2024 IEEE 14th International Conference
"Nanomaterials: Applications & Properties" (IEEE NAP-2024)
Riga, Latvia, Sep. 8-13, 2024

Design and Research of Thermo-Responsive Gelatin-Alginate-Humic Nanocomposite Hydrogels for Controlled Drug Delivery

Katerina Lebedeva
The department of plastics and biologically active polymers technology
National Technical University «Kharkiv Polytechnic Institute»
Kharkiv, Ukraine
oazis.ruk@gmail.com

Natalia Klochko
The department of micro- and nanoelectronics
National Technical University «Kharkiv Polytechnic Institute»
Kharkiv, Ukraine
klochko.np16@gmail.com

Denis Miroshnichenko
The department of Technologies of Oil, Gas and Solid Fuel Processing
National Technical University "Kharkiv Polytechnic Institute"
Kharkiv, Ukraine
dvmir79@gmail.com

Anna Cherkashina
The department of plastics and biologically active polymers technology
National Technical University «Kharkiv Polytechnic Institute»
Kharkiv, Ukraine
annikcherkashina@gmail.com

Olena Bogoyavlenska
The department of Technologies of Oil, Gas and Solid Fuel Processing
National Technical University "Kharkiv Polytechnic Institute"
Kharkiv, Ukraine
evbsob@gmail.com

Volodimir Lebedev
The department of plastics and biologically active polymers technology
National Technical University «Kharkiv Polytechnic Institute»
Kharkiv, Ukraine
vladimirlebedev1980@ukr.net

2024 IEEE 14th International Conference Nanomaterials: Applications & Properties (NAP) | 979-8-3503-8012-5/24/$31.00 ©2024 IEEE | DOI: 10.1109/NAP62956.2024.10739758

Abstract— In this work, thermo-responsive gelatin-alginate-humic nanocomposite hydrogels with physiological melting temperatures were developed. These hydrogels exhibit optimal rheological characteristics suitable for hemostasis and controlled drug delivery, achieved through the combination of gelatin (GN) and sodium alginate (ALG) with humic acids (HANA and HACA) as modifiers. The addition of humic acids enhanced the gel-sol transition temperatures, contributing to their stability and suitability for medical applications. Structural investigation using X-ray diffractometry revealed alterations in nanocrystallinity, while Fourier transform infrared spectroscopy confirmed non-covalent interactions between the hydrogel components, affecting the conformational structure. The resulting hydrogels exhibited enhanced mechanical properties, biocompatibility, and controlled transition temperatures suitable for targeted drug delivery and wound healing applications. The study demonstrates that the integration of humic acids into gelatin-alginate hydrogels significantly improves their thermo-responsive properties, making them promising candidates for biomedical applications.

Keywords—humic acid, gelatin, alginate, hydrogel, drug delivery

I. Introduction

Hydrogels are three-dimensional polymeric materials whose matrix consists primarily of water (usually 70–90 wt.%). The association of molecular chains in hydrogels occurs through weak non-covalent bonds such as hydrogen bonds, π–π stacking, van der Waals connections, metal coordination, electrostatic interactions, and host–guest interactions. Therefore, they can respond to various small external stimuli, such as pressure, light, electric and magnetic fields, and temperature, by changing their shape and form, volume and state of aggregation (reversible transition from sol to gel and vice versa). The above smartness and excellent diffusion properties of hydrogels have recently been widely used in the creation of multifunctional soft robots and actuators, in particular, for targeted delivery of drugs for wound healing and hemostasis [1-3]. Biopolymers are materials that can be extracted from biological sources such as microorganisms, vegetables, plants, trees, algae, animals, fish, crustaceans, etc. Biopolymer smart hydrogels have many unique advantages such as natural abundance, strong structure, hydrophilicity, water solubility, multiple active functional groups, as well as mechanical flexibility, biocompatibility, non-toxicity, biodegradability and renewability [5-6]. For example, thermosensitive, or thermo-responsive biopolymer hydrogels are capable of containing drugs at room temperature and delivering drugs at physiological temperature (i.e., 37 °C) when applied to human skin or when attached to a wound. Due to this, thermo-responsive biopolymer hydrogels have also long been used as carriers for targeted drug delivery systems for *in vivo* immobilization due to their excellent biocompatibility with the body environment and controlled degradation period for self-destruction [3-6]. Typically, the principle of operation of thermo-responsive biopolymer hydrogels is the transition from gel to sol due to the weakening or breaking of intermolecular and intramolecular bonds when the temperature rises to the gel-sol transition temperature T_{GS}, the so-called melting temperature of the hydrogel. Hydrogels with a critical temperature T_{GS} close to physiological temperature are particularly preferred for drug transport and release [6].

