

Preparation of Chitosan/Collagen Blend Membranes for Wound Dressing: A Study on FTIR Spectroscopy and Mechanical Properties

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Abstract. The effect of different gamma-ray irradiations on the functional groups and the mechanical properties of chitosan, collagen, and chitosan/collagen blend have been studied. Commercially available chitosan and extract of collagen from bovine tendon by acid dissolution method were employed. Chitosan, collagen and chitosan/collagen blend membranes were prepared using the solvent evaporation method. The dried membranes were subjected to gamma-ray irradiation using 0, 15, and 25 kGy. The membranes then characterized by Infrared spectroscopy and measured in tensile and elongation at break. The irradiated chitosan membranes displayed reduced intensity of -OH, -CH, -NH and -C-O-C groups, whereas, the collagen membranes revealed amide I, II and III groups. The irradiated chitosan/collagen (50/50%) blend membranes demonstrated the decreased intensities in the -OH and amide groups. The application of gamma-ray irradiation had produced chitosan/collagen blend membranes with a tensile strength and elongation at break between the chitosan and collagen membranes. It was further determined that the decrease in the mechanical properties of the membranes was likely to be due to some changes of the functional groups.

Keywords: Chitosan, collagen, gamma-ray, tensile strength, elongation.

1. Introduction

Skin plays a major role in body protection. Severe damage to the skin may cause disruption or injury to skin structure and function, and gives the damage to intrinsic skin barriers [1]. In healing the wound, an inflammatory response occurred; the cells behind the dermis start building collagen, and it continues up to the end of establishing the epithelium. The healing process of skin damage may be sufficient with the promotion of wound dressing [1-3]. Wound dressings prepared from natural polymers have the capacity to uptake water [3-4]. The moist environment of wound dressings is aimed to maintain the existence of moisture around the wound, so it can accelerate tissue restoration and alleviates pain [1].

Natural polymers are widely acceptable for biomedical applications. Considerable research interest



is focused on blend polymers that may produce new polymeric materials for which has little been existing as a blend of nature. Nowadays, it has become common to blend biopolymers to produce new materials with better properties [5-7]. The blend of biopolymers, such as chitosan and collagen (chitosan/collagen), have been widely applied as wound dressings about their potentially beneficial biological properties. As an addition, spectroscopic techniques have been used to better identify the interactions and structural changes in the biopolymer network [8-9].

Chitosan is a nitrogenous polysaccharide deacetylated from chitin, found in cell walls of crustaceans, such as crabs [10]. Chitosan contains aliphatic ($-\text{CH}$), ether ($-\text{C}-\text{O}-\text{C}$) and hydroxyl ($-\text{OH}$) groups of the primary alcoholic group and a number of basic amino amide ($-\text{NH}$) resulting in cationic polyelectrolyte properties and act as an ion exchanger [11-12]. Similar to many cationic polymers, chitosan can disrupt the outer membrane of bacteria that possesses anions, and subsequently hamper the growth of infectious substances [13]. Wound dressing from chitosan may prevent penetration of bacteria through the membranes [13-14]. However, the hard and inelastic linear polysaccharide structure of chitosan is known to cause the limitation of its application [10]. One way to overcome the poor mechanical strength of this biopolymer is to modify the physical properties of the membrane.

