The Effect of Glutaraldehyde on the Properties of Gelatin Films

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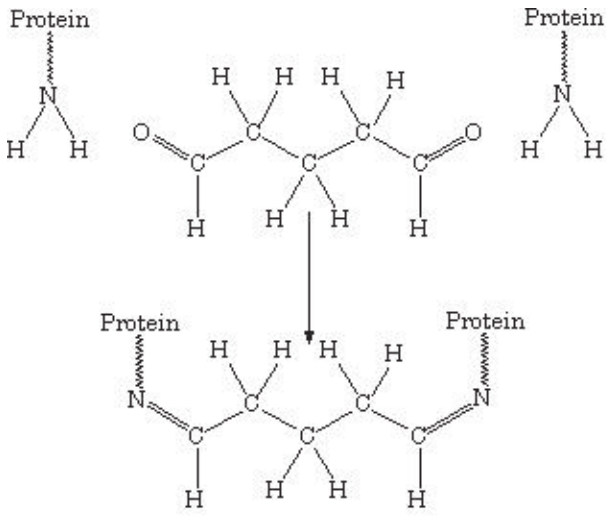
In this work gelatin film was prepared from cow's bone. In order to increase mechanical stability of the prepared transparent film as well as decrease its swelling, glutaraldehyde (GTA) was used. Different mass fractions of GTA were utilized. It was observed that at $w = 0.18$ % of GTA the load at break of the film is $\delta = 53.7$ N and the solubility decreased. The solubility of the film was measured as a dependant parameter of the swelling behavior. In this case it was decreased from 389 % to 156 % at 5 min for gelatin films without GTA and with 0.18 % GTA, respectively. FTIR spectroscopy results showed a peak for crosslinked gelatin at $\tilde{\nu} = 1650$ cm^{-1}. It means the crosslinking between gelatin and GTA has taken place. SEM micrographs confirm the porosity has decreased by increasing the GTA fraction, which is an indication of higher strength.

Key words: *Gelatin, glutaraldehyde, mechanical properties, FTIR, SEM*

Introduction

In recent years, the development of environment-friendly and biodegradable materials based on natural polymers has received increasing attention. This is due to attempt to substitute petroleum-based plastics, which present concerns in terms of pollution and sustainability.[1] Among other biopolymers, gelatin has been extensively studied due to its low cost, biodegradability, biocompatibility and non-immunogenic. Gelatin is obtained by thermal denaturation or physical and chemical degradation of collagen from animal skin and bones.[2–4] Four processes are used: Acid, Alkaline, Enzymatic and Heat/Pressure process. Among all of these processes, heat/pressure process is done at a short time and no chemicals, but the extracted gelatin has low bloom index.[5,6] Gelatin is widely used in food, pharmaceutical industries as well as in the biomedical field: hard and soft capsules, microspheres, sealants for vascular prostheses, wound dressing, adsorbent pads for surgical use and implantable devices.[2,3,5,7] In spite of gelatin advantages, gelatin films have poor mechanical properties and are dissolvable in aqueous solutions very fast, which limit their possible applications as a biomaterial especially for long-term applications.[2,3] Thus, to modify mechanical properties and delay the solubility of gelatin films, the formation of crosslinks among the macromolecular chains has been proposed.[8] Aldehydes, such as formaldehyde and glyoxal,[9] glutaraldehyde[3] and other crosslinking agents like genipin[2] and transglutaminase,[9] were used to produce modified gelatin films. Among the chemical crosslinking agents, glutaraldehyde (GTA) is by far the most widely used chemical because it is inexpensive, easily available and its aqueous solutions can effectively crosslink collagenous tissues in a relatively short period[3] that the crosslinking reaction between GTA and protein (like gelatin) is shown in Fig. 1.[10,11]



F i g. 1 – *The crosslinking reaction between glutaraldehyde and protein*[10,11]

S l i k a 1 – *Reakcija umrežavanja između glutaraldehida i proteina*[10,11]

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Many researchers[2,3,7,8,9] studied functional properties of modified films based on the best quality gelatin, but in this study we have studied the effect of GTA mass fraction on mechanical properties, swelling, amount of free glutaraldehyde and fine structure of gelatin films based on the cow's bone.

Experimental

Gelatin preparation

The bone of Holstein cow was crushed and washed with water. The crushed bones were treated in a high-pressure tank along with water at a mass ratio of $\zeta = 1{:}3$ (bone: water) at $\vartheta = 104$ °C and p = 1.2 bar for $t = 5$–6 h. The gelatin solution was then filtered using a multi-layered gauze (cloths filter) to remove all solid materials. This solution was first defatted by phase separation method then the remaining gelatin solution was boiled to achieve a concentrated gelatin solution. The solution was dried at room temperature for $t = 48$ h and milled. The produced powder (CBG: Cow's Bone Gelatin) was used for the experimental work.