The most widely used biopolymer hydrogels are protein gelatin (GN) and polysaccharide alginate (ALG), the important features of which are low cost, wound healing and bleeding control [7-13]. Gelatin is a thermo-responsive hydrogel widely used in wound dressings because it provides immediate homeostasis and also prevents wound contracture and contour deformities associated with normal wound healing [10,13]. When GN sols are cooled to temperatures

below 30 °C, a sol-gel transition occurs as the GN chains undergo a progressive conformational change known as the coil-to-helix transition, resulting in the formation of a triple helix network [13]. However, the melting points T_{GS} of GN hydrogels are lower than the physiological temperature. For example, for a gelatin hydrogel containing from 2.5 wt.% GN up to 5 wt. % GN, the T_{GS} values are within the range of 32.4°C – 34°C [10]. In addition, the hygroscopicity of GN films makes it difficult to use them in conditions of high humidity [7]. In [13], the addition of humic acids was used as a way to strengthen gelatin hydrogels in order to increase the melting point, as well as to improve their wound-healing ability.

Alginate is a hydrophilic polysaccharide anion, which structurally is a linear copolymer consisting of alternating blocks of (1,4)-α-Lguluronate (unit G) and (1,4)-β-d-mannuronate (unit M) [7]. Pure sodium alginate hydrogels do not exhibit temperature effects [10]. However, ALG is capable of forming hydrogels through ionotropic gelation with divalent and multivalent cations. In accordance with [7, 14], in the presence of calcium ions, carboxyl groups in the G-units of neighboring polymer chains of alginates are cross-linked due to interaction with Ca^{2+} to form calcium alginate according to the "egg box" model, which, due to its ionic cross-links and chain-chain stabilization, has excellent mechanical properties. ALG exhibits increased hemostatic properties, because when it comes into contact with blood, platelet aggregation accelerates and the blood clotting process starts [15]. The disadvantages of alginate are its low mechanical strength and cell adhesion, low drug loading, microbial degradation and burst release [9]. Therefore, according to [9], to overcome such problems, ALG must be mixed with other biopolymer hydrogels, which will improve its properties due to additive non-covalent interactions. Recently, a large number of studies [10,12,16] have been devoted to the formation of complex double-network and multiphase gelatin-alginate hydrogels (GN_ALG), the structure of which is formed by homopolymer and heteropolymer blocks. According to [10,12,16], the GN_ALG hydrogels demonstrate the joint synergistic effect of alginate and gelatin on rheological and thermo-responsive properties. On the other hand, local agents, which can prevent infection of wounds by pathogenic microorganisms, should be added to ready-made complex hemostatic and wound-healing biopolymer hydrogels [11,13]. For this purpose, in this work, gelatin-alginate composite hydrogels were modified with the addition of humic acids, which have antioxidant, antifungal, antibacterial and anti-inflammatory properties [17]. Here we study the structure and rheological properties of bioactive smart thermo-responsive gelatin-alginate-humic nanocomposite hydrogels, which at a physiological temperature of 37°C are reversibly converted into sols, due to which they can be used for the introduction of drugs into hard-to-reach places for local and long-term delivery of drugs, and also to reduce the doses of delivered drugs.

