphylococcus aureus (RCMB 010028), as Gram-positive bacteria and Escherichia coli (RCMB 010052) as Gram-negative bacteria and the antifungal activity against Aspergillus fumigatus (RCMB 02568), Candida albicans (RCMB 05036) and Geotrichum candidum (RCMB 05097) were examined using the diffusion agar technique. The obtained data proved that modified chitosan by grafting show better antimicrobial activities than Chitosan. Also Schiff base derivatives showed better antimicrobial activities than grafted chitosan.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction
The most abundant biopolymers found in the shells of crustacean, e.g. crab and shrimp, and cuticles of insects and also in the cell walls of some fungi and microorganisms is Chitin [1]. Chitin consists of N-acetyl-d-glucosamine repeating units linked by β-(1 → 4) bonds. Due to its inherent intractability, it is often converted to chitosan, 2-amino-2-deoxy-(1 → 4)-β-d-glucan by hot alkali treatment [1]. Chemical modification of chitosan is an important topic for the production of bifunctional materials. Work on graft copolymerization based on chitin and chitosan and their applications has been reported [1]. Chitosan has good Properties such as antimicrobial activity, biocompatibility, non-toxicity and biodegradability, chitosan can be applied in many fields, such as pharmaceutical and medical applications, environmental protection, textiles, waste water treatment, biotechnology, cosmetics, food processing and agriculture [2–4]. Although it has poor physical properties such as high brittleness and poor solubility, its requires improvement to widen its medical applications particularly in drug delivery as a carrier matrix.

Chitosan has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions [5]. The presence of much groups leads to the possibility of several chemical modifications, including graft copolymerization. Examples for graft copolymerization we can mentioned the grafting of novel monomer 2-(furan-2-carbonyl)acrylonitrile (FCAN) onto chitosan which was carried out under heterogeneous conditions using potassium persulfate and sodium bisulfite as a redox system [6], the graft-polymerization of 2-hydroxyethyl acrylate (HEA) onto chitosan (Ch) using ammonium persulfate (APS) as an initiator [7], the graft copolymerization of methyl methacrylate (PMMA) and glycidyl methacrylate (GMA) onto chitosan [8], and the graft copolymerization of acrylonitrile onto chitosan in the presence of ceric ammonium nitrate as redox initiator [9].

Poly(acrylonitrile) PAN is one of the most important fiber-forming polymers and has been widely applied in textiles because of its excellent physical and chemical properties. To endue PAN textiles with antibacterial functions from chitosan, the methods of blending PAN with chitosan or its derivatives such as carboxymethyl chitosan have been investigated [10]. However, this kinds of products are often accompanied with the problem of water stability, in which some of antibacterial reagent may be released during laundering. Therefore, graft copolymerization of PAN with...
chitosan is a novel and promising attempt due to the chemical binding between macromolecules [11].

The presence of amino groups in the repeated unites of chitosan macromolecules leads to the possibility of several chemical modifications, including the preparation of Schiff bases (−N=N−CH−R) by reaction with aldehydes, ketones and acylation using acid chloride. The reaction of chitosan with aromatic aldehydes to produce the corresponding Schiff bases has been described [12–14]. Schiff bases characterized by the −N=N−CH− (imine) groups are active against a wide range of organisms including bacteria, fungi and even algaes [15]. Moreover, a large number of papers based on Schiff bases and its antimicrobial activity such as synthesis the Schiff base of chitosan with cinnamaldehyde (or citral), sorbyl chitosan and p-aminobenzoyl chitosan work in high-intensity ultrasound, on the basis of which the antimicrobial activities against Escherichia coli, Staphylococcus aureus and Aspergillus niger have been investigated [16]. Preparation of soluble p-aminobenzoyl chitosan ester by Schiff’s base and antibacterial activity of the derivative has been also reported [17].

In the present work, chitosan was modified by grafting with acrylonitrile using potassium persulfate as initiator. The variables in grafting reaction were investigated including initiator and monomer concentrations, reaction time and temperature of the polymerization process. Further modification was done by the synthesis of Schiff’s base derivatives using different aldehydes. The prepared derivatives were characterized by FT-IR, X-ray diffraction, thermal analyses and scanning electron microscope. Their antibacterial activities against some gram-positive and gram-negative bacteria and against some fungi were also examined using the diffusion agar technique.

