Effects of Neutralization Process on Preparation and Characterization of Chitosan Membranes for Wound Dressing

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Summary: Neutralization process effects on preparation and characterization of chitosan membranes were evaluated by differencial scanning calorimetry and scanning electron microscopy. Water solubility and humidity of chitosan membranes were also studied. Swelling behavior in different pH media was evaluated and mechanical properties such as tensile strength and elongation at break were measured. Neutralization process increased glass transition temperature of chitosan membranes and decreased their water solubility, humidity and water sorption. An improvement in mechanical properties of chitosan membranes was also observed after neutralization process.

Keywords: biomaterials; chitosan; membranes; neutralization process; wound dressing

Introduction

Wound dressing is an area of current research interest due to its great importance in treatment of traumas, burns, diabetes wounds, skin ulcers and other skin damages, such as surgical wounds.[1]

An ideal wound dressing should replace the function of lost skin, protect wounds from fluid and protein losses, prevent bacterial invasion, and dissipate mechanical stress (external), and finally, improve and stimulate wound healing.[2] Moreover, it should promote adequate gaseous and heat exchange with environment and be biocompatible and non-antigenic to avoid body rejection.[3]

Developments in the area of biomaterials offer several commercial wound dressings like Bioprocess®, Opsite® and Biobrane II®, based on collagen, hyaluronic acid and other biomaterials. However, they do not have a very accessible cost.

Chitosan is a biopolymer obtained by the deacetylation of chitin, which is the second more abundant polysaccharide found in nature. Chitin is mainly found in external skeleton of insects and in shells of crustaceans. Thus, chitosan production process is ecologically interesting and economically viable due to the use of seafood industry by-products.[4]

Moreover, chitosan exhibits various interesting biological activities, which made this polysaccharide increasingly important. Typical activities include antitumor, immunoadjuvant, antibacterial and hemostatic activities.[5] Chitosan is metabolized by certain human enzymes, especially lysozyme, and is considered biodegradable.[6] It also can accelerate wound healing.[7] In addition, due to its positive charges at physiological pH, chitosan is bioadesive, which increases retention at the site of application.[8] For all those reasons, chitosan is one of the most important biomaterials for wound management in the recent years.

In this present study, chitosan membranes for wound dressing were prepared

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by a solvent evaporation technique and then neutralized, in order to evaluate the effects of neutralization process on preparation and characterization.

Experimental Part

Materials

High molecular weight chitosan ($M_w \sim 100\,000$), being more than 75% deacetylated, was purchased from Aldrich Chemical Company (USA). Glacial acetic acid was purchased from Synth (Brazil). Chitosan was dissolved in acetic acid (1.0% w/w) and then filtered to remove any undissolved impurities.

Preparation of the Membranes

Chitosan membranes were prepared by a solvent evaporation technique by using aqueous acetic acid (1.0% w/w) as solvent. Chitosan solution was prepared by dissolving 1.5 g of chitosan in 100 ml of solvent. After complete dissolution, it was filtered and cast on Petri plates. Following this, chitosan membrane (C1) was obtained by solution evaporation in an oven at 40 °C. Neutralized chitosan membrane (C2) was prepared by immersion of C1 into NaOH (2% w/w) – Na_2CO_3 (0.05% w/w) aqueous solution for 1 h. After the immersion, C2 was repeatedly washed with distillated water and dried at 40 °C.

Modulated Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurements were performed on a DSC 2920 –TA Instruments. DSC curves of chitosan membranes were obtained by heating samples from −130 °C up to 200 °C, at heating rate 2 °C · min^{-1} and modulation rate ±1 °C · min^{-1}, under nitrogen atmosphere, in order to estimate glass transition and melting temperatures.

Scanning Electron Microscopy

Chitosan membranes samples were cut and kept in a vacuum desiccator for 48 h. Then, samples were coated with gold/paladium using a SC 7620 Sputter Coater—POLARON under high vacuum and 4 mA for 180 s. Coated samples were examined using a LEO 440i Scanning Electron Microscope.

Water Solubility and Humidity

Percentage of humidity in chitosan membranes was determined by gravimetric method. Circular membranes samples ($D = 2$ cm) were kept in a vacuum desiccator for 24 h and weighed to obtain the initial mass (M_i). After this procedure, they were dried at 100 °C for 24 h and weighed again in order to determine the final mass (M_f). The percentage of humidity was calculated according to the following Equation:

$$H(\%) = (M_i - M_f) \times 100/M_i \quad (1)$$

Chitosan membranes water solubility was determined according to Gontard et al.[9]

Circular samples ($D = 2$ cm) were dried at 100 °C for 24 h and weighted to obtain their dry mass (W_i). After this procedure, they were immersed into 50 ml of water for 24 h at 25 °C by slowly string. Samples were then taken out and dried (100 °C for 24 h) to determine the membrane weight not soluble in water (W_f). The percentage of water solubility (W) was calculated from the following Equation:

$$W(\%) = (W_i - W_f) \times 100/W_i \quad (2)$$

Each experiment was repeated three times and the average value was calculated.

