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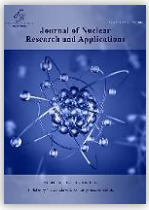

Fabrication of Hemostatic Powder Based on Modified Starch by Electron Beam Irradiation Method

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A B S T R A C T

In the present work, a hemostatic powder based on the starch (St) biopolymer was synthesized using an innovative production method involving electron beam irradiation (EBI). Compared to conventional chemical methods, irradiation methods offer several advantages, including high production speed (suitable for industrial-scale production), the elimination of common chemicals in polymerization processes (e.g., initiators or crosslinkers), simultaneous sterilization and production, no waste generation, and operation at room temperature. In the first stage, a novel approach was introduced involving the chlorination and amination of starch, followed by grafting plant polyphenols onto the starch. Under high-energy electron beam irradiation, crosslinking reactions were found to dominate over chain-breaking reactions. The chemical and physical structures of the synthesized product were characterized using FTIR, SEM, EDX, XRD, and TGA analyses. The effect of electron beam irradiation dose on swelling, gel percentage, and mechanical properties of the hemostatic product was also investigated. Among the tested doses, the 15 kGy irradiation dose yielded samples with superior swelling and suitable mechanical properties, making it the optimal dose for product synthesis. Notably, the absorption rate and capacity of the hemostatic powder synthesized by this irradiation method exceeded those of commercial samples.

Keywords: Electron beam irradiation; Hemostatic powder; Modified biopolymers; Starch.

1. Introductions

One of the main concerns of surgeons during surgery is maintaining blood flow in the body and minimizing blood loss as bleeding can often pose a life-threatening risk to the patient. Therefore, it is essential to prepare a suitable hemostatic agent with high blood coagulation efficiency to achieve optimal surgical outcomes. Often, surgeons' first choice to control bleeding

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is to apply direct pressure or compact the bleeding site, however, compression or other mechanical methods may not be appropriate in all surgical procedures. For example, if the bleeding site is unidentifiable or dispersed, or if the patient has an inherent coagulation disorder, these methods may not effectively control the bleeding. Recently, thermal methods such as lasers have become suitable surgical options to reduce bleeding. However, the frequent application of burning or other thermal techniques can lead to complications, limiting their use in clinical practice [1,2].

Hemostatic powders which are effective in controlling all types of bleedingare among the most common, fastest, easiest, and most accessible methods for surgeons to stop bleeding. These hydrophilic polymer powders, with their network structure, absorb blood plasma, leading to the concentration and accumulation of platelets and coagulation factors at the bleeding site. In addition, upon contact with blood, these powders rapidly form a gel matrixthat creates a mechanical barrier by binding to blood tissue. Hemostatic powders are usually used as an absorbable (degradable) powder in heart, vascular, orthopedic, spinal, and nerve surgeries to prevent bleeding from capillary, venous, or vessels under pressure. In addition to surgical applications, these powders are used in military settingsand war casualties, accidents, and general injuries that lead to bleeding [3].

Hemostatic powder products are primarily made from natural biopolymers or synthetic polymers, such as (polycyanoacrylate, polyacrylate, polyalkyne oxides). The use of biopolymers compared to all kinds of synthetic polymers in the manufacture offers several advantages over synthetic polymers, includingnot causing sensitivity or toxicity to the body, more similar to the molecular structure of body tissues, biocompatibility and degradability (faster absorption in the body) [1-5]. Additionally, biopolymers are cost-effective and derived from readily available resources.Since hemostatic powders will remain in the body for a considerable period, they must exhibit excellent biocompatibility to avoid triggering immune or chronic inflammatory responses, tissue adhesion, or infections. Therefore, the best choice of raw materials to make them is biopolymers such as starch. However, no previous studies have investigated the synthesis of hemostatic compounds based on biopolymers, particularly starch modified through chlorination and amination with polyphenols, using the electron beam irradiation (EBI) method, and the present study introduces a novel formulation and innovative production technique for manufacturing hemostatic powder, offering a significant advancement in this field.