Determination of color

The color of the CBG powder was determined using a colorimeter (color eye 7000A-Gretagmacbeth), working with D65 (day light) at 10 visual angle, being used CIELab color parameters (L^*, a^*, b^*). The color parameters were measured and calculated by computer and color of gelatin was expressed as values of L^*, a^*, b^* and also color difference (ΔE^*) expressed as compared with standard sample (Merck gelatin powder).

Determination of moisture content

Ceramic beaker was dried at $\vartheta = 104$ °C for $t = 24$ h. The beaker was cooled down in a vacuum incubator, and precise weight was recorded. Approximately $m = 1$ g of CBG was put in the ceramic beaker, and dried at $\vartheta = 104$ °C for $t = 24$ h, cooled down in a vacuum incubator and the exact weight was recorded and the moisture content in bone gelatin was calculated.

Determination of viscosity

The viscosity of a $w = 6.67$ % gelatin solution (as reported by Muyonga,[12] Comez-Guillen[13]) at $\vartheta = 25$ °C was determined with rotary viscometer (Especialidades, Medieas, MYR S. L., Type V2–R, Spain) at speed of $n = 30$ min^{-1}.

Film preparation

Film-forming solutions were prepared by dissolving $m = 10$ g of CBG and $m = 4.5$ g glycerin in 100 ml distilled water. Then from $m = 0.01$ to 0.2 g glutaraldehyde was added to the solutions. The solutions were poured on to Plexiglas plates (15 · 20 cm) and dried at room temperature for 48 h. The gelatin films were stored in 28 % relative humidity and 25 °C conditions for 3 days before testing.

Mechanical properties

The load (L) and elongation (E) at break of gelatin films, average of five determinations, were determined using an Instron Testing Machine Micro250 (SLD) according to method ASTM D882–91. A speed of $v = 50$ mm min^{-1} was used with an initial grip separation of 100 mm.

Swelling

Gelatin films were weighed in air-dried condition. They were then immersed in distilled water for different periods of time. Wet samples were wiped with filter paper to remove excess liquid and weighed again. The amount of adsorbed water was calculated as: $m_{H_2O} = m_w - m_d$

Dividing m_{H_2O} by m_d gives the percentage od adsorbed water:

$$S(\%) \text{ or } m_{H_2O}(\%) = 100\,(m_w - m_d)/m_d$$

Where m_w and m_d are the masses of the wet and air-dried samples.

Glutaraldehyde release

The release of GTA from crosslinked gelatin films was determined according to the method proposed by *Bigi* et al.[3] 50 mg of gelatin film was immersed in $V = 3$ ml of a phosphate buffer solution at 37 °C for 24 h. 7 ml of a $c = 0.1$ mol l^{-1} glycine solution was added to the release buffer and the absorbance of the solution at $\lambda = 436$ nm was measured using a UV2101PC spectrophotometer (SHIMADZU). GTA concentration in the release solution was determined through comparison with a calibration curve.

Fourier transform infrared spectroscopy

FTIR spectra were obtained from very thin gelatin films and potassium bromide (KBr) discs containing the gelatin powder. All spectra were obtained using a Bomem-MB-100 infrared spectrophotometer over a range of $\tilde{\nu} = 4000$–500 cm^{-1} with a resolution of $\tilde{\nu} = 4$ cm^{-1}.

Scanning electron microscopy

The morphology of the gelatin films was determined by observation of the samples on a scanning electron microscope (SEM) (LEO440i) with an acceleration voltage of 10 kV. The gelatin films were mounted on stubs using double side sticky tab and coated with a thin layer of gold to avoid charging in the microscope.

Results

In order to produce gelatin from cow's bone by heat/pressure method the required time for treatment and concentrate steps is less than 10 h and drying at room temperature takes 48 h. The duration of drying can be reduced by warm air method. At the used methods for producing gelatin,[6,12–14] the time is necessary for producing gelatin of bone is too long. Also most of them need chemicals during the processing. The method used in this research for producing gelatin of cow's bone, was done with short time and no chemicals, but in the severe conditions.

The results of the determination of cow's bone gelatin color, expressed as the color parameters L^*, a^*, b^* and the difference of color (ΔE^*) in relation to the standard gelatin powder are shown in Table 1.

