RESEARCH ON PREPARATION OF FISH GELATIN HYDROGEL BY ELETRON BEAM IRRADIATION TO APPLY AS BIO MATERIAL

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Abstract: Hydrogel based on fish-derived gelatin was prepared by electron beam (EB) irradiation under the linear accelerator in TARRI, QST, Japan. The gelatin derived from the Tilapia fish with different molecular weights was mixed in high concentration range from 10 – 50 wt.% in distilled water and Phosphat buffer solution (PBS) of 1M at 30 °C, and then irradiated by gamma and EB in the dose range of 10 – 160 kGy. The heat-resistance of fish gelatin was determined by differential thermal analysis (DTA). And the results indicated that heat-resistance of the gelatin was improved by EB. The gel fraction of the fish gelatin at the initial concentration of 30 wt.% increased with absorbed dose, and reached up to 80 % at dose of 100 kGy. The elastic modulus of the gelatin gels prepared at concentration of 30 wt.% solution by EB irradiation with the doses from 20 to 100 kGy were 100 – 500 kPa, which are suitable for the use as bio-materials.

Key words: *gelatin, hydrogel, electron beam, irradiation*

I. INTRODUCTION

Gelatin is a kind of protein obtained by the partial hyrolysis of collagen derived from bone, skine and scale of animals (such as pork, beef, fish…). It has been widely used as gelling agent in many fields such as food, cosmetic, pharmaceutical, and medical because of its specific characteristics: biological origin, non-antigenicity, biocompatibility and biodegradability [1, 2]. Depending on the source of collagen and the hydrolytic treament used, there are several varieties of gelatins with different compositions. Most commercial gelatins are porcine and bovine gelatins extracted from the mamalian source because of abudant resource and cheap price. Addition, the high gel strength is also an advantage of these gelatins. However, products made of mamalian gelatins have concern with ethic problem or religious issue (Judaism, Islam and Hindu). From this necessary demand, fish gelatin can replace and resolve this disadvantage of porvine and bovine ones [1].

Gelatin derived from fish has some disadvantages such as weak strength, low gelling and melting temperature because of the difference in content of proline and hydroxyproline. In recent years, there are many researchs that improve the gel strength of fish gelatin through the crosslinking by the enzymatic, chemical and physical processes or irradiation method [3 – 9]. There are specific advantages in irradiation method compared with other ones due to it can not required any crosslinking agents, organic solvents, but can sterile product. With this method, gelatin hydrogel will be useful to apply for clinical medicine and tissue engineering [2, 12, 13]. Nanogels or micellar gels of gelatin prepared by irradiation method was also studied to apply for drug delivery in pharmaceutical applications [9 – 11].

The purpose of this research is the preparation of hydrogel from fish-derived gelatins with different molecular weights (M_w) by EB irradiation for the biomedical applications.

II. EXPERIMENT

II.1. Materials

- Gelatin derived from Tilapia fish with various bloom strength: Bloom 134 (BL134), Bloom 222 (BL222), Bloom 297 (BL297), Nitta Gelatiin Inc.

- Buffer phostphate powder (PBS), Wako Pure Chemical Insustries, Ltd., Japan

II.2. Methods

- **Preperation of gel**: The gelatin was mixed in high concentration range from 10% to 50% in diluted water (Dw) and buffer phostphate (PBS) of 1M, and then irradiated by γ – ray or electron beam (EB) in the dose range of 10 – 160 kGy.

- **Determination of gel fraction:** The gel was dried at 30 °C in the atmosphere pressure for 24h and then in vacuum for 24h. After measuring the weight of dried gel, it was soaked in the water at 30 °C in 48h to elute the soluble part to water. The insoluble part was seperated by a stainless steel net (150 mesh) and measure its weight after drying.

- **Determination of swelling ratio:** The weight of dried gel was measured. Then it was soaked in diluted water at room temperature in 48h to obtain the hydrogel and then its swollen weight was determined.

