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Microarticle

Improvement of the physical properties of chitosan by γ-ray degradation for wound healing

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ABSTRACT

Improving the physical properties of chitosan films by irradiation with different doses (5, 10, 15, 20 and 25 kGy) of γ-rays to be used as a wound healing material. Mechanical properties and dielectric spectroscopy were measured for the films. Results showed that at low γ-dose, tensile strength (TS) elongation percent (E%) increase, while E-modulus, ε', ε'' and $\delta_{A.C}$ decrease due to the accumulation of ions between the chitosan chains which make a bulk network due to hydrogen bonds. This will make the film more elastic and high biodegradable which is preferred in wound healing. While the contrary was observed by increasing the radiation dose, chain scission occur resulting in weak chitosan interchain bonds and this will increase the mobility of the segments which increase the rigidity. In conclusion, the dose of 5 kGy was the most optimum for the properties of chitosan films used in the application of wound healing.

Introduction

Skin is considered the largest immunologically organ in humans which protects the body against pathogens and microorganisms and also protects the internal organs beneath it from the surroundings. Skin damage due to different types of wounds such as burns, acute wounds, chronic diabetic wounds and infected wounds need to choose an appropriate dressing for a fundamental treatment [1–4].

During the wound-healing process, dressings are used for the regeneration and repair of dermal and epidermal tissues. To accelerate the wound healing process, it is preferred to use a dressing that increase the wound moist environment, make gas exchange, be non-toxic, be antimicrobial, be biocompatible and biodegradable and act as a barrier against dust and microorganisms [4].

Chitosan is one of the most used materials for wound dressing. Chitosan is known as a polysaccharide polymer obtained from the deacetylation of a natural polymer (chitin) derived from the exoskeleton of shrimps and craps [5]. Positively charged glucosamine groups (NH_3) give chitosan a cationic nature that can interact with the negative charge of the bacterial cell surface. This interaction causes the bacterial cell wall to rupture. This can give chitosan an antibacterial activity [6].

Chitosan is known as a film forming polymer which have different mechanical and biomedical properties like relieving pain, biocompatibility, ability to absorb exudates, biodegradability, nontoxic, inhibiting growth of microorganisms and promoting haemostasis and epidermal cell growth. All these properties make chitosan a good dressing for burn and wound management [2–4,6]. Chitosan increases the rate of wound healing and scar prevention by the reaction of N-acetyl glucosamine (NAG) which is a major component of dermal tissue [7,8].

Mechanical properties are important requirements for wound dressing because it is close to the skin surface and it should not be torned [9]. Wound dressing must be mechanically durable and elastic having a high value for tensile strength and elongation at break, thus it easily adapts to the shape of the part of the body [10,11].

Chitosan used as wound dressing can be modified through the reduction of its molecular weight to enhance its lower mechanical properties. Various radiation processing techniques such as electron beam, gamma radiation, UV, and X-rays have been demonstrated to be very effective in improving the properties of chitosan. These radiations interact with chitosan chain leading to crosslinking or chain scission which alter the macromolecular structure. Reduction of chitosan molecular weight by gamma radiation was studied by several techniques [12,13].

Also, the electrical properties of chitosan can be improved by chain scission, crosslinking and breaking of bonds which occur in dielectric spectroscopy [13]. The interactions of the hydroxyl and the amino groups of chitosan play an important role in enhancing its physical properties [14].

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The aim of this study is to improve some physical properties of chitosan films; such as: mechanical and electric properties by γ-ray irradiation at different doses (0, 5, 10, 15, 20 and 25 kGy) to be used as wound dressing.

Materials and methods

Materials

Chitosan from Oxford Laboratory/Mumbai-400002 India, with deacetylation (90–95%) and solubility 97% in 1% acetic acid, pH 6.5–8 and moisture 10% max, glacial acetic acid (CH_3COOH) and $NaHCO_3$ from ADWIC (Laboratory chemicals).

Methods

Films preparation

Chitosan films were prepared by means of solvent casting technique, 4 wt% chitosan solution was prepared and left on the magnetic stirrer Jenway 1000 for 24 h for complete solubility of chitosan. After adjusting the pH to 5.5 by adding sodium bicarbonate, the mixture was allowed to dry at room temperature (≈ 25 °C) for 3 days.