2. Materials and methods

2.1. Materials

Chitosan (Bio Basic Canada INC) degree of deacetylation 96%, acrylonitrile (LOBA chemie), potassium persulfate (Oxford Laboratory), acetaldehyde (Central Drug House (P) LTD), P-ansaldehyde (ALDRICH), benzaldehyde (LOBa chemie), salicyaldehyde (LOBa chemie), cinnamaldehyde (LOBa chemie), Ethyl alcohol (Biochem) and Propan-2-ol/Oxford Laboratory) have been used in the study.

2.2. Experimental

2.2.1. Preparation of chitosan graft-poly(acrylonitrile)

Chitosan solution was prepared by dissolving 1 g of chitosan in 100 mL of 1% aqueous acetic acid solution and was placed in a flat bottomed three necked flask. Throughout the reaction time, nitrogen was purged at a constant temperature (60 °C) through the stirred solution. Freshly prepared potassium persulfate solution (1−3.5 × 10−2 mol L−1) was added followed by dropwise addition (1−4 mol L−1) of acrylonitrile. The reaction was conducted for 2 h with stirring and continued for another 15 min at room temperature. Then the reaction product was precipitated out with 2 vol of isopropyl alcohol, filtered, and dried. It was again subjected to Soxhlet extraction for 8−12 h using N,N-dimethylformamide (DMF) to solubilize and remove any homopolymer and finally lyophilized [1].

The graft yield (G%), the grafting efficiency (GE%) and the amount of homopolymer (H%) formed were calculated according to the following equations:

Graftyield(G%) = [(W1 − W0)/W0] × 100

Homopolymer(H%) = [(W2 − W1)/W1] × 100

Grafting efficiency (GE%) = (W1/W2) × 100

Where W0, W1 are the weights of initial matrix and grafted matrix (i.e., weight of the product after extraction, respectively, where as W2 is the crude product before extraction and W3 is the weight of monomer [18].

2.2.2. Modification of the ch-g-PAN with some aldehyde derivatives

In 20 mL of absolute ethanol, 1 mmol of Ch-g-PAN, 2 mmol of aldehyde and 1 mL glacial acetic acid were added. The reaction was stirred at room temperature for 48 h and then the system was fitted to reflux at 85 °C for 24 h. The product was filtered and washed with methanol to remove the excess aldehyde and acetic acid, then the products were collected and dried in vacuum oven at 40 °C for 24 h [19].

3. Instrumentations

3.1. Infrared spectroscopy

FT-IR spectra were recorded in ATR discs on (VERTEX 70 FT-IR spectrometer) at room temperature within the wave number range of 4000−600 cm−1.

3.2. Scanning electron microscope

Scanning electron microscopy was (SEM) images were obtained using Jeol (JSM-5200). Samples were prepared by placing a small part of film on carbon tube on a stub, which was coated with thin layer of gold.

3.3. X-ray diffraction

It was done on 2020964 PANalytical Empyrean which is technique uniquely provides phase identification (e.g. graphite or diamond), along with phase quantification and crystallite size.

3.4. Thermogravimetric analysis

It was done on TGA-50H thermogravimetric analyzer. Samples were heated from 10 to 600 °C in a platinum pan with a heating rate of 10 °C min−1 under N2 atmosphere with flow rate of 25 ml min−1.

3.4.1. Antimicrobial activity measurements

3.4.1.1. Antibacterial activity measurement

The disks of Whatman filter paper were prepared with standard size (50 mm diameter) and were kept into 10 screw capped wide mouthed containers for sterilization. These bottles were kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMSO (1 mg ml−1) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard conditions of 106 CFU ml−1 (Colony Forming CFU ml−1) were used for testing antibacterial activity. Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. The utilized test organisms were Streptococcus pneumoniae (RCMB 010010) and Staphylococcus aureus (RCMB 010028) as example of Gram-Positive bacteria and Escherichia coli (RCMB 010052) as example of Gram-negative bacteria. Ampicillin and Ciprofloxacin were used as reference drugs against Gram-positive bacteria and Gram-negative bacteria, respectively. DMSO alone was used as control at the same aforementioned concentration and during this, there was no visible change in bacterial growth. The plates were incubated at 37 °C.
for 24 h. The derivatives that showed significant growth inhibition zones using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

