

# NEW MATERIALS BASED ON HYALURONIC ACID AND EGG ALBUMIN MIXTURE

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## Abstract

*In this work, new materials based on the mixture of hyaluronic acid and albumin from chicken eggs have been studied. Tests were carried out to determine the molecular weight of the tested hyaluronic acids. The properties of hyaluronic acid were investigated and significant differences were found in the mechanical properties of the tested compound, depending on its molecular weight. It was found that egg albumin can be combined with hyaluronic acid and thin films can be obtained. Spectrometric tests were performed both for pure compounds and for mixtures of hyaluronic acid with chicken egg albumin. IR spectroscopy showed that interactions between hyaluronic acid and egg albumin are mainly by hydrogen bonds, as the shifts in the main bands in IR spectra were observed. The addition of egg albumin to hyaluronic acid leads to the decrease of its mechanical properties. The deterioration of the mechanical properties of polymer films from HA-albumin mixtures may be due to interactions between compounds which were shown in the IR spectra. The thin films based on hyaluronic acid and egg albumin blend can be used as adhesive materials in biomedicine and cosmetics. Both biopolymers are biocompatible and biodegradable so we can expect a biocompatible and biodegradable material for potential application as adhesives.*

**Keywords:** hyaluronic acid, albumin from chicken egg, mechanical properties, polymer films

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## Introduction

The definition of a biomaterial states that it is a chemically or pharmacologically inert substance intended for implantation or contact with a living organism. The biomaterial must meet several basic conditions. It must be non-toxic, biocompatible, and non-carcinogenic. It cannot react with a living organism causing unwanted reactions. Biomaterials can be divided into several basic groups: bioinert (any material that does not cause a negative response by the body's immune system), bioactive, and bioabsorbable [1]. Biomaterials are used in tissue engineering to create healing materials and also as drug delivery systems. Natural polymers, such as proteins, nucleic acids, polysaccharides, are most often used as scaffolds or substrates in biomaterials [2].

The new trend in materials sciences is the fabrication of new biomaterials based on the mixtures of biopolymers. Within the last three decades, an increasing interest in new materials based on blends of two or more polymers has been observed [3-9]. The potential applications of biopolymer blends in the biomedical field can be wide and may include drug delivery systems, tissue engineering, wound healing, or gene therapy. Biopolymer blends can be also applied as edible packaging materials.

Polysaccharides and their modifications have found wide application in the medical and pharmaceutical industries as drug carriers [10]. They may show anti-inflammatory, antiviral, and antibacterial properties, which makes them good raw materials for the production of biomedical materials. They can be of plant or animal origin. The most common natural polysaccharides are cellulose, starch, chitosan and hyaluronic acid [11]. The above-mentioned polysaccharides can be blended with another biopolymer. One of the most commonly used polysaccharides is hyaluronic acid (HA). HA is a water-soluble compound whose structure includes D-glucuronic acid and N-acetyl-d-glycosamine [12]. Hyaluronic acid has been used in ophthalmology, embryo protection, and drug delivery because it is a non-toxic, non-inflammatory, non-allergenic compound, as well as a highly elastic hydrophilic substance [1]. Hyaluronan is also used in eye drops because its sodium salt is present in the vitreous body of the human eye [13]. Hyaluronic acid can be blended with another polymer and/or a biopolymer and in this way, new materials can be obtained [14-20].

In the biomaterials industry, apart from polysaccharides, proteins occupy a special place due to their low cost, high availability, and biodegradability [21]. Proteins can also be considered as a component of the blend with polysaccharides. The most common types of albumin in the biomaterials industry are human albumin, bovine albumin, and egg albumin. Egg albumin is composed of glycoprotein and amino acid residues. Polymer films obtained from chicken egg white have the appropriate properties, thanks to which they can be used as food packaging material. Moreover, egg albumin is biocompatible and biodegradable [22]. It can be blended with water-soluble polysaccharides and in such a way new materials can be fabricated. Biopolymer blends preparation by dissolution in the same solvent allows avoiding protein denaturation. The specific interaction between the blend components is often called miscibility. The most common interactions in the blends are: hydrogen bonding (when polymers contain chemical groups capable of forming hydrogen bonds), ionic and dipole, pi-electrons and charge-transfer complexes. The interactions between HA and egg albumin can determine the properties of the blend. Moreover, the interactions between hyaluronic acid and albumin can be important from biomedical point of view because the mixture of hyaluronic acid and albumin can mimic the synovial fluid and it may influence the tribological properties of cartilage. The blends of hyaluronic acid and albumin can be used in drug delivery systems so it is crucial to know the physico-chemical properties of such blends.

