Development and Characterization of Cross-Linked Poly(acrylic acid) Hydrogel Containing Drug by Radiation-Based Techniques

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Abstract: Poly(acrylic acid) (PAAc) hydrogels possess good bioadhesive properties and allow enhanced penetration of drugs. In addition, it is possible to localize the absorption site of the drug in the hydrogel and increase the drug residence time. As opposed to other cross-linking processes radiation-induced polymer cross-linking can be easily and rapidly carried out without the use of cross-linking agents and other chemical additives. In this study, we fabricated metronidazole (MD) containing PAAc hydrogel (MD/PAAc) with different MD contents (0.1, 0.25, 0.5 wt%) using varying radiation doses (25, 50, 75 kGy) by gamma-irradiation. The physical and thermal properties were determined by gel content analysis, swelling ratio measurements, compressive strength measurements, differential scanning calorimetry, and thermogravimetric analysis. The properties of the hydrogel degraded due to the crystalline nature of MD. The properties of the hydrogel degraded due to the crystalline nature of MD. Cumulative release observed after 50 min in the case of 0.5MD/PAAc and 0.1MD/PAAc was 50% and 10%, respectively. Our findings suggest that MD/PAAc could be a suitable drug delivery carrier for use with radiation-based techniques.

Keywords : poly(acrylic acid); metronidazole; hydrogel; crosslinking; radiation

1. Introduction

A hydrogel is defined as a three-dimensional polymeric network that can be contained in water or body fluids [1]. Hydrogels have been widely used in biological applications such as wound dressings, adhesion prevention films, and contact lenses because of their marked similarity of human tissue, excellent biocompatibility, and controllable properties [2-4]. In addition, hydrogels retain stable volume in the body owing to the formation of stable structures that show little fluidity under the application of external forces. Hydrogels are fabricated by physical and chemical cross-linking processes [5, 6]. Physical crosslinking is achieved by the formation of ionic bonds, hydrogen bonds, crystallization between the polymer chains, or freezing/thawing method. In the freezing/thawing method, cross-linking is achieved by the formation of crystallites in the polymer-rich domain between water and the polymer chain during repeated freeze/thaw cycles. However, the inherent disadvantage is the long process time of 3 or 4 days [7]. In contrast, chemical cross-linking processes are fast in nature. In this case, cross-links are formed by chemical and radical reactions. Irradiation of polymer solutions results in polymer radicals, which combine through covalent bonds to form insoluble hydrogels. [8].

In the case of radiation-induced cross-linking, cross links are formed at the carbon atom of the C-C bond in the polyvinyl polymer, because one or more hydrogen atoms could be attached to the carbon atom. The radiation cross-linking method has many advantages: cross-linking agents are not required [9, 10]; no residual toxic substances are found in the hydrogel; hydrogels having diverse properties can be fabricated by simply controlling radiation dose, polymer concentration, and atmosphere of radiation exposure [11-13].

Hydrogels are typically composed of polymers such as natural polymers (*e.g.*, collagen, gelatin, hyaluronic acid, and chitosan) and synthetic polymers (*e.g.*, poly(acrylic acid) (PAAc),

polyethylene oxide (PEO), and poly(vinyl alcohol) (PVA)) [8,9,14,15]. The synthetic polymers allow for a higher degree swelling, and impart superior mechanical properties and adhesion strength to the hydrogels as compared to those in the case of natural polymers. For example, PAAc hydrogels show good adhesion strength and are used in bioadhesive drug delivery systems [16, 17]. PAAc is a hydrophilic and a highly absorbent polymer that has been widely applied to biomaterials due to its high solubility and biodegradability [5]. PAAc hydrogels have been widely used as drug carriers because of their good bioadhesive properties and enhanced drug penetration. It is possible to localize the absorption site of the drug in the PAAc hydrogel and increase the drug residence time [18].

Metronidazole (MD) is a nitroimidazole compound that has been widely used for treating infections caused by anaerobic bacteria and otitis media. The nitro group of MD is activated by nitroreductases such as ferredoxin in the body. High antibacterial property is indicated by disturbed DNA division and nucleic acid synthesis, which can be due to the destruction of the helical structure of the bacterial DNA [19].

In this study, PAAc and MD mixtures were exposed to γ -radiations, and MD containing PAAc hydrogels were verified by gel content analysis, swelling ratio measurements, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), compressive strength tests, and cumulative release of MD analysis. Past studies have reported the fabrication of hydrogels under to γ -irradiation. This study focuses on the MD containing PAAc hydrogel and can reveal a promising approach for the development of radiation cross-linking processes.

2. Experimental

2.1. Materials

Poly(acrylic acid) (PAAc, molecular weight = 100,000) was purchased from Waco Pure Chemical Industries, Ltd (Osaka, Japan). Metronidazole (MD) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents were of analytical grade and used as received.