II. Experimental Procedures

In this work we used edible gelatin grade R-11 (TM "Mriya", PJSC "Ukroptbakaliya", Ukraine) and sodium alginate (Lianyungang Fengyun Seaweed Manufacturing Co., Ltd., China) as sources of GN and ALG, respectively. Two types of humic acids, HANA and HACA, were obtained in accordance with [17] from brown coal by primary extraction using a solution of sodium pyrophosphate. The difference consisted in subsequent extractions with aqueous solutions of 1% NaOH for HANA and 0.2% $Ca(OH)_2$ for HACA, followed by precipitation with hydrochloric acid with a density of 1.15-1.19 g/cm^3 (DSTU 3118, Ukraine). To prepare hydrogel GN_ALG, containing 14 wt. % GN and 6.4 wt. % ALG, a defined amount of gelatin was first placed in distilled water, preheated to 90°C and stirred with a VEVOR 85-2 magnetic stirrer in a water bath until a pure gelatin sol was obtained. Then, sodium alginate was added to the gelatin sol and mixed with a VEVOR 85-2 magnetic stirrer with a heating plate to obtain a homogeneous GN_ALG sol contained approximately 80 wt.% of water. Humic acids were partially dissolved in aqueous alkaline solutions to obtain a suspension of HANA in a solution of 1 wt.% NaOH, and a suspension of HACA in a solution of 0.2 wt.% $Ca(OH)_2$, before their addition to the biopolymer sol GN_ALG. After their adding to GN_ALG, gelatin-alginate sols modified with humic acids. Once cooled, they turned into hydrogels GN_ALG_HANA2.5, GN_ALG_HANA5 and GN_ALG_HANA7.5, containing 2.5 wt.% HANA, 5 wt. % HANA and 7.5 wt. % HANA, respectively. The modified with humic acids gelatin-alginate hydrogels GN_ALG_HACA2.5, GN_ALG_HACA5 and GN_ALG_HACA7.5 contained 2.5 wt. % HACA, 5 wt. % HACA and 7.5 wt. % HACA, respectively.

To reveal the crystal structure, X-ray diffraction analysis (XRD) was carried out on films of these gelatin-alginate hydrogels, including those modified with humic acids, deposited on glass substrates. For this purpose, a Shimadzu XRD-6100 diffractometer with monochromatic CuKα radiation (λ_x = 1.54060 Å), was used working in the Bragg–Brentano geometry (θ–2θ). To avoid sample drift and to preliminarily determine the sample zero point, a standard sample holder was used and a standard diffractometer setup was performed, after which the instrument was calibrated against polycrystalline silicon. Errors in the determination of Bragg angles θ were corrected by applying analytical methods to the measured Bragg peaks. For this purpose, the X-ray patterns were processed by methods of background removal, smoothing, splitting the Kα1 – Kα2 doublet, as well as calculating the diffraction profile parameters using the "New_Profile v.3.4 (486)" program. The approximate average size of nanocrystallites D (more precisely, the size of coherent scattering regions in the direction normal to the reflecting plane) in the hydrogel films was estimated by the method of broadening X-ray lines using the Scherer equation, as in [18]:

$$D = (k \cdot \lambda_x) / (\beta \cdot \cos\theta), \quad (1)$$

where Scherrer constant for spherical nanocrystals k = 0.9; β is the line broadening at half maximum intensity (full width at half maximum, FWHM) after subtracting the instrumental line broadening in radians.

To obtain the experimental interplanar spacing d, we applied Bragg's law using a positive integer n = 1 [18]:

$$n\lambda_x = 2d\sin\theta \quad (2)$$

The rheological measurements were carried out to study the gel-sol transition temperature of the obtained hydrogels. Herein, we used a standard method for determining kinematic viscosity (mm^2/s) using a glass viscometer VPZh-2 3.35 with a capillary diameter of 3.35 mm and a viscometer constant of 10. The measurement of kinematic viscosity using a VPZh-2 viscometer consisted of determining the time of flowing of a certain volume of sol through a capillary from a measuring container. The T_{GS} temperature was obtained from the moment the sol began to flow freely through the widening in a capillary with a diameter of 3.35 mm, when kinematic viscosity drops to about 450 mm^2/s.

To study the non-covalent interactions between GN, ALG and humic acids HANA and HACA in the thermo-responsive and wound-healing biopolymer hydrogels with physiological melting points, Fourier transform infrared spectroscopy (FTIR) was used. FTIR spectra were recorded on a Nicolet 380 IR spectrophotometer (USA) at temperatures 20-25 °C in the frequency range 500 - 4000 cm^{-1}.

