



Water Absorption of Chitosan, Collagen, and Chitosan/Collagen Blend Membranes Exposed to Gamma-Ray Irradiation

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Abstract

This study investigated the water absorption of chitosan, collagen, and chitosan/collagen blend membranes exposed to gamma-ray irradiation. These membranes were produced via the solvent evaporation method. All membranes then underwent irradiation at 15 or 25 kGy gamma-ray doses, while membranes without irradiation were used as controls. After immersing the membranes in distilled water for up to 180 min, water absorption was determined by calculating the percentage weight increase. The results demonstrated some changes in the water absorption curves with differing membranes and irradiation doses. However, after 60 min, all of the water absorption curves plateaued. With respect to membrane type, the chitosan membranes exhibited the highest water absorption; the blend displayed the lowest; and also the collagen was in the mid range. With the use of higher radiation doses, the chitosan membranes displayed lower water absorption, which was also true of the blend but not collagen membranes. To conclude, the water absorption of chitosan, collagen, and chitosan/collagen blend membranes with and without gamma-ray irradiation initially increased steadily and then plateaued. The water absorption values of the irradiated blend membranes were the lowest; however, the values were relatively steady.

Keywords: chitosan; collagen; gamma-ray; irradiation; water absorption

1. Introduction

Wound care is important, and wound dressing is an important part of the wound care process. A wound dressing should ideally provide an optimal healing environment, with the removal of excess exudate being an important part of providing this environment. To increase the absorption of excess exudate,

wound dressings should have the ability to absorb water [1]. Polysaccharides have been shown to be hydrophilic, thus are capable of absorbing water.

In the last decade, suitable polysaccharides, such as chitosan and collagen, have been studied to determine their potential for use in wound healing [1-3]. Chitosan (N-acetyl-D-

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glucosamine) is produced by the deacetylation of chitin, which is the structural element in the cell walls of fungi, insects, and in the exoskeletons of crustaceans, mainly crabs. Chitosan contains several functional groups, such as hydroxyl ($-OH$), ether ($-COC$), and amide ($-NH$), that are hydrophilic. These functional groups are also known to act as ion exchangers, allowing the formation of electrostatic complexes with other negatively charged polymers, such as collagen. Furthermore, due to their anti-bacteriostatic properties, chitosans have been widely explored for use in wound dressing [4, 5].

Collagen consists of three polypeptides chains: glycine, proline, and hydroxyproline, arranged as a triple helix. An H atom in glycine links the NH peptide bond (amide II) and CH (amide III) of the peptide carbonyl ($C=O$; amide I) group in an adjacent polypeptide, and this helps hold the three polypeptide chains in place [6]. The hydrophilic groups contained in glycine, proline, and hydroxyproline are carboxyl ($-COOH$), amide ($-NH$), and also $-OH$ in hydroxyproline. Collagen controls numerous cellular functions, thus plays a key role in the wound-healing cascade [7, 8].

Collagen is found in mammal skin [9], in squids [10], and in fish scales [11], as well as in the tendons of many animals [12, 13]. Compared to fish scales and squids, the bovine tendons contain approximately 85% [14-16].

To be used in clinical applications, biomedical materials must be sterilized to avoid the transmission of diseases. Irradiation, such as with ultraviolet (UV) and gamma-ray, is used to sterilize biomedical materials. Gamma-ray is preferred over UV for this purpose because it involves a lower level of atmospheric pollution and does not involve the introduction of any chemical reagents [17]. Furthermore, the gamma-ray irradiation process is cost-effective. According to ISO 11137, the gamma-ray irradiation dose needed to sterilize medical devices ranging from 15 to 25 kGy [18].

The water absorption of wound membranes exposed to gamma-ray irradiation has been studied over the last decade. Bajpai *et al.* studied chitosan films containing poly (acrylamide-co-itaconic acid) and found a decrease in water absorption [19]. With 25 kGy gamma-ray applied to collagen/polyglycolic acid blend membranes, Mazor and Zilberman [20] reported that water absorption was reduced from 110% to 60%. Similarly, Bano *et al.* [21] increased gamma-ray irradiation from 0 to 25 kGy and noted a reduction in the water absorption of chitosan/poly (vinyl alcohol) membranes from 120.9% to 62.2%.

