PREPARATION AND CHARACTERIZATION OF CHITOSAN/PVA POLYMERIC FILM FOR ITS POTENTIAL APPLICATION AS WOUND DRESSING MATERIAL

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ABSTRACT

In this work, chitin flakes were deacetylated with 50% (w/v) sodium hydroxide under nitrogen atmosphere at 120 °C for 80 min to obtain chitosan. The chitosan produced was characterized for degree of deacetylation (DD) and molecular weight. Chitosan with the DD of 78-80 % was reproducibly obtained. Molecular weight showed a decreasing trend as the concentration of NaOH increased. Further, chitosan polymeric film was prepared from chitosan and polyvinyl alcohol (CH/PVA) blend using aqueous citric acid solution. The CH/PVA film was analyzed by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Thermo gravimetric analysis (TGA). XRD and FTIR demonstrated that there was strong intermolecular hydrogen bonding between the molecules of chitosan and PVA. The swelling studies, tensile properties and porosity of polymeric film were also studied. In vitro biocompatibility and biodegradation of CH/PVA polymeric film have been studied in simulated body fluid (SBF) and phosphate buffer (PBS) respectively to prove its potential for possible application as wound dressing material.

KEYWORDS: Chitin Deacetylation, Chitosan, Polyvinyl Alcohol, Biocompatibility, Wound Dressing

Chitosan is a copolymer of 2-glucosamine and N-acetyl-2-glucosamine units, produced from deacetylation of chitin which is the structural element of the exoskeleton of crustacean's arthropods such as insects, crabs, lobster and shrimps etc. [Qu et. al., 2014]. It is a nontoxic, biodegradable, biocompatible and cationic polymer having significant role in biomedical application [Du and Hsieh, 2009]. Chitosan is insoluble in water, organic solvents and aqueous bases and it is soluble after stirring in acids such as nitric, perchloric, phosphoric, hydrochloric, formic, acetic and citric acid solutions [Ahmed et. al., 2015].

Chitosan have been examined to be used in a wide variety of biomedical application, such as drug delivery carriers, surgical thread, bone healing materials, especially wound dressing [Su et. al., 1997]. Chitosan could achieve haemostasis and allow the promotion of normal tissue regeneration [Tomihata and Ikada, 1997]. Besides, the biodegradable chitosan itself provides bacteriostatic and fungistatic activities. Intrinsic antibacterial and wound healing properties of chitosan make it suitable for wound dressing application [Rasaee et. al., 2016].

An ideal wound dressing material should be biocompatible, absorb exudates and provide moist environment [Eaysmine et. al., 2016]. Considering degree of wound exudation and injury location, each type of wound requires specific dressing; hence, the physicochemical and mechanical properties of the dressing should be adjusted [Tuzlakoglu and Reis, 2007]. Film forming ability is another special aspect of chitosan in comparison with other biopolymers. The film properties of chitosan depend on several parameters such as its molecular weight, the degree of deacetylation, organic acid used, and the possible presence of plasticizer [Xie et. al., 2013].

The work presented in this research paper covers the extraction of chitosan from chitin and preparation of polymeric film from blend of CH/PVA using solvent casting technique. The preparation and characterization of this film has been discussed in this work. To improve processability of chitosan, it was blended with synthetic biocompatible polymer PVA. PVA is a bioresorbable polymer with potential applications in tissue engineering for bone and cartilage repair. The aim of this study is to investigate some significant properties of the CH/PVA blend film to apply in wound dressing, including the porosity, tensile strength, swelling behavior, in vitro biocompatibility and biodegradation properties.

EXPERIMENTAL

Materials

Chitin flakes were supplied by S D Fine Chemicals Ltd., Mumbai. Chitin samples were dried at 60^0C for 2hrs in an oven and then grounded before use. Polyvinyl alcohol LR (Mw. 125000) was purchased from S D Fine Chemicals Ltd. Sodium hydroxide, hydrochloric acid and citric acid was supplied by S D Fine Chemicals Ltd., Mumbai. pH meter from Equiptronics EQ-634 was used for pH measurement.

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Deacetylation of Chitin

The deacetylation of chitin was carried out by treatment of chitin flakes with 50% (w/v) NaOH at 120^0C for 80 mins in presence of inert atmosphere of nitrogen using a solid to solvent ratio of 1:40. After reaction, the material produced was washed several times with distilled water until near to neutral pH and dried at 50^0C in a vacuum oven for overnight [Moura et. al., 2015].