Collagen is the substance that holds the whole body together and found in the extracellular matrix. Collagen consists of a repeating amino acid that constructs the connective tissue throughout the body, acting as the triple helix structure in tissues. An H atom of the side chain of glycine, links the NH peptide bond (amide II) and CH (amide III) of a glycine to a peptide carbonyl ($\text{C}=\text{O}$; amide I) group in an adjacent polypeptide and helps hold the three chains together and, therefore, can be involved in all 3 phases of the wound-healing cascade [15-16]. Collagen is the most abundant protein found in mammals; parts of an animal that can be the main sources of collagen for biomedical uses are skin or tendon, such as from rat-tail or bovine [17-18]. Collagen builds a network of macromolecules that provide structural support, in relation to strength and degree of elasticity. For clinical applications, the use of non-sterilized biomedical materials is associated with the risk of infectious disease transmission. Wound dressing needs to be perfectly sterile to avoid the transmission of any illness [2]. The physical methods including UV or gamma irradiation have been shown to be effective in sterilizing material for biomedical applications. Gamma-ray irradiation is preferred for sterilization of biomaterials due to some advantages. Gamma irradiation, which has less atmospheric pollution, is an easy and cost-effective process, is regarded for sterilization and the application is without the introduction of chemical reagents [19-21]. Gamma irradiation doses required for sterilizing medical devices are 15 to 25 kGy (ISO 11137) [22]. Gamma irradiation at 25 kGy was found to be suitable for sterilization of the dressings, and infrared spectral scanning has shown that papain was stable on gamma irradiation at 25–35 kGy [21]. Studies have also shown that gamma-ray irradiation may alter the mechanical properties of some biopolymers [19, 23]. Growing irradiation doses up to 60 kGy applied to gelatin films have raised their tensile strength, although, the chitosan/gelatin blend films lowered the elongation at break [23].

In relation to their potentially beneficial biological properties for wound dressings, chitosan and collagen are mostly environmentally friendly because they do not produce harmful residues. Previous research has shown the extractions of chitosan from crabs and collagen from bovine tendon to produce chitosan/collagen membranes [3, 14]. Here, chitosan/collagen blend membranes with or without gamma-irradiation up to 25 kGy have shown a capacity to uptake water [3] and also demonstrated the prevention of bacteria penetration power through the blend membranes [14]. On the other hand, the mechanical strength of wound dressings is of great importance because they give influence on the performance of the result. However, they have not been studied. Tensile strength and elongation at break are necessary to be investigated for unirradiated (initial) and irradiated wound membranes of single and blend components. Therefore, the purpose of the present study was to innovate wound dressing prepared with chitosan/collagen membranes by enhancing the properties of those obtained from chitosan or collagen membranes alone and analyze the effect of gamma-ray irradiation doses on their functional groups, tensile strength, and elongation at break.

2. Experimental Method

2.1. Materials

Commercial medical grade chitosan, extracted from crabs having 90 % degree of deacetylation (DDA) and molecular weight of 170 kDa was obtained from the Biotech Surindo (Cirebon, Indonesia). The bovine tendon was available from the Research Laboratory of the National Nuclear Agency, Indonesia. Reagents, i.e. glacial acetic acid (100 % pure) and sodium chloride were purchased from Merck (Darmstadt, Germany) and used without any further purification.

2.2. Methods

2.2.1 Chitosan membrane preparation

In the attempt to prepare chitosan, methods as provided in [5] and [7] were selected. Firstly, the chitosan powder was dissolved in 0.7 M acetic acid under a constant stirring of 750 rpm for 1 hour at 40 °C and continued without stirring at room temperature for 12 h to obtain a chitosan solution. This membrane-forming solution was cast in $7.5 \times 7.5 \text{ mm}^2$ and subjected to solvent evaporation at 25 °C for 48 h in order to obtain chitosan membranes. Each dried chitosan membrane was peeled off manually using a spatula and then stored in a polyethylene bag before characterization.

2.2.2. Collagen membranes preparation

Collagen was obtained by enzymatic hydrolysis and chemical hydrolysis [17-18]. Collagen achieved by biological processes used the addition of enzymes [6]; which is more promising, but more expensive. This study used the chemical hydrolysis process, which is more commonly used. Collagen was extracted from bovine tendon using the acid-soluble method following the study of [5]. After all washes, clean collagen bundles were immersed in a 0.7 M acetic acid solution, then remained at -4 °C for 12 h and after that, they were folded until they gelled. The gel was reconstituted by adding 4M NaCl and then stirred at 750 rpm for approximately 1 hour, until it was white cotton-like. Next, the fibers were separated from the NaCl solution. The precipitated fibers were washed using a phosphate buffer solution to neutralize the pH. They were then subjected to freezing at -20 °C for 12 h and immediately freeze-dried at -109 °C at 5×10^5 bar for 24 h. The dried collagen was dissolved in 0.7 M acetic acid and then stirred at room temperature to produce the collagen solution. The preparation of collagen membrane was identical to the previously described preparation of the chitosan membranes.