3.4.1.2 Antifungal activity measurement. The disks of Whatman filter paper were prepared with standard size (50 mm diameter) and were kept into 10 screw capped wide mouthed containers for sterilization. These bottles were kept into hot air oven at a temperature of 150 °C, then, the standard sterilized filter paper disks impregnated with a solution of the test compound in DMSO (1 mg ml⁻¹) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard conditions of 104 CFU ml⁻¹ were used for testing antifungal activity. Petri dishes (9 cm in diameter) were used and two disks of filter paper were inoculated in each plate. They were also evaluated for their in vitro antifungal potential against Aspergillus fumigatus (RCMB 02568), Candida albicans (RCMB 05036) and Geotrichum candidum (RCMB 05097). Amphoterin B was used as a reference drug against fungi. The plates were incubated at 37 °C for 48 h. The derivatives that showed significant growth inhibition zones using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

4. Results and discussion

4.1 Effect of the different reaction parameters on the grafting process

The effect of reaction conditions on the acrylonitrile grafting onto chitosan under inert atmosphere using PPS as initiator was studied. The reactive C-2 amino group in chitosan is important in several of the structural modifications because the deprotonated amino group acts as a powerful nucleophile readily reacting with electrophilic of the polymerization reagents [18]. Even in free radical initiated copolymerization, NH₂ groups of chitosan involve in macroradical formation [11]. In the present investigation, potassium persulfate was used as a free radical initiator to induce grafting. The graft reaction conditions were investigated in detail to obtain a high efficiency of grafting, including initiator (PPS) and monomer (AN) concentrations, reaction time and temperature of the polymerization process.

4.1.1 Effect of initiator concentration

The effect of initiator concentration (PPS) on the graft copolymerization of acrylonitrile onto chitosan by keeping the other reaction conditions constant is shown in Fig. 1. Both graft yield (G%) and grafting efficiency (GE%) showed an increase with increasing initiator concentration at first, while homopolymer (H%) decreased. The increase of grafting percentage may be ascribed to the increase of macroradicals generated by the attack of more PPS on the saccharide units of chitosan and therefore, the more active sites of chitosan react with acrylonitrile monomer. Further increase of initiator concentration resulted in a decrease of grafting yield and grafting efficiency. This might be due to the possible chain-transfer to initiator at high initiator concentration. The maximum yield was found to be 150% at initiator concentration 0.03 mol l⁻¹ for this grafting reaction at [AN] = 2 mol l⁻¹ at 60 °C for 2 h.

4.1.2 Effect of monomer concentration

The effect of acrylonitrile concentration on the graft copolymerization reaction is shown in Fig. 2. The grafting yield (G%) and grafting efficiency (GE%) were found to increase with increasing acrylonitrile concentration, while the homopolymer H% decreased. The maximum grafting yield was found to be 188% at monomer concentration 3.5 mol l⁻¹ for this grafting reaction at [I] = 0.01 mol l⁻¹ at 60 °C for 2 h. The increase of grafting percentage may be ascribed to the higher acrylonitrile concentration [20], as the increase of monomer concentration, will increase the opportunity for further addition on the growing chains, thus increasing the G% graft. The decrease in the amount of homopolymer is attributed to the sequence of the reactant in the copolymerization procedure as we allowed the interaction of the initiator molecule with chitosan matrix (considering the OH groups of chitosan as a part of the redox initiator system) before adding the monomer. The portion wise addition of the monomer is another factor favoring the grafting yield.

4.1.3 Effect of the reaction temperature

The influence of reaction temperature on the graft parameters was studied by changing the reaction temperature from 40 to 70 °C at [AN] = 2 mol l⁻¹, [I] = 0.01 mol l⁻¹ for 2 h. The result of the effect of the reaction temperature are illustrated in Fig. 3. G% and GE% increased with increasing the reaction temperature till 60 °C, while the homopolymer H% decreased. The increase in the%G and%GE with the increase in the copolymerization reaction temperature for 40–60 °C is attributed to the increase in the mobility of the monomers as a function of temperature raise and consequently more collision took place between the monomer and the growing chains which led to the increase in the%Graft, while the decrease in the homopolymer is once again attributed to the sequence of addition of reaction mixture. Increasing the reaction temperature above 60 °C showed a decrease in both the%G and%GE. This is probably due
to the fact that increasing the copolymerization temperature above a certain value (ceiling temperature) which according to our result was 60°C, the rate of depolymerization exceeded the rate of polymerization and consequently more monomer units were detached for the growing side chains which results in the observed decrease in both the% graft and the% grafting efficiency [21].