- Gel strength: The gels obtained by EB irradiation with the dose rate of 10 kGy/pass in the dose range of 20 – 100 kGy. Their compressive elastic modulus were analyzed by Creep Meter RE-3305C, Yamaden co.,ltd. with a rate of 0.05 mm/s and load cell of 20 N at room temperature. And their compressive elastic modulus were plotted as the funtions of radiation dose.

- **Thermal analysis:** TGA and DTA was analysed by the DTG-60, Shimadzu, Japan with the heating speed of 10 °C/minute in the temperature range of 30 – 600 °C and in nitrogen gas condition.

III. RESULTS

III.1. Gel fraction and swelling ratio

The Fig.1.1 showed the effect of absorbed dose on the gel fraction of BL222 irradiated by gamma (a) and EB (b).

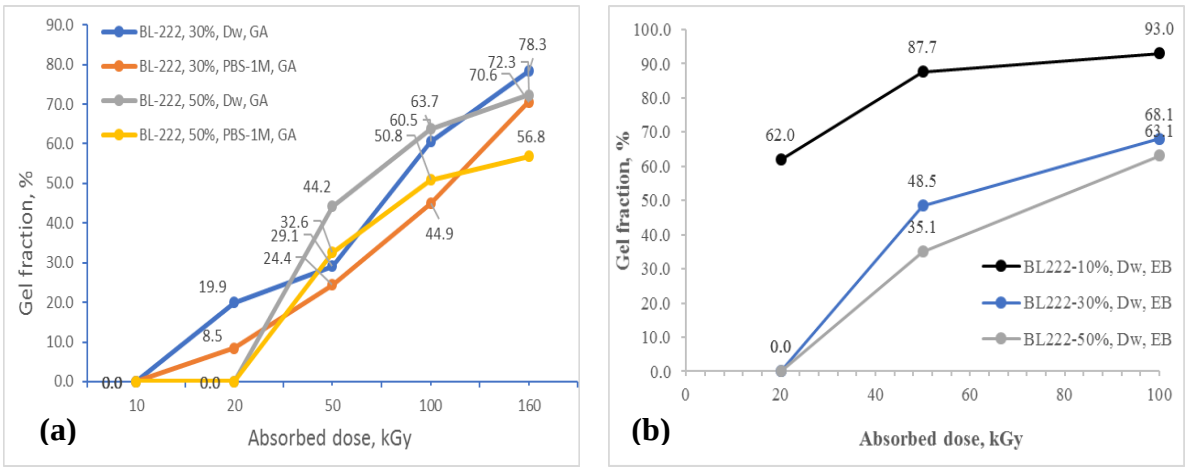

Fig.1.1. *Effect of absorbed dose on the gel fraction of BL222 irradiated by γ-ray (a) and EB (b)*

There are no gel can be observed with the gamma irradiated samples of 10% gelatin, namely that the fish-derived gelatin BL222 couldn't crosslinked in the analysed condition, so it was not presented in the Fig1.1a. For the samples that having gelation concentration higher than 30% in both DS and PDS solution, the resuts (Fig.1.1a) showe that BL222 started crosslinking by gamma irradiation at the dose of 20 kGy. It had the same tendency that gel fraction increased steadily in the increase of dose. The figure also indicated that the

crosslinking of BL222 was easier at higher doses. However, the gels obtained from the gelation disolved in water were higher than that dissolved in PBS with the same concentration at the same dose. For example, with concentration of 30% at the dose of 100 kGy, the gel fraction of BL222 gelatin in water and PBS were 60.5% and 40.9%, respectively. With the presence of PBS, the gelatin mixture was partially seperated, so that preventing the combination of free radicals, which formed by irradiation.