In this work polymer thin films made of pure hyaluronic acid of three different molecular weights and their mixtures with egg albumin have been researched. The mechanical and physicochemical properties of the obtained membranes were investigated and compared. To the best of the authors' knowledge, there is very limited information about the structural features of hyaluronic acid-albumin molecular assemblies, including intermolecular interaction characteristics.

## Materials and Methods

### Materials

High-molecular-weight hyaluronic acid (HA), low-molecular-weight HA, and ultra-low-molecular HA were purchased from the cosmetic company (Prochowice, Poland). Albumin from chicken egg was purchased from Sigma-Aldrich (Merck Life Science, Poznań, Poland).

### Preparation of solutions

To determine viscometric average molecular weights of HA, a stock solution of NaCl was prepared in which the three tested hyaluronic acids were dissolved. The following solutions were obtained: a solution 0.1% of high-molecular HA in NaCl, a 0.1% solution of low-molecular HA in NaCl, and a 1% solution of ultra-low-molecular HA in NaCl.

For the study of mechanical properties and FTIR analyses, thin films were manufactured using the 1.5% high-molecular HA, the 1.5% low-molecular HA, and the 2% ultra-low-molecular HA solutions which were prepared in water.

### Determination of viscometric average molecular weights

The prepared stock solution of hyaluronic acid in NaCl was poured into the Ubbelohde viscometer (Ubbelohde viscometer is a standard glass device commercially available). Then, the time of the solution flow between the two marked places was measured several times. The stock solution was then diluted with successive portions of the NaCl solution and the flow times were measured each time. The measurements were performed for three different hyaluronic acids. The molecular weights of the tested hyaluronic acids were determined with the help of a computational program.

The molecular weight of HA used in this study was as follows:

$$M_{\text{HAhigh-molecular}} = 8,434 \cdot 10^5 \text{ (g/mol)}$$

$$M_{\text{HALow-molecular}} = 6,327 \cdot 10^5 \text{ (g/mol)}$$

$$M_{\text{HAultra-low-molecular}} = 3,445 \cdot 10^4 \text{ (g/mol)}$$



FIG. 1. Image of the film made from the 1.5% low molecular weight HA solution.

### Preparation of polymer blends and films

Each of the three hyaluronic acid solutions was mixed with powdered egg albumin. The amount of 0.3 g powdered albumin was added to 30 g of HA solution and then stirred for about 15 min on a magnetic stirrer. Then 25 g of the resulting mixtures and the pure solutions of the three test HAs were poured onto plastic plates. It took a week for the solvent to evaporate and the polymer film to form. The images of the films obtained in such a way are shown in FIG. 1 and FIG. 2.

### IR spectroscopy

The infrared spectra were registered by Nicolet iS10 spectrophotometer equipped with an ATR device with a germanium crystal (Thermo Fisher Scientific, Waltham, MA, USA). All the spectra were recorded with the resolution of  $4 \text{ cm}^{-1}$  with 64 scans. The spectra were evaluated in the range of  $400\text{--}4000 \text{ cm}^{-1}$ . The data was obtained using the Omnic Spectra 2009 program.

### Mechanical properties

Fittings of the same size were cut from all the obtained polymer films. Mechanical properties, including tensile strength force and Young's modulus, were tested on a Zwick & Roell Z.0.5 testing machine (ZwickRoell Group, Ulm, Germany). 10 trials were made for each film. Initial parameters of the research program were as follows: initial force 0.1 MPa, initial force speed 5 mm/min, testing speed 50 mm/min, the load cell is 0.5 N. The data was collected in the testXpert II 2017 program. The statistical analysis of the obtained results was performed using the Q-Dixon test in MS Excel.

## Results and Discussions

### Physicochemical properties

The IR spectra were recorded to determine the interactions between hyaluronic acid and albumin. The individual spectra of pure compounds and mixtures are presented in the figures below (FIGs. 3-8). The positions of the individual bands are presented in the tables below (TABLES 1-3).