III. Results and Discussion

The X-ray diffraction patterns of the biopolymer samples in Fig. 1 and Fig. 2 show two peaks and an amorphous halo, which, according to literature data [7,19,20], belong to the alginate anion, that is built from the residues of β-D-mannuronic (M) and α-Lguluronic (G) acids. The nanocrystallinity of alginate is explained [19] by the strong interaction between alginate chains through intermolecular hydrogen bonds. According to [2], the diffraction peak at values of the Bragg angles 2θ of approximately 14º belongs to the crystal plane (110) of the polyguluronate unit (G), the peak at 2θ at approximately 22º corresponds to the crystal plane (200) of polymannuronate (M), and the halo at 2θ at approximately 40º corresponds to the amorphous phase of alginate. According to [21], the structure of gelatin is essentially amorphous with a halo at 2θ angles of about 20º. On the contrary, the authors of [13] argued that a broad X-ray diffraction peak at an angle of 2θ ≈ 20° is typical for the partially crystalline structure of gelatin and is associated with the presence of an α-helix in the protein.

As can be seen in Fig. 1 (b), (c), (d), and Table I, the addition of HANA humic acids to the gelatin-alginate hydrogel reduces the number of G nanocrystalline areas, which evidenced by a decrease in the intensity of the (110)G peak in the corresponding X-ray diffractions at 2θ of approximately 14º. Calculations according to Scherer's formula (1) presented in Table I, showed a decrease in the average size of nanocrystallites of polyguluronate units of alginate *D* from 5.3 nm to 2.3 nm with an increase in the concentration of HANA in the gelatin-alginate hydrogel to 7.5 wt.%. At the same time, the analysis of the effect of NANA on the polymannuronate component of alginate, i.e., on the (200)M peak in X-ray diffractions (Fig. 1, Table I), demonstrates a decrease in the size of the crystal lattice of nanocrystals of the polymannuronate component of alginate.

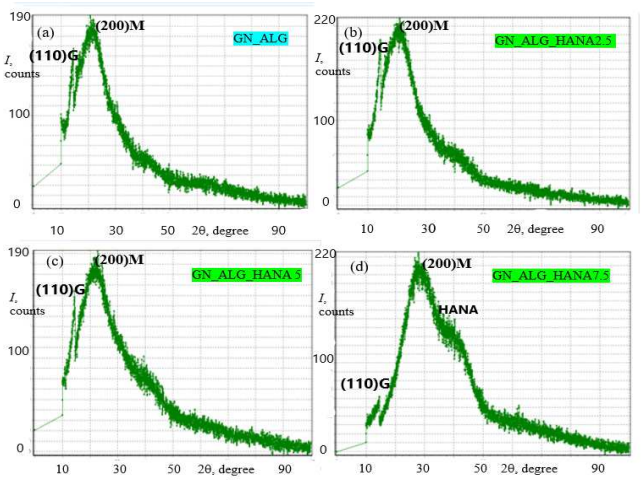

Fig. 1. X-ray diffraction patterns of dried gelatin-alginate hydrogel films on glass substrates: (a) GN_ALG; (b) GN_ALG_HANA2.5; (c) GN_ALG_HANA5; (d) GN_ALG_HANA7.5

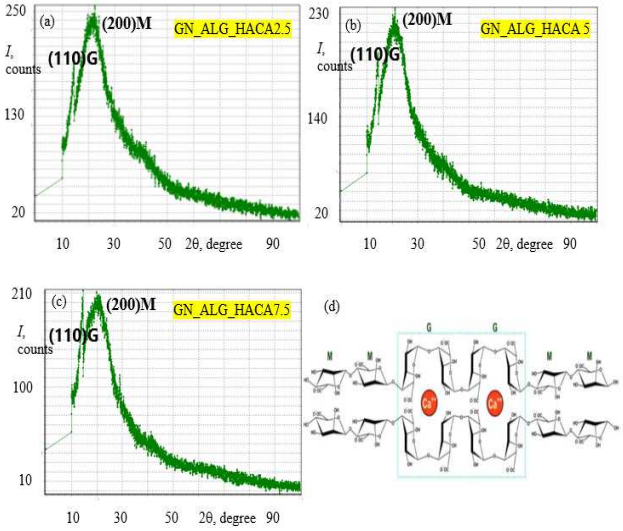

Fig. 2. (a)-(c) X-ray diffraction patterns of dried gelatin-alginate hydrogel films on glass substrates: (a) GN_ALG_HACA2.5; (b) GN_ALG_HACA5; (c) GN_ALG_HANA7.5. (d) – Scheme of “egg-box” structure in alginate hydrogel crosslinked with calcium ions.

