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Biopolymer Modifications for Biomedical Applications

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1. Introduction

Chitosan is typically obtained by deacetylation of chitin under alkaline conditions, which is one of the most abundant organic materials, being second only to cellulose in the amount produced annually by biosynthesis. Chitosan is a linear polysaccharide, composed of glucosamine and *N*-acetyl glucosamine units linked by (1–4) glycoside bonds. The content of glucosamine is called the degree of deacetylation (DD). In fact, in a general way, it is considered that when the DD of chitin is higher than about 50% (depending on the origin of the polymer and on the distribution of acetyl groups along the chains), it becomes soluble in an aqueous acidic medium, and in these conditions, it is named chitosan. The DD also affects the biodegradability of this polymer, and for DD above 69% a significant decrease of *in vivo* degradation has been found (1). Chitosan displays interesting properties such as biocompatibility, biodegradability (3, 4) and its degradation products are non-toxic, non-immunogenic and non-carcinogenic (5, 6). Therefore, chitosan has prospective applications in many fields such as biomedicine, waste water treatment, functional membranes and flocculation. However, chitosan is only soluble in few dilute acid solutions, which limits its applications.

Recently, there has been a growing interest in the chemical modification of chitosan in order to improve its solubility and widen its applications (7–9). Derivatization by introducing small functional groups to the chitosan structure, such as alkyl or carboxymethyl groups (10, 11) can drastically increase the solubility of chitosan at neutral and alkaline pH values without affecting its cationic character.

Substitution with moieties bearing carboxylic groups can yield polymers with polyampholytic properties (12). The antimicrobial activity of chitosan increases with decreasing pH (13–17). This is due to the fact that the amino groups of chitosan become

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ionized at pH below 6 and carry a positive charge. Unmodified chitosan is not antimicrobially active at pH 7, since it does not dissolve and also since it does not contain any positive charge on the amino groups (18, 19). The antimicrobial activity of chitosan also increases with increasing degree of deacetylation, due to the increasing number of ionisable amino groups (19). Several approaches were done to increase the antimicrobial activity of chitosan by introduce amino groups, on the primary amino groups of the back bone of chitosan polymer chains but it was failed (20). The obtained results were explained based on the remote position of the new introduced amino groups.

In this work, we aim to increase both the solubility and antimicrobial activity of chitosan via increase the amino groups on the polymer back bone by attaching amino groups directly on the hydroxyl groups of polysaccharide to wide its applications.

A new technique has been used to avoid the consumption of the original amino groups of the chitosan as sites of grafting, so chitin was first grafted with amino groups in separate step then it was de-acetylated to have the aminated chitosan. Aminated chitosan was tested as antimicrobial agent and aminated chitosan membranes were prepared, characterized and evaluated for wound dressing applications.

2. Modified chitosan membranes

Modified chitosan was prepared through introducing extra amino groups to its structure. A new chemical route was used to graft selectively the extra amino groups on the hydroxyl groups of chitosan rather than its amine ones. To achieve this goal, chitin has been activated first with PBQ then amino groups were grafted using ethylene diamine as source of amino groups. The obtained aminated chitin was finally de-acetylated (21-22) to have modified chitosan with extra amino groups as shown in the following schema (figure 1). Different concentrations of PBQ were used to have modified chitosan with different amine content (table1). From the table it is clear that increasing PBQ concentration increased the nitrogen content in an obvious manner. This increment starts in linear way then turns to level off.