FIG. 2. Image of the film made from the mixture of 1.5% low molecular weight HA with egg albumin.

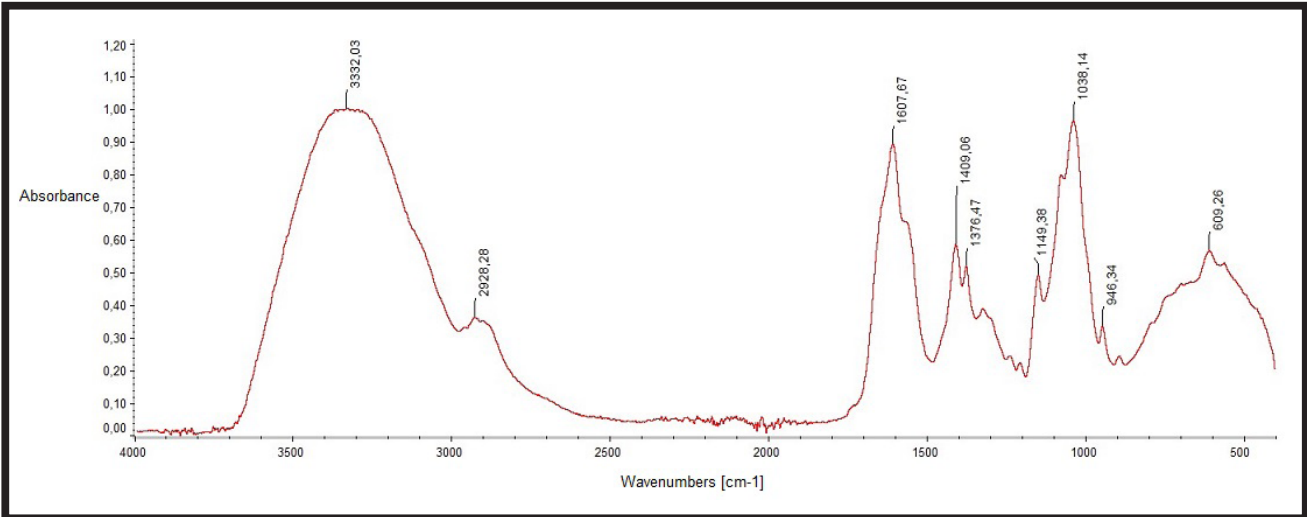


FIG. 3. IR spectrum of the 1.5% HA high molecular weight.