As observed in the Fig.1.1b, the samples irradiated by EB showed higher gel fraction than that irradiated by by γ-ray in the same condition of concentration and absorbed dose. For example, the samples of 30% BL222 irradiated at the dose of 50 kGy by γ-ray and EB have the gel fraction of 29.1 % and 48.5 %, respectively. Even the crosslinked gels can be obtained with the 10% gelatin sample irradiated by EB at the dose of 20 kGy. The figure also revealed that the gel fraction of BL222 irradiated by EB decreased in the upward trend of concentration at the same dose.

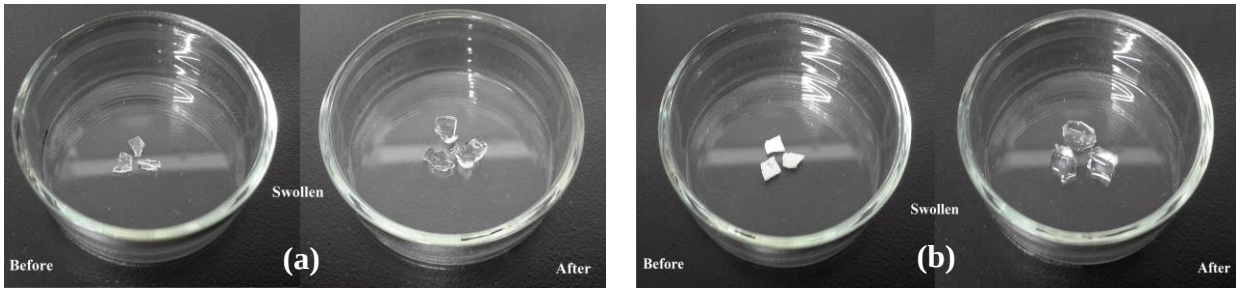

Fig.1.2. *BL222 with concentration of 30% in diluted water (a) and PBS 1M (b) irradiated by γ - ray at the dose of 50 kGy*

Bloom test is a measure of the gel strength of gelatin, reflecting the average molecular weight of its constituents. So the higher values of bloom, the higher molecular weight of gelatin. In this experiment, the gelatins derived from Talipia fish with difference M_w included BL134, BL222 and BL297 were dissolved in diluted water and then irradiated by EB in the dose range of 20 – 100 kGy with the dose rate of 10 kGy/pass.

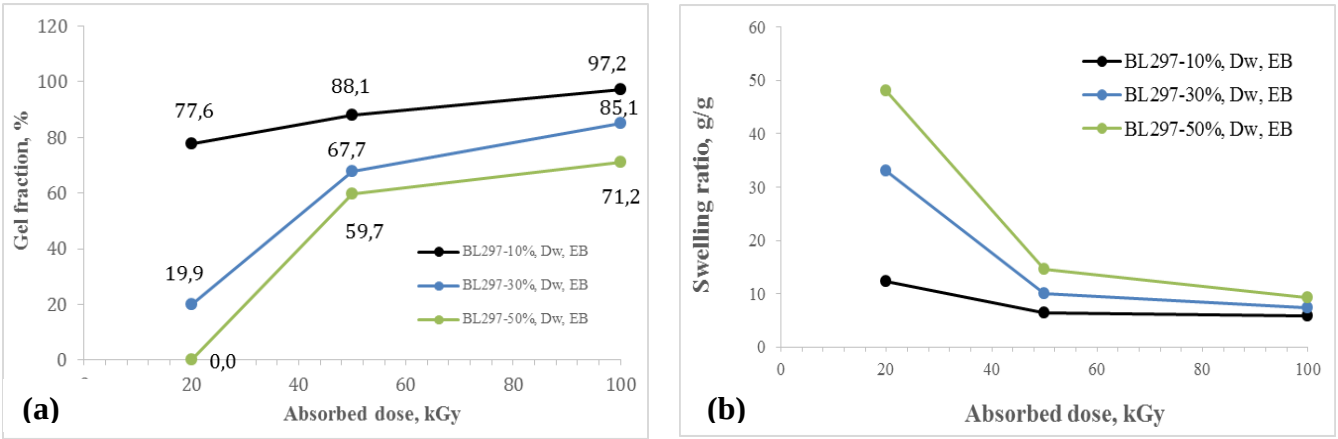

Fig.1.3. *Effect of absorbed dose on the gel fraction of BL297 (a) and swelling ratio of BL297 (b) with different concentrations*