Determination of Degree of Deacetylation (DD) using pH-metric Titration

The chitosan obtained after deacetylation (0.2 gm) was initially dissolved in 20 ml of 0.1 M hydrochloric acid and further addition of 25 ml distilled water was done. After 30 minutes of continuous stirring, next portion of distilled water (25 ml) was added for adjusting the molarity of the solution and stirring continued for 30 minutes. When the dissolution procedure was completed, the chitosan solution was titrated with a 0.1 M sodium hydroxide solution. The titrant was added until the pH value reaches to neutral, and the pH values of solution were recorded for deflection point [Biskup et. al., 2012].

Degree of deacetylation (DD) of chitosan was calculated using formula:

$$DD\ (\%) = 2.03 \times \frac{V2 - V1}{m + 0.0042(V2 - V1)} \qquad (1)$$

where: m– weight of sample, V_1, V_2– volumes of 0.1 M sodium hydroxide solution corresponding to the deflection points, 2.03 – coefficient resulting from the molecular weight of chitin monomer unit, 0.0042 – coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

Characterization

Fourier-transform infrared spectroscopy (FTIR) (SHIMADZU FTIR-8400S) was used to confirm the modification in chitin, chitosan and CH/PVA blend polymeric film. The X-ray diffraction pattern of chitin and deacetylated chitosan was recorded at room temperature on X-ray diffractometer (LabX XRD-6100 SHIMADZU). The data was collected in the 2θ range 5–40^0 with a step size of 0.02^0 and a counting time of 5 sec/step. Thermo gravimetric analysis (TGA) was used to evaluate the thermal stability and the decomposition temperature of chitin, chitosan and CH/PVA blend polymeric film. Thermo gravimetric measurements were made using TG/DTA Simultaneous Measuring Instruments (DTG-60H SHIMADZU).

Preparation of polymeric solution

2% (w/v) concentration of chitosan solution was prepared by dissolving in 2% (v/v) of citric acid. 2% (w/v) PVA solution was prepared in distilled water with continuous stirring for 2 hrs at 60^0 C. CH/PVA blend solution was then prepared by mixing the two solutions at 80/20 CH/PVA mass ratio. Chitosan and PVA mixing were performed at room temperature using a laboratory magnetic stirrer for 5-6 hrs to obtain homogenous solution. The prepared solution was left overnight for degassing at room temperature.

Physical properties

The porosity of CH/PVA polymeric film was measured by liquid displacement method, similar to a published method [Gupta et. al., 2012]. The swelling and tensile properties of the CH/PVA polymeric film were examined by weighing method and tensile testing (Instron type universal testing machine), respectively [Abdeen, 2011].

Biocompatibility experiment in simulated body fluid

Bioactive properties of the CH/PVA polymeric film were examined by in vitro soaking in simulated body fluid (SBF). Polymeric film of dimension 20 x 40 mm^2 was soaked in SBF solution following the method of Kokubo and Takadama, 2006. The bioactivity of material was determined by the formation of hydroxyapatite on its surface upon immersion in SBF. Vacuum dried film was placed in SBF solution at 37 ^{0}C in a humidified incubator and the SBF was refreshed every day. The sample was collected after 2 days, 4 days and 6 days of incubation. The samples were carefully rinsed in distilled water and then vacuum dried overnight. The bioactivity and possible hydroxyapatite formation on the samples were examined using FTIR and XRD analysis [Zhang, 1999].

In vitro biodegradation

CH/PVA polymeric film was cut into a rectangular shape of dimension 20 x 20 mm^2 for the biodegradation test. The specimens were placed in closed bottles containing 30 ml of PBS and incubated at temperature of 37 ^{0}C for 6 weeks. After each degradation period, the specimens were rinsed with distilled water and dried in vacuum oven at room temperature. The weight loss percentages of the specimens were calculated from

the weights obtained before and after degradation as below [Zhang, 1999]:

Weight loss (%) = $(W_1 \times W_2)/W_1 \times 100$

Where, W_1 and W_2 are the samples weight before and after degradation, respectively.

RESULTS AND DISCUSSION

An ideal wound dressing should be able to create a favorable environment to promote the healing process. The dressing must present flexibility, porosity, good swelling behavior, adequate tensile properties and adherence to the tissue.