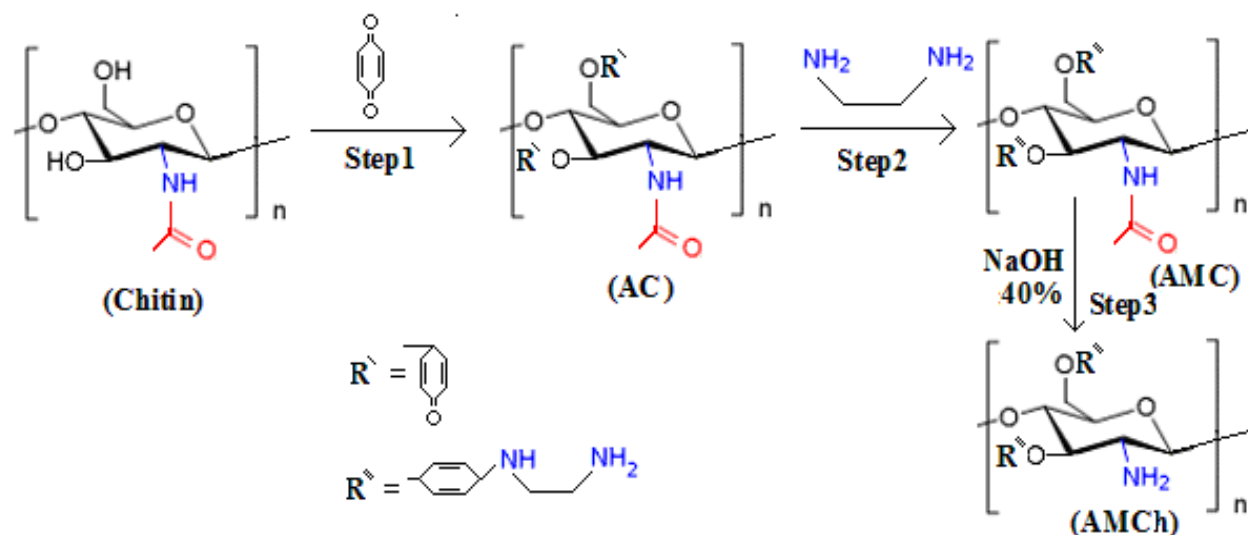


Fig. 1. Schematic diagram for synthesis of aminated chitosan.

PBQ :chitin ratio	N %
Chitosan control	7.42
0.0935	7.50
0.187	7.56
0.374	8.73
0.545	9.60
0.747	9.62

Table 1. Effect of PBQ concentration on the nitrogen content of modified chitosan

2.1 Characterization of modified chitosan membranes

In the characterization of modified chitosan membranes, different characters were monitored to show the effect of modification process on their properties. The occurrence of amination process was verified through examination of the chemical structure changes using FT-IR, TGA analysis and solubility test.

The FTIR spectrum of the modified chitosan and intermediates to verify structure changes was obtained using FTIR-8400S SHIMADZU, Japan. As shown in figure 2, the major differences are the wide peaks at 3431 cm^{-1} , (I) corresponding to the stretching vibration of $-\text{NH}_2$ and $-\text{OH}$ groups became more sharp at modified chitosan as a result of alternation of $-\text{OH}$ groups with $-\text{NH}_2$ groups. Absorption band intensity at $1560, 1649\text{ cm}^{-1}$ (II) corresponding to carbonyl bands have been increased in (AC), curve (b), via introducing further carbonyl groups of PBQ as illustrated in schema1, then return to normal at aminated chitin, curve (c). Finally, peaks will be reduced after deacetylation as a result of removal of the acetyl groups in modified chitosan. This observation confirmed the occurrence of the modification process with different steps indicated in figure 1.

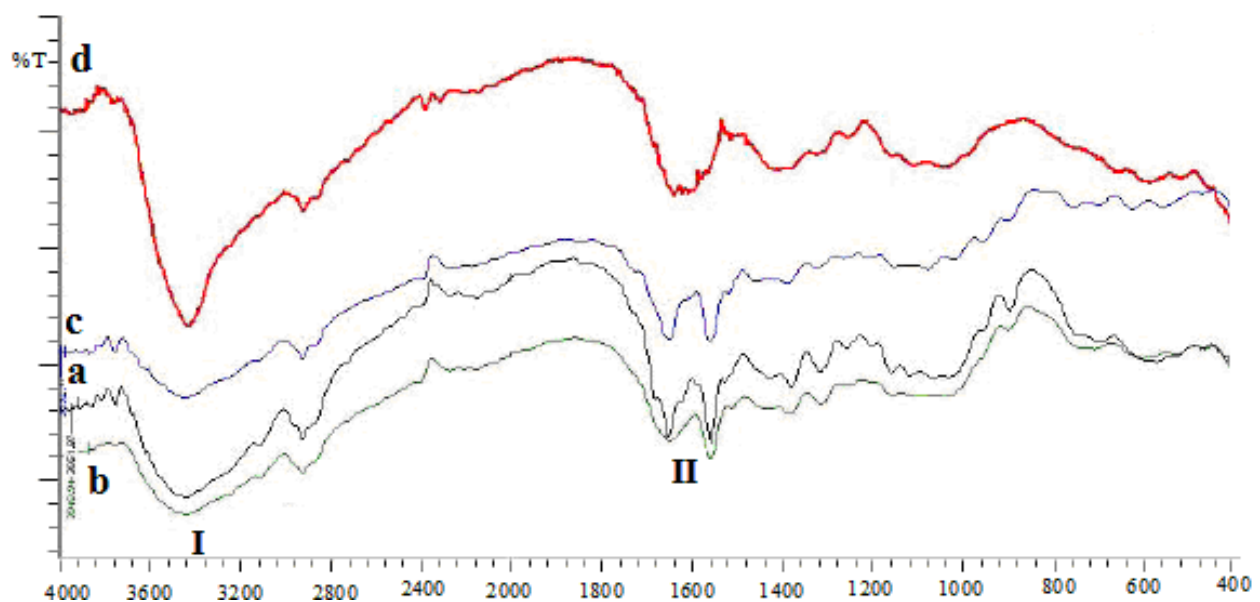


Fig. 2. FTIR spectra of chitin (a), activated chitin (AC) (b), aminated chitin (c) and modified chitosan (d).