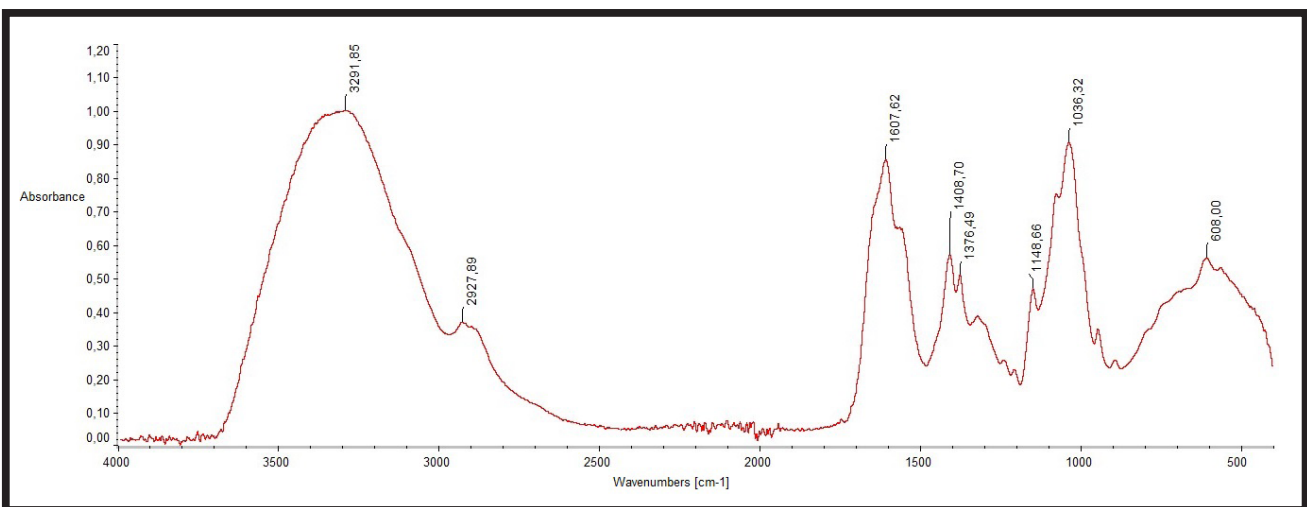


FIG. 4. IR spectrum of 1.5% HA low molecular weight.

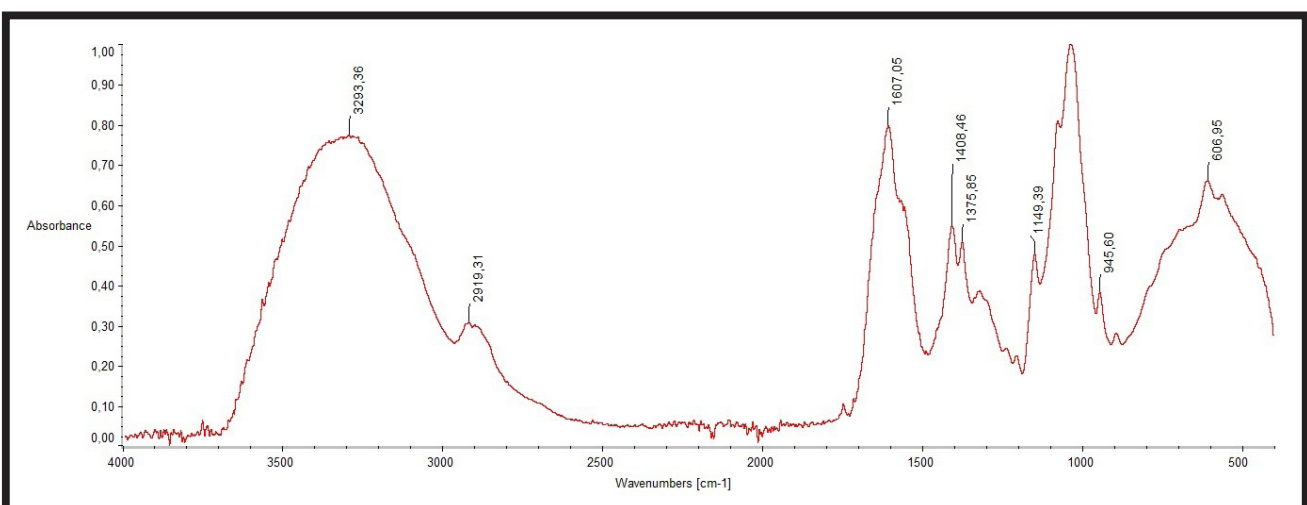


FIG. 5. IR spectrum of the 2% ultra-low molecular weight HA.