In this work, a polymeric film composed of chitosan and polyvinyl alcohol was prepared for application as wound dressing materials. Key properties were tested to evaluate the potential of these films for wound dressings, such as porosity, tensile strength, swelling behavior, in vitro biocompatibility and biodegradation.

Preparation of chitosan

Degree of deacetylation (DD) and molecular weight are the important parameters for application of chitosan in various fields. From this regard, certain researchers [Dutta et. al., 2004 & Shahidi et. al., 1999] suggested that the term chitosan should be used when the degree of deacetylation is above 70 %. In the process of deacetylation, the chitin was treated with different concentrations of NaOH for the removal of acetyl group and formation of amino group which makes the chitosan water soluble. However the process of removal of the acetyl groups lead to the depolymerisation of the polysaccharide chains causing degradation. Due to this degradation, the molecular weight of the chitosan formed decreases. Also during the study, it was observed that with increase in NaOH concentration the molecular weight of chitosan decreases [Abdou et. al., 2008 & Sila et. al., 2014].

The deacetylation degree of the extracted chitosan was found to 78-80 % using pH-metric titration method. The deacetylation was carried out using 50% (w/v) sodium hydroxide at 120^0C. Reactions were performed under nitrogen atmosphere to minimize decrease in molecular weight of the chitosan obtained after deacetylation. The molecular weight of chitosan was calculated from solution viscosity measurement as 1.112526×10^5 gm/mole [Weska et. al., 2007 & Yen et. al., 2009].

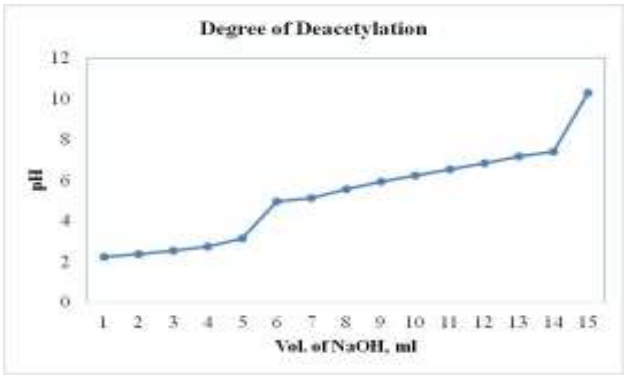

Figure 1: Graph of Degree of deacetylation (DD) of chitosan solution by pH-metric titration

FTIR Analysis

The FTIR spectra obtained for Chitin, Chitosan, and CH/PVA blend polymeric film is shown in fig.2. The intensity of 1560 cm^{-1} in chitin decreases with deacetylation, becoming weak for deacetylated chitosan. As seen in the fig.2, the intensity of the band at 1560 cm^{-1} is almost absent in the chitosan spectrum fig.2b while a significant broad peak is observed in the chitin spectrum fig.2a.

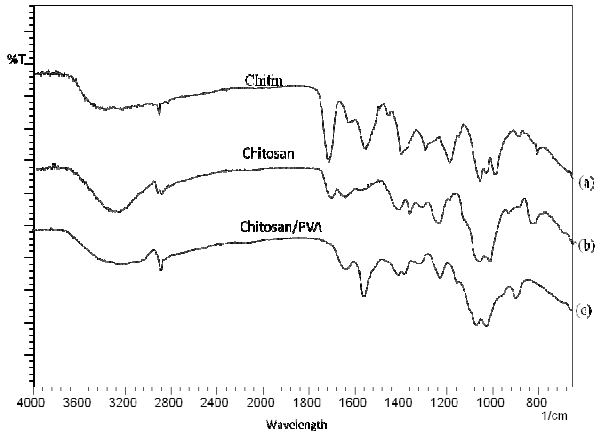

Figure 2: FTIR spectrum of chitin (a), deacetylated chitosan (b) and CH/PVA film with blend ratio (80/20) (c)

With increase in deacetylation, the intensities of the characteristic peaks attributed to the amide group at 1700 cm^{-1} decreases as shown in fig.2b. In the IR spectra of chitosan the peaks at 3336 cm^{-1} (N-H stretching), 2921 and 2867 cm^{-1} (C-H stretching), 1652 cm^{-1} (amide II band N-H stretching), 1425 cm^{-1} (asymmetrical C-H bending of

the CH_2 group) and 1066 and 1024 cm^{-1} (O- bridge stretching) represent the glucosamine residue. In the IR spectra of CH/PVA blend fig.2c, the large band observed between 3550 and 3200 cm^{-1} is linked to the O–H stretching from the intermolecular and intramolecular hydrogen bonds overlapping with N-H stretching. The peak is gradually broadened and shifted to lower wave numbers by increasing the PVA content in blend. The CH/PVA blend spectrum showed the characteristic bands of both chitosan and PVA which indicated presence of chitosan and PVA in the polymeric film.