4.1.4. Effect of reaction time

The effect of reaction time on copolymer formation was studied at [AN] = 2 mol l−1, [I] = 0.01 mol l−1 at 60°C is shown in Fig. 4. The grafting yield (G%) and grafting efficiency (GE%) were found to increase with increasing the reaction time, while the homopolymer (H%) decreased. Increasing the time of copolymerization reaction gave the opportunity of more monomer units to added to be the polymeric matrix via the formation of new graft chains. However, the lowering in the H% as a function of reaction time again was attributed to the possible coupling of macro radicals grafted onto chitosan especially at the later stages of polymerization [18].

4.2. Synthesis of ch-g-PAN Schiff base derivatives

The Schiff base formation between grafted chitosan and various aldehydes was carried out in absolute ethanol. Series of five different chitosan-graft-PAN was prepared by condensation of acetaldehyde (Ch-g-PAN I), benzaldehyde (Ch-g-PAN II), P-anisaldehyde (Ch-g-PAN III), salicylaldehyde (Ch-g-PAN IV) and cinnamaldehyde (Ch-g-PANV) in presence of glacial acetic acid as catalyst at 85°C with stirring (Scheme 1).

4.3. Characterization

4.3.1. Infrared spectroscopy

FTIR spectrum of chitosan showed four strong peaks at 1149, 1063, 1024, and 893 cm−1 which are characteristic peaks of the saccharide structure. The very strong broad peak 3352 cm−1 should be assigned to the stretching vibration of the –OH groups, the extension vibration of the –NH2, and the intermolecular hydrogen bonds of the polysaccharide.

FTIR spectrum of chitosan-g-polyacrylonitrile (Ch-g-PAN) showed the appearance of –CN (nitrile) absorption at around 2244 cm−1 and its intensity increase as function of the increasing in the G% (at 16%, 53% and 155%) which gave a confirmation for the grafting reaction [21] as show in Fig. 5.

FTIR spectrum of grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives data are listed in Table 1 and illustrated in Fig. 6(A and B). The appearance of –CH3 band at 1375 cm−1 corresponding to confirm Ch-g-PAN Schiff base by acetaldehyde, the appearance of C–H aromatic at 3065 cm−1 corresponding to confirm Ch-g-PAN Schiff base by benzaldehyde, also the appearance of a weak band of the methoxy group (–OCH3) at 2848 cm−1 confirming the Ch-g-PAN Schiff base by P-anisaldehyde, while the appearance of the stretching vibration of the –OH group at 3745 cm−1 confirm the Ch-g-PAN Schiff base by salicylaldehyde. On the other hand the appearance of (C=) stretch at 1625 cm−1 the confirm Ch-g-PAN Schiff base by cinnamaldehyde.

4.3.2. Thermal analysis

TGA analysis of the original chitosan and the Ch-g-PAN with different grafting percentages are shown in Fig. 7. For example at
300 °C, the residual weight percent in case of ungrafted chitosan was 52%, while in Ch-g-PAN with different grafting percentages 16%, 53% and 155%, the residual weight percent was 51%, 52% and 89%, respectively. Also at 500 °C the residual weight in case of ungrafted chitosan was 24%, while in Ch-g-PAN with different grafting percentages 16%, 53% and 155% the residual weight percent was 31%, 36% and 70%, respectively. Also at 600 °C the residual weight in case of ungrafted chitosan was 10%, while in Ch-g-PAN with different grafting percentages 16%, 53% and 155%, the residual weight percent was 22%, 27% and 62%, respectively. This improvement in the thermal stability of grafted chitosan samples was mainly attributed to the nitrile groups of the acrylonitrile units grafted...
onto Chitosan which are known to undergo cyclic oligomerization with gradual heating [22] which – with no doubt – led to a delay in the degradation process. So all Ch-g-PAN with different grafting percentages were more thermally stable than ungrafted chitosan.

TGA analysis of grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives are shown in Fig. 8(A and B) and summarized in Table 2. For chitosan and Ch-g-PAN Schiff base derivatives taken 300 °C, 500 °C and 600 °C as examples, showed that all Ch-g-PAN Schiff base derivatives are less thermally stable than grafted chitosan, but more stable than native chitosan. The first thermal event occurred around 50–110 °C observed for all samples was due to water evaporation. The second thermal event was due to the decomposition process. The other stages of weight loss were dependent on the chemical structure of the derivatives and attributed to thermal decomposition. The results showed that all aromatic derivatives were found to be less stable than grafted chitosan which were due to the presence of aryl group which decrease stability [23]. The residual weight at 600 °C could be arranged in the following order Ch-g-PAN I < Ch-g-PAN II < Ch-g-PAN V < Ch-g-PAN IV < Ch-g-PAN III. The instabilities of the Schiff base polymers compared with chitosan were due to the formation of –N=N– groups in the prepared Schiff's derivatives which are more sensitive toward thermal decomposition than the amino groups of chitosan [24].

