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Study of the Mechanical Properties of Gelatin Films with Natural Compounds of *Tamarix hispida*

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Abstract

In this work, an extract of the *Tamarix hispida* (TH-10) was prepared in 10 vol.% water-ethanol solution. Based on the properties of antioxidant, and anti-inflammatory activity, various gelatin films containing (10–15 wt.%) gelatin were obtained. The fabricated gelatin films containing TH-10 extract were used as wound dressings for burns. Their deformation properties, physical and mechanical properties, and SEM micrographs were defined. An additional film structure was determined by using a Fourier transform infrared spectrometer (FT-IR). The strength of polymer films containing TH-10 depends on gelatin content. This indicates that the gelatin film binds the TH-10 with the help of hydrophobic interactions. It was found that the higher amount of gelatin leads to lower tear resistance of films. This is due to the formation of micro-cracks on the surface of films leading to their easy rupture. During the study of tensile strength, it was proven that the optimal amount of glycerin in the film is 5 vol.%. The polymer film containing 12 wt.% gelatin and 1 wt.% TH-10 was recognized as the most effective composition in terms of strength and elasticity properties for application as wound dressing materials.

1. Introduction

In most cases, the main disadvantages of using traditional dressings in the treatment of skin injuries or burns, inflammatory skin diseases, chronic wounds, and the prevention of infectious diseases with skin lesions include the short duration of their pharmacological action, toxicity, low selective effect or the appearance of side effects on the body [1]. With the elimination of these shortcomings, the joint use of drugs with polymers, the creation of polymer derivatives, and the immobilization of drugs in polymers are promising. The use of polymer forms of medicines creates certain advantages over conventional forms, namely, it allows you to obtain a long–term therapeutic effect, reduce side effects, and reduce the total consumption of the drug for the course of treatment while maintaining a uniform concentration of the drug in the body for a long time [2]. Therefore, polymers used for pharmacological purposes are set to the following requirements: absolutely harmless material to the body, do not exhibit carcinogenic or allergenic properties, biocompatibility, do not undergo changes and do not cause denaturation, are easy to process, have bactericidal properties [3].

In this work, a gelatin-based film as a polymer carrier was obtained in accordance with the above requirements. Gelatin–based polymer carriers are used in many studies [4]. It is because of obtaining by partial hydrolysis and denaturation of collagen,

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which is the most common component of the extracellular matrix. In addition, the low cost and availability of gelatin promotes its use in medicine, in the food industry, and even in the production of machinery [5]. Gelatin is a natural protein that forms chemical bonds according to the functional groups $–NH_2$, and –COOH that facilitate obtaining polymer film. Plasticizers are usually used to improve the thermal and mechanical properties of gelatin-based films.

Gelatin is widely used in the biomedical field, in particular, for wound healing [6–8]. For example, it has been shown that in the treatment of complications of diabetes mellitus, the most severe form of skin injuries, it is possible to use films containing nanoscaled microneedles based on gelatin and polycaprolactone (PCL/Gel), which include the diabetes drug multifunctional nanosystem metformin, calcium peroxide/polydopamine (CaO_2/PDA) [9]. Scientific data also shows the use of gelatin-based biomaterial in the treatment of burn wounds. Burns are usually difficult to treat in the clinic, as the wounds are susceptible to bacterial infections and produce large amounts of pus and serum. Therefore, it is envisaged to use a biodegradable cryogel based on gelatin with silver nanoparticles, which promotes the healing of burn wounds, absorbs a large amount of water, and has good bactericidal activity [10]. In many research papers, gelatin biomaterials have been used not only as drug carriers but have also been considered in tissue engineering as the basis for 3D/4D bioprinting [11, 12].

Gelatin films treated with glycerin can be used as carriers of compounds that exhibit antimicrobial activity. In this context, a biodegradable, biocompatible polymer film, such as gelatin, is added to natural plant extracts to produce various biological activities [13].

Many scientists are interested in the study of plant raw materials containing biologically active compounds (dietary supplements). These dietary supplements are toxic, have a low cumulative effect, and prevent allergic reactions in the body compared to synthetic compounds. Medicinal products of plant origin are inexpensive, affordable, low toxic, can serve as a therapeutic service for a long time, and are easy to use [14].

Thus, one of the most pressing tasks today is the production of new domestic products from plant resources with medicinal properties. Therefore, it is important to study biologically active substances that are part of medicinal raw materials.