2.2. Preparation of metronidazole containing poly(acrylic acid) film using γ -irradiation

PAAc was dissolved in distilled water using a magnetic stirrer to a final concentration of 7 wt%. MD (0.1, 0.25, 0.5, and 1 wt%) was dissolved in the PAAc solution by using a shaking incubator at 50 °C at 100 rpm until perfect dissolution, under dark conditions. The MD containing PAAc solution was irradiated using a ^{60}Co source (ACEL type C-1882, Korea Atomic Energy Research Institute) with different radiation doses of 25, 50, and 75 kGy (10 kGy/h) to prepare the MD containing PAAc hydrogel film (MD/PAAc). The general schematic and abbreviated MD/PAAc samples are shown in Figure 1 and Table 1.

Figure 1. Schematic diagrams of MD containing PAAc hydrogel films using γ -irradiation.

Table 1. Characteristics of MD containing PAAc hydrogel films at different radiation doses

Sample	PAAc content (wt%)	MD content (wt%)	Radiation dose (kGy)
PAAc	7	0	0
MD/PAAc	7	1	0
MD/PAAc25	7	1	25
MD/PAAc50	7	1	50
MD/PAAc75	7	1	75
0.1MD/PAAc	7	0.5	25
0.25MD/PAAc	7	1	25
0.5MD/PAAc	7	2	25

2.3. Differential scanning calorimetry

The thermal properties of MD/PAAc were investigated by DSC analysis (sQ100, TA instrument, New Castle, PA, USA) under nitrogen flow. The analysis was performed at a heating rate 10 °C/min from 100 to 180 °C.

2.4. Thermogravimetric analysis

Thermogravimetric analyses were performed using a TA Q600 system (TA Instrument, New Castle, PA, USA). 15.0 mg of the MD/PAAc sample was placed in a platinum pan. The analysis was performed at a heating rate 10 °C/min from 35 to 600 °C under nitrogen flow

2.5. Determination of gel content

Prior to gel content measurements, the MD/PAAc samples were dried until they were completely free of water. MD/PAAc test specimens were prepared using an 8 mm biopsy punch (Integra, USA). The initial weight of the samples was recorded, and the samples were stirred in distilled water for 24 h at room temperature to remove insoluble components. Then, the MD/PAAc samples were dried and weighed for calculating the gel content. The gel content in the film was determined from the following equation:

$$\text{Gel content (\%)} = (W_f / W_i) \times 100$$

where W_i and W_f represent the initial and final weights of the dried samples, respectively.

2.6. Determination of swelling ratio

To measure the swelling ratio, the dried film was prepared by an 8 mm biopsy punch (Integra, USA) and then immersed in distilled water for different time intervals at room temperature, until the equilibrium swelling state was reached. The swelling ratio of the film was determined from the following equation:

$$\text{Swelling ratio (\%)} = [(W_w - W_d) / W_d] \times 100$$

where W_d and W_w represent the weights of dry and wet film, respectively.

2.7. Compressive strength

The MD/PAAc sample was prepared by using a 12 mm biopsy punch and then subjected to compressive strength measurements using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd., Godalming, UK) with a load of 50 N and a working distance of 20 mm from the punch. The compressive strength was measured under 50% compression, from the decomposition of the hydrogel film between the plates of the test machine, at a crosshead speed of 5 mm/s.

2.8. Attenuated total reflection Fourier transform infrared spectroscopy

The chemical properties of the MD/PAAc samples were measured using an FTIR instrument in ATR mode (ATR-FTIR, Bruker TEMSOR 37, Bruker AXS. INC., Karlsruhe, Germany) over the range 650 to 4000 cm^{-1} at a resolution of 6 cm^{-1} averaged over 64 scans.

2.9. Release test

MD/PAAc hydrogels were dried at room temperature. Each dried sample was immersed in phosphate buffered saline (PBS) and incubated in a water bath (BS-21, JEIO TECH, Daejeon, Korea) at 37 °C for 120 min, with gentle shaking at 50 rpm. Samples were acquired at pre-determined time intervals (0, 10, 20, 30, 40, 50, 60, and 120 min), and the released MD was measured using a UV-Vis spectrophotometer (S-3100, Scinco, Seoul, Korea). The absorbance was recorded at 319 nm.

2.10. Statistical analysis

All data were presented as mean standard deviation (SD) for $n = 4$. The t-test (Excel, Microsoft) was used to assess the statistical significance of the results ($p < 0.05$).