2. Experimental

2.1. Reagents and instrumentation

All reagents and chemicals with analytical grade were prepared from Sigma-Aldrich (St. Louis, MO, USA). For the preparation of the sorbents, a high-energy Rhodotron industrial electron accelerator device, model TT200, with energies of 10 MeV (located in Yazd, Iran), was used. Irradiation dosimetry was done by cellulose triacetate film. The irradiation dose rate was 7.5 kGy s^{-1} and the electron beam current was 5.0 mA.

2.2. Chlorination and amination of raw starch

For starch chlorination (St-Cl), 0.2 g of raw starch was added to 100 mL of alkaline ethanol mixture and to create a suspension. Then 10 mL of epichlorohydrin (ECH) was introduced into the suspension, which was then refluxed for 6 hours. At this stage, ECH bonded to the surface of the starch. The starch deposits were then separated using filter paper, washed three times with ethanol to eliminate unreacted reagents, and air-dried at room temperature. For the amination of starch, 0.2 g of St-Cl was added to 50.0 mL of water/ethylene diamine (EDA) mixture (1:1 v/v) and then it was stirred at 55°C for 4 hours by a magnetic stirrer. After the completion of the St-Cl aminolysis reaction, 200 mL of ethanol was added to the solution and stirred at room temperature using a mechanical stirrer to remove unreacted EDA. The resulting St-N deposits appeared in the form of a white suspension, which was gently stirred in an ice water bath for 2 hours to complete the St-N deposition process. Then the resulting mixture was centrifuged at 5000 rpm for 5 minutes. Finally, the St-N precipitates were separated, washed three times with ethanol, and air-dried at room temperature.

2.3. Grafting of caffeic acid (CA) on St-N

0.2 g of St-N and 0.6 g of chlorogenic acid (CGA) were added to 100 mL of distilled water. The pH of the mixture was adjusted to 7 using NaOH solution, and the mixture was refluxed at 60 ºC for 6 hours under vigorously stirring. Upon completingof the aminolysis reaction of CGA ester groups and the capture of the CA part of CGA on St-N, 200 mL of ethanol was added to the above solution and stirred at room temperature using a mechanical stirrer to remove unreacted CGAs with St-N and St-N-CA particles appear as yellow deposits. The resulting suspension was placed in an ice-water bathand gently stirred for 2 hours to finalize the deposition process of St-N-CA. The resulting sediments were separated by centrifuging the suspension for 5 minutes at 5000 rpm. Then the St-N-CA deposits were washed three times with ethanol and dried at room temperature.

2.4. Preparation of hemostatic powder based on St-N-CA by crosslinking process through EBI

0.2 g of St-N-CA was added to 50 mL of distilled water and the mixture was stirred for 2 hours at room temperature by a mechanical stirrer until it was fully dissolved. Subsequently, 20 mL of the resulting gel solution was transferred to a Petri dish and covered with plastic film to prevent water evaporation and subjected to EBI in the range of 5-20 kGy. The resulting crosslinked biopolymers were washed several times with distilled water to remove unreacted biopolymers or biopolymers formed with low molecular mass and then dried in a vacuum oven at 50 °C for 2 days. Finally, the dried film was converted into fine granules (below 100 microns) by a hammer mill and stored in a desiccator until use.

3. Results and discussion

3.1. Mechanism of production

In this project, involving the chlorination and amination of starch (St), followed by the grafting of plant polyphenols such as chlorogenic acid (CGA) onto starch. This process ensured that cross-linking reactions predominated over chain breakage reactions