Changes in the structure of modified chitosan via introducing extra hydrophilic amine groups were expected to influence its solubility. The solubility of chitosan and the modified chitosan in different pH was measured as in table 2. The improving of the solubility of modified chitosan over chitosan itself was attributed to grafted amino groups. The aminated chitosan solubility at pH range from 5.0 to 6.0 was almost double of its unmodified chitosan counter part. These results, along with the obtained data from FT-IR analysis, confirmed the occurrence of amination process.

pH	Chitosan solubility (%)	Modified chitosan solubility (%)
4	97.2	99
5	41.7	81.1
6	17.9	29.6
7	0	3.9
8	0	0

Table 2. Solubility percent of chitosan and modified chitosan in different pH

Thermal graph metric analysis of chitosan and aminated chitosan membranes with different content of glycerol was done; figures 3. The data of the TGA was summarized in table (3). From the table a number of informations were abstracted. First notice is the increase of weight loss in the temperature range 0 - 118°C which refers to loss of water. The water loss

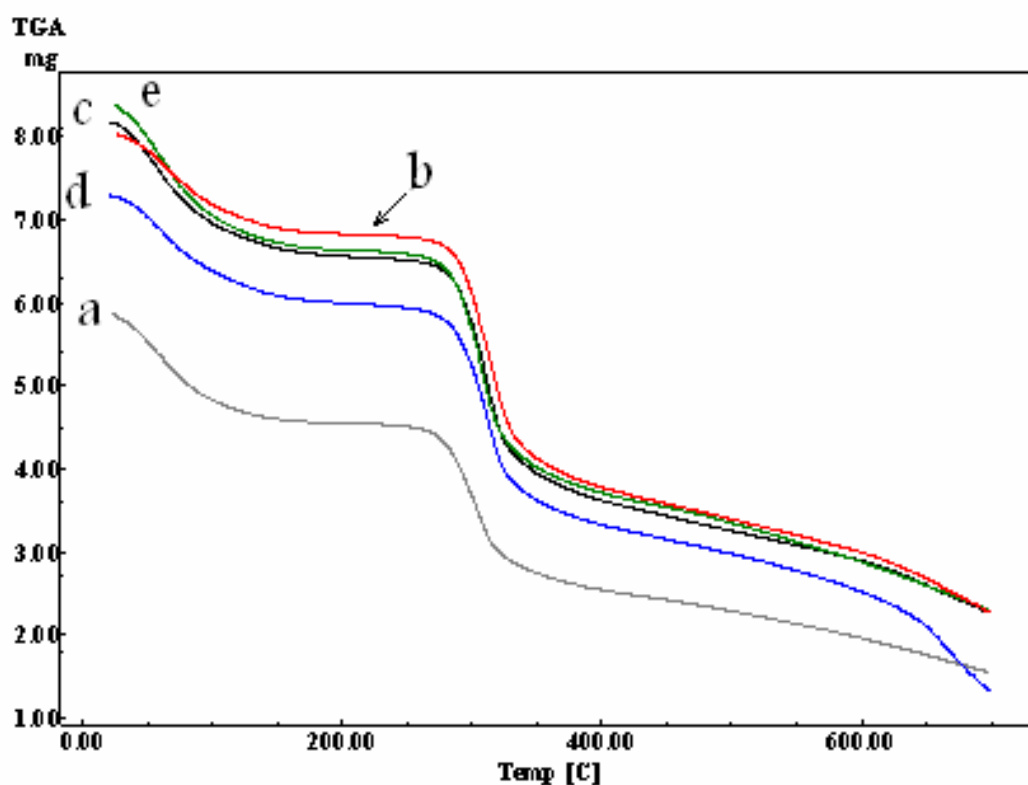


Fig. 3. Thermal graph metric diagrams of chitosan membrane (a), chitosan modified membrane (b), chitosan modified membrane (glycerol 2%)(c), chitosan modified membrane (glycerol 4%)(d), and chitosan modified membrane (glycerol 6%)(e).