Films irradiation

Irradiation of chitosan films of diameter 9 cm and thickness 0.31 mm was performed at Egyptian Atomic Energy Authority (EAEA) using ^{60}Co γ-radiation source in the Gamma Irradiation Facility (Cobalt-60), at a dose rate 1.8 kGy/h. Chitosan was irradiated with different gamma doses (5, 10, 15, 20 and 25 kGy), at room temperature (≈ 25 °C) under atmospheric air.

Mechanical measurements

Mechanical measurements for un-irradiated and irradiated chitosan films were measured at the National Institute for Standards-Giza-Egypt. The measurements were made by using Zwick Tensometer (ASTM method D822-02) with calibrated load cell (100 N). Each sample of the chitosan films was cut into five pieces each of 1 × 2.5 cm, and the results were the averages of these five readings. The environmental conditions: Temperature: 23 ± 2 °C, Relative humidity: 50 ± 5%.

Dielectric measurements and A.C. electrical conductivity

Dielectric measurements for the un-irradiated and irradiated chitosan films were made in Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt. The chitosan films were cut into circles (diameter 2.2 cm). The measurements of the dielectric parameters (the capacitance (C) and the dissipation factor (tan δ)) were performed with a Dielectric Thermal Analyzer (PL-DETA), England, at room temperature (23 °C) by varying the frequency from 50 Hz till 5 MHz.

Results

Mechanical properties

Fig. 1 shows the stress-strain curves for un-irradiated and irradiated chitosan films with different doses of γ-radiation (5,10, 15, 20 and 25 kGy).

The stress (σ) is defined as the load (F) per unit cross-sectional area (A).

$$\sigma = F/A \tag{1}$$

The strain (ε) is the amount of deformation per unit length of the material due to the applied load.

$$\varepsilon = (l_i - l_o)/l_o = \Delta l/l_o \tag{2}$$

where l_o is the original length of the film before any load is applied, l_i is the instantaneous length, and Δl is the amount of elongation. From these curves, the tensile strength (T.S.),

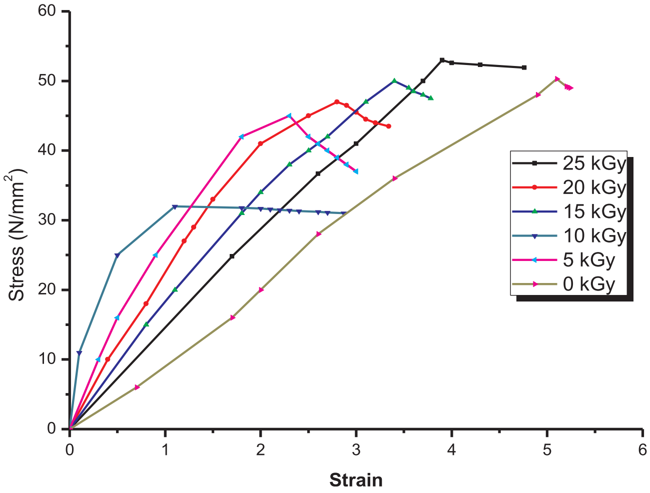

Fig. 1. Stress-strain main value curve for un-irradiated and irradiated chitosan films with different gamma doses (0, 5, 10, 15, 20 and 25 kGy).

$$\text{Tensile Strength (T.S)} = \text{The load at break/Original cross - sectional area} \tag{3}$$

The elongation at break % (E%)

$$\text{Elongation at Break (E\%)} = \text{Elongation at break/original length} \times 100 \tag{4}$$

and E-modulus (Young's Modulus) which can be provided from the slope of the curves.

$$\text{E - modulus} = \text{Stress/strain.} \tag{5}$$

Table 1 and Fig. 2 show the measured parameters tensile strength, elongation at break % and E-modulus of un-irradiated and irradiated chitosan films.