under high-energy electron beam irradiation (EBI)[6-10]. It is not possible to graft polyphenolic reagents on starch to create suitable sites for radiation crosslinking reactions by raw starch biopolymer chains. Therefore, before carrying out polyphenol grafting and radiation crosslinking, modification reactions such as chlorination and amination of raw starch must be done. In the first step, to chlorinate raw starch, St was chlorinated by ECH during a heterogeneous chemical reaction (Fig. 1a). Here, ECH can react as a cross-linker with the hydroxyl groups of St, and it can also be attached and stabilized on the surface of St. The use of ethanol solvent mixture reduces more cross-linking reactions and causes more ECH binding on the St surface. First, ECH is attached to the St surface through the attack of St hydroxyl groups on the epoxy ring of ECH and its opening. A summary of the mentioned chlorination reaction for St is shown schematically in Fig. 1a. In the second step, in order to amination the St-Cl, EDA is added to the St-Cl aqueous solution and refluxed. EDA, through the anionic attack of its amine groups on the residual chloride end of ECH immobilized on St, removes chlorine and binds to the St surface. A summary of the starch amination mechanism and St-N preparation is shown in Fig. 1b. The grafting of the phenolic part of CGA (CA) on St-N takes place through the nucleophilic attack of the amino groups of St-N on the ester groups of CGA (Fig. 1c) and thus the CA part of the CGA molecule is grafted on the St-N. The color change of St to yellow indicates the successful grafting of CA on St. Finally, as shown in Fig. 2, St-N-CA in an aqueous medium, when exposed to EBI, generates stable, non-destructive polyphenoxy macroradicals. As the biopolymeric chains approach each other, these macroradicals bond covalently (via C-C or C-O bonds), resulting in the cross-linking of the biopolymer chains.

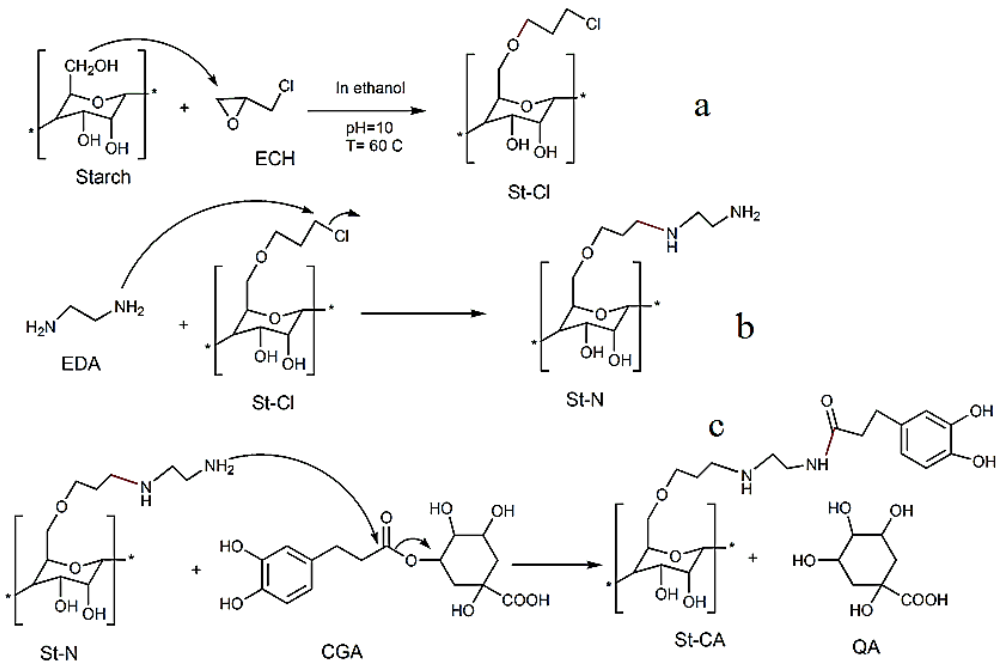

Fig . 1. View of St chlorination reaction (a), St-Cl amination (b) and CA grafting on St-N (c).