Sample	T50	Weight loss at	
		T ₍₀₋₁₁₈₎	T ₍₂₄₀₋₃₈₀₎
Chitosan	351.6	12.28 %	42.9 %
Aminated chitosan (0% glycerol)	325.89	19.638 %	42.47 %
Aminated chitosan (2% glycerol)	333.06	16.523 %	43 %
Aminated chitosan (4% glycerol)	346.72	14.415 %	42.68 %
Aminated chitosan (6% glycerol)	335.86	17.741 %	42.57 %

Table 3. Thermo-gravimetric data of chitosan and aminated chitosan membranes with different percent of glycerol

increases by about 50% upon amination. This observation confirms the modification of membranes hydrophilicity as a result of the amination process. The second notice is the shift of T50, temperature needed to lose 50% of sample weight, to lower temperature which came as a result of chemical structure modification and as a result its hydrophilicity improvement. The last notice is the insignificant changes of the characteristic thermo-gram of pristine chitosan in the temperature range 240 -380°C, which indicates the absence of crosslinking role of the glycerol content between aminated chitosan chains.

The membrane was prepared as the following 1 gm of chitosan or aminated chitosan was dissolved in 50 ml 2% acetic acid. Glycerol was added to the solution as a plasticizer. The solution was strained through cheesecloth to remove any un-dissolved particulates. The solution was then casting in a clean Petri dish and left at room temperature for 48 hours to ensure complete solvent evaporation. The humidity of the room was not controlled. Once the membrane was dried and separated from the Petri dish it was rinsed with 500 ml of 1 M of NaOH. The rinsing of the membrane in a caustic solution gives the films water-resistance by neutralizing and removing any acetic acid anions present in the membrane then the membrane was washed with distilled water to remove the traces of alkali and neutralized it.

Finally, the wet membranes were spread out and attached to the clean glass support with clamps and allowed to dry for 24 hours at room temperature. The resulting membranes were transparent and flexible.

Physico-chemical properties like water uptake, tensile strength, elongation (23), surface roughness, SEM and finally water vapor permeability have been conducted for the casted membranes. The obtained results show the impact of modification process on the properties of chitosan membranes through evaluation of their bio-characters such as; antimicrobial activity (24-25), hemocompatibility (26), cytotoxicity (27), and biodegradability (28).

Water up-take

The introduction of biomaterials surface in blood creates a new interface between cellular and fluid components of blood and material. This results in a thermodynamic driving force that acts to reduce the solid-liquid interfacial free energy at this interface. Ignoring interactions with blood cellular components, the blood plasma-biomaterial interfacial free energy is a thermodynamic quantity that incorporates the surface free energy contributions of both solid and liquid phases and provides a measure of the driving force for the adsorption of blood components on solid surfaces. The configuration of the initially

adsorbed proteins on the solid surface may be determined by the magnitude of the blood plasma-biomaterial interfacial free energy (29). Andrade proposed the minimum interfacial free energy hypothesis of biocompatibility (30). Water plays an important role in determining the biocompatibility of synthetic materials. Ratner et al. (31) have recognized that high water levels within the surface of materials will help provide a low interfacial free energy with blood and will reduce both protein adsorption and cell adhesion on the polymeric surface. Therefore, a surface with a hydrated polymer (hydrogel) coating would be expected to be more compatible with body fluids than a non-polar or less hydrated type of surface. The water up take of chitosan and aminated chitosan membranes with 0, 2, 4 and 6 % glycerol content was recorded in table (4).

Sample	Water uptake %
Chitosan	183.14
Aminated chitosan (glycerol 0%)	197.83
Aminated chitosan (glycerol 2%)	208.94
Aminated chitosan (glycerol 4%)	220.62
Aminated chitosan (glycerol 6%)	255.59

Table 4. Water uptake of chitosan and aminated chitosan membranes with different percent of glycerol

The water sorption of the chitosan was attributed to the hydrophilic groups of the polysaccharide chains; hydroxyl and amino groups. From the table, it is clear that increase of the water sorption of aminated chitosan over the chitosan it self. This was explained by increase the hydrophilic groups on the chitosan via grafted with amine groups. In the other hand, the increase of the water sorption of the aminated chitosan as increase the plasticizer percent was attributed to the hydrophilic power of the glycerol as polyols and also results from the effect of the plasticizer on the limitation of crystallinity of the membrane. The obtained results are in accordance with published results by other authors in which they added poly propylene glycol as a plasticizer to chitosan (32).

Mechanical properties

The tensile properties of chitosan and aminated chitosan membranes with different percent of plasticizer were measured and recorded in table (5). It was determined from the critical breaking point of the stretching test pieces. The maximum stress σ_{\max} (Nm⁻²) was evaluated as the ratio of the stretching force divided by the cross-sectional area of broken membrane piece. The maximum strain λ_{\max} was measured as the elongation ratio of the initial length of the test piece.