2.2.3. Chitosan/collagen blend membrane preparation

Before irradiation, the chitosan, collagen-chitosan/collagen blend membranes were packaged in polyethylene film to prevent oxidation during the process and to maintain sterility after irradiation. Chitosan and collagen solutions obtained from the previous step were mixed in the ratio of 50:50 and the stirring was maintained at 750 rpm at room temperature for 24 h. The preparation of the chitosan/collagen membrane was identical as previously described preparation of the chitosan membranes.

2.2.4. Irradiation Treatment

Before irradiation, the chitosan, collagen-chitosan/collagen blend membranes were packaged in polyethylene film to prevent oxidation during the process and to maintain sterility after irradiation. The membranes were irradiated under a ^{60}Co gamma-ray source using a linear electron accelerator of 7.9 kGy/h dose rate at room temperature. Doses required for irradiation in this study were 15 and 25 kGy. The membranes without irradiation were used as controls.

2.2.5. FTIR spectroscopy

The Fourier Transform Infrared (FTIR) spectrum from each membrane was obtained using a spectrometer UVmini-1240 (Shimadzu, Japan). The transmittance spectral profiles were determined at the wavenumbers ranging from 4000 cm^{-1} to 400 cm^{-1} .

2.2.6. Mechanical properties

The mechanical properties of chitosan, collagen and chitosan/collagen blend membranes were assessed by the measurements of tensile strength and elongation at break. The measurements were carried out according to ASTM Standard D882-91 [23]. The membranes were cut precisely to get rectangular ($2.5 \times 8 \text{ cm}^2$) test piece specimens. The specimens were placed in an extension grip of the Instron universal testing machine (Groove City, Pennsylvania, U.S) and stretched uniaxially with a 300 N load cell at a rate of 10 mm/min until breaking. The stress and strain curves obtained from the machine were used to determine the tensile strength and elongation at break [24]. The tensile strength was measured using the equation (1)

$$\sigma = F / A \quad (1)$$

Where A is the area of the specimen, and the elongation at break was calculated using Equation (2).

$$E = \left(\frac{L_1 - L_0}{L_0} \right) 100\% \quad (2)$$

Where L_0 is the specimen area before the test and L_1 is the extension when broken up. The measurements were carried out at room temperature, and five specimens were tested for each membrane.

3. Results and Discussion

3.1. Data

This study has shown chitosan, collagen and chitosan/collagen blend membranes regarding their functional groups and their mechanical properties. Visual inspection to the membranes showed that all membranes were in good homogeneity. FT-IR profiles for the non-irradiated and irradiated chitosan, collagen and the chitosan/collagen blend membranes in spectral ranged from $4000\text{--}400 \text{ cm}^{-1}$, X axis, wavenumbers in cm^{-1} , Y axis, the transmittance in % are presented in Figures 1, 2, and 3.