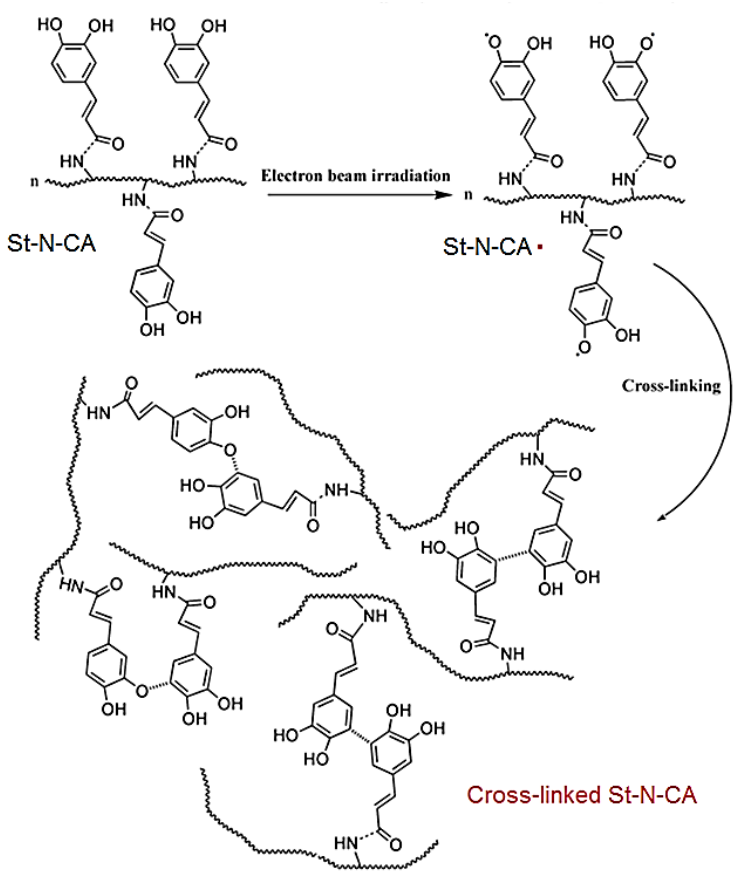

Fig. 2. Schematic of crosslinking mechanism of St-N-CA biopolymeric chains due to electron irradiation and synthesis of the final product of hemostatic.

3.2. Characterizations of synthesized hemostatic powder

FTIR analysis was conducted to confirm the amination of starch (St) and the grafting of chlorogenic acid (CA) onto St-N (Fig. 3). The appearance of amine bands in spectrum St-N and the appearance of amide bands in spectrum St-N-CA (C=O stretching peaks at 1652 cm^{-1}, C-N stretching at 1150 cm^{-1}, N-H stretching at 3095 cm^{-1} and N-H bending at 1534 cm^{-1}) respectively, confirming the amination reaction of starch and the successful grafting of the CA part of CGA on St-N. The presence of C=C aromatic stretching peaks of the benzene ring of the CA part of CGA at 1601 cm^{-1} The increased intensity of the peak at 1652 cm^{-1} in the St-N-CA spectrum, compared to the pure starch spectrum, indicates the overlap of the CA vinyl group and the newly formed amide C=O groupsalso indicates the successful grafting of the CA part of CGA on St-N.

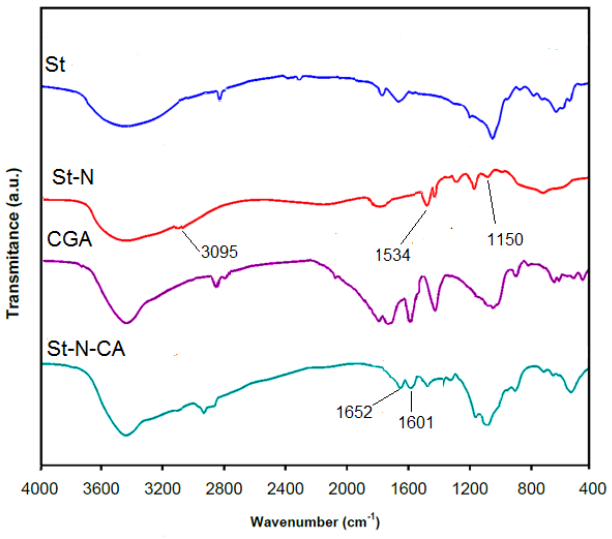

Fig. 3. FTIR spectra of components of hemostatic powder.

The surface morphology of the freeze-dried sample of St-N-CA, both before and after electron beam irradiation (EBI) at 15 kGy, was analyzed using SEM images. (Fig. 4a, b). The distinct differences in morphology, porosity, and surface unevenness observed between the pre-EBI sample (Fig. 4a) and the post-EBI sample (Fig. 4b) suggestthe formation of a 3-D network polymer structure (which is distributed heterogeneously) in hemostatic powder. The open and porous structure related to the created networks polymer increases the permeability and easier transfer of the molecules of the aqueous media of the blood (during the swelling phenomenon) to the depth of the network structure of hemostatic powder and thus increases its absorption capacity and rate.