The Fig.1.3a showed that all Blooms prepared in the low concentration of 10% could be crosslinked by EB irradiation. They had the same tendency that gel fraction increased steadily in the growing trend of dose in the range of 20 – 100 kGy. However, their gel fraction decreased in the increase of concentration at the same absorbed doses. For example, at the absorbed dose of 50 kGy, gel fraction values of BL297 are 88.1%, 67.7% and 59.7% in concentration of 10%, 30% and 50% respectively. The results suggested that the higher

concentration, the lower ratio of free radical in the dissociation of aqueous molecular after irradiated.

In addition, the result of Fig.1.3b showed that the swelling ratio was opposite with the gel fraction. The higher gel fraction got, the lower swelling ratio became. It was totally suitable with the previous results. The results of BL134 and BL222 were similar with that of BL297.

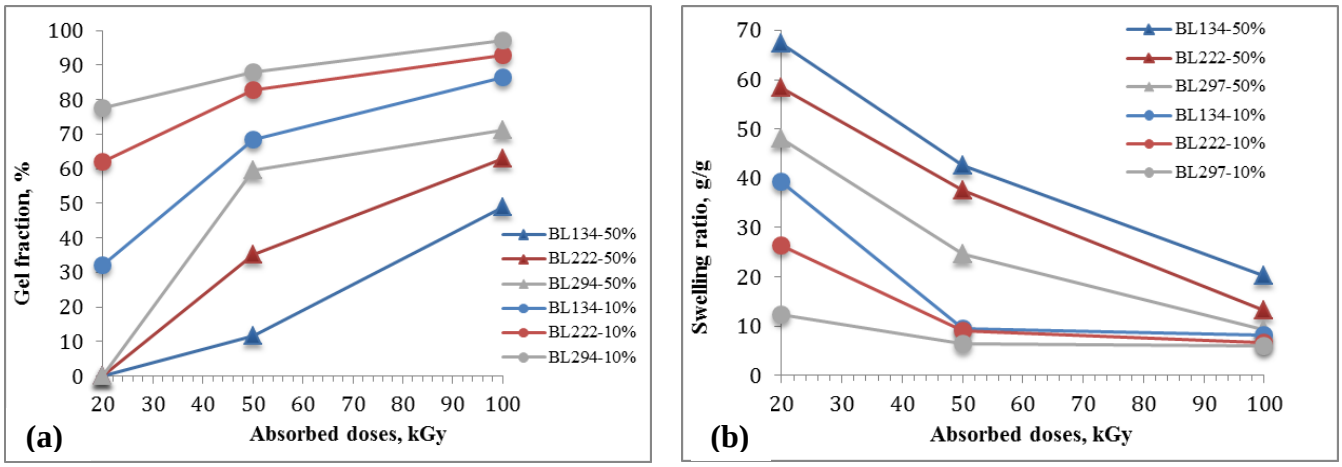

Fig.1.4. *Effect of absorbed dose and M_w on the gel fraction (a) and swelling ratio (b) with concentrations of 10 % and 50 %*

From the results presented in the Fig.1.4a, the M_w had determined the gel fraction of gelatin sample in the same concentration. In particular, the higher molecular weight, the higher gel fraction. For example, at the dose of 50 kGy and concentration of 10 %, the gel fraction values were 11.7 %, 35.1 % and 67.7 % for the BL134, BL222 and BL297 gelatin, respectively. Fig.1.4b also revealed that the swelling ratio decrease in the growth trend of M_w in the low concentration. It was suitable the result of the Fig.1.4a. The higher gel strength, the lower swelling ratio. However, with the concentration of 50%, the swelling ratio of all samples got saturated at the absorbed dose higher than 50 kGy.