The effect of the stress on the elongation of the membranes was found more clear for aminated chitosan higher than chitosan. The change of elastic intensity suggested that the number of the functional groups significantly affected the network elasticity of chitosan. This was reflected on increase the maximum stress and decreases the maximum strain.

By added the glycerol, the elongation of the aminated chitosan was increased with increase the percent of glycerol in polymer. Glycerol is miscible easily with chitosan, so the introduction of glycerol moisture resulting in drastic chain flexibility which reduced the

Sample	Max force N	Max strain λ_{\max} %	Max stress σ_{\max} N/mm ²
Chitosan	19.80 N	24.36	37.72
Aminated chitosan (0% glycerol)	20.60 N	31.85 %	43.32
Aminated chitosan (2% glycerol)	26.88 N	28.60 %	46.66
Aminated chitosan (4% glycerol)	25.78 N	29.30 %	36.83
Aminated chitosan (6% glycerol)	28.75 N	34.33 %	42.91

Table 5. The maximum stress and strain of chitosan and aminated chitosan membranes with different percent of glycerol

rigidity of native chitosan. Other authors used poly propylene glycol as a plasticizer and obtained similar results. They claimed the obtained results to the fact that propylene glycol is containing several OH groups, which can form intermolecular bonds with the excess of NH_3^+ groups in the chitosan polymer (32).

Surface roughness

It is known that biomaterials have to fulfill many conditions. However, the surface quality is one of the most important properties of biomaterial, which limits its applications. It is so important because most of biological reactions occur on the surface and at interface. Among other things the hydrophilicity, chemical structure, topography and roughness of surface create the response of the host tissue to presence of implant. The adhesion and proliferation of cells is also determined by the surface properties. They play a significant role in biocompatibility as well as for tissue engineering.

The surface roughness of chitosan and aminated chitosan membranes was evaluated (table 6). It was found that the roughness of aminated chitosan membranes is less than chitosan. Also the table shows a decrease in the film roughness with increase of glycerol concentration. This behavior could be referred to the changes in chemical structure, hydrophilicity and as well as the surface energy which separately or in combination affect the arrangement of the macromolecules and hence its surface roughness. It can be seen the role of glycerol in improving the surface smoothness. In natural skin, the dense skin layer has a surface roughness of 20 to 165 nm. This means that increase of the glycerol

Sample	Surface roughness μm		
	Front face	Behind face	average
Chitosan	3.853	3.57	3.7133
Aminated chitosan (0% glycerol)	2.34	2.28	2.31
Aminated chitosan (2% glycerol)	1.92	1.3	1.611
Aminated chitosan (4% glycerol)	1.73	1.25	1.49
Aminated chitosan (6% glycerol)	1.4	1.28	1.35

Table 6. Surface roughness of chitosan and aminated chitosan membranes with different percent of glycerol

concentration in the membrane cast solution, brings the membrane roughness close to that of skin which increases the contact area and hence preventing frictions and simultaneously reduces the healing time.

Scanning electron microscope

The surface of chitosan membrane and aminated chitosan membranes with different percent of plasticizer was scanned using scan electron microscope (SEM) (figure 4). The surface became smoother with amination and furthermore with glycerol addition. This observation is in agreement with roughness test obtained data in table 6.