Due to a decrease in interplanar distances *d* in the polymannuronate nanocrystals, which, in accordance with equation (2), is demonstrated by the shift of the (200)M peak towards larger angles 2θ from approximately 21º for GN_ALG to almost 28º for GN_ALG_HANA7.5, we can confirm the appearance of large tensile microstrains.

In addition, with higher concentrations of NANA in the geltin-alginate hydrogel, X-ray diffraction patterns show an increase in amorphous halos at 2θ of approximately 40º, which is due to the defective structure of the gelatin-alginate hydrogel, which is aggravated by the addition of HANA. According to [13], high amounts of HANA affect the secondary structure of the protein, preventing the organization of gelatin chains into three-helical domains and causing the formation of a more disordered organization. At the concentration of HANA of 7.5 wt.%, the X-ray diffraction pattern (Fig. 1 (d)) exhibits an additional peak at 2θ ≈ 40°, which correspond to small HANA nanocrystals with *D* of 1 nm.

TABLE I. ANALYSIS OF THE NANOCRYSTALLINE STRUCTURE OF GELATIN-ALGINATE HYDROGELS, INCLUDING THE ONES MODIFIED WITH HUMIC ACIDS, ACCORDING TO XRD DATA

Sample	(110) G plane from polyguluronate			(200)M plane from polymannuronate		
	Position 2θ	*Intensity, counts*	*Crystal size, D, nm*	*Position, 2θ, degree*	*Intensity, counts*	*Crystal size, D, nm*
GN_ALG	14.54º	71	5.3	20.82	190	0.68
GN_ALG_HANA2.5	14.38	113	5.3	21.14	218	0.71
GN_ALG_HANA 5	14.34	105	3.6	22.86	192	0.66
GN_ALG_HANA7.5	14.52	48	2.3	27.94	224	0.60
GN_ALG_HACA2.5	14.52	134	2.3	22.44	256	0.73
GN_ALG_HACA 5	14.36	113	3.0	21.08	219	0.84
GN_ALG_HACA7.5	14.78	160	8.9	20.92	342	0.83

The influence of NACA humic acids on the crystalline structure of gelatin-alginate hydrogel is shown in Fig. 2 as increasing the intensity of the (110)G peak at 2θ ≈ 14º. According to [7, 20], in the presence of calcium ions, carboxyl groups in polyguluronate units G of neighboring alginate polymer chains interact with Ca^{2+}, due to which cross-linking occurs with the formation of calcium alginate through the "egg-box" model shown in Fig. 2(d), which contributes to an increase in the number of nanocrystalline regions (110)G.

Also, as shown by the calculations according to Scherer's formula (1), presented in the Table. I, there is a significant increase in the average size of the nanocrystallites of the polyguluronate units of alginate *D* to 9 nm when the concentration of NACA in the gelatin-alginate hydrogel increases to 7.5 wt.%.

At the same time, as follows from the analysis of the (200)M peaks on the X-ray diffractions in Fig. 2 and Table I, there is no noticeable effect of NACA on the polymannuronate component of alginate.

Thus, X-ray diffraction studies revealed the strengthening of a hydrogel containing 14 wt. % GN and 6.4 wt. % ALG and modified with aqueous solutions of 2.5-7.5 wt.% HACA and 0.2% $Ca(OH)_2$ due to the expansion of crystalline regions and an increase in the size of nanocrystals. Studies of their rheological properties by measuring kinematic viscosity have shown that these hydrogels are more viscous and thermally stable, having a T_{GS} in the range of 55-60 ºC. Therefore, the gel-sol transition temperature of the biopolymer hydrogels GN_ALG_HACA2.5, GN_ALG_HACA5 and GN_ALG_HACA7.5 turned out to be much higher than the physiological temperature, which does not allow their use as smart thermo-responsive hydrogels for controlled drug delivery.