III.2. Gel strength

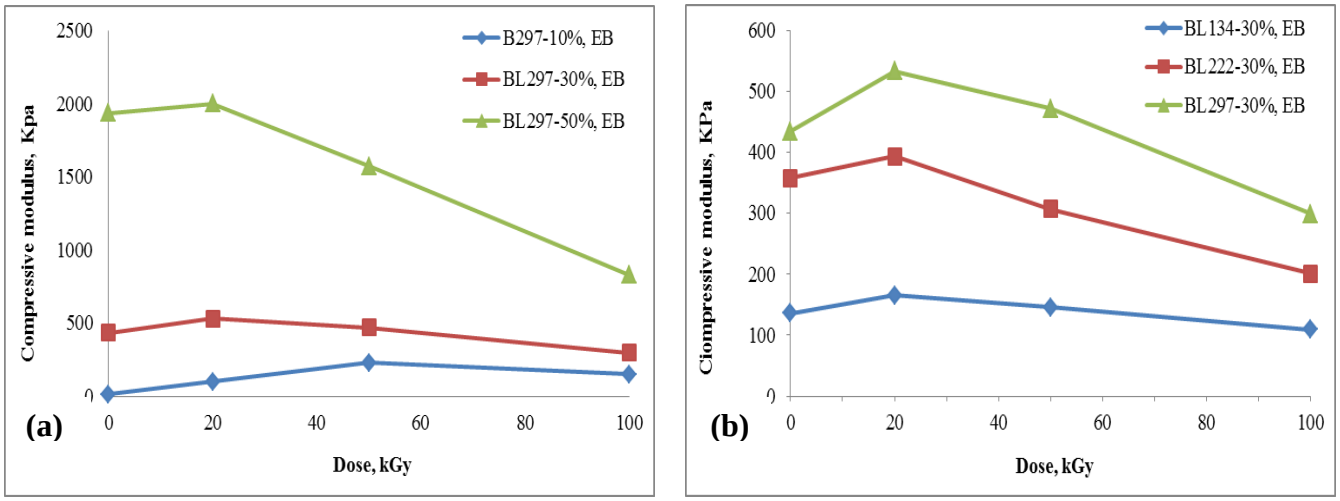

Fig.2.1. *Effect of absorbed dose on the compressive modulus of BL297 at different concentration (a) and of BL134, BL222, BL297 with the same concentration of 30 % (b)*

The Fig.2.1a presented the effect of absorbed dose on the compressive modulus of BL297 gels obtained with different concentrations. The result showed that the gel strength of

10% BL297 increased slowly with the increase of absorbed dose and remained steady at the dose higher than 50 kGy. It was clear that the higher gel fraction, the higher gel strength. However, with higher concentration of 30 % and 50 %, the highestgel strength of samples prepared from 30 % and 50 % gelatin obtained by EB irradiation at the dose of 20 kGy and then decreased with further increasing of dose.

As one can see in the Fig.2.2, the bubbles appeared inside the sample during EB irradiation, in particularly, at the high dose of 50 kGy and 100 kGy. The higher absorbed dose, the higher amount of bubble. These bubbles may influence to the structure of resulting gels, so that reduced their elastic modulus at the same absorbed dose.

The compressive molulus of the gels increase with gelatin concentration. For example, the modulus of the gels obtained by irradiated at the dose of 20 kGy were 102 kPa, 533 kPa and 2000 kPa corrsesponding with the samples of 10 %, 30% and 50% gelatin, respectively. Similar results were also obtained with BL134 and BL222.