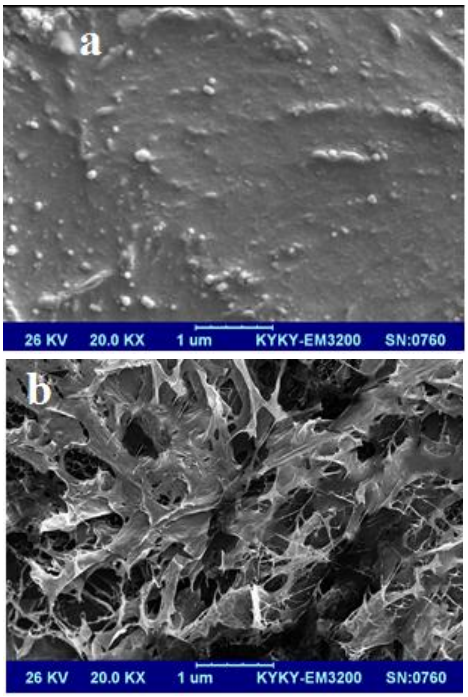

Fig. 4. SEM images of St-N-CA samples before (a) and after (b) EBI.

EDA immobilized to the structure of hemostatic was also investigated by EDX analyzes (Fig. 5). The presence of the nitrogen signal and the disappearance of the chlorine peak in the St-N and St-N-CA spectra indicate the successful stabilization of EDA with St-Cl through the removal of chlorine.

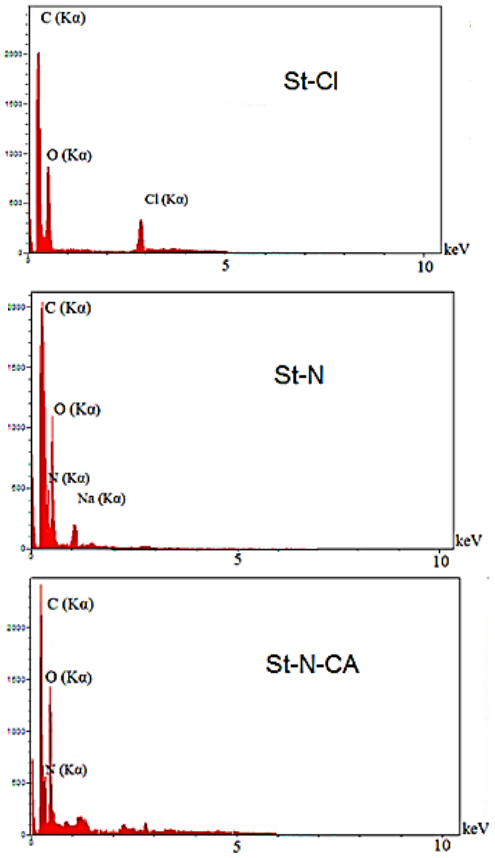

Fig. 5. EDX spectra of St-Cl, St-N and St-N-CA.

The thermal stability of raw St, St-N-CA before and after EBI (15 kGy) was investigated by TGA analysis (Fig. 6). The results indicate a reduction in the thermal stability of St-N-CA compared to raw St. This reductioncan be due to breakage of starch caused by the successive process of chlorination, aminolysis and grafting. The thermal stability of hemostatic powder based on cross-linked St-N-CA compared to raw St is improved. For example, the residual weight of raw St at 350 ºC is equal to 32%, which is less than the residual weight of hemostatic powder (42.5%) at the same temperature. In general, the thermogram of hemostatic powder shows a gradual decrease in weight with less intense

weight loss at different temperatures compared to the thermograms of raw St and St-N-CA. This improvement in thermal stability is due to the random crosslinking between St-N-CA chains through EBI, which can act as a thermal barrier (Because of the hydrophilic network structure of the samples).