4.3.3. Scanning electron microscope (magnification × 1000)

Scanning electron microscope of the original chitosan and the Ch-g-PAN with different grafting percentages are shown in Fig. 9. It was found that the surface appearances were drastically changed upon reaction as compared with the surface of Chitosan which is fibrous in nature, whereas, the surfaces of the grafted samples appeared more soft and porous.

Scanning electron microscope of grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives are shown in Fig. 10. It was found that the surface morphology of the Ch-g-PAN Schiff base derivatives was changed due to the Schiff base product which differ in size and shape from a derivative to another. This obvious change in the surface morphology of the modified chitosan gave an additional proof for both the grafting and Schiff base modification.

4.3.4. X-ray diffraction

The XRD spectra of the original chitosan and the Ch-g-PAN with different grafting percentages are shown in Fig. 11. The diffractograms of pure chitosan sample show the characteristic peaks at around 2θ = 11° and 20° which were attributed to the overlapped diffraction from the chitosan's crystal planes. The typical crystalline peak of chitosan that appeared at 2θ = 20° decreased when poly(acrylonitrile) was grafted onto chitosan which showed change in the crystalline structure of chitosan. Thus at 16% Ch-g-PAN 2θ became 19.3° and at 155% Ch-g-PAN it become 16.06°, this is in addition to the appearance another weak absorption at 29.04°. PAN had grew into enough long chain to form the regular crystalline region during the graft copolymerization.

The XRD spectra of grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives are shown in Fig. 12(A and B). It is clear that the diffractograms of Ch-g-PAN Schiff base derivatives were different than the parent Ch-g-PAN. Where the characteristic peaks of Ch-g-PAN Schiff base using salicylaldehyde were at 2θ = 16.6° and 20.2°, while in case of Ch-g-PAN Schiff base P-anisaldehyde a single peak appeared at 2θ = 16.9° and for Ch-g-PAN Schiff base acetaldehyde a single peak appeared at 2θ = 16.9°. On the other hand, Ch-g-PAN Schiff base using benzaldehyde had five peaks at 2θ = 19.1°, 17.1°, 21.2°, 25.9° and 44.5°. Also Ch-g-PAN Schiff base using cinnamaldehyde had six peaks at 2θ = 9.6°, 14.9°, 19.2°, 21.4°, 23.6°, 25.17° and 29.2°. The difference in the x-ray diffraction peaks of the different investigated Schiff's base derivatives of the grafted chitosan gave an additional proof for the synthesis of these materials (Fig. 13).

4.4. Antimicrobial activity

The antimicrobial activity of the original chitosan, the Ch-g-PAN with different grafting percentages and Ch-g-PAN Schiff base derivatives were evaluated against S. pneumoniae (RCMB 010010) and S. aureus (RCMB 010028) as gram-positive bacteria, against E. coli and against S. aureus (RCMB 010028) as gram-negative bacteria.
coli (RCMB 010052) as gram-negative bacteria and against A. fumigatus (RCMB 02568), G. candidum (RCMB 05097) and C. albicans (RCMB 05031) as fungi. Agar disk diffusion method was used for the determination of the preliminary antibacterial and antifungal activity. Ampicillin, gentamicin and Amphotericin B were used as reference drugs against Gram-positive bacteria, Gram-negative bacteria and fungi, respectively.

All of the synthesized substituted derivatives under investigation showed, in vitro, antimicrobial activity against the tested microorganisms.

The results of antibacterial activity of the original chitosan, the Ch-g-PAN with different grafting percentages and Ch-g-PAN Schiff base derivatives using inhibition zone method are listed in Tables 3 and 4. Several mechanisms elucidating the antimicrobial activity of chitosan have been postulated. The most acceptable mechanism is the interaction between positively charged chitosan...
molecules and negatively charged microbial cell membrane. The interaction is mediated by the electrostatic forces between the protonated –NH\(^+\) groups of chitosan and the electronegative charges on the microbial cell surface \[25\].

This electrostatic interaction results in two fold interferences: (1) by promoting changes in the properties of membrane wall permeability, thus provoke internal osmotic imbalances and consequently inhibit the growth of the microorganisms, and (2) by the hydrolysis of the peptidoglycans in the microorganism wall, leading to the leakage of intracellular electrolytes such as potassium ions, and other low molecular weight proteinaceous constituents (e.g. protei, nucleic acid, glucose and lactate dehydrogenase) \[26\].
Fig. 11. XRD of (1) chitosan, (2) Ch-g-PAN at 16% and (3) Ch-g-PAN at 155%.