One of the promising plant sources of biologically active substances is the *Tamarix hispida*, belonging to the Tamaricaceae family. This plant contains biologically active substances polyphenols, terpenoids, and a complex of steroids. It has been proven that extracts and individual components have antimicrobial, antifungal, antiamnestics, cytotoxic, and antioxidant properties and are active as hemostatic agents, as well as against diabetes mellitus [15–18].

In different papers [15–18], it was found that the *Tamarix hispida* plant has high antioxidant properties in vitro due to the presence of terpenoid compounds in its composition [8]. In addition, the qualitative and quantitative component composition of the *Tamarix hispida* plant has been studied.

As a result, it was shown that plant extracts contain a significant amount of alkaloids of various groups, as well as biologically active compounds containing halogen and nitrogen [16]. Moreover, it was found that the main component of biologically active substances contained in the *Tamarix hispida* are tannins [17].

2. Experimental part

Gelatin powder (Ph.Eur., NF) pure, (128–192 Bloom batch) from AppliChem, Germany, was used without additional cleaning. Type–A in the acid treatment of soft collagen–containing raw materials from the processing of pig skins in the – pH range between – 3.8–7.6, with an average molecular weight of about – 15000–250000 g/mol.

Potassium sorbate (Sigma Aldrich, USA) with a purity > 99% was used without additional cleaning. Potassium sorbate extends shelf life and is a highly effective agent against yeasts and molds.

Glycerin (Pharmpur, Ph. Eur, BP, USP) from Scharlab, S.L. Spain, with a purity > 99% was used without additional cleaning.

In this work, a dry extract of the natural biological compound of the *Tamarix hispida* TH-10 was prepared. First, raw materials (100 g) and a water-ethanol solution in a ratio of 1:4 vol.%, consisting of 10 vol.% ethanol and 90 vol.% water, were prepared in an ultrasonic bath «Sapphire – 1,3» for 25 min. Because of the high water content in the resulting concentrate, it was distilled on a rotary evaporator at a temperature of 40 °C. The obtained initial concentrate was lyophilized until a completely dry powder state. The dry extract was applied for the preparation of gelatin films possessing anti-inflammatory and antioxidant activity. TH-10 is a hygroscopic brown crystalline powder with a specific odor and sweet taste.

In order to investigate the mechanical properties of gelatin films, 10-15 wt.% gelatin solutions were prepared. First, the gelatin was swollen in cold water for 30 min, and then it was placed in a water bath and stirred at a temperature of 35–40 °C until complete dissolution of gelatin [19–21]. A pre-dissolved drug TH-10, glycerin, and potassium sorbate were added to the cooled polymer mixture. The resulting solution was poured onto a glass surface [22] and dried at room temperature until the constant mass. The total area of the obtained film was 38.4 cm^2, the thickness was 0.1–0.5 mm, the average weight of each film was 1.5–1.7 g, and 0.004 g of TH-10 was applied per 1 cm^2 of the film area. The mechanical strength indicates the ability of films to resist destruction under the influence of various deformations (stretching, compression, bending) when applied to the skin.

The most important physical and chemical characteristics of polymer films include mechanical properties, transparency, thickness, elasticity, and plasticity. The mechanical properties of the films imply the degree of its stress σ, the strain ε. These parameters reflect the deformation properties of the polymer film [23].

Texture analyzer for determining deformation properties on the TA.XTPlus device (Stable Micro Systems, UK) [24] mechanical tests were carried out on rectangular samples of 1x5 cm^2 with a load of 7.5 kg. As a result of the tests, a graph of the dependence of stress and strain, strength is obtained which is determined by the equation below. The strength and deformation of the film were determined by the formulas given below:

$$\delta = \frac{F}{S} \quad (1)$$

$$\varepsilon = \frac{\Delta l}{l_0} \times 100 \quad (2)$$

$$\Delta l = l - l_0 \quad (3)$$

where, σ is the longitudinal stress, MPa; F is the deforming force, H; S is the area of cross–section of the film, mm^2; ε is the longitudinal strain, %; l_0 – is the original length of the bar being stretched, mm; l is the length after it has been stretched, mm; the extension of the bar, the difference between these two lengths, mm.

3. Results and discussions

Important characteristics of medical films are transparency, thickness, and elastic properties. To ensure these properties, it is necessary to choose the right plasticizer. Due to the similarity of nature with the natural components of TH-10 (flavonoids, tannins), glycerin was chosen as a plasticizer. In order to determine the mechanical characteristics of the obtained medicinal films, stress, ultimate unloading force, and tensile strength were determined for each sample.