TABLE II. RHEOLOGICAL STUDIES OF THERMO-RESPONSIVE GELATIN-ALGINATE-HUMIC NANOCOMPOSITE HYDROGELS

Temperature, °C	Kinematic viscosity, mm²/s		
	GN_ALG	*GN_ALG_HANA2.5*	*GN_ALG_HANA5*
32	1400	1405	1410
32.5	960	990	1010
33	900	985	995
33.5	760	975	985
34	730	890	910
34.5	700	870	890
35	660	855	910
35.5	630	815	825
36	620	760	770
36.5	600	735	682
37	440	460	611
37.5	310	370	410
38	300	360	370
38.5	260	330	340
39.0	210	310	330
39.5	160	260	280
40.0	90	200	220

Studies of the rheological properties of the biopolymer hydrogel GN_ALG_HANA7.5 by measuring its kinematic viscosity showed a gel-sol transition at T_{GS} in the range of 45-50 ºC. According to XRD data, the strengthening of this hydrogel may be a result of the appearance of small HANA nanocrystals at such high concentrations of humic acids of this type in the gelatin-alginate hydrogel matrix.

Table II shows data on the kinematic viscosity of biopolymer hydrogels GN_ALG, GN_ALG_HANA2.5 and GN_ALG_HANA5 at temperatures below and around the physiological temperature of 37ºC. It can be seen that thermo-responsive gelatin-alginate hydrogels modified with not too high concentrations of HANA have only slightly increased gel-sol transition temperatures. Precision kinematic viscosity measurements showed that the GN_ALG_HANA2.5 hydrogel has a gel-sol transition temperature T_{GS} of ~36.9°, and the GN_ALG_HANA5 hydrogel has a T_{GS} of ~37.2°C. Thus, both of these thermosensitive biopolymer hydrogels are suitable for hemostasis and wound healing.

The results of the analysis of thermo-responsive gelatin-alginate hydrogels and gelatin-alginate-humic nanocomposite hydrogels with a gel-sol transition temperature of about 37°C using Fourier transform infrared spectroscopy are presented in Fig. 3. The infrared spectral characteristics of the hydrogels developed in this work can be described in accordance with the literature data [12,13]. The band at 3400 cm^{-1} belongs to the νN-H and νO-H stretching vibration modes of GN and ALG. The bands at 1643 cm^{-1}, 1557 cm^{-1} and 1453 cm^{-1} belong to the stretching vibrations νC=O, νNH, νC-N, νC-C and ν-COO in the amide groups of gelatin and are associated with the presence of intermolecular associations: triple helix, single α-helix, β-sheets, β-turns and random coils. The bands at 2853 cm^{-1} and 2925 cm^{-1} belong to the asymmetric and symmetric vibrations of νCH_2 in GN and GN-HANA hydrogels [13]. The band at 1253 cm^{-1} correlates with the bending vibrations δNH and stretching vibrations νCN in GN and GN-HANA hydrogels in [13]. The band at 1093 cm^{-1} corresponds to the νC-O stretching vibrations of the mannuronic units of ALG [12].

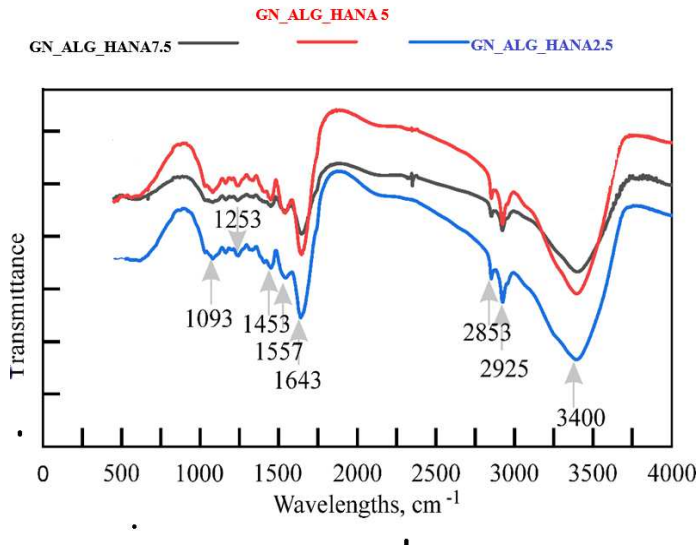

Fig. 3. FTIR spectra of the developed thermo-responsive gelatin-alginate hydrogels modified with HANA humic acids

In general, all FTIR spectra have small red and blue shifts in the positions of stretching and bending vibrations occurred due to GN-ALG, GN-HANA and ALG-HANA interactions similar to those observed in [12,13]. These shifts are due, in particular, to a conformational change in the secondary structure of the GN, as well as the interaction of NANA with ALG, which was shown by X-ray diffraction studies.