Swelling Behavior

Swelling behavior of chitosan membranes was determined by the gravimetric method. Square membranes samples ($L = 2$ cm) were kept in a vacuum desiccator for 24 h and their dry mass (W_D) were determined. After this procedure, they were immersed in pH 7.4 and 5.6 of phosphate buffer saline solution at 37 °C. After pre-determined periods (5, 20, 40, 60, 80, 100, 120 and 180 min), wet weights (W_W) of chitosan membranes were determined by first blotting the membranes with filter paper to remove adsorbed water on the surface, then

immediately weighted on an electronic scale. The swelling ratio (S) of chitosan membranes in the media was calculated from the following Equation:

$$S(\%) = (W_W - W_D) \times 100/W_W \quad (3)$$

Each experiment was repeated three times and the average value was taken as the swelling ratio.

Mechanical Properties

Eletronic digital caliper (Fowler & NSK—Max-Cal) was used to measure membranes thickness (δ). TA-XT2 (SMS, Surrey, UK) instrument was used to measure tensile strength (TS) and percent elongation at break (E). Tests were carried out according to ASTM D-882,[10] with initial grip separation of 50 mm and cross head speed of 60 mm $\cdot$ min^{-1}. TS was calculated by dividing the maximum load for breaking film by cross sectional area and E was determined by dividing film elongation at rupture by initial gauge length and multiplying by 100%.

Results and Discussion

Differential Scanning Calorimetry

DSC results are presented in Table 1.

DSC analysis presented lower value of glass transition temperature for C1 and lower values of enthalpy and melting temperature for C2. It can be attributed to acetic acid molecules that act as plastcizer in C1, increasing its mobility. On the other side, neutralization process removes acetic acid molecules from C2 and promotes a molecular rearrangement of polymer chains, which is responsible for a decrease in the free volume of the system and an increase in its glass transition temperature.

Table 1.
Thermal properties of chitosan membranes.

Sample	T_{g1}	T_{g2}	T_{g3}	T_m	ΔH
	°C	°C	°C	°C	J $\cdot$ g^{-1}
C1	−81.70	−84.10	−73.69	101.95	458.70
C2	−76.90	−75.63	−73.16	88.39	418.50

Scanning Electron Microscopy

C1 and C2 scanning electron micrographs are presented in Figure 1 and 2, respectively.

SEM micrographies showed that C1 surface is more regular than C2 surface, which presented some rugosity. It may be attributed to the neutralization process that removes superficial acetic acid molecules from C2. Moreover, they showed that both C1 and C2 are dense and packed membranes.

Water Solubility and Humidity

Some parameters such as percentage of humidity (H) and of water solubility (S), thickness (δ), tensile strength (TS) and percent elongation at break (E) obtained for C1 and C2 membranes are presented in Table 2.

Chitosan is insoluble in neutral and alkaline media, but it is soluble in acid one. Residual acetic acid of C1 interacts with water and forms a weak acid solution. For this reason, C1 is much more soluble than C2. The largest humidity of C1 compared to C2 one can be attributed to the presence of acetic acid molecules in C1, which increases its hydrophilic character.

Swelling Behavior

Swelling behavior of C1 and C2 at different pHs is presented in Figure 3.

According to Figure 3, C1 presented higher water absorption in acid medium (pH 5.6) than in physiological medium (pH 7.4). It can be attributed to the protonation of chitosan amine groups in acid medium that increases its hydrophilicity. As soon as C1 was immersed in pH 5.6 solution, it swelled and presented a gel aspect, which had lead to a more difficult removal of adsorbed water on the surface. Samples immersed in that solution for 120 and 180 min could not be totally taken off, since part of them were dissolved. This fact can explain the swelling ratio decreasing for those times. When immersed in pH 7.4

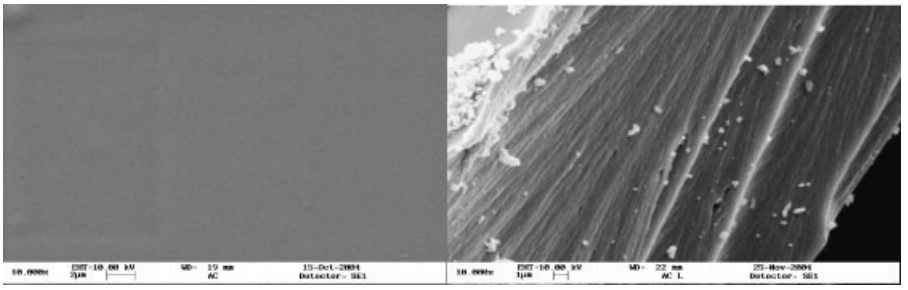

Figure 1.
Superficial and internal morphologies of C1.