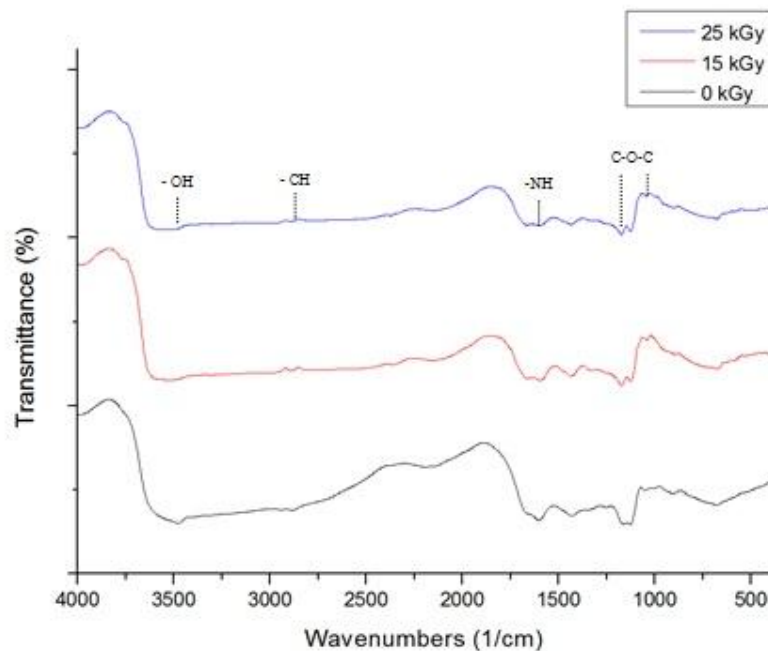


Figure 1. FTIR spectra for chitosan membranes with gamma-ray irradiation doses of 0,15 and 25 kGy

Figure 1 displays the spectra of characteristic peaks obtained from the chitosan membrane with and without gamma-ray irradiation that was similar. However, there were some changes in the peaks. The irradiated (0 kGy) chitosan membranes showed a peak of around 2993 cm^{-1} which corresponded to —CH of the aliphatic groups, a broadband around 3458 was due to —OH groups, whereas, the characteristic peaks were located at 1664 and 1041 cm^{-1} which represented the —NH (amide) and C—O—C groups, respectively. When gamma-ray irradiation was given in the doses of 15 or 25 kGy , the intensity of the peaks remained unchanged, with the exception of the —NH group that had its intensity decreased, as shown by visual inspection of the spectra.

The FTIR spectra in figure 2 showed characteristic peaks of the collagen membranes. Three different bands in the collagen membranes in position for amides I, II, and III characterizing the peptide bond. The peak of 1685 cm^{-1} was mainly associated with C=O (amide I) groups, the peak of 1573 appears from C—N , and N—H (amide II) groups, and the peak of 1249 cm^{-1} corresponded to CH_2 (amide III) groups of glycine backbone. After the implementation of 15 or 25 kGy gamma irradiation, they did not show any changes in their intensities.

The spectra of the chitosan/collagen blend membrane without gamma-ray irradiation can be seen in Figure 3. The chitosan/collagen blend membranes showed peaks from both the chitosan and collagen membranes that demonstrated changes. Three different bands showed a general shift in the positions of approximately 1692 , 1597 , and 1265 cm^{-1} due to amide I, II and III, respectively, as compared to those in the collagen membrane alone. Visual inspection of the spectra of the blend membranes irradiated with 15 kGy indicated a lower intensity of —OH peak, for which the heights were less evident when considering a combination spectrum obtained from differences in absorbance values and higher irradiation at 25 kGy showed further reduced the intensity of the peaks.

In terms of mechanical strength, figure 4 summarized the tensile strength for the resulting chitosan, collagen and chitosan/collagen blend membranes with variations in the irradiation doses of 0 , 15 and 25 kGy .