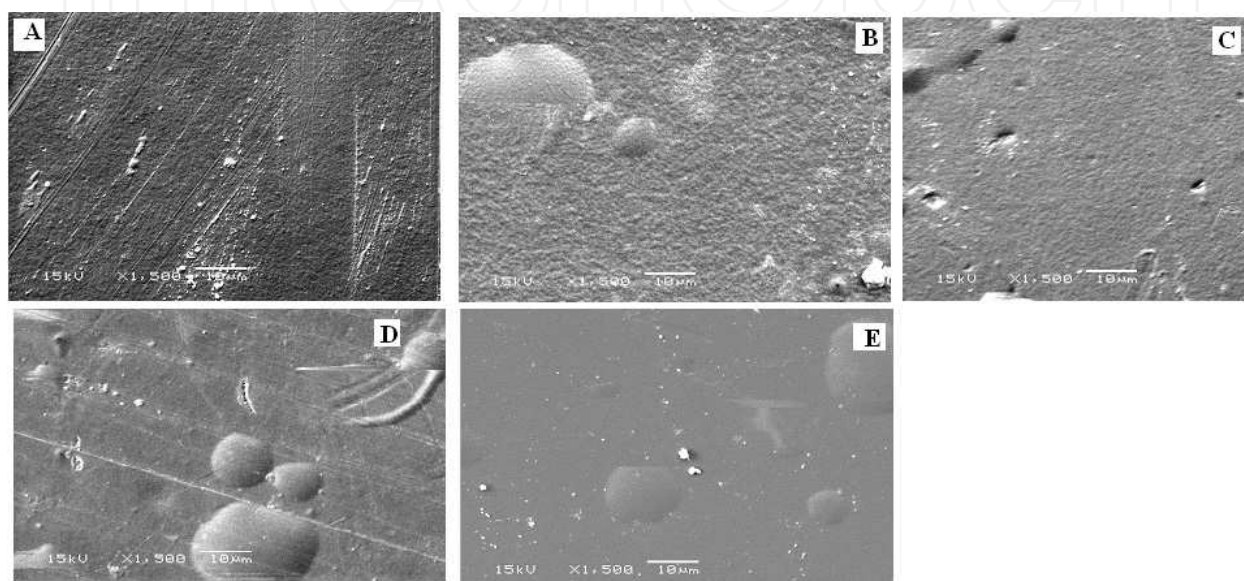


Fig. 4. Surface scan electron microscope photo of chitosan membrane (a), aminated chitosan membrane (b), aminated chitosan membrane with 2% glycerol (c), aminated chitosan membrane 12 with 4% glycerol (d), and aminated chitosan membrane with 6% glycerol (e).

Water vapour permeability

One of the main functions of wound dressing membrane is maintains a moist environment at the wound/ dressing interface. This characteristic was measured for chitosan and modified chitosan membranes, with different glycerol content, through measuring of its water vapor permeability (table 7).

Sample	Permeability gm - mil / m ² -day
Chitosan	3.843057
Aminated chitosan (glycerol 0%)	3.372898
Aminated chitosan (glycerol 2%)	2.476318
Aminated chitosan (glycerol 4%)	1.178036
Aminated chitosan (glycerol 6%)	0.586429

Table 7. Water permeability of chitosan and aminated chitosan membranes with different percent of glycerol

The decrease of the membrane permeability as exposed to water vapor was attributed to two factors. First, the swelling of polymer chains which decreases the pores diameter of the membrane and second is the reduction of the pore volume due to the presence of bulk PBQ benzene ring in the structure. The decrease of the aminated chitosan membranes permeability proved the changes in the chemical structure which in consequence affects the hydrophilicity of the membranes. In the same way, the addition of glycerol decreases the permeability of membranes gradually with its content. These results are in accordance with the water uptake percentage measurements. This future enables us to control the water vapor permeability.

2.2 Bio-evaluation of modified chitosan

For application as wound dressing, modified chitosan membranes have been evaluated from Bio-point of view. Properties such as cytotoxicity, Hemocompatibility and Biodegradability have been evaluated.

Antimicrobial activity

Chitosan derivatives present interesting properties for biomedical applications, because such materials can exhibit enhanced bacteriostatic activity with respect to pure chitosan. Ethylene diamine tetraacetic acid (EDTA) grafted onto chitosan increases the antibacterial activity of chitosan by complexing magnesium that under normal circumstances stabilizes the outer membrane of gram-negative bacteria (33). The increase in chitosan antimicrobial activity is also observed with carboxymethyl chitosan, which makes essential transition metal ions unavailable for bacteria (34) or binds to the negatively charged bacterial surface to disturb the cell membrane (35). Therefore, these materials are used in wound healing systems, such as carboxymethyl chitosan for the reduction of periodontal pockets in dentistry (34) and chitosan-grafted with EDTA as a constituent of hydro-alcoholic gels for topical use (33). Chitosan and chitooligosaccharide-grafted membranes showed antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, methicilin-resistant *Staphylococcus aureus* (MRSA), and *S. aureus* (36). Also, it was observed that the antimicrobial activity of chitosan and graft copolymers against *Candida albicans*, *Trichophyton rubrum*, and *Trichophyton violaceum* depends largely on the amount and type of grafted chains, as well as on the changes of pH (37).