T a b l e 1 – *The color parameter of cow's bone and standard gelatins*

T a b l i c a 1 – *Parametar boje kravlje kosti i standardnih želatina*

Characteristics Karakteristike	Cow's bone gelatin Želatine od kravlje kosti	Standard gelatin Standardna želatina
L*	57.5	71.7
a*	5.1	0.5
b*	27.7	19.6
ΔE*	15.7	

CBG obtained, was dark yellow in color and its solution was turbid. The colorimetric results of CBG compared to standard gelatin showed that the CBG has a lower lightness because the L* for CBG is 57.5 and for standard gelatin and Nile Tilapia film is 71.7 and 90.02,[15] respectively. The CBG with higher a* (5.1) than standard gelatin a* (0.5) looks more reddish. This difference can be observed visually, and its yellowness b* (24.7) is higher than standard gelatin b* (19.6) and fish gelatin b* (11.34).[15] Color difference (ΔE*) of CBG and standard gelatin was mainly due to the variation of L*, that it means the CBG has the lower lightness. This is probably resulted of either existence of metal ions in gelatin or more hydrolysis of peptide bonds due to heat and pressure treatment.

Moisture content of CBG is $\chi = 9.6$ % that is in the range of $\chi = 8$–15 % moisture content reported [9,12,16] for other gelatins.

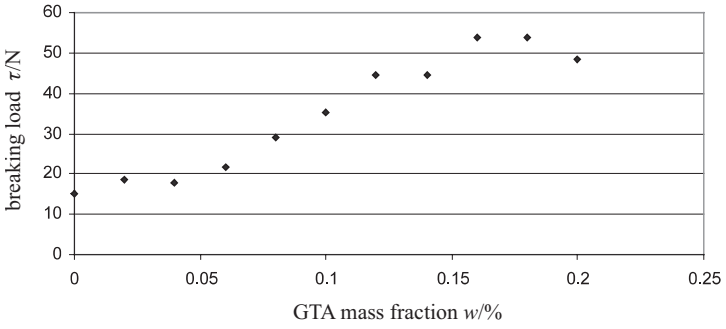
The viscosity of 6.67 % CBG solution was $\eta = 1.2$ mPa s. The viscosity of the gelatin is lower than the viscosity of commercial gelatins because the sever condition for the gelatin extraction has been caused to decreasing the molecular mass. It is in agreement with researchers' results that gelatin viscosity is influenced by raw material and production process and viscosity of hydrolyzed gelatin is increased with an increase in molecular mass.[7,12,13,17,18]

Aldehydes (glutaraldehyde and formaldehyde) crosslinking influence the stress at break and the extensibility of films based on high bloom gelatin.[3,9] In gelatin films increasing the bloom index from 80 to 270 g causes to increasing the stress at break from 1.3 to 5.1 MPa.[7] The results of mechanical properties of the CBG gelatin films show that the load at break increases (Fig. 2) and the elongation at break increases slightly (Fig. 3) on increasing GTA mass fraction. It is evident that the main variations in breaking load of gelatin films are between w(GTA) = 0.05 % and w(GTA) = 0.18 %, but a significant increasing in breaking load is at w(GTA) = 0.16 %. It means that the strength of gelatin films can be improved just with 0.12% GTA, while the elongation increases slightly and the films are flexible and easily handled.

Fig. 4 reports the swelling variations of gelatin films after different time of storage in distilled water, as a function of GTA mass fraction.

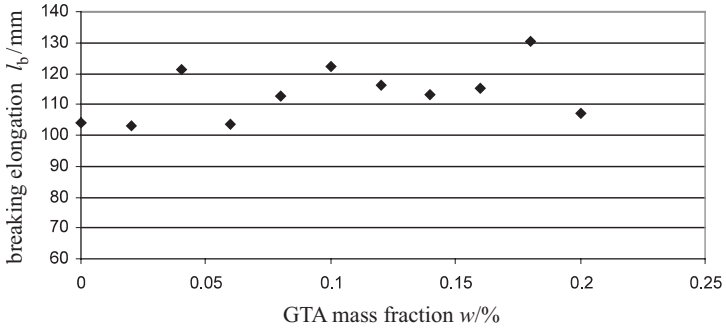
The swelling percentage of uncrosslinked gelatin film is about 390 % after 5 min. swelling measurements at longer times are hindered by the solubility of the film which begins to dissolve in water. In general, increasing GTA mass fraction provokes to decrease the swelling percentage and increase the time of films solubility. The major variations occur up to w(GTA) = 0.12 %.