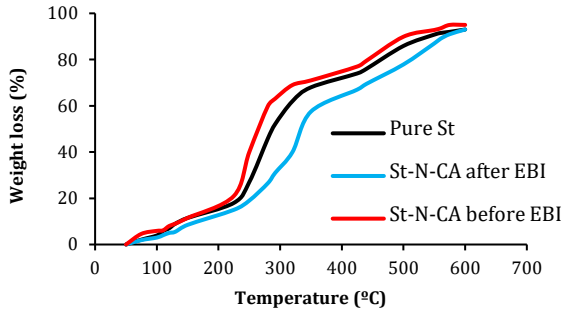

Fig. 6. TGA graphs of St and St-N-CA before and after EBI.

Raw St usually has a granular structure with 15 to 45% crystallinity, which corresponds toits type A structure, which can be recognized by the sharp peaks at the positions of 15.5º, 17.1º, 18.3º and 23.4º (Fig. 7). The XRD patterns of raw St, St-N-CA before and after the cross-linking process using EBI (15 kGy) are shown in Fig. 7. However, when St is subjected to the successive reactions of chlorination, aminolysis, and polyphenolic grafting, the crystal structure is significantly altered. The created amine or polyphenolic groups play an important role in the order and stabilization of linear starch amylose molecules and minimizing retrogression. The reduction and disappearance of some crystal peaks for St-N-CA before EBI and only the observation of 2 peaks at the positions of 17.0 ºand 20.1º indicate the change like part of the St crystallinity due to the mentioned reactions. However, the intensity of these crystal peaks in the XRD pattern of St-N-CA hemostatic powder has been greatly reduced or disappeared due to the random crosslinking process with EBI and the formation of an amorphous structure.

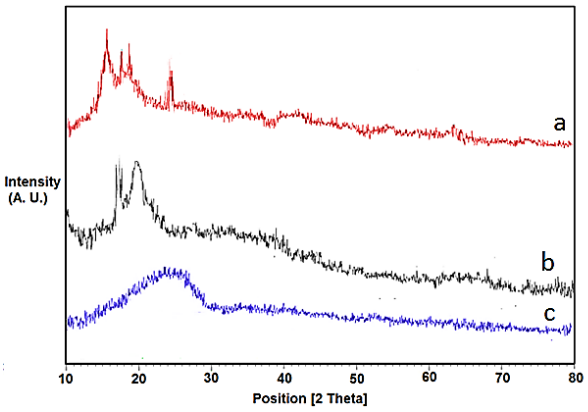

Fig. 7. XRD patterns of raw St (a), St-N-CA before (b) and after (c) the cross-linking process using EBI.

3.3. Effect of EBI dose on swelling, gel percentage and mechanical properties of synthesized hemostatic powder

As illustrated in Figure 8a, the swelling capacity of the hemostatic powder decreases with increasing EBI dose. These observations can be related to the production of more free macroradicals of polyphenoxy grafted on St followed by the increase of cross-linking density at higher EBI doses. As a result of this process, the mobility of biopolymer chains is reduced and water molecules hardly penetrate the hemostatic networks. Additionally, the effect of EBI on the mechanical properties of hemostatic hydrogel films (prior to the drying and grinding processes) was evaluated., gel percentage and mechanical properties (compressive and tensile strength, Fig. 8b) of hemostatic samples increased due to the formation of polyphenoxy-free macroradicals and as a result of increasing the density of crosslinking between St biopolymer chains. The compressive strength of more than 60% for samples synthesized at EBI dose values higher than 10 kGy indicates the appropriate hardness of the prepared hemostatic powder for practical applications.

Considering more economical in production with lower irradiation doses, the higher swelling and also appropriate mechanical properties of hemostatic samples synthesized at 15 kGy irradiation dose, this dose was used for product synthesis and further studies.