From the table, it is observed that the tensile strength increases at irradiated chitosan film with 5 kGy and decreases by increasing the radiation doses. Also, the results show that the Young's modulus increases by increasing the radiation dose in contrary to the elongation at break (it decreases).

Dielectric measurements

The dielectric constant which is due to the polarization of molecules and the energy loss are essential parameters for dielectric material science [23]. From the measured parameters, dielectric constant (ε′), dielectric relaxation loss (ε″) and A.C. electrical conductivity were calculated for the un-irradiated and irradiated films from the following equations:

$$\varepsilon' = CL/\varepsilon_0 A \tag{6}$$

where C is the capacitance measured, L is the thickness of the film, ε_0 is the permittivity of free space (8.85×10^{-12}) and A is the cross-

Table 1
Tensile strength, elongation at break and E-modulus for un-irradiated and irradiated chitosan films with different doses of γ-radiation.

Sample	Tensile Strength (MPa)	Elongation at Break (%)	E-Modulus (N/m^2)
Chitosan (CS) (0 kGy)	49.42 ± 1.23	5.49 ± 0.31	1040.35 ± 107.12
CS + (5 kGy)	51.99 ± 1.58	4.26 ± 0.68	1318.49 ± 278.65
CS + (10 kGy)	47.58 ± 1.53	3.66 ± 1.64	1418.17 ± 147.86
CS + (15 kGy)	43.28 ± 9.06	3.53 ± 0.68	1631.79 ± 314.86
CS + (20 kGy)	37.18 ± 7.96	3.18 ± 0.75	1739.17 ± 111.79
CS + (25 kGy)	31.01 ± 5.63	2.87 ± 0.98	1893.62 ± 389.47

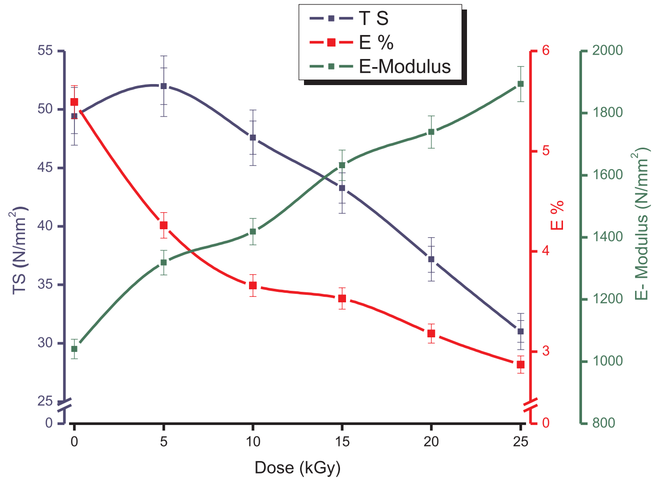

Fig. 2. The values of T.S., E% and E-modulus of un-irradiated and irradiated chitosan films with different gamma doses (the data are represented as mean value ± S.D.)

sectional area of the film.

$$\varepsilon'' = \varepsilon' \cdot \mathrm{Tan}\delta \tag{7}$$

where Tan δ is the dissipation factor measured.

$$\sigma_{A.C.} = \omega \varepsilon_0 \varepsilon' \mathrm{Tan}\delta \tag{8}$$

where ω is the angular frequency.

Dielectric constant (ε′)

Fig. 3 illustrates the change of ε′ in the frequency range of (50 Hz to 5 MHz) for un-irradiated and irradiated chitosan films with different doses (5, 10, 15 20 and 25 kGy) of γ-rays.

From the figure, it is observed that at constant dose, ε′ decreases by increasing the frequency, while it increases by increasing the radiation dose at constant frequency.

Dielectric relaxation loss (ε″)

Viscoelastic (dipoles on the side chains) and conductivity (ionic translational movement) relaxations are the two types of relaxations found in polymers [26]. Fig. 4 illustrates the variation of dielectric relaxation loss (ε″) within the frequency range (50 Hz–5 MHz) for un-irradiated and irradiated chitosan films at different doses (5, 10, 15 20 and 25 kGy) of γ-rays.