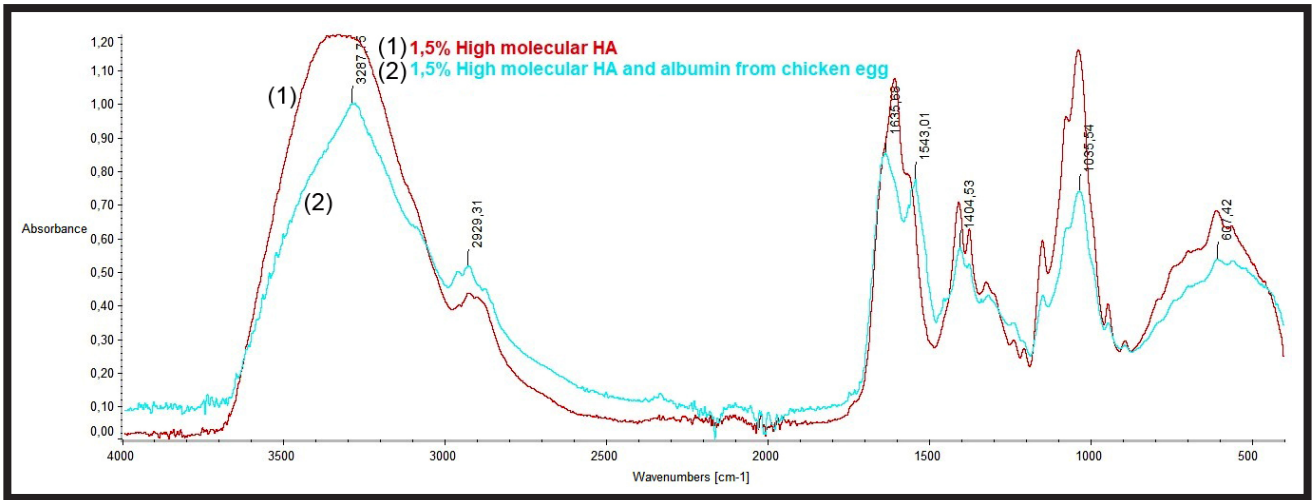


FIG. 6. IR spectrum of the mixture of 1.5% HA high molecular HA with albumin and IR spectrum of the pure high molecular HA.

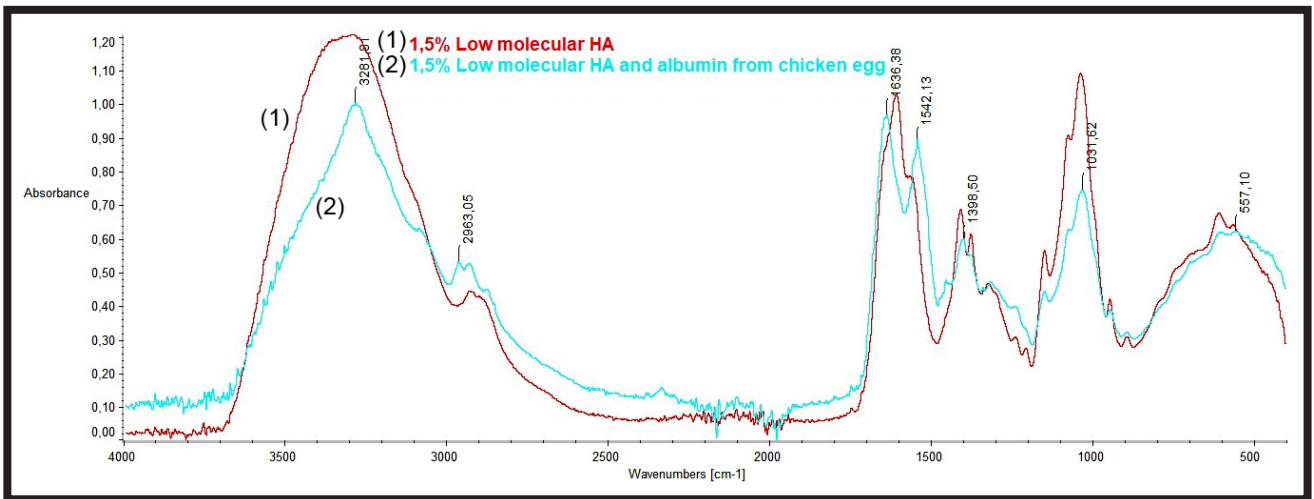


FIG. 7. The IR spectrum of the mixture of the 1.5% HA low molecular weight HA with albumin and the IR spectrum of pure low molecular weight HA.