Fig. 12. A. XRD of grafted chitosan (Ch-g-PAN 155%) with some aldehydes, (1) ch-g-PAN at 155%, (2) Ch-g-PAN I, (3) Ch-g-PAN II. B. XRD of grafted chitosan (Ch-g-PAN 155%) with some aldehydes, (4) Ch-g-PAN III, (5) Ch-g-PAN IV and (6) Ch-g-PAN V.
Fig. 13. Antibacterial activity of grafted chitosan Schiff base derivatives against Streptococcus pneumonia, Staphylococcus aureus and Escherichia coli where, C = negative control (DMSO), I = Ch-g-PAN I, II = Ch-g-PAN II, III = Ch-g-PAN III, IV = Ch-g-PAN IV and V = Ch-g-PAN V.

Table 4
Antibacterial activity of the original chitosan, grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tested organisms</th>
<th>Gram-positive bacteria</th>
<th>Staphylococcus aureus (RCMB 010028)</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition zone (mm)</td>
<td>Minimum inhibitory concentration (MIC) (µg ml⁻¹)</td>
<td>Inhibition zone (mm)</td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
<td>13.5</td>
<td>62.20</td>
<td>16.2</td>
</tr>
<tr>
<td>Ch-g-PAN at 155%</td>
<td></td>
<td>19.3</td>
<td>3.90</td>
<td>21.3</td>
</tr>
<tr>
<td>Ch-g-PAN III</td>
<td></td>
<td>23.4</td>
<td>0.98</td>
<td>24.6</td>
</tr>
<tr>
<td>Ch-g-PAN V</td>
<td></td>
<td>23.1</td>
<td>1.95</td>
<td>24.4</td>
</tr>
<tr>
<td>Ch-g-PAN IV</td>
<td></td>
<td>22.1</td>
<td>1.95</td>
<td>23.6</td>
</tr>
<tr>
<td>Ch-g-PAN II</td>
<td></td>
<td>22.3</td>
<td>1.95</td>
<td>23.5</td>
</tr>
<tr>
<td>Ch-g-PAN I</td>
<td></td>
<td>20.4</td>
<td>3.90</td>
<td>22.3</td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td>23.8</td>
<td>0.98</td>
<td>27.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Since such mechanism is based on electrostatic interaction, it suggests that the greater the number of cationized amines, the higher will be the antimicrobial activity.

Another proposed mechanism is the binding of chitosan with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via penetration of chitosan into the nuclei of the microorganisms [27].

Table 3 illustrates that the antimicrobial activity of the original chitosan and Ch-g-PAN with different grafting percentages against the gram-positive bacteria and gram-negative bacteria increased compared to the pristine chitosan. Thus Ch-g-PAN (155%) caused the most inhibition zone diameter against S. pneumonia, S. aureus and E. coli being 19.3, 21.3 and 22.1 mm, respectively. Ch-g-PAN (53%) which caused inhibition zone diameter against the same bacteria being 16.3, 18.3 and 19.3 mm, respectively, while Ch-g-PAN with low graft percentage (16%) caused the least inhibition zone diameter against the same bacteria being 14.8, 16.3 and 17.2 mm in diameter, respectively. The reason for these findings may be due to the nitrile groups which are know to enhance the antibacterial activity [28]. Therefore increasing the amount of nitrile groups (as a result of increasing the%G) increase the antimicrobial activity of the grafted materials. An additional evidence for the increase of the antimicrobial activity of Ch-g-PAN with the increase in grafting percentages is through the determination of minimum inhibitory concentration (MIC) values. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. As shown, the MIC values of Ch-g-PAN at 155% against S. pneumonia, S. aureus and E. coli were 3.9, 1.95 and 0.98 µg ml⁻¹, respectively, where as the MIC values of Ch-g-PAN at 53%G against the same bacteria were 31.25, 7.81 and 3.9 µg ml⁻¹, respectively, while for the MIC values of Ch-g-PAN at 16%G against the same bacteria were 31.25,31.25 and 15.63 µg ml⁻¹, respectively.