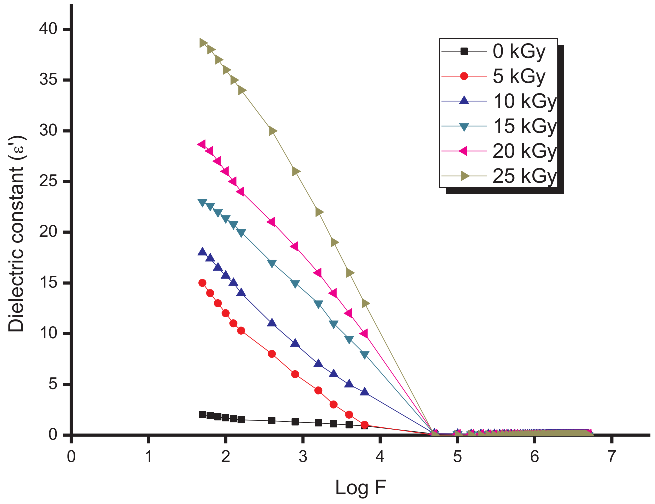

Fig. 3. Variation of the dielectric constant (ε′) of un-irradiated and irradiated chitosan films with different doses in the frequency range (50 Hz–5 MHz).

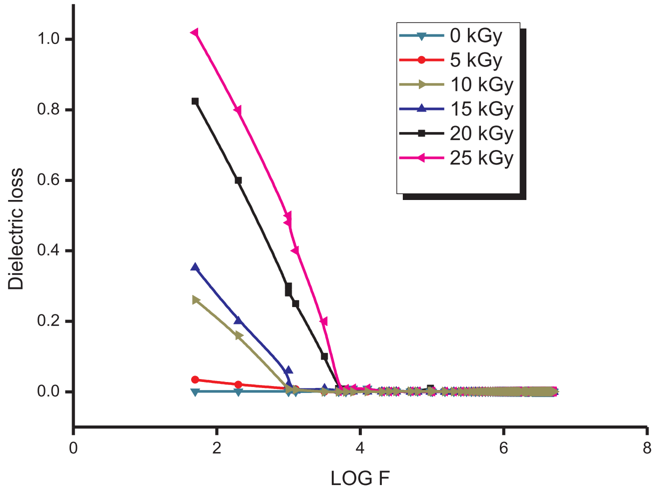

Fig. 4. Variation of the dielectric loss (ε″) of un-irradiated and irradiated chitosan films with different doses in the frequency range (50 Hz–5 MHz)..

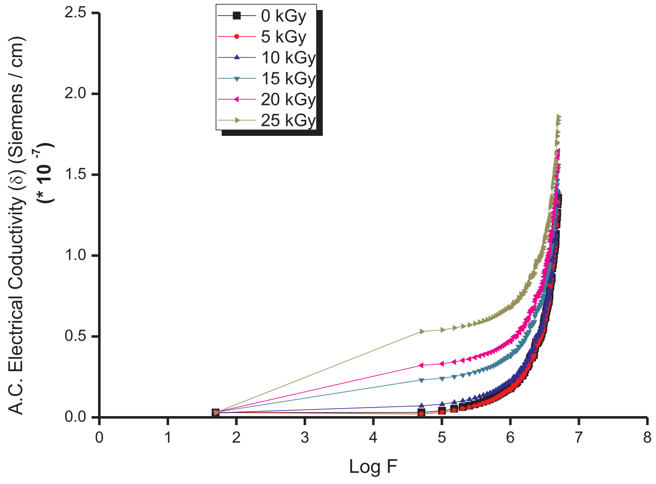

Fig. 5. Variation of A.C. Electrical conductivity $\delta_{A.C.}$ of un-irradiated and irradiated chitosan films with different doses in the frequency range (50 Hz–5 MHz).

Fig. 4 shows that at constant dose, ε″ decreases by increasing the frequency and it increases by increasing the radiation dose.

A.C. electrical conductivity

Fig. 5 illustrates the variation of A.C. electrical conductivity ($\delta_{A.C.}$) within the frequency range (50 Hz–5 MHz) for un-irradiated and irradiated chitosan at different doses (5, 10, 15 20 and 25 kGy).