Moreover, the result of Fig.2.1b showed that the M_w also effect on the compressive modulus of sample. For example, with the same concentration of 30% and at absorbed dose of 50 kGy, the gel strength values are 230 kPa, 470 kPa and 1575 kPa for the gels prepared with BL134, BL222 and BL297, respectively. It indicated that the higher molecular weight, the higher gel strength obtained.

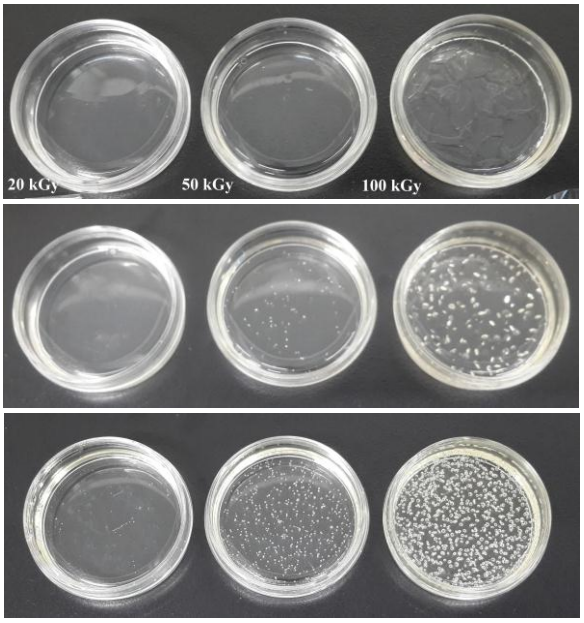

Fig.2.2. *BL297 irradiated at 20 kGy, 50 kGy and 100 kGy with concentration of 10% (a), 30% (b) and 50% (c)*

III.3. Thermal analysis

The Fig.3.1 illustrated the DTA curves of 30% BL297 at different absorbed doses. The results showed that degradation temperature of BL297 increased steadily with the increase of absorbed dose. In particularly, its values are 274 °C, 282 °C and 287 °C for the gels irradiated at the absorbed doses of 0 kGy, 20 kGy and 100 kGy, respectively. It suggested that crosslinking degree had effect on the heat-resistance of gelatin gels. In these analyzed conditions, the higher crosslinking degree got, the better heat-resistance of gelatin became.

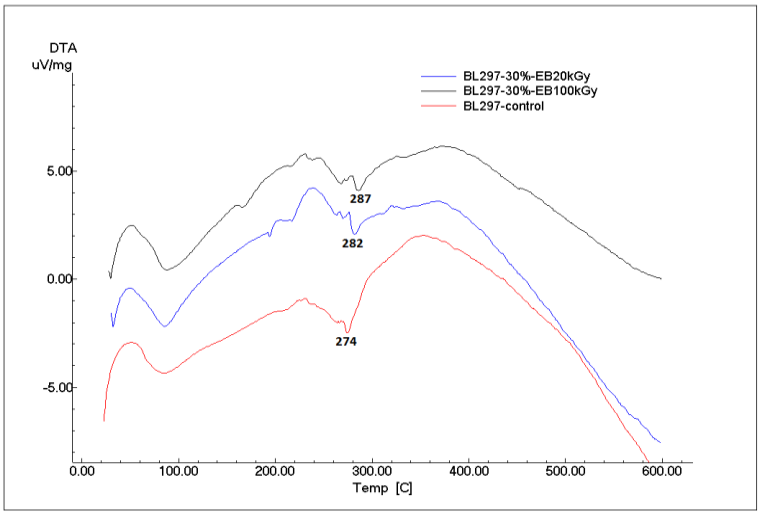

Fig.3.1. *Effect of absorbed dose on DTA results of BL297 with concentration of 30 %.*