Table 8 shows the effect of chitosan amination degree variation on its antimicrobial activity. It was found that antimicrobial activity of the aminated chitosan increased as a result of increase grafted amine groups due to selective grafting of this external amine groups on the hydroxyl groups of chitosan. This leads consequently to increase the positive charges on the polymer backbone. The antibacterial activity was improved against two gram negative bacteria, *Escherichia coli* and *pseudomonas aeruginosa*, by 50% and 40%, respectively. At the same time, it was improved against two gram positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, by 114% and 45%, respectively. In microbial profile, the powerful effect of modified chitosan on the gram negative rather than the gram positive bacterium was explained by the difference on the pathological composition of the cell wall. Gram negative bacteria have thick layer of phospholipids rather than the peptidoglycan comparing to the gram positive which has thick layer of peptidoglycan. The negative charges of the phospholipids enhance the adhesion power of poly cationic polymer such as

PBQ :chitin ratio	N content (relative percentage)	Maximum inhibition (%)			
		<i>E.coli</i>	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>Stapylococcus</i>
Chitosan control	100	62.6298	35.689	68.73178	67.047076
0.0935	101	77.4394	46.2014	80.53936	69.258203
0.187	102	79.8616	57.3322	86.22449	73.18117
0.374	117.7	83.1834	58.2155	87.17201	85.805991
0.545	129.3	88.7889	62.3675	92.34694	94.293866
0.747	129.6	90.7958	76.7668	96.13703	97.360913

Table 8. Effect of the degree of amination on the antimicrobial activity of modified chitosan against different microorganisms

chitosan on the cell wall. Increasing the cell inhibition capability of modified aminated chitosan affects directly on the time needed for wound healing and almost eliminates the possibility of bacterial infections. This idea was the base for preparation and synthesis of different quaternary chitosan derivatives (38). Improvement of the antibacterial activity has been achieved by other authors by adding silver to chitosane polyphosphate membranes (39). However, the cytotoxicity of this derivative was found very high.

The effect of adding glycerol as plasticizer to aminated chitosan on the antimicrobial activity was studied. Four samples with different percent of glycerol 0, 2, 4 and 6 % were tested. It was found that the antibacterial activity is nearly constant and does not affect with increase of the glycerol content in the membranes.

Hemocompatibility

Hemolysis is regarded as an especially significant screening test, once it provides quantification of small levels of plasma hemoglobin, which may not be measurable under in vivo conditions. As reported in literature (ISO 10993-4 (1999)), it is not possible to define a universal level of acceptable or unacceptable amounts of hemolysis. Although by definition a blood-compatible material should be non-hemolytic, in practice several medical devices cause hemolysis. This means that when such hemolytic effect takes place, it is important to make sure that clinical benefits overcome the risks and that the values of hemolysis are within acceptable limits. It has been shown that chitosan derivatives have great potential to be used in other biomedical applications. As a result of the biocompatible properties such as good blood compatibility and cell growth efficiency, grafted chitosan materials have potential to be used in cardio-vascular applications (40-41).

According to ASTM F 756-00 (2000) materials can be classified in three different categories according to their hemolytic index (hemolysis %). Materials with percentages of hemolysis over 5% are considered hemolytic; while the ones with hemolytic index between 5% and 2% are classified as slightly hemolytic. Finally, when the material presents a hemolysis percentage below 2% it is considered as a non-hemolytic material. Table 9 represents the values of hemolysis obtained for all membranes. The obtained results indicate that the chitosan and chitosan derivatives are non hemolytic compounds and compatible with human body. Addition of glycerol with any percent of studied range reduced the

Sample	Hemolytic percent
Chitosan	1.933816
Aminated chitosan (glycerol 0%)	2.109617
Aminated chitosan (glycerol 2%)	1.447777
Aminated chitosan (glycerol 4%)	1.168563
Aminated chitosan (glycerol 6%)	1.013444

Table 9. Hemolytic percent of chitosan and aminated chitosan membranes with different percent of glycerol

hemolysis to less than 2%, so the presence of glycerol is beneficial from this point of view. Best results obtained with 6% sample; 1.01 hemolytic percent. The effect of glycerol addition may be explained according to reduce the surface roughness and accordingly the contact area between the blood vessels and the membranes surface. It is worthy to mention here that the reduction of hemolytic effect is directly proportional with surface roughness mentioned in table (6). Reduction of surface roughness by 50% relative to chitosan has been obtained with aminated chitosan with 2% glycerol. This reduction was aligned with reduction in hemolytic percentage by about 25%. Critical value of surface roughness obtained with aminated chitosane with 2% glycerol, 1.61, which beyond the hemolytic percent reduced linearly and with higher rate. Addition of glycerol as plastizer may also contribute in increasing of the porous structure.