From the figure, it is observed that the A.C. electrical conductivity ($\delta_{A.C.}$) increases with increasing the frequency. Also, $\delta_{A.C}$ increases by increasing the radiation dose at constant frequency.

Discussion

Mechanical properties

From Table 1, the increase of tensile strength at 5 kGy in comparison with the control film may be due to the crosslinking resulting from the formation of more polar groups (—OH) [10,15–18]. As the tensile strength increases the biodegradability increases which is promising in wound dressing material [19]. Also, the increase in tensile strength is a promising advantage because chitosan can increase the tensile strength of wounds [4]. While the reduction of tensile strength by increasing the radiation doses may be due to the degradation (chain scission) of chitosan chains resulting from radiation. This reduction results from the break of molecular chains and less chain entanglement. So, chitosan can no longer sustain any force on it due to these small chain entanglements [15,16,20].

The results of the Young's modulus and the elongation at break indicates that there is an increase in stiffness, rigidity and a decrease in elasticity of chitosan films by increasing the radiation dose [20,21].

At low gamma dose (5 kGy), the elasticity increases in comparison to the un-irradiated film and in comparison to the films irradiated with high doses. The crosslinking which occurs may reduce the ability of the chains to move [22].

Dielectric measurements

Dielectric constant (ε′)

From Fig. 3 and at low frequency, the decrease of ε′ may be due to that all the dipoles can orient in the same direction of the electric field without any delay. These dipoles make a cloud of charges around the electrodes (electrode polarization) which in turn inhibit the high frequency dielectric [24–26]. At high frequency, the induced dipoles begin to lag behind the applied field or the alignment dipoles with the applied oscillating field gradually failed and hence, the dielectric constant doesn't change [13].

The increase of ε′ by increasing the radiation dose is due to the strong orientation and the build-up of charges forming the acetate and the NH_3^+ ions. Radiation causing chain scission may increase the mobility of the small entanglements by weakening the interchain bonds [24,27].

High value of dielectric constant is preferred for antimicrobial activity because of NH_3^+ ions. These positive ions play an important role in damaging the microbial cell wall [27,28].

Dielectric relaxation loss (ε″)

In Fig. 4 the decrease of ε″ by increasing the frequency may be due the build-up of charge at the surface of each phase (Maxwell–Wagner–Sillars effect) [29]. Also, it may be due to the formation of space charge at low frequency followed by a sharp increase in dielectric constant and dielectric loss. No relaxation peaks are observed leading to a conduction process which means that the viscoelastic and the conduction relaxations are coupled [30].

The increase of ε″ by increasing the radiation dose at a constant frequency is attributed to higher relaxations due to conduction in chitosan electrolyte. This conduction results from charge migration between coordinate sites of chitosan [26,31].

A.C. electrical conductivity

In Fig. 5 the increase in $\delta_{A.C}$ due to increasing the frequency reveals that there may be more free charges involved in the hopping process through the defective sites along the macro polymer chains.

The increase of $\delta_{A.C.}$ by increasing the radiation dose can be ascribed to the high charge carrier motion assisted by larger segmental motion of the chitosan backbone due to chain scission (degradation) of the chitosan chains [13]. This result confirms the results of dielectric constant.

At low radiation dose (5 kGy), there is an accumulation of ions (crosslinking) between chitosan chains which make an immobile network due to hydrogen bonds resulting in a decrease in conductivity [30,31].

Conclusion

It is concluded that exposing chitosan films to gamma radiation causing degradation, due to the cleavage of β-1–4 glycosidic bonds of chitosan which reduce the molecular weight without changing its chemical structure.

Also, it is concluded that at low dose (5 kGy), the tensile strength of the films is improved which is preferred for the biomedical application especially as a wound healing dressing. While at high dose (25 kGy), the dielectric constant is improved which is preferred for the antibacterial property of the dressing. Thus, chitosan film with improved properties can be prepared by varying the radiation dose.

Finally, it is observed that irradiation of chitosan with 5 kGy gives optimum conditions in the properties studied and this can be needed in wound healing.

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