The dependence of the tensile strength of polymer films on the concentration of the 10–15 wt.% gelatin composition is shown in Figs. 1 and 2.

The maximum force for the studied films is observed in the gelatin film at 15 wt.% (see Table 1). It was found that films have increased stretchability with the addition of plant extract at low concentrations of gelatin, this effect is more pronounced.

With increasing gelatin content in the films, an increase in strength and a decrease in elastic properties were observed (Fig. 1).

The dependence of the tensile strength on gelatin film in the presence of 1wt.% of [TH-10] is shown in Fig. 2.

Film strain in dependence on the gelatin concentration in absence of [TH-10] is shown in Fig. 3 and the presence of 1 wt.% of [TH-10] can be seen in Fig. 4.

With increasing gelatin content in the films, a decrease in elastic properties was observed (Fig. 3).

Table 1

Stress in dependence of the film composition

Film composition, wt.%	σ stress, MPa
[Gelatin]=10, [TH-10]=1	0.1528±0.006
[Gelatin]=11, [TH-10]=1	0.4841±0.040
[Gelatin]=12, [TH-10]=1	0.8026±0.030
[Gelatin]=13, [TH-10]=1	0.6624±0.090
[Gelatin]=14, [TH-10]=1	0.8575±0.020
[Gelatin]=15, [TH-10]=1	1.3416±0.060
[Gelatin]=10	0.59±0.030
[Gelatin]=11	0.61±0.070
[Gelatin]=12	0.59±0.030
[Gelatin]=13	0.94±0.030
[Gelatin]=14	1.12±0.020
[Gelatin]=15	1.41±0.004

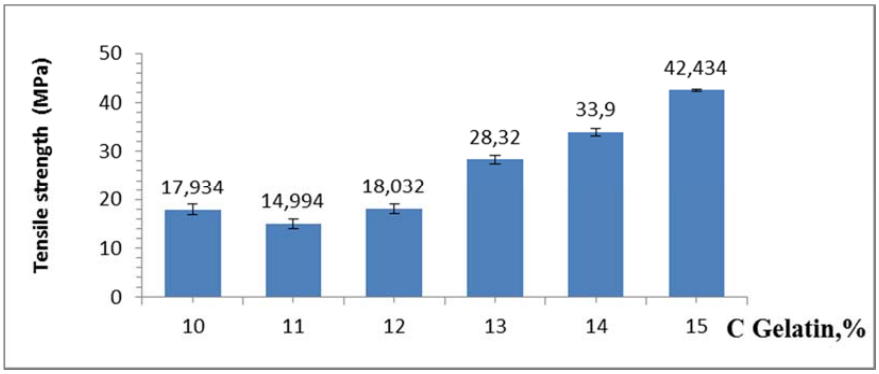

Fig. 1. Concentration dependent strength of gelatin film.

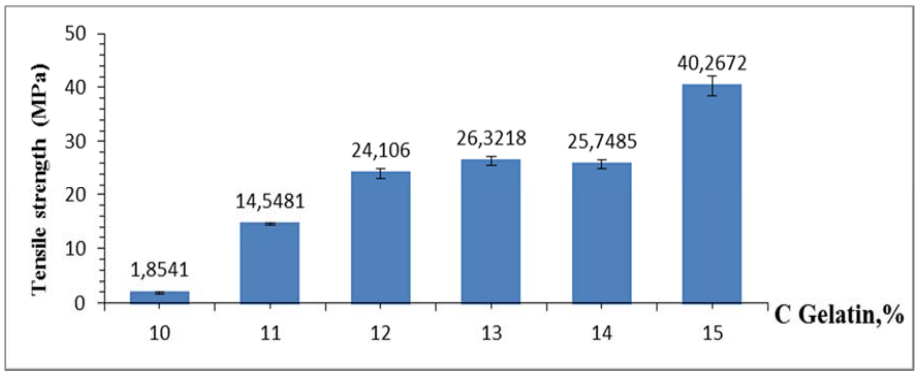

Fig. 2. Concentration dependent strength of gelatin film in the presence of 1 wt.% of [TH-10].