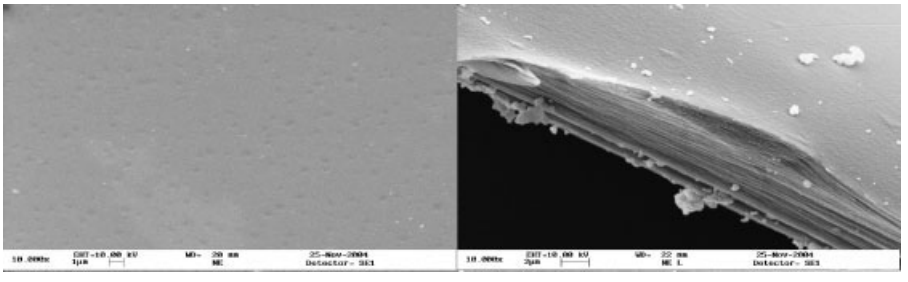

Figure 2.
Superficial and internal morphologies of C2.

solution, C1 also swelled and got a gel consistence. However, it was only observed in the first three points (5, 20 and 40 min). After those periods, C1 probably reached the equilibrium state with the medium and its water absorption was decreased.

C2 swelling ratio was also higher in acid medium due to protonation of amine groups. Nevertheless, when compared to C1 swelling ratio, C2 one was lower in both pH solutions. This can be attributed to neutralization process that removed acetic acid molecules from C2 and decreased its hydrophilicity.

Swelling behavior is an important parameter for wound dressing management. An ideal wound dressing should absorb fluids excreted by the wound to avoid accumulation of fluid between wound and dressing because of the risk of infection.[13] However, wound dressing should maintain its properties to continue replacing skin lost functions. In that way, C2 presented better results than C1, because it maintained its shape and consistence when swollen.

Mechanical Properties

Skin is a viscoelastic material and is permanently subjected to slight stress. Tensile strength of skin is very dependent on the rate of loading and varies with the direction of the stress. Moreover, it is different depending on the gender, age and site. An average tensile strength of 1.8 kgf $\cdot$ mm^{-2} ($\sim$17.6 MPa) was quoted by Wohlish.[11] However, tensile strength of wounds is weaker than the skin's one and varies during wound healing process.[12]

Tensile strength (TS) and percent elongation at break (E) of C1 and C2 are presented in Table 2. C1 presented lower tensile strength and higher elongation at break than C2, what can be explained by the presence of acetic acid

Table 2.
Properties of chitosan membranes.

Sample	H	W	δ	TS	E
	%	%	μm	MPa	%
C1	17.42	20.28	40	122.31	23.45
C2	12.11	2.34	50	140.54	8.04

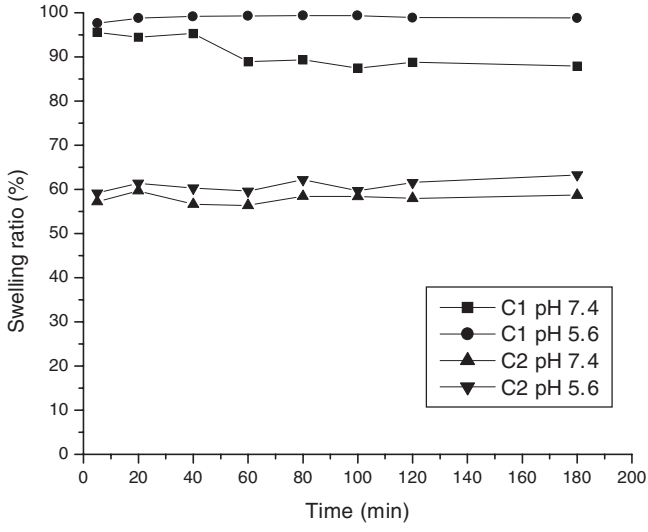

Figure 3.
Swelling behavior of chitosan membranes at pH 7.4 and 5.6.

molecules in C1, that act as plasticizer, lowering the tensile strength and improving the elongation at break.

According to skin tensile strength average, both chitosan membranes can replace mechanical functions of lost skin, due to their tensile strength almost 10 times higher than the skin.

Conclusions

Neutralization process promoted a rearrangement on chitosan membrane due to acetic acid molecules removal and decreased free volume around the polymer chains. In addition, neutralization process reduced water solubility, humidity and water sorption of chitosan membrane by removing hydrophilic groups (acetic acid). An improvement on chitosan membrane tensile strength and a decrease on its elongation at break were observed after neutralization process and they were also attributed to the removal of acetic acid molecules that acted as a plasticizer. Finally, neutralization process is an important step on preparation of chitosan membranes as a biomaterial for wound dressing; and also this process retards acid hidrolysis of chitosan.

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