In our previous studies, we investigated chitosan/collagen blend membranes made with bovine tendon-based collagens, exposed to

gamma-ray irradiation in doses appropriate to sterilize medical devices, i.e., 15 kGy and 25 kGy. We explored the intensities of the –OH, –NH, and –COO functional groups in blended membranes, including their effects on tensile strength and elongation at the break [22]. When the chitosan/collagen blend membranes exposed to 15 kGy were placed in an environment that contained *Staphylococcus aureus*, they reduced the bacterial penetration power [23].

Due to the soluble nature of polysaccharides; however, the membranes may begin to lose their physical integrity on exudating wounds during prolonged application. However, the absorption of exudate by sterilized wound dressings is of great clinical importance. Nevertheless, few previous studies have explored the water absorption of chitosan, collagen, and chitosan/collagen blend membranes after exposing these membranes to gamma-ray irradiation at 15 or 25 kGy. Therefore, the aim of this study was to observe the water absorption of chitosan, collagen, and chitosan/collagen blend membranes exposed to gamma-ray irradiation at 15 or 25 kGy.

2. Materials and Methods

2.1. Reagents

The reagents used in this study: glacial acetic acid (100% purity), sodium chloride, and sodium hydroxide were obtained commercially (Merck, Darmstadt, Germany). Chitosan powder extracted from crabs with 90% DDA and bovine tendon was obtained

from the Research Laboratory of the National Nuclear Energy Agency of Indonesia.

2.2. Preparation of Chitosan, Collagen, and Chitosan/Collagen Membranes

First, we prepared the collagen powder, which was extracted from bovine tendon using the modified acid-soluble method described by Fernandes et al. [8] and Ramasamy and Shanmugam [9]. Then, the chitosan, collagen, and chitosan/collagen blend membranes were prepared using the method described by Uriarte-Montoya [24]. In brief, a chitosan or collagen solution that was obtained by dissolving the powder in a 0.7 M acetic acid aqueous was stirred at 700 rpm for 2 h then maintained without stirring for 12 h at room temperature. The solution was then cast in a plastic plate (15 x 10 mm²) and left to dry for 48 h to produce the chitosan or collagen blend membrane. To produce the chitosan/collagen blend membrane, chitosan and collagen powders were mixed at a 50:50 ratio, then the preparation procedure described above was followed. All dried membranes were peeled off and maintained in clean plastic bag prior to usage.

2.3. Irradiation

The membranes were packaged in a specific plastic container provided for irradiation. The irradiating gamma-ray of 15 or 25 kGy at a rate of 7.9 kGy/h was applied to each membrane. Membranes without irradiation (0 kGy) were used as controls.

2.4. Water Absorption Determination

We determined the water absorption according to ASTM D570-98 [25]. The chitosan, collagen, and chitosan/collagen blend membranes were cut into precise rectangular ($1.5 \times 1 \text{ cm}^2$) test pieces. All membranes were first dried in an oven at 40°C for 24 h and immediately weighed (0 min) using an analytical digital balance that was accurate to the nearest 10^{-4} g. Each type of membrane was then immersed in distilled water at room temperature for 60, 120, or 180 min. After removal from the immersion, each membrane was swabbed with a dry cloth and immediately weighed. The water absorption (Wabs) was calculated using the following formula [19]:

$$Wabs = \frac{Wa - Wo}{Wo} \times 100\%$$

where Wo was the initial dry mass and Wa was the weight after immersion.

3. Results and Discussion

The water absorption values for the chitosan, collagen, and chitosan/collagen blend membranes that underwent gamma-ray irradiation at 15 or 25 kGy or did not undergo

irradiation (0 kGy) are shown in tables 1, 2, and 3, respectively. Values are given for the immersion times 0, 60, 120, and 180 min.