Table 3, illustrates that the antimicrobial activity increased with the increase in the% graft. Therefore, the chemical modifications by Schiff bases were performed using the highly grafted chitosan (Ch-g-PAN at 155%). Table 4, represents the antimicrobial activity of the original chitosan, grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives. The data clearly revealed that all Ch-g-PAN Schiff base derivatives were more active against the gram-positive bacteria than against the gram-negative bacteria. This may be attributed to their different cell walls. The cell wall of gram-positive bacteria is fully composed of peptide polyglycogen. The peptidoglycan layer is composed of networks with plenty of pores, which allow foreign molecules to come into the cell without difficulty and allows more rapid absorption of ions into the cell. But, the cell wall of gram-negative bacteria is made up of a thin membrane of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein and phospholipids. Because of the complicated bilayer cell structure, the outer membrane is a potential barrier against foreign molecules with high molecular weight. Therefore, the derivatives have different effects on the two kinds of bacteria [25].

Table 4 also illustrates that the Ch-g-PAN Schiff base using P- anisaldehyde caused the most inhibition zone diameter against S. pneumonia, S. aureus and E. coli being 23.4, 24.6 and 23.2 mm, respectively. This is attributed to the methoxy group which is highly electron donating group where the electron donating group, as a substituent, showed a greater activity. The same was found to be true for the Ch-g-PAN Schiff base using cinnamaldehyde and salicylaldehyde that contain vinyl group and hydroxyl group, respectively, as the electron donating group, but the vinyl group
is more electron donating than the hydroxyl group, so the Ch-g-PAN Schiff base using cinnamaldehyde caused more inhibition zone diameter against S. pneumoniae, S. aureus and E. coli more than the Ch-g-PAN Schiff base using salicylaldehyde. On the other hand, the Ch-g-PAN Schiff base using benzaldehyde have lower antimicrobial activity than the other aromatic Ch-g-PAN Schiff base derivatives. The obtained data also showed that the Ch-g-PAN Schiff base using acetaldehyde, had the lowest antimicrobial activity compared to aromatic Ch-g-PAN Schiff base derivatives as the aryl ring play an important role in the antibacterial activity. Lipophilicity, which correlates well with the bioactivity of chemicals, is a very important molecular descriptor and different lipophilic behavior of compounds plays an important role in their biological activity mechanisms. The n-octanewater partition coefficient ( log Pow) is widely used as a general measure of lipophilicity. Compounds with benzyl groups have relatively higher log Pow values and hence shows more lipophilic character as compared to the compounds with aliphatic groups. Thus, the aliphatic derivative does not penetrate into the bacteria as easily as the derivatives with benzyl group do. This behavior could be attributed to the lower lipophilicity of the aliphatic derivatives [25]. Table 4, also show that the MIC values of the original chitosan and grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivatives gave an additional proof that Ch-g-PAN Schiff base derivatives had better antimicrobial activity as compared to native chitosan and Ch-g-PAN (155%).

Table 5
Antifungal activity of the original chitosan and Ch-g-PAN with different grafting percentages.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tested organisms</th>
<th>Aspergillus fumigatus (RCMB 02568)</th>
<th>Candida albicans (RCMB 05036)</th>
<th>Geotrichum candidum (RCMB 05097)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition zone (mm)</td>
<td>Minimum inhibitory concentration (MIC) (μg ml⁻¹)</td>
<td>Inhibition zone (mm)</td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
<td>13.2</td>
<td>62.50</td>
<td>11.1</td>
</tr>
<tr>
<td>Ch-g-PAN at 155%</td>
<td></td>
<td>20.1</td>
<td>3.90</td>
<td>18.3</td>
</tr>
<tr>
<td>Ch-g-PAN III</td>
<td></td>
<td>23.1</td>
<td>0.98</td>
<td>21.4</td>
</tr>
<tr>
<td>Ch-g-PAN V</td>
<td></td>
<td>22.4</td>
<td>1.95</td>
<td>21.3</td>
</tr>
<tr>
<td>Ch-g-PAN IV</td>
<td></td>
<td>21.2</td>
<td>1.95</td>
<td>20.3</td>
</tr>
<tr>
<td>Ch-g-PAN II</td>
<td></td>
<td>21.1</td>
<td>3.90</td>
<td>19.6</td>
</tr>
<tr>
<td>Ch-g-PAN I</td>
<td></td>
<td>20.3</td>
<td>3.90</td>
<td>19.2</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td>23.7</td>
<td>0.98</td>
<td>25.3</td>
</tr>
</tbody>
</table>