XRD Analysis

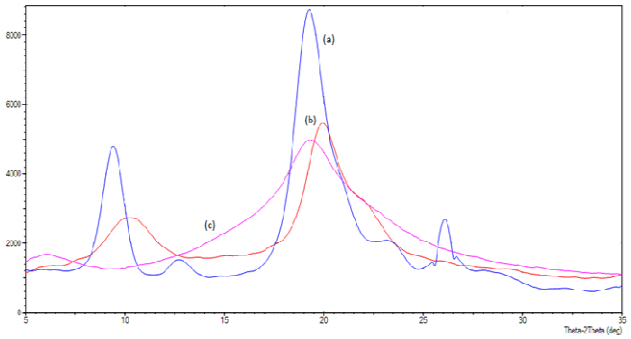

Figure 3: XRD patterns of chitin (a), chitosan (b), and CH/PVA (c) polymeric film

Fig.3 shows the XRD pattern of chitin and deacetylated chitosan. The peak intensity was obtained for chitin at $2\theta=9.39^0$ and $2\theta=19.35^0$ and it decreases with the increase in degree of deacetylation, and moved to a higher angle i.e. the peak of chitosan at $2\theta= 10.17$ and $2\theta= 20.19^0$. In the literature, many XRD patterns of chitosan have two characteristic peaks which are usually around $2\theta = 10^0$ and $2\theta = 20^0$ [Yen et. al., 2009 & Zhang et. al., 2005]. The strong interaction between chitosan and PVA molecules in blend is evident as there are no individual peaks which can be seen from fig.3c. The peak of chitosan at $2\theta= 20.19^0$ becomes weaker and gets shifted towards lower degree with increase in composition of PVA in the blend.

Thermal properties of chitin, chitosan and CH/PVA polymeric film

In fig.4, two stages of weight losses were observed in the TGA. The first stage at around 90^0C, the weight loss of about 10% was observed in all the three samples. The weight loss in chitin and chitosan is due to the release of hydrogen bonded water. The second stage weight loss of about 60% around 250^0C- 320^0C temperature is attributed to the dehydration of saccharide rings and the depolymerization and decomposition of the chitosan [Valderruten et. al., 2014].

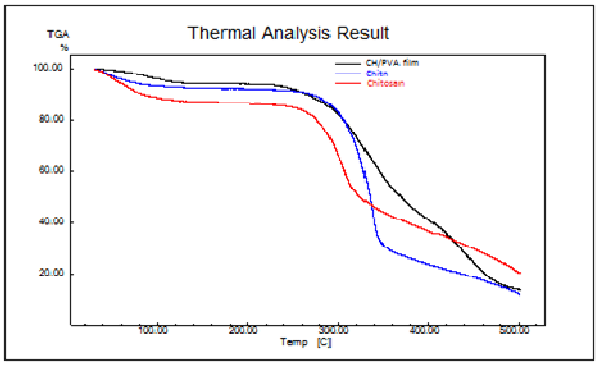

Figure 4: TGA curve of chitin, chitosan and CH/PVA polymeric film

Analysis of physical properties of CH/PVA polymeric film

Table I: Analysis of breaking strength, porosity and swelling degree of PVA, chitosan and CH/PVA (80/20) blend polymeric film

Specimen	Breaking strength (Kgf)	Porosity (%)	Swelling degree (%)
PVA	30.06	25.5	285.1
Chitosan	36.71	40.8	450
Chitosan/PVA	38.65	44.1	514

The enhancement of the tensile strength in the CH/PVA blend as compared to chitosan and PVA is due to the strong hydrogen bonding between -OH and - NH_2 in chitosan and -OH groups in PVA.

It is fundamental to investigate the swelling behavior of the hydrophilic films for wound dressings, as it determines the capacity of the films to absorb exudates from the wound, avoiding maceration. From table I, it is seen that, increase in the percentage of chitosan in CH/PVA blend significantly increases both the water absorption and the time to reach the equilibrium. The increase of swelling degree is due to the presence of amino groups in chitosan. In an ionic environment chitosan molecules become uncoiled and assume more elongation or exist in a rod like shape. In presence of ions, the electro-neutrality condition increases and creates an additional osmotic pressure that expands the CH/PVA polymeric film.