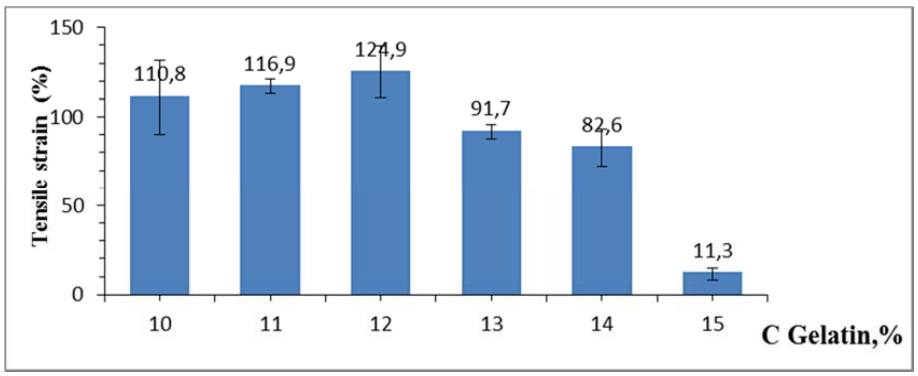

Fig. 3. Film strain in dependence on the gelatin concentration in absence of [TH-10].

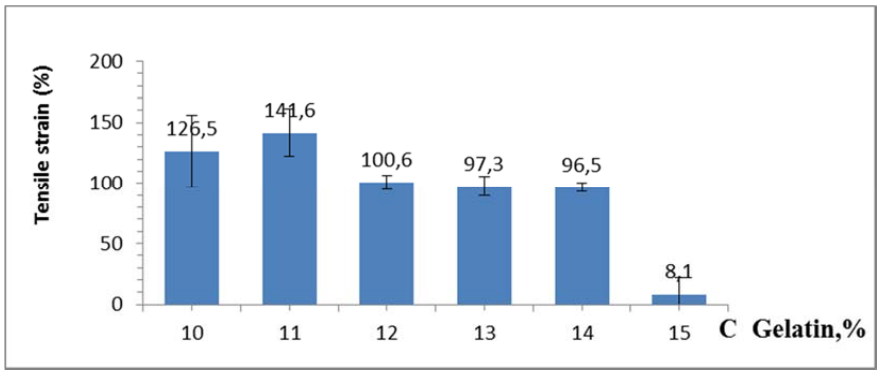

Fig. 4. Film strain in dependence of the gelatin concentration in the presence of 1 wt.% of [TH-10].

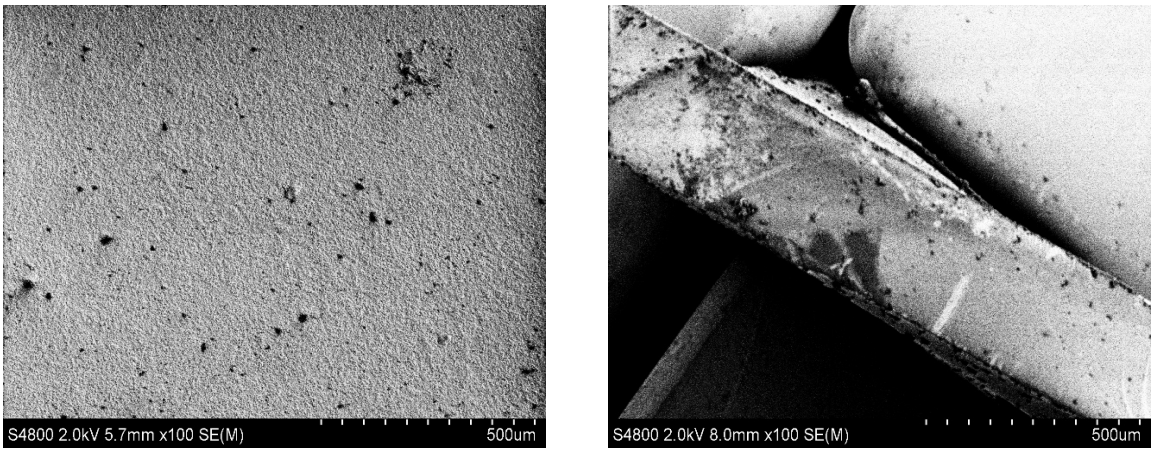

Fig. 5. SEM micrographs of gelatin film surface (left) and cross sections (right).

However, when adding the extract TH-10 to the polymer film, a strong influence dependence on the content of gelatin can be noticed. The addition of TH-10 to the gelatin film increases its elasticity and softens the film. This effect was especially observed in the 10 wt.% gelatin film. Accordingly, it has been observed that the strength of the films deteriorates. The highest strength of the gelatin film was obtained at the gelatin concentration of 15 wt.%.