In table 1, for each immersion time, the chitosan membranes that were not exposed to irradiation (0 kGy) displayed higher water absorption values than those irradiated at 25 kGy. As shown in table 2, the opposite was true for the collagen membranes. In other words, the collagen membranes that were not exposed to irradiation (0 kGy) had lower water absorption values than those irradiated at 25 kGy. Regarding table 3, the chitosan/collagen blend membranes yielded results that were similar to those of the chitosan membranes in that the blended membranes not exposed to irradiation (0 kGy) displayed higher values than those irradiated at 25 kGy. For all membranes irradiated at 15 kGy, the water absorption values were between the corresponding water absorption results without irradiation (0 kGy) and at 25 kGy. The plots of time versus the water absorption values in tables 1, 2, and 3 are displayed as water absorption curves in figures 1, 2, and 3, respectively.

Table 1. Water absorption of the chitosan membranes with gamma-ray irradiation doses of 0, 15 or 25 kGy.

Time of immersion (min)	Water absorption (%)		
	Gamma-ray irradiation doses 0 kGy	Gamma-ray irradiation doses 15 kGy	Gamma-ray irradiation doses 25 kGy
0	$123,3 \pm 8.16$	$110,9 \pm 10.18$	$69,5 \pm 10.66$
60	$290,5 \pm 10.79$	$290,8 \pm 12.27$	$260,9 \pm 15.85$
120	$290,8 \pm 13.57$	$261,6 \pm 10.20$	$260,5 \pm 10.69$
180	$290,1 \pm 9.89$	$261,2 \pm 13.87$	$260,2 \pm 15.85$

Table 2. Water absorption of the collagen membranes with gamma-ray irradiation doses of 0, 15 or 25 kGy.

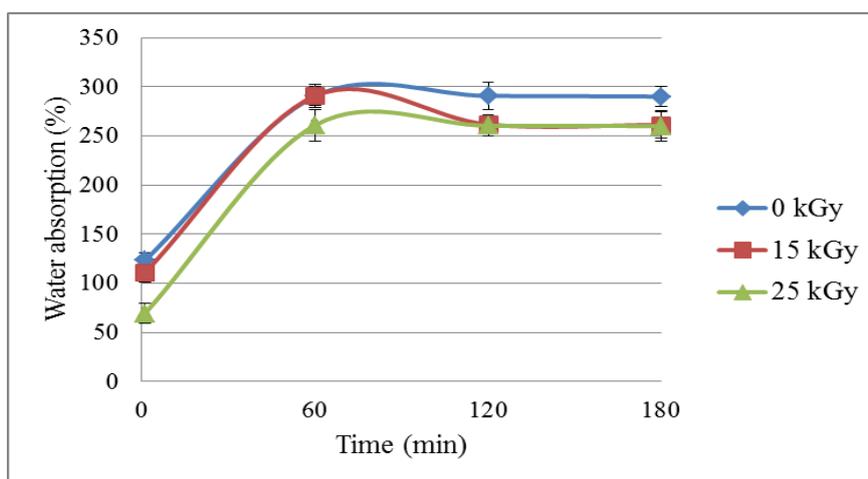
Time of immersion (min)	Water absorption (%)		
	Gamma-ray irradiation doses 0 kGy	Gamma-ray irradiation doses 15 kGy	Gamma-ray irradiation doses 25 kGy
0	76,0 ± 19.20	80,2 ± 20.79	113,2 ± 19.65
60	162,5 ± 18.50	179,4 ± 22.88	215,4 ± 18.12
120	155,5 ± 17.50	166,8 ± 23.60	203,2 ± 23.54
180	146,6 ± 18.93	168,8 ± 21.37	188,9 ± 20.80

Table 3. Water absorption of the chitosan/collagen blend membranes with gamma-ray irradiation doses of 0, 15 or 25 kGy.