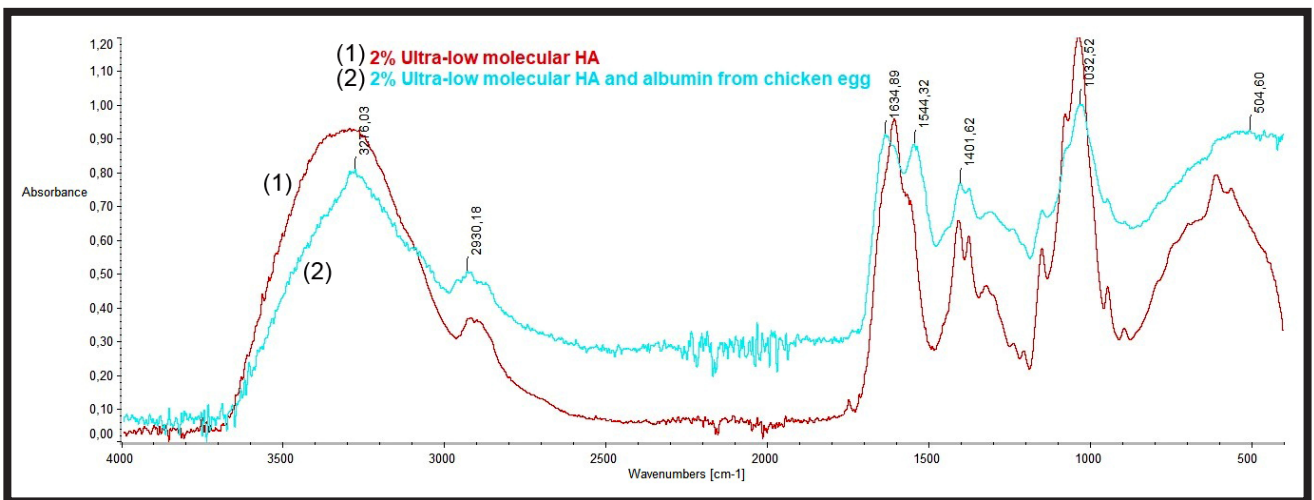


FIG. 8. The IR spectrum of the mixture of 2% HA of ultra-low molecular weight with albumin and the IR spectrum of the pure ultra-low molecular weight HA.

**TABLE 1. Wavenumbers in IR spectra for bonds occurring in the high molecular weight hyaluronic acid and its mixture with egg albumin.**

No.	Function group	Vibrations	Band position for hyaluronic acid [cm <sup>-1</sup> ]	Band position for the mixture of hyaluronic acid and albumin [cm <sup>-1</sup> ]
1	O-H; N-H	Stretching	3332	3287
2	C-H	Stretching	2928	2929
3	N-H	Deformative	1607	1635
4	C-N; N-H	Stretching; Bending	-	1543
5	C=O	Stretching	1409	1404
6	C=O	Stretching	1376	-
7	COH	Stretching	1038	1035
8	COC	Stretching	946	-
9	COC	Stretching	609	607

**TABLE 2. Wavenumbers for bonds occurring in the low molecular weight hyaluronic acid and its mixture with egg albumin.**

No.	Function group	Vibrations	Band position for hyaluronic acid [cm <sup>-1</sup> ]	Band position for the mixture of hyaluronic acid and albumin [cm <sup>-1</sup> ]
1	O-H; N-H	Stretching	3291	3281
2	C-H	Stretching	2927	2963
3	N-H	Deformative	1607	1636
4	C-N; N-H	Stretching; Bending	-	1542
5	C=O	Stretching	1408	1398
6	C=O	Stretching	1376	-
7	COH	Stretching	1036	1031
8	COC	Stretching	608	557

**TABLE 3. Wavenumbers for bonds occurring in the ultra-low molecular weight hyaluronic acid and its mixture with egg albumin.**

No.	Function group	Vibrations	Band position for hyaluronic acid [cm <sup>-1</sup> ]	Band position for the mixture of hyaluronic acid and albumin [cm <sup>-1</sup> ]
1	O-H; N-H	Stretching	3293	3276
2	C-H	Stretching	2919	2930
3	N-H	Deformative	1607	1634
4	C-N; N-H	Stretching; Bending	-	1544
5	C=O	Stretching	1408	1401
6	C=O	Stretching	1375	-
7	COH	Stretching	945	1032
8	COC	Stretching	606	504

The IR spectra for hyaluronic acid are almost identical, regardless of its molecular weight. Minor differences in the wavenumber may be caused by a slight change in conditions when performing spectrometric measurements. The obtained IR spectra confirm the presence of amide, hydroxyl, and carboxyl groups in the tested compounds.

The addition of egg albumin to hyaluronic acid changed the infrared spectra of this polysaccharide. The characteristic band for albumin, confirming the presence of protein in the tested film, is the band corresponding to the wavenumber 1543, 1542, and 1544 cm<sup>-1</sup>. This band corresponds to the stretching of the C-N group and bending of the N-H group [23]. Reducing the intensity of the bands in mixtures and shift of the bands, e.g. 3332 cm<sup>-1</sup> in HA and 3287 cm<sup>-1</sup> in the mixture of HA with albumin, may suggest the interactions between the components of the mixtures. The interaction occurs mainly via hydrogen bonds.