Gelatin films with extracts of TH-10 were examined by scanning electron microscopy (SEM) using the S–4800 from Hitachi to characterize the film morphology. Figure 5 shows SEM micrographs of the film surface and the cross-section of the film. The absence of pores in the SEM micrographs verifies that the film structure is uniform and dense. Nevertheless, the black point indicates micro-heterogeneities.

The FT-IR spectra of the films were recorded on an "Equinox 55" FT-IR Fourier spectrometer (Broker) in the range 4000–500 cm^{-1}. Spectral data were analyzed according to the instructions. In the FT–IR spectrum of the gelatin film, the stretching shifts of the N–H and C–H bonds are clearly visible in the absorption region of 1640–1200 cm^{-1}. 2900–3600 cm^{-1} corresponds to the formation of a hydrogen interaction due to the displacement of the O–H bond in the carboxyl group –COOH of gelatin.

The FT–IR spectra of films in Fig. 6 show a very intense peak in 3.560 cm^{-1}. It is due to the simultaneous displacement of interactions in the –COOH and $–NH_2$ –amino groups of the polymer (O–H and N–H). As evidence of this, the 1638 cm^{-1} did not show intense activity beyond the zone. Therefore, it is noticeable that the displaced of C–H connections were formed from the appearance of a shoulder–shaped swallowing image. Hydroxo and imine groups were bound to the next drug substance, causing the simultaneous formation of charged ions, and complexes. In addition, gelatin film contains all the characteristic bands of functional groups which were observed at 3.392 cm^{-1} (phenyl-OH), 2.926 cm^{-1} (Alkane C–H), and 1.638 cm^{-1} (ketone C=O). C=O elongation (amide I) peak and 1.638 cm^{-1} and N–H bending (amide II) peak at 1.523 cm^{-1}, 1.215 cm^{-1}. In addition, the peak of 2.926 cm^{-1} in the C–H stretching of the FT-IR spectrum can be shifted to the asymmetric mode of CH_2.

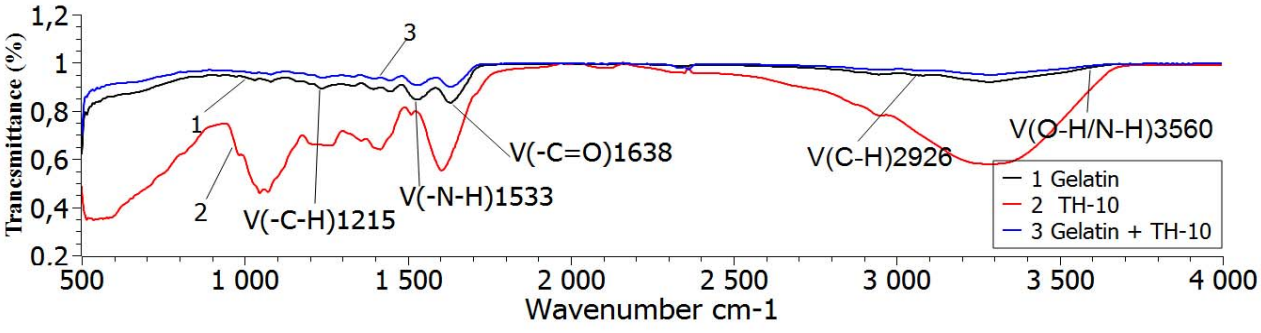

Fig. 6. FT-IR spectra of films [Gelatin] (1), [TH-10] (2), [Gelatin + TH-10] (3).