In vitro biocompatibility and biodegradation

In fig.5, the corresponding FTIR spectrum of CH/PVA polymeric film confirms the presence of hydroxyapatite (HA) after its immersion in SBF fluid. The characteristic phosphate picks for HA at wavenumbers of 1084 cm^{-1} and 1042 cm^{-1} are present. The two peaks at 1415 cm^{-1} and 1564 cm^{-1} are probably of carbonate group which are commonly found in synthetic HA and natural bone.

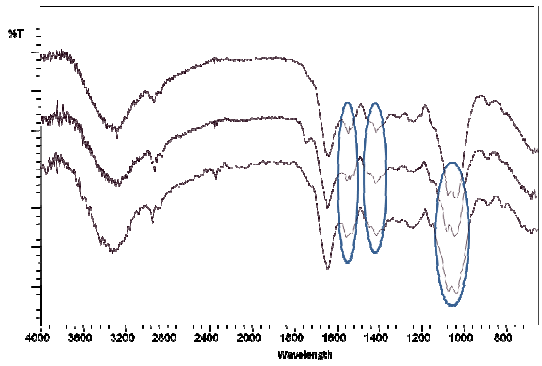

Figure 5: FTIR spectra of SBF incubated CH/PVA polymeric film as function of time

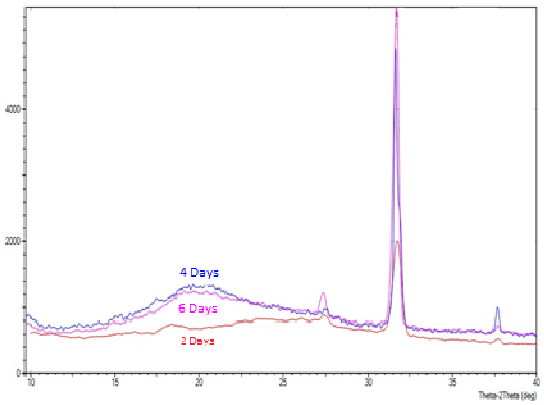

Figure 6: XRD patterns of the SBF incubated CH/PVA film as a function of time

Hydroxyapatite crystal (HA) formation on CH/PVA film has been confirmed through XRD measurements as shown in fig. 6. The broad peak that appeared around 20° was assigned to chitosan (20.19°) and the sharp diffraction characteristic peaks that appeared at around 31.8° and 25.8° corresponding to the peaks of HA. Further, the peak intensities of HA increases with increasing the exposure time in SBF.

Weight loss has been taken as a measure of biodegradation when samples were placed in PBS. Initially, due to swelling of film, the degradation was slower but as the time increases significant weight losses were observed as: 14±0.5% and 16±0.5% in CH/PVA and chitosan polymeric film, respectively. The weight loss is lower in CH/PVA blend as compared with chitosan. This may be due to PVA has greater stability in water; it does not degrade unless higher temperature conditions (≥ 60 ^{0}C).

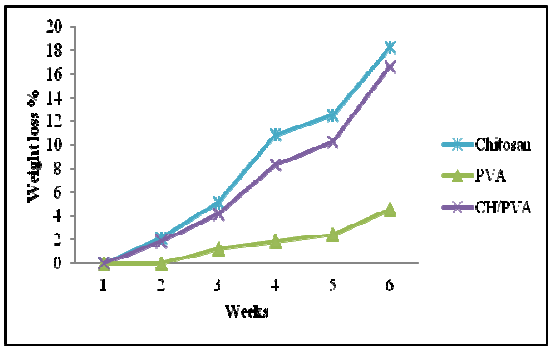

Figure 7: Weight loss of polymeric films in PBS as a function of time

CONCLUSION

Chitosan was made successfully from chitin flakes by chemical deacetylation using 50% NaOH. CH/PVA (80/20) polymeric film was prepared from solvent casting method for potential application as wound dressing material. These films exhibit excellent swelling behavior, porosity, tensile strength, and we, therefore, conclude that adequate swelling and strength retention are promised during the anticipated duration of usage as a wound dressing material. The CH/PVA polymeric films with high percentage of chitosan present both improved in vitro biocompatibility and biodegradation properties. These results also suggest that chitosan and CH/PVA polymeric films can be potentially explored as wound dressing material.

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