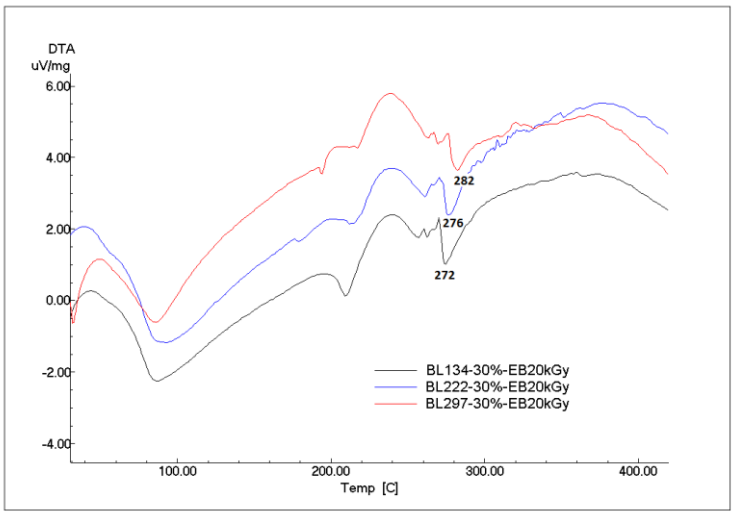

Fig.3.2. *Effect of absorbed dose on DTA results of BL134, BL222 and BL297 with the same concentration of 30 %.*

The effect of gelatin M_w on the heat-resistance of resulting gels was also presented in the Fig.3.2. The heat-resistance increased steadily with the increase of M_w. For example, for the gels obtained from the same concentration of 30 % by irradiated at the dose of 20 kGy, the degradation temperatures of BL134, BL222 and BL297 were 272 °C, 276 °C and 282 °C, respectively. It was clear that the heat-resistance of gelatin gels was much improved by EB irradiation.

IV. CONCLUSION

Different hydrogels can be prepared from fish-derived gelatin by gamma and EB irradiation. Crosslinking conditions of fish gelatins depend on their molecular weights, concentrations, solutions and absorbed doses. BL134, BL222 and BL297 gelatins dissolved in water were crosslinked by EB irradiation at the low absorbed dose of 20 kGy with low concentration of 10%.

The strength of fish gelatin gels were improved by EB irradiation. And the strength of resulting gels could get from several hundreds to thousands of kPa. It was suitable to apply for bio device.

In addition, heat-resistance of fish-derived gelatin also was improved by irradiation.

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NGHIÊN CỨU CHẾ TẠO HYDROGEL TỪ GELATIN CÁ BẰNG PHƯƠNG PHÁP CHIẾU XẠ EB ỨNG DỤNG LÀM VẬT LIỆU Y SINH

Tóm tắt: Các vật liệu hydrogel được chế tạo từ gelatin cá bằng cách chiếu xạ các dung dịch gelatin nồng độ khác nhau trên máy gia tốc chùm tia điện tử (EB) tại Viện TARRI, QST, Nhật Bản. Trong nghiên cứu này, các loại gelatin được chiết xuất từ cá rô Tilapia với khối lượng phân tử khác nhau được hòa tan trong nước cất và dung dịch đệm Photphat (PBS) 1M ở nhiệt độ 30 °C trong khoảng nồng độ cao từ 10 – 50 wt.%, sau đó được chiếu xạ EB trong khoảng liều xạ 10 – 160 kGy. Khả năng chịu nhiệt của gelatin hydrogel được xác định bằng phương pháp phân tích nhiệt vi sai DTA và kết quả cho thấy tính bền nhiệt của nó được cải thiện đáng kể. Hàm lượng gel của gelatin chiết xuất từ cá tại nồng độ tối ưu 30 % wt tăng theo liều xạ và đạt giá trị 80 % tại liều xạ 100 kGy. Modul đàn hồi của gel gelatin đạt giá trị từ 100 đến 500 KPa trong khoảng liều 20 – 100 kGy tại nồng độ 30 % wt, điều kiện phù hợp sử dụng làm vật liệu y sinh.

Key words: *gelatin, hydrogel, electron beam, irradiation*