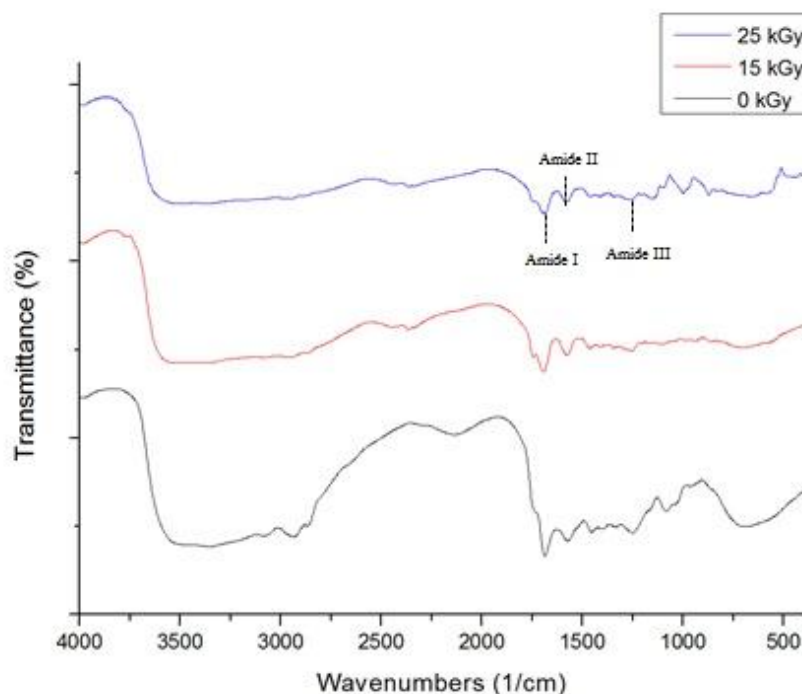


Figure 2. FTIR spectra for collagen membranes with gamma-ray irradiation doses of 0 , 15 , and 25 kGy

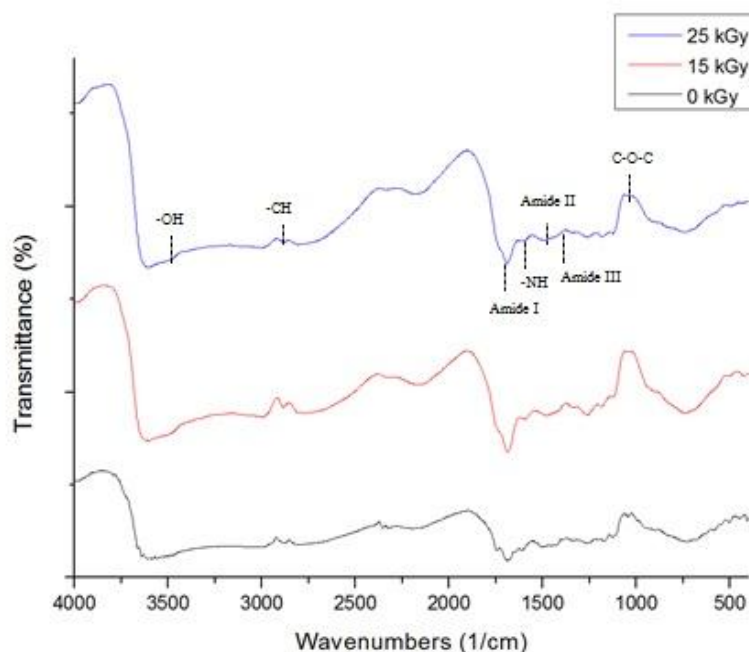


Figure 3. FTIR spectra for chitosan/collagen blend membranes with gamma-ray irradiation doses of 0, 15, and 25 kGy

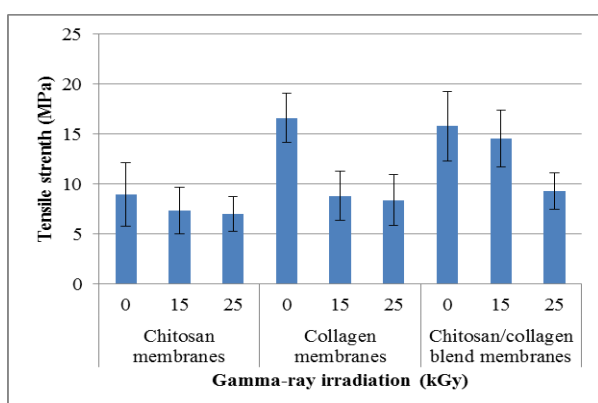


Figure 4. Tensile strength of chitosan, collagen and chitosan/collagen blend membranes irradiated with gamma-ray irradiation doses of 0, 15, and 25 kGy