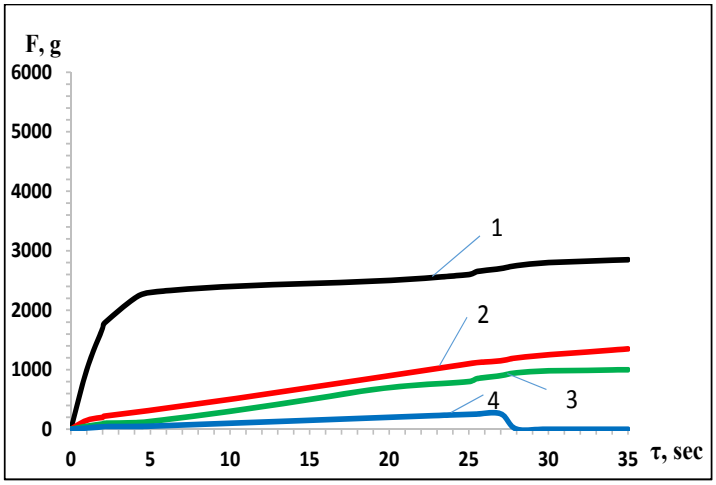

Fig. 7. Deforming force F independence on load to time of films by varying the glycerin concentration: [TH–10] – 1 wt.%, [Gelatin] – 12 wt.%, [Glycerin] – 5 vol.%, (1); [TH–10] – 1 wt.%, [Gelatin] – 12 wt.%, [Glycerin] – 10 vol.%, (2); [TH–10] – 1 wt.%, [Gelatin] – 12 wt.%, [Glycerin] – 15 vol.%, (3); [TH–10] – 1 wt.%, [Gelatin] – 12 wt.%, [Glycerin] – 20 vol.%, (4).

On the other hand wide band was observed in the area of 2.926–3.292 cm^{-1}, which may be related to free –NH groups, hydroxyl groups and intramolecular hydrogen interactions between the components of the gelatin film.

Unfortunately, the characteristic interactions of TH-10 are not detectable in the corresponding Gelatin + TH-10 sample due to the low concentration.

Comparing the numerical values in Table 1 and the curves, in Figs. 1 and 2, it turned out that as the amount of gelatin in the film increased, its strength also increased. The polymer gelatin and the natural extract TH-10 form a complex through hydrogen interactions, increasing its strength depending on the amount of gelatin, compaction of hydrogen interactions, film compaction and macromolecules shrinkage. The reason is that gelatin is a natural polyampholyte.

With the addition of natural extract TH-10 to films, a decrease in elasticity and strength properties can promote the formation of micro-crack on the surface of films, which can result in their rupture. Thus, it was found that the larger the sizes of the gelatin film with the addition of TH-10 in the film, the lower its resistance to breakage.

The effect of glycerin concentration on the gelatin film is shown in Fig. 7.

It has been noted that the variation of the plasticizer concentration in the structure of gelatin film affects the physical properties of the film. In other words, the film becomes less soft, less stretched, less tacky, and less durable as the concentration of glycerin increases. Glycerin is of great importance as a plasticizers that increases the elasticity properties of polymer film [25]. It is important to know the amount of plasticizer when creating film forms. Since gelatin molecules are sensitive to external factors, glycerin molecules penetrate the intermolecular space and affect the mechanical elasticity of films [13]. The most important issue in the development of film material with *Tamarix* is the regulation of the content of the plasticizer (glycerol) in the film composition. Films of *Tamarix* with glycerin content from 5 to 20 wt.% were obtained. To establish the optimal content of glycerol, tensile strength was taken as a criterion for the mechanical properties of polymer films, which was studied using a TA.XTPlus texture analyzer (Stable Micro System, UK), equipped with a load of 7.5 kg. For analysis, rectangular samples were taken with a size of 1x5 cm, with an effective area of the analyzed sample of 3 cm^2. The top clamps allow the film to be drawn out at a speed of 0.5 mm/sec. Another thing to consider is that the increase in the content of glycerin in the film also increases its elasticity, which can give long-term stability to the applied load. On the contrary, in samples with low glycerin concentration, the polymer films were torn in a short time, nevertheless, it kept high strength properties. Thus, with an increase in the amount of glycerin, the load weight for stretching the film can be reduced from 5.5 to 1.5 kg. As for the composition of the polymer film (1:7) vol.%, it was found that the composition with 5 vol.%, glycerin is more effective in a short time at maximum performance, and high mechanical and strength properties of the film. This is due to

the fact that with an increase in the amount of glycerin, the film softens and becomes elastic. This can be evidenced by the fact that the 20 vol.%, glycerin film is not load-resistant, but quickly tears.

4. Conclusion

The best composition for obtaining films based on gelatin containing extract TH-10 was proposed. The components included in the dosage forms were obtained in the following quantities: TH-10 extract – 1 wt.%, glycerin – 5 vol.%, potassium sorbate – 0.02 wt.%, polymer (Gelatin) – 10-15 wt.%. When determining the tensile limit, it was shown that the optimal glycerin content in the film is 5 vol.%. It was found that the deformation properties of the film are directly related to the chemical bond between gelatin and the natural substance TH-10, and the conformation is different in each composition, depending on whether the bond is weak or strong. The result of the SEM photographs showed that the surface of the gelatin film was homogeneous and tightly interacted. As a result 12 wt.% gelatin film was chosen as the most effective composition.

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