The gelatin films with $w = 0.01$ up to 0.1 % GTA have been dissolved after 24 h of storage in buffer solution, so the amount of free GTA couldn't be determined. However on increasing GTA fraction from $w = 0.1$ to 0.2 % in gelatin films, the amount of free glutaraldehyde in buffer solution decreases.



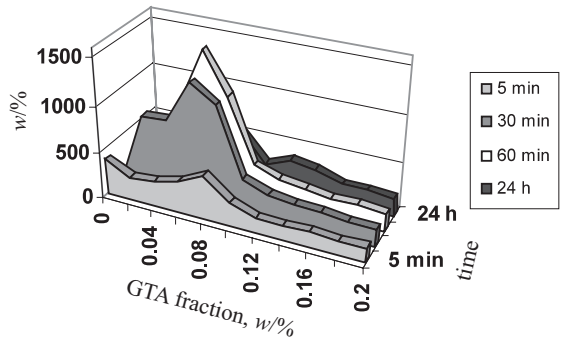
F i g. 2 – *Effect of glutaraldehyde on breaking load of gelatin films*

S l i k a 2 – *Utjecaj glutaraldehida na prekidnu silu želatinskih filmova*



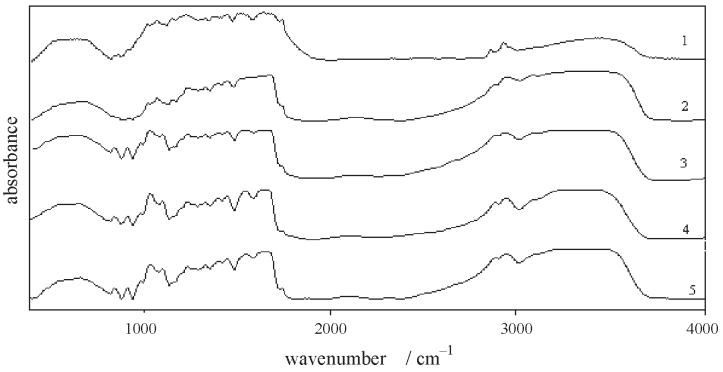
F i g. 3 – *Effect of glutaraldehyde on breaking elongation of gelatin films*

S l i k a 3 – *Utjecaj glutaraldehida na prekidno rastezanje želatinskih filmova*



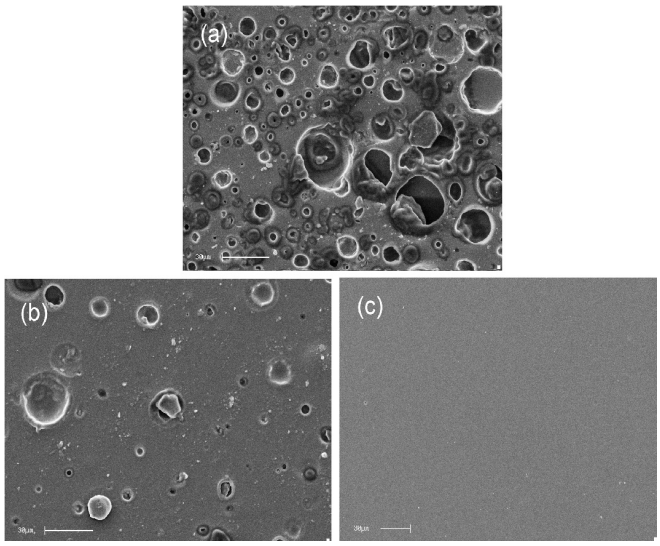
F i g. 4 – *Swelling (w, %) of gelatin films after different times of storage in distilled water as a function of GTA mass fraction*

S l i k a 4 – *Bubrenje (w, %) želatinskih filmova nakon različitih vremena zadržavanja u destiliranoj vodi kao funkcija udjela GTA*



F i g. 5 – *FTIR spectra for: (1) bone gelatin powder, (2) bone gelatin film, (3) bone gelatin film contains glycerin, (4) bone gelatin film contains glycerin and w = 0.01 % GTA and (5) bone gelatin film contains glycerin and w = 0.12 % GTA*

S l i k a 5 – *FTIR spektri za: (1) prah od koštane želatine, 2) film od koštane želatine, 3) film od koštane želatine sadrži glicerin, 4) film od koštane želatine sadrži glicerin i w = 0,01 % GTA i 5) film od koštane želatine sadrži glicerin i w = 0,12 % GTA*



F i g. 6 – *SEM micrographs for gelatin films: (a) without GTA; (b) with w = 0.01 % GTA; (c) with w = 0.12 % GTA (original magnification × 1000)*