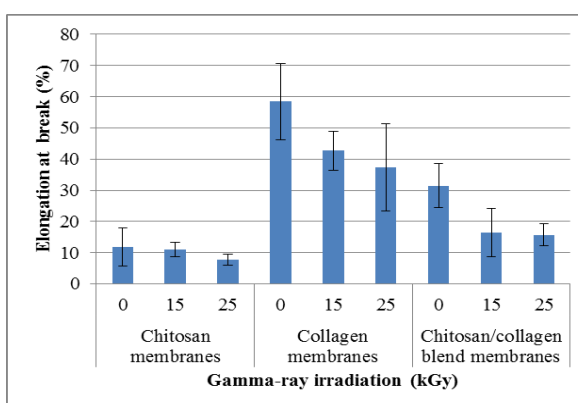


Figure 5. Elongation at break of chitosan, collagen and chitosan/collagen blend membranes irradiated with gamma-ray irradiation of 0, 15, and 25 kGy

As shown in Figure 4, the chitosan membranes without irradiation (0 kGy) showed the lowest tensile strength (8.97 ± 3.21 MPa). In contrast, the collagen had the highest tensile strength (16.61 ± 2.45 MPa), whereas, chitosan/collagen blend was between the chitosan and the chitosan/collagen (15.8 ± 3.48 MPa). With 15 kGy, the tensile strength of the chitosan, collagen and chitosan/collagen membranes decreased by 18.1%, 46.8% and 8.0% respectively, whereas, with 25 kGy, further decreased by 21.6%, 49.5% and 41.3%, respectively, when compared to that of each value without irradiation (0 kGy).

Regarding the elongation at break obtained from the chitosan, collagen and chitosan/collagen blend membranes against gamma irradiation doses, the values are plotted in Figure 5. In Figure 5, the chitosan membranes without irradiation demonstrated the lowest elongation at break (11.9 ± 6.1 MPa), the collagen had the highest elongation at break (58.5 ± 12.3 MPa), whereas, chitosan/collagen blend

was between the chitosan and the chitosan/collagen membrane blend (31.4 ± 7.1 MPa). Similar to those revealed from the tensile strength of all membranes. With irradiation of 15 kGy, the value from the chitosan, collagen and chitosan/collagen membranes decreased by 6.7%, 27.0%, and 47.6%, respectively, and a higher irradiation of 25 kGy occurred with lowering values, i.e. 33.9%, 36.1%, and 49.8%, respectively, in comparison to those without irradiation.

3.2. Discussion

Immersing the bovine tendon in an acidic solution has caused the solution to penetrate throughout the causing material swelling to two or three-time-bigger compared to its initial volume. The acetic acid used in this study was a common solvent to dissolve pure collagen partially. The non-cross-linked collagen, which was sensitive to the pH of the solvent, experienced dissociation. It may cause the non-covalent molecular bonds to disrupt.

The FTIR spectra of the chitosan/collagen blend membranes showed some peak changes in the characteristic peaks. The shifting of the amides I, II, and III groups of the blend membranes might indicate a new linkage between the chains contained the chitosan and collagen. A molecular interaction was likely to occur due to hydrogen bonding (H-O-H) and to electronic interaction between the cationic chitosan ($-\text{NH}_3^+$ groups) and neighboring anionic collagen ($-\text{COO}^-$ groups); these might produce a network of the blend membranes. Regarding the chitosan/collagen blend membranes irradiated with 15 kGy, the observed shifting in amides I, II, and III showed by lowering the intensity of each peak, indicating changes in the structure. With a higher irradiation of 25 kGy, further lowering of the peak intensities was likely to relate to further changes in the structure. This result was consistent with that of reported in [25] that utilized chitosan/starch films irradiated with gamma-ray.