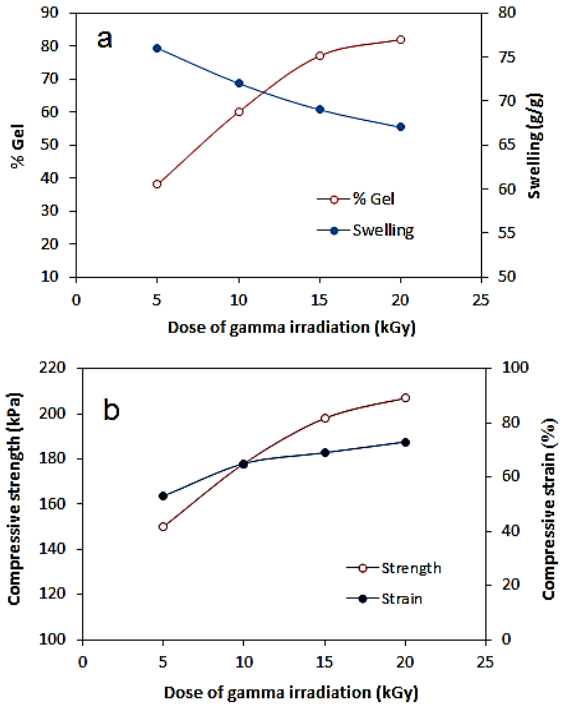

Fig. 8. Effect of EBI dose on swelling, gel percentage and mechanical properties of hemostatic powder.

3.4. Study and comparison of absorption rate of synthesized hemostatic powder with other commercial products

The mechanism of action for hemostatic products in stopping bleeding primarily involves the absorption of the aqueous component of blood plasma, resulting in the concentration of blood coagulation factors and accelerating the hemostasis process.. evaluate and comparethe effectiveness of the synthesized product (at 15 kGy) as a hemostatic agent, the results of the time profile of blood absorption capacity by commercial hemostatic powders (Perclot, Celox, Arista, and Chitohem) and the synthesized hemostatic product are shown in Fig. 9. As it is known, the synthesized product has a better absorption capacity and rate (less time) than the introduced commercial products, which is caused by the synergistic effect of EBI in creating porosity (to carry out the crosslinking reaction in the aqueous media and the confinement of water molecules in polymer networks and finally the exit of water in the drying stage) in nano and micro dimensions during the crosslinking reaction of the biopolymer chains. For example, the synthesized product achievesthe maximum swelling capacity of 33 g/g in 35 seconds, while the Perclot and Chitohem products (within this time) reach swelling values of 18 and 7 g/g, respectively. Furthermore, the Celox product attains its maximum swelling capacity of 15 g/g at 55 seconds, and the Arista product achieves a swelling capacity of 16 g/g at 60 seconds.

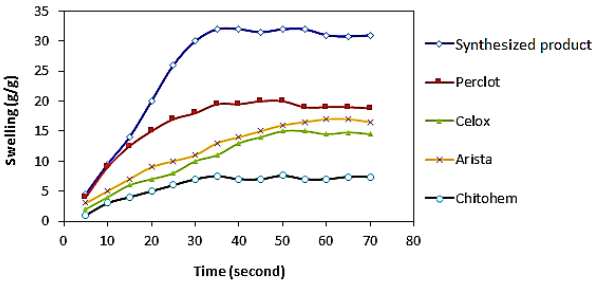

Fig. 9. A comparison of the time profile of absorption capacity of commercial hemostat powders and synthesized product in the blood media.

4. Conclusions

In this study, hemostatic powder was prepared by introducing a new production method based on modified starch biopolymers using the EBI method. The use of the clean and sterile EBI method, as opposed to conventional polymerization processes that rely on toxic and allergenic chemicals, enhancesthe biocompatibility of the synthesized hemostatic powder for the human body. The chemical and physical structure of the synthesized product was confirmed by FTIR, SEM, EDX, XRD, TGA. With increasing EBI dose, gel percentage and

mechanical properties of hemostatic samples increased. Considering the higher swelling and appropriate mechanical properties of hemostatic samples synthesized at a 15 kGy irradiation dose, this dose was selected for product synthesis. The hemostatic powder synthesized through this method demonstrated superior absorption capacity and rate compared to commercial products. Full compatibility with green chemistry production processes, no waste generation, high production speed, eco-friendly, and reaction at room temperature and pressure are among the advantages of this synthesis method for hemostatic products.

Conflict of interest

The authors declare no potential conflict of interest regarding the publication of this work.

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