Table 6
Antifungal activity of the original chitosan, grafted chitosan (Ch-g-PAN at 155%) and its Schiff base derivative.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tested organisms</th>
<th>Aspergillus fumigatus (RCMB 02568)</th>
<th>Candida albicans (RCMB 05036)</th>
<th>Geotrichum candidum (RCMB 05097)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition zone (mm)</td>
<td>Minimum inhibitory concentration (MIC) (μg ml⁻¹)</td>
<td>Inhibition zone (mm)</td>
</tr>
<tr>
<td>Chitosan</td>
<td></td>
<td>13.2</td>
<td>62.50</td>
<td>11.1</td>
</tr>
<tr>
<td>Ch-g-PAN at 155%</td>
<td></td>
<td>15.3</td>
<td>15.63</td>
<td>14.3</td>
</tr>
<tr>
<td>Ch-g-PAN at 53%</td>
<td></td>
<td>17.3</td>
<td>15.30</td>
<td>15.2</td>
</tr>
<tr>
<td>Ch-g-PAN at 15%</td>
<td></td>
<td>20.1</td>
<td>3.90</td>
<td>18.3</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td></td>
<td>23.7</td>
<td>0.98</td>
<td>25.3</td>
</tr>
</tbody>
</table>

4.5. Antifungal activity

Generally the antifungal mechanism of chitosan involves all wall morphogenesis with chitosan molecules interfering directly with fungal growth, similarly to the effects observed in bacteria cells. The microscopic observation reported that chitosan molecules diffuse inside hyphae interfering on the enzymes activity responsible for the fungus growth [29].

Table 5 represents the antifungal activity of the original chitosan and Ch-g-PAN with different grafting percentages against A. fumigatus (RCMB 02568), C. albicans (RCMB 05036) and G. candidum (RCMB 05097). The results showed that the antifungal activity increased as the grafting percentage increased compared to the original chitosan, which is probably due to the presence of the nitrile groups which enhanced the antifungal activity [28]. Thus, Ch-g-PAN with higher grafted percentage (155%) had the greater antifungal activity (greater inhibition zone diameter and lower MIC values) with inhibition zone against A. fumigatus, C. albicans and G. candidum were 20.1, 18.3 and 21.3 mm and with MIC values 3.9, 7.81 and 1.95 μg ml⁻¹, respectively.

On the other hand, Table 6, represents the antifungal activity of the original chitosan and the Ch-g-PAN (155%) and its Schiff base derivatives. Again, the results also demonstrated how the antifungal activities were affected by the nature of substituents in the aryl ring of the Schiff bases, so methoxy group in the Ch-g-PAN Schiff base using P-anisaldehyde gave the greater antifungal activity compared to the other aromatic Ch-g-PAN Schiff base derivatives, that gives inhibition zone against A. fumigatus, C. albicans and G. candidum were 23.1, 21.4 and 25.1 mm and with MIC values 0.98, 3.9 and 0.98 μg ml⁻¹, respectively. Also, the aliphatic Ch-g-PAN Schiff base using acetaldehyde showed the lower antifungal activity than the derivatives having aryl rings.

5. Conclusion

The obtained results from this study revealed that the optimum conditions for grafting PAN onto chitosan were as follows: [M] is 3.5 mol l⁻¹, [I] is 3 × 10⁻² mol l⁻¹, reaction temperature is 60 °C and reaction time 2 h. Chemical modification of grafted chitosan was
done by Schiff’s base reaction using different aldehydes. The results of the thermal stability of chitosan, Ch-g-PAN and its Schiff’s base derivatives showed that Ch-g-PAN Schiff’s base derivatives were less thermally stable than grafted chitosan, but more thermally stable than native chitosan. X-ray diffraction showed changes in crystallinity of Ch-g-PAN and its Schiff’s base derivatives compared to chitosan. Also scanning electron microscope showed changes in the surface morphology of Ch-g-PAN and its Schiff’s base derivatives compared to chitosan. The native chitosan, Chitosan-g-PAN with different grafting percentages and Schiff’s base derivatives of Ch-g-PAN (155%) using various aldehydes were tested against the bacterial species (Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli) and the fungal species (Aspergillus fumigatus, Candida albicans, Geotrichum candidum). The antimicrobial activity showed that the antimicrobial activity increased with the increase in the percent grafting, so the modified Schiff base was run out on the highly grafted Ch-g-PAN at 155%. All Ch-g-PAN Schiff base derivatives were more active against the Gram-positive bacteria than against the Gram-negative bacteria. Also all Ch-g-PAN Schiff base derivatives were of more antifungal activity compared to native chitosan and Ch-g-PAN (155%).

References