3. Results and Discussion

Under γ -irradiation, PAAc formed free radicals on the polymer chain [17]. The water molecule formed a hydroxyl radical ($\text{OH}\cdot$) and a hydrogen radical ($\text{H}\cdot$). The C-H bond of PAAc was broken by the hydroxyl radical ($\text{OH}\cdot$) to form a free radical on the PAAc chain. The released hydrogen radical ($\text{H}\cdot$) combined with $\text{H}\cdot$ from water, leading to hydrogen gas evolution. In addition, the free radical of PAAc can cross-link with other PAAc molecules, resulting in the formation of a PAAc hydrogel [22]. The overall reaction mechanism of MD/PAAc is shown in

Figure 2.

Figure 2. Chemical mechanism of MD containing PAAc hydrogel films using γ -irradiation

MD has 4 main physical characteristics: (1) it is difficultly soluble in common solvents; (2) it has a crystalline structure and the crystals are off-white in color; (3) it is temperature-sensitive; and (4) its color is easily changed upon exposure to light [20, 21]. The color of the MD/PAAc hydrogel films changed to dark yellow with an increase in the radiation dose from 25 to 75 kGy, due to prolonged exposure to high-energy γ -radiation. The optical images and thicknesses of the MD/PAAc films are shown in Figure 3 and Table 2.

Figure 3. Optical images of MD containing PAAc hydrogel films using γ -irradiation

Table 2. Thickness of MD containing PAAc hydrogel films at different radiation doses

Sample	Thickness (mm)
MD/PAAc25	0.207±0.030
MD/PAAc50	0.243±0.047
MD/PAAc75	0.317±0.029
0.1MD/PAAc	0.280±0.052
0.25MD/PAAc	0.243±0.047
0.5MD/PAAc	0.277±0.035

The thermal properties of MD/PAAc were confirmed using DSC and TGA data, as shown in Figure 4 and Figure 5. The melting temperature (T_m) of MD/PAAc increased with an increase in MD content. T_m of 0.1MD/PAAc, 0.25MD/PAAc, and 0.5MD/PAAc was 132.68, 135.33, and 135.74 °C respectively, and the difference between these values increased by approximately 3 °C after exposure to γ -radiation. In addition, the latent heat increased with an increase in MD content, suggesting that the high crystallinity of MD, as shown in Table 3. The initial

degradation temperature of MD/PAAc was approximately 60 °C, and further degradation peaks were observed at 210 and 340 °C owing to the decomposition of MD [22]. The residual amounts of PAAc were 89.91% and 63.87% at 210 and 340 °C, respectively. The MD/PAAc content was 86.67% and 61.08% at the same temperatures, and the weight decreased with an increase in the MD content. Furthermore, the remaining amounts of MD/PAAc75 were 83.71% and 58.86%, which were lower than that of MD/PAAc, as shown in Table 4. The thermal stability decreased slightly as the radiation dose was increased because MD slightly affects the thermal stability.

Figure 4. Thermal properties of MD containing PAAc hydrogel films by DSC analysis: (a) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation doses at 25 kGy, (b) 1wt% MD containing PAAc hydrogel films with different radiation doses (0, 25, 50, and 75 kGy).

Figure 5. Thermal properties of 1wt% MD containing PAAc films using TGA, at different radiation doses of 0, 25, 50, and 75 kGy.

Table 3. Melting point and latent heat of MD containing PAAc hydrogel films with different radiation doses, as determined by DSC

	Tm (°C)	Latent heat (J/g)
0.1MD/PAAc	132.68	2.68
0.25MD/PAAc	135.33	3.36
0.5MD/PAAc	135.74	3.86
MD/PAAc25	142.94	4.11
MD/PAAc50	141.47	3.57
MD/PAAc75	140.19	2.75

Table 4. Residual amounts (210 °C and 340 °C) of MD containing PAAc hydrogel films at different radiation doses determined by TGA.

Sample	210 °C	340 °C
PAAc	89.91	63.87
MD/PAAc	86.67	61.08
MD/PAAc25	86.13	60.96
MD/PAAc50	85.68	60.14
MD/PAAc75	83.71	58.86

The gel content of MD/PAAc at different radiation doses and MD contents is shown in Figure 6. The gel content of PAAc was as high as 88.65%, with a dense three-dimensional network. However, as the MD content was increased from 0.1 to 0.5 wt%, the gel content significantly decreased at 25 kGy: the gel content for the 0.1, 0.25, and 0.5 wt% MD containing samples was 82.58 %, 71.33%, and 53.06 %, respectively, as shown in Figure 3(b). The gel content of the only dried MD/PAAc was 64.29%, indicating no cross-linking. The MD/PAAc samples exposed to increased radiation dose at 25, 50, and 75 kGy had significantly lower gel contents of 49.38%, 40.71%, and 29.54%, respectively, as shown in Figure 3(a). This result suggests that the crystallinity of MD increased with increased radiation dose, as the samples were kept at room temperature for a long period of time during the irradiation process.