Time of immersion (min)	Water absorption (%)		
	Gamma-ray irradiation doses 0 kGy	Gamma-ray irradiation doses 15 kGy	Gamma-ray irradiation doses 25 kGy
0	88,5 ± 7.61	78,3 ± 7.03	47,5 ± 11.76
60	128,5 ± 8.30	98,4 ± 10.27	74,5 ± 8.35
120	128,8 ± 9.90	98,5 ± 10.26	74,5 ± 8.35
180	126,7 ± 8.05	98,7 ± 7.28	73,5 ± 12.29

All membranes exposed to 0, 15, or 25 kGy gamma-ray irradiation demonstrated water absorption curves that rose sharply within 60 min. Further immersion after 60 min up to 180 min induced a plateau in all curves. Early in the

immersion period, instantaneous water absorption was observed for all membranes. After 60 min, all curves plateaued, showing that all membranes could only absorb a certain number of water molecules.

**Figure 1.** Water absorption of the chitosan membranes with gamma-ray irradiations doses 0, 15 or 25 kGy.

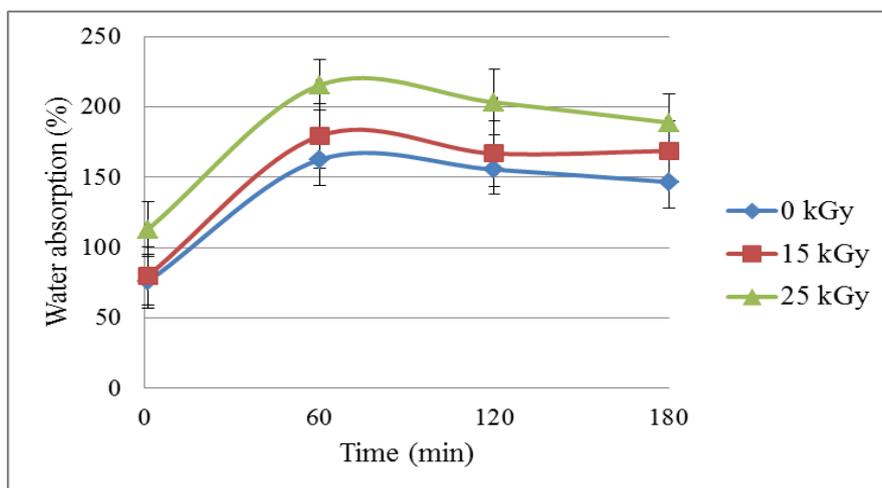


Figure 2. Water absorption of the collagen membranes with gamma-ray irradiations doses 0, 15 or 25 kGy.

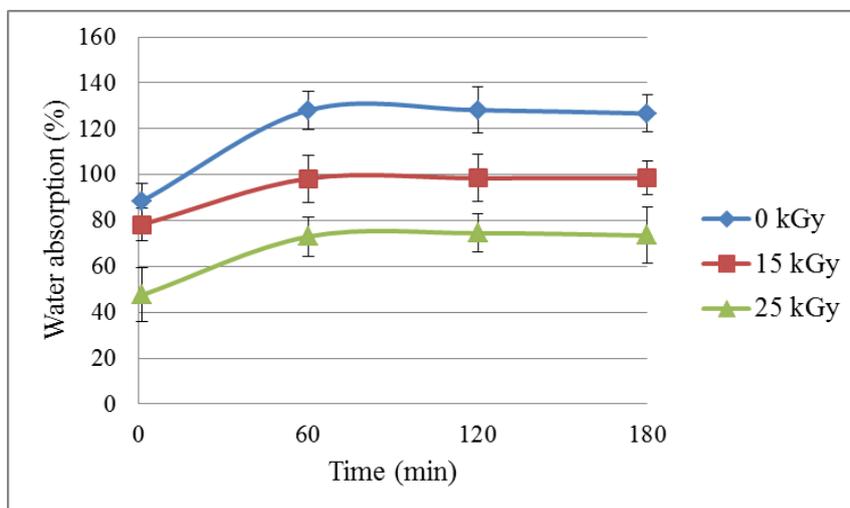


Figure 3. Water absorption of the chitosan/collagen blend membranes with gamma-ray irradiations doses 0, 15 or 25 kGy.