The tensile strength or the elongation at break of the chitosan/collagen blend membranes were between each of the chitosan and the collagen membrane alone. The chitosan membranes showed a lower tensile strength or elongation at break when compared to those of the collagen membranes. It meant that the chitosan membranes were mechanically weaker than the collagen membranes. When chitosan was combined with collagen, the chitosan/collagen blend membranes could improve its tensile strength or its elongation at break. The improved values were probably related to the formation of attractive interactions between the chitosan and the collagen in the blend membranes. These attractive interactions were likely to cause additional interactions between chitosan and collagen, resulting in more stable network polymers, consequently, some enhancement in the strength. This meant that the collagen has a larger role in the blend membrane compared to that of chitosan. It agrees with the work reported [19] that showed the mechanical properties of chitosan membranes were improved by blending it with gelatin membranes. A previous study performed in [9] showed that there was also an increase in their tensile strength when collagen was added to chitosan to produce chitosan/gelatin blend membranes. Also, studies also showed that the mechanical properties of protein-based membranes are generally better than those of polysaccharide-based membranes [26].

The improvement of tensile strength and elongation at break of the blend membranes were likely to be due to an addition of a plasticizer. It is known that the addition of a plasticizer, in this case: collagen, can modify some of the physicomechanics of the blend membranes. In a previous study, [9] showed that there was also an increase in the tensile strength when collagen was added to chitosan to produce their blend membranes. Similarly, other researchers showed the mechanical properties of chitosan membranes raised by blending them with gelatin membranes [19].

When gamma-ray irradiation was applied, the chitosan, collagen or chitosan/collagen blend membranes demonstrated decreasing effects on their tensile strength and elongation at break. Regarding the characteristic peaks, this can be related to the lowering intensities of the amide I, II, and III peaks in the irradiated chitosan/collagen blend membranes. With irradiation of 15 kGy application, the main chains in each biopolymer might experience radiation degradation. After attaining the 25 kGy, the cross-linking probably showed further reduction and degradation increased gradually and the structure might have degraded into fragments. Observing the work reported in [9] of up to 5 kGy

doses, the tensile strength arose with the irradiation. However, at a higher dose of 10 kGy the tensile strength decreased. The authors proposed that the initial increase using the 5 kGy was due to a crosslink of both polymers. Then at a higher dose, the degradation of the chitosan chain (C-O-C) occurred. The irradiation might also destroy hydrogen bonds between those two macromolecules.

On the chitosan/collagen blend membranes, the values of the mechanical strength were between the chitosan and collagen membranes. The rise in the tensile strength and elongation at break of the blend membranes were likely to be due to the addition of a biodegradable plasticizer, in this case, the collagen. Therefore, it is required to overcome the relatively low mechanical strength of the chitosan membranes. The application of gamma-ray irradiation to the chitosan/collagen blend membranes, nevertheless, has lowered both tensile strength and elongation at break. However, when compared to the collagen membranes, the increase of both measurements in chitosan/collagen blend membranes may be an advantage. However, since chitosan has an antimicrobial property, it seemed that this added value might be produced from a chitosan/collagen blend membrane. Therefore, the chitosan/collagen blends may be applied as an alternative due to the combination of bacteriostatic of chitosan and the relatively high mechanical strength of collagen.

4. Conclusion

The addition of the collagen has improved both tensile strength and elongation at break of the chitosan/collagen blend membranes when compared to the chitosan membranes. It is supported by the infra-red spectroscopy results, which showed the shifting of the amide groups I, II, and III, in carbonyl and amino groups of collagen. The applications of gamma-ray irradiation with the doses of 15 or 25 kGy to the chitosan/collagen blend membranes has lowered the tensile strength and elongation at break. However, they were higher when compared to the chitosan membranes.

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