S l i k a 6 – *Mikroskopske slike SEM želatinskih filmova: (a) bez GTA; (b) s w = 0,01 % GTA; (c) s w = 0,12 % GTA (originalno uvećanje × 1000)*

The FTIR spectra (Fig. 5) exhibited by bone gelatin powder differ from those exhibited by bone gelatin films especially in the amide I (about 1650 cm^{-1}), amide II (about 1550 cm^{-1}) and amide III (about 1240 cm^{-1}) regions. Compared to the spectra for bone gelatin powder, the gelatin films containing GTA show higher intensity amide I and amide II bands. It means the extent of order in GTA gelatin films may be higher than that in gelatin powder. The intensity of amide III band has been associated with the triple helical structure[17] that the intensity of amide III band for GTA gelatin films is higher than that for gelatin powder. It seems that the GTA gelatin films have more intermolecular associations, as a result of GTA crosslinking. As shown in Fig. 5 with increasing the GTA fraction the intensity of peaks increase at about 1110 cm^{-1}, which related to C–O vibration, show crosslinking has been occurred. Absorption in the region of 1000 to 1100 cm^{-1} is attributed to C–O vibration due to carbohydrates in collagen are associated with glycation of collagen and carbohydrates are required in the formation of pentosidine crosslinks.[17]

Regards to SEM micrographs (Fig. 6a), there are a lot of porous on uncrosslinked gelatin films. The number of porous reduces as GTA fraction increases and a smooth and avoid free surface induces (Fig. 6b, 6c), that it confirms the increasing of film strength and decreasing the swelling percentage of the gelatin films.

Conclusion

In this research, gelatin was obtained from cow's bone by heat/pressure method. The advantages of this method compared to other conventional methods, are short processing time and no need chemicals. However the viscosity of the diluted gelatin solutions is $\eta = 1.2$ mPa s, the flexible gelatin films could be produced. The mechanical properties of gelatin films are improved by using glutaraldehyde as a crosslinking agent. $w = 0.18$ % GTA is caused to increase the breaking load by a factor of about 3.5 times with respect to uncrosslinked gelatin film. The breaking elongation of gelatin films is increased slightly, as well as the swelling percentage and the amount of free GTA in buffer solution is decreased. According to the SEM micrograph, the porosity in gelatin films that contained GTA is reduced dramatically. FTIR spectroscopy shows that conversion of gelatin powder to gelatin film and addition GTA to the films leads to increase crosslinking formation and molecular order.

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List of symbols
Popis simbola

c – concentration, mol l^{-1}
– koncentracija, mol l^{-1}

CBG – cow's bone gelatin
– želatina kravlje kosti

GTA – glutaraldehyde
– glutaraldehid

l_b – breaking elongation, mm
– prekidno rastezanje, mm

m – mass, g
– masa, g

n – relation speed, min^{-1}
– brzina rotacije, min^{-1}

t – time, min
– vrijeme, min

v – speed, mm min^{-1}
– brzina, mm min^{-1}

V – volume, mL
– obujam, mL

w – mass fraction, %
– maseni udjel, %

χ – moisture content, %
– vlažnost, %

ζ – mass ratio
– maseni omjer

η – dynamic viscosity, mPa s
– dinamična viskoznost, mPa s

ϑ – temperature, °C
– temperatura, °C

$\tilde{\nu}$ – wave number, cm^{-1}
– valni broj, cm^{-1}

τ – breaking load, N
– prekidna sila, N

λ – wave length, nm
– valna duljina nm

SAŽETAK

Utjecaj glutaraldehida na svojstva želatinskih filmova

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U ovom je radu pripremljen želatinski film od kravlje kosti. Da bi se povećala mehanička stabilnost pripremljenog transparentnog filma kao i smanjilo njegovo bubrenje, upotrijebljen je glutaraldehid (GTA). Upotrijebljeni su različiti maseni udjeli mase GTA. Uočeno je da kod 0,18 % GTA prekidna sila filma iznosi 53,7 N i da se povećala topljivost. Topljivost filma izmjerena je kao parametar ovisan o ponašanju pri bubrenju. U ovom slučaju smanjio se s 389 % na 156 % kod 5 minuta za želatinske filmove bez GTA odn. s 0,18 % GTA. Rezultati spektroskopije FTIR pokazali su maksimum za umreženu želatinu kod 1650 cm^{-1}. To znači da je došlo do umrežavanja između želatine i GTA. Mikroskopske slike SEM potvrđuju da se poroznost smanjila povećanjem dijela GTA, što je pokazatelj veće čvrstoće.

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