The water absorption of the chitosan/collagen blend membrane exposed to 25 kGy gamma-ray irradiation could be compared to results from other studies that used a similar dose. In 180 min of immersion, this study determined the percentage water absorption to be 73.5%, which was higher than the 60% obtained using the collagen/poly(DL-lactic-co-glycolic acid) by Mazor and Zilberman [20] and higher than the 62.2%

obtained from the chitosan/poly(vinyl alcohol) blend membranes tested by Bano *et al.* [21]

In this study, immersion of the chitosan, collagen, and chitosan/collagen blend membranes in water for up to 180 min caused increased water absorption. The water absorption values revealed were due to weight gain that likely occurred in relation to diffusion during immersion. We proposed a diffusion mechanism in the presence of water molecules, with regards to being ionized into H⁺ and OH⁻,

and were considered polar. They were trapped at the absorption sites, i.e., $-OH$, $-COC$, and $-NH$ functional groups in the chitosan membrane and $-OH$, $-COOH$, and $-NH$ in the collagen membranes. This absorption might encompass the hydrogen-bonded and/or hydrogen-unbonded state of the polar groups. The uneven water absorption of some membranes might be due to the uneven behavior of the fractional absorbed volume. This effect may also be explained by water transfer depending on the surface diffusion and, consequently, the sensitivity of the diffusion coefficient to the activation energy [26, 27].

Different water absorption was seen in different membranes. This may be due to the stranded structure of the glycine, proline, and hydroxyproline in collagen, as some of their functional groups, which are considered polar, may not be absorption sites. When immersed in water, the absorption sites that are located within the inner parts of the stranded structure may not make contact with water, and consequently, this reduce the water absorption of the collagen membranes. In the chitosan/collagen blend membranes, the relatively low water absorption (Table 3) may be due to the cross-linking between chitosan and collagen. Here, the $-NH$ of the chitosan membranes and the $-COO$ of collagen membranes may form a linkage, resulting in a $-COONH$ group, which is not considered polar. Thus, a decrease in the number of polar groups due to cross-linking between the two membranes reduced the number of water

absorption sites, resulting in steady water absorption curves for longer immersion times.

Exposure of the membranes to gamma-ray irradiation changed the water absorption of the membranes. In our previous study [22], after such exposure, the chitosan and chitosan/collagen blend membranes showed decreased intensities of the functional groups. When related to this study, it seemed that the reduced intensities of the functional groups caused decrements in the sites available for water absorption, thus lowering the water absorption. In contrast, when the higher gamma-ray irradiation dose was used on the collagen membrane, the hydrogen bonds that link the $-NH$ and $O=C$ groups in the polypeptide chains may have broken, as was explained in a previous study [28]. Consequently, more water absorption sites were created causing an increase in water absorption

With respect to the water absorption of the chitosan/collagen membranes exposed to gamma-ray irradiation at 15 kGy, it appears that the water absorption values ($98.4 \pm 10.27\%$ to $98.7 \pm 7.28\%$), were close to the value of 91.63% reported in the European Standard 13726 [29]. The data provided by the water absorption in this study, in addition to the mechanical properties [22] and bacterial penetration power [23] reported in previous studies, may serve as a baseline for wound dressing simulations and designs. Further research is therefore needed to examine the application of chitosan/collagen blend membranes after exposure to gamma-ray irradiation at 15 kGy in clinical settings.

4. Conclusion

The percentage water absorption of the chitosan, collagen, and chitosan/collagen blend membranes exposed to gamma-ray irradiation was initially high; however, after reaching peak values, the curves plateaued. The chitosan membranes showed the highest water absorption and the chitosan/collagen blend membranes showed the lowest. However, variations in gamma-ray irradiation doses resulted in steady water absorption curves in the chitosan/collagen blend membranes.

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