Figure 6. Gel content of MD containing PAAc hydrogel films: (a) 1 wt% MD containing PAAc hydrogel films with different radiation doses (25, 50, and 75 kGy), (b) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation dose of 25 kGy.

Figure 7. Swelling ratio of MD containing PAAc hydrogel films: (a) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation dose of 25 kGy, (b) 1 wt% MD containing PAAc hydrogel films with different radiation doses (25, 50, and 75 kGy).

Figure 7(a) shows that the swelling degree of MD/PAAc increased with an increase in MD content, as the porous three-dimensional networks were affected by MD incorporation. While the swelling degree of MD/PAAc 25 was 2,829%, the MD/PAAc75 sample showed a swelling

of 4,963%. This suggests that MD/PAAc exhibits increased gel content with increasing radiation dose. However, after 40 min, the swelling degree decreased due to the destruction of the hydrogel structure by the crystalline nature of MD.

Figure 8. Compressive strength of MD containing PAAc hydrogel films: (a) 7 wt% PAAc hydrogel films with different radiation doses at 0, 25, 50, and 75 kGy, (b) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation dose at 25 kGy. (c) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation dose at 50 kGy. (d) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation dose at 75 kGy. (N.D = not detected)

Figure 8 shows the compressive strength of PAAc and MD/PAAc for different radiation doses and MD contents. In this study, a 50% compression was chosen for the compressive strength of the β -glucan hydrogel. The compressive strength of PAAc was found to increase with the radiation dose at 25 kGy (204.08 ± 42.78 MPa), 50 kGy (242.17 ± 12.01 MPa), and 75 kGy (254.89 ± 15.02 MPa), because of the larger degree of cross-linking in the hydrogels.

At 25 kGy, the compressive strength of 0.1MD/PAAc 25 was 130.24 MPa (± 53.16 MPa), while that of 0.5MD/PAAc25 was 63.72 MPa (± 22.86 MPa), indicating a decrease of approximately 60 MPa. The same trend in compressive strength was observed at the other radiation doses.

Figure 9. Chemical properties of MD containing PAAc hydrogel films using ATR-FTIR: (a) MD containing PAAc hydrogel films with different contents of MD (0, 0.1, 0.25, and 0.5 wt%) and radiation dose at 25 kGy, (b) 1wt% MD containing PAAc hydrogel films with different radiation doses (0, 25, 50, and 75 kGy).

Chemical characterization of the MD/PAAc samples was carried out using ATR-FTIR, and the results are shown in Figure 9. The presence of PAAc was confirmed by -COOH (carboxylic acid group) and -OH (hydroxyl group) stretching vibrations at 1700 cm^{-1} and 3400 cm^{-1} , respectively [14, 23]. New peaks from the nitrite group of MD/PAAc were observed at 1363 cm^{-1} and 1541 cm^{-1} due to the presence of MD [24]. The peak intensity of the nitrite group increased with the MD concentration. In contrast, for MD/PAAc, there was no change in the intensity of the nitrite peak with an increase in the radiation dose from 25 to 75 kGy.

Figure 10. Drug release profile of MD containing PAAc hydrogel films.

The MD/PAAc hydrogels were dried at room temperature. The drug release was determined by placing it in PBS (pH 7.4). The release of MD was determined at various times by UV spectroscopy. The absorbance was recorded at 319 nm. The MD drug release profiles from the PAAc hydrogels were investigated and are shown in Figure 10. As shown in Figure 10, the drug release confirmed that MD was slowly released without any initial burst. The drug was consistently released from the PAA hydrogel (0.5MD/PAAc25), reaching 60% at approximately 60 min in PBS. However, 0.1MD/PAAc exhibited a release of approximately 10 % at approximately 60 min in PBS.

4. Conclusion

In this study, MD containing PAA hydrogel films were synthesized by mixing various quantities of MD and PAA, followed by γ -irradiation at different doses. The physical, chemical, and thermal properties of the MD/PAA samples were observed and characterized. When the

fabricated 0.5MD/PAA25 was exposed to γ -irradiation, the gel content was significantly decreased as compared to that for MD/PAA. The presence of MD in MD/PAA was confirmed by the observation of nitrite group peaks at 1363 cm^{-1} and 1541 cm^{-1} , with an increase in peak intensity with the MD content. In addition, the thermal properties of MD/PAA degraded with an increase in the radiation dose and MD content. After 50 min, the cumulative release of 0.5MD/PAA was more than 50%, whereas that of 0.1MD/PAA was 10%. Therefore, MD/PAA could be a viable candidate for a drug delivery carrier, with radiation-based techniques.

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