The band of about 3300 cm<sup>-1</sup> is attributed to vibrations stretching the OH bond and to vibrations stretching the NH bond, while the band of about 1600 cm<sup>-1</sup> corresponds to deformation vibrations of the NH bond (presence of an amide functional group in the structure of hyaluronic acid). The band at 2930 cm<sup>-1</sup> corresponds to the stretching vibration in the C-H group. The bands appearing around 1030 cm<sup>-1</sup> and from 607 to 505 cm<sup>-1</sup> may indicate stretching vibrations in the COC group. The C = O stretching vibration corresponds to the band around 1408-1375 cm<sup>-1</sup> and confirms the presence of the carboxyl group in hyaluronic acid. The band appearing around 1038 cm<sup>-1</sup> corresponds to the COH stretching vibrations [24,25]. Having added albumin to hyaluronic acid, the shift of the above-mentioned bands in the IR spectra clearly shows the interactions between the mixture components.

### Mechanical properties

The mechanical properties of films made of pure hyaluronic acid solutions of three different molecular weights and of films produced by mixing hyaluronic acid with egg albumin were investigated. The obtained results are presented in the tables below (TABLES 4-6).

The mechanical properties of obtained polymeric films vary, depending on the molecular weight of the hyaluronic acid. The highest value of  $F_{max}$  was obtained for the high-molecular weight HA, and the lowest for the ultra-low-molecular HA. The highest value of Young's modulus ( $E_{mod}$ ) was obtained for the ultra-low molecular weight HA.

The addition of ovalbumin to the hyaluronic acid solution significantly affects the mechanical properties of polymer films. The tested films made of mixtures of HA and albumin had lower  $E_{mod}$  and  $F_{max}$  values. The  $F_{max}$  values for the mixtures decrease with the decreasing molecular weight of HA.

Changes in the mechanical properties following the addition of albumin may suggest interactions between hyaluronic acid and albumin. This has been confirmed in spectrometric tests. The weaker mechanical properties after the addition of egg albumin may suggest that the hydrogen bonds between HA and egg albumin are weaker than hydrogen bonds between HA molecules.

### Conclusions

Egg albumin can be combined with hyaluronic acid and thin films can be obtained. The addition of albumin reduces the mechanical properties of the hyaluronic acid films. The tensile strength of the HA films decreases when albumin is added to hyaluronic acid. This may be due to the interaction of albumin and hyaluronic acid and the formation of bonds weaker than those between HA molecules. The spectrometric studies confirmed the interactions between the hyaluronic acid molecule and albumin. The obtained polymer films based on hyaluronic acid and their mixtures with albumin can be used in the biomaterials and cosmetics industry, e.g. as biomimetic coatings and adhesives. The blends of hyaluronic acid and albumin can also be used in drug delivery systems. However, more research is needed to investigate the biological activity as well as the cosmetic and biomaterial potential of albumin-containing hyaluronic membranes. Nevertheless, it should be emphasized that the obtained biomaterial should be biocompatible and biodegradable as no cross-linking agent has been used for the materials preparation and the thin films were formed spontaneously via self-assembly.

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**TABLE 4. Mechanical properties of the film made of the 1.5% high molecular weight hyaluronic acid solution and its mixture with egg albumin.**

No.	Material	$E_{mod}$ [GPa]	$F_{max}$ [MPa]
1	HA	0.708	61.5
2	HA with albumin	0.298	48.07

**TABLE 5. Mechanical properties of the film made of the 1.5% solution of low molecular weight hyaluronic acid and its mixture with egg albumin.**

No.	Material	$E_{mod}$ [GPa]	$F_{max}$ [MPa]
1	HA	0.196	58.37
2	HA with albumin	0.220	38.02

**TABLE 6. Mechanical properties of the film made of a 2% solution of ultra-low molecular weight hyaluronic acid and its mixture with egg albumin.**

No.	Material	$E_{mod}$ [GPa]	$F_{max}$ [MPa]
1	HA	1.44	53.13
2	HA with albumin	0.303	17.85

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