Cytotoxicity

Several searches were tested and measured the cytotoxicity of Chitosan (39, 42). Cytotoxicity of Chitosan and aminated Chitosan was measured using mouse fibroblasts with the direct connect method and the data are shown in table 10. According to the tabulated results, the viability of the live cell decreased by increase the amine group substitution.

sample	Live cells X 10 ⁵	Dead cell X 10 ⁵	Total cells X 10 ⁵	viability %
Control	6.75	1.2	7.95	89.9
Chitosan	5.35	0.8	6.15	86.9
aminated Chitosan	5	1.5	6.5	76.9
aminated Chitosan with plasticizer glycerol (4%)	4.3	1.6	5.9	72.9

Table 10. Cytotoxicity of chitosan and modified chitosan (0.6 x 10⁵ cells incubated for five days)

Several authors have discussed the role of amine substitute on polymers in influencing toxicity. Dekie et al. (43) noted that the presence of primary amines in poly(L-glutamic acid) (PGA) derivatives has a significant toxic effect on red blood cells. Based on studies with modified poly(L-lysine) (PLL), Ferruti et al, (44) conclude that polymers with tertiary amine groups exhibit a lower toxicity than those with primary and secondary residues. They have also synthesized tertiary amine group containing poly (amidoamine)s (PAAS); these substitutes polymers have good biocompatibility and can form complexes with heparin

(45-47). Fischer et al (48) confirmed these observations for PLL and PEI, but argued that cationized human serum albumin and starburst dendimer, which also contain primary amino groups, showed only moderate cytotoxic effects. They conclude that not only the type of amino function but also the charge density and arrangement is an important factor for determining cytotoxicity and hence biocompatibility (48). This may be explains the moderate increment of cytotoxicity of modified chitosan, table 10, in which the cell viability % decreases by about 10%, although the nitrogen content increases by about 30%. Only part of this increment is referred to primary amino groups and the other part is referred to secondary amino groups (figure 1), which is less toxic.

Biodegradability

The biodegradability of chitosan and aminated chitosan was measured as produced reduced sugar results from the cellulase enzyme action on the chitosan. Table 11 represents the OD of the total reduced sugar produced from chitosan and aminated chitosan hydrolysis with cellulase enzyme for 30 minutes at 50°C. It was observed that the degradation in the case of chitosan is higher than the aminated chitosan. This may be attributed to the steric effect result from increase the amine content and presence of benzene ring of PBQ on the polymer chains which consequently reduced the accessibility of the aminated chitosan to enzyme hydrolytic activity.

Polymer	OD
Chitosan	0.747
Aminated chitosan	0.643

Table 11. Biodegradability effect of cellulase enzyme on the chitosan and aminated chitosan (30 minute, at 50°C and pH 7)

3. Conclusion

Modified chitosan with antibacterial potentials superior to native chitosan was prepared. To achieve this goal, new route of chemical modification to graft extra amine groups onto native chitosan has been presented. As a result of modification, the nitrogen content of modified chitosan increased by about 30%.

FT-IR, TGA analysis and solubility test confirmed the occurrence of amination process. The solubility near neutral media, pH 6.0, increases by about 65%. Using glycerol as plasticizer improved the surface roughness, water uptake, and the mechanical properties of aminated chitosan membranes while its water vapor permeability was reduced.

The modified chitosan membranes were evaluated as wound dressing biomaterial and show high profile. Its bacterial cell inhibition capability against gram negative and gram positive was improved in the range of 40 - 100%. Moreover, no hemolytic effect was observed. In addition, its biodegradability was not affected significantly. However, the cytotoxicity was increased, not in a dramatic way, and the water vapor permeability also reduced which limit the application of modified chitosane membranes as wound dressing. In the meanwhile, further investigations are conduction in this direction to overcome these drawbacks through quaterinization process.

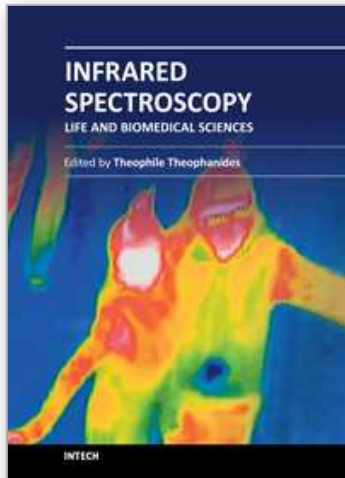
In conclusion the new route of modification was found successful and is recommended for preparation of novel chitosan biomaterials.

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This informative and state-of-the art book on Infrared Spectroscopy in Life sciences designed for researchers, academics as well as for those working in industry, agriculture and in pharmaceutical companies features 20 chapters of applications of MIRS and NIRS in brain activity and clinical research. It shows excellent FT-IR spectra of breast tissues, atheromatic plaques, human bones and projects assessment of haemodynamic activation in the cerebral cortex, brain oxygenation studies and many interesting insights from a medical perspective.

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