IV. Conclusion

In this study, we investigated the structure of a pure biopolymer hydrogel containing 14 wt.% gelatin (GN) and 6.4 wt.% sodium alginate (ALG), as well as its modifications with various concentrations of humic acids in aqueous-alkaline solutions containing sodium or calcium cations. This allowed us to discern the nature of the strengthening or disordering effects on the modified hydrogels.

X-ray diffraction studies revealed that the biopolymer hydrogels GN_ALG_HACA2.5, GN_ALG_HACA5, and GN_ALG_HACA7.5 exhibited structural reinforcement due to the expansion of crystalline regions and an increase in nanocrystal size. Rheological analysis through kinematic viscosity measurements indicated that these hydrogels possess high viscosity and thermal stability, with gel-sol transition temperatures in the range of 55-60 ºC, rendering them unsuitable as smart thermo-responsive hydrogels for controlled drug delivery.

In contrast, the incorporation of humic acids from HANA into the gelatin-alginate hydrogel introduced additional tensile microstresses and disrupted the crystalline structure. Fourier transform infrared spectroscopy revealed conformational changes in the secondary structure of gelatin, as well as interactions between HANA and ALG. Consequently, kinematic viscosity measurements showed that the hydrogels GN_ALG_HANA2.5 and GN_ALG_HANA5 demonstrated gel-sol transitions at physiological temperatures, making them suitable for applications in hemostasis, wound healing, and controlled drug delivery.

Acknowledgment

The authors are grateful to the Ministry of Education and Science of Ukraine for funding this work under project No. 230D dated January 16, 24, topic 8203 (P).

References

[1] H. Banerjee, M. Suhail, H. Ren, “Hydrogel actuators and sensors for biomedical soft robots: Brief overview with impending challenges”, Biomimetics, vol. 3(3), pp. 15–41, July 2018.

[2] M. Sobczyk, S. Wiesenhütter, J.R. Noennig, T. Wallmersperger, “Smart materials in architecture for actuator and sensor applications: A review”, Journal of Intelligent Material Systems and Structures, vol. 33(3), pp. 379-399, February 2021.

[3] Q. Shi, H. Liu, D. Tang, Y. Li, X.J. Li, F. Xu, “Bioactuators based on stimulus-responsive hydrogels and their emerging biomedical applications”, NPG Asia Materials, vol. 11, pp. 64–21, November 2019.

[4] V. Lebedev, K. Lebedeva, A. Cherkashina, A. Voronkin, V. Kopach, S. Petrushenko,·A. Fedonenko, N. Klochko, “Biopolymer-based sustainable Internet of Things for smart homes”, Discover Civil Engineering, vol. 1, pp. 20–32, June 2024.

[5] J. Baranwal, B. Barse, A. Fais, G.L. Delogu, A. Kumar, “Biopolymer: A sustainable material for food and medical applications”, Polymers, vol. 14, pp. 983–22, February 2022.

[6] L. Chen, F. Liu, T. Abdiryim, X. Liu, “Stimuli-responsive hydrogels as promising platforms for soft actuators”, Materials Today Physics, vol. 40, pp. 101281–22, January 2024.

[7] L. Lan, J. Ping, J. Xiong, Y. Ying, “Sustainable natural bio-origin materials for future flexible devices”, Adv. Sci., vol. 9(15), pp. 2200560–34, March 2022.

[8] Y. Xie, P. Gao, F. He, C. Zhang, “Application of alginate-based hydrogels in hemostasis”, Gels, vol. 8, pp. 109–21, February 2022.

[9] A. Ahmad, N. Mubarak, F.T. Jannat, T. Ashfaq, C. Santulli, M. Rizwan, A. Najda, M. Bin-Jumah, M.M. Abdel-Daim, S. Hussain Ali Shafaqat, “A critical review on the synthesis of natural sodium alginate based composite materials: an innovative biological polymer for biomedical delivery applications”, Processes, vol. 9, pp. 137–27, 2021.

[10] T. B. Goudoulas, N. Germann, “Phase transition kinetics and rheology of gelatin-alginate mixtures”, Food Hydrocolloids, vol. 66, pp. 49–60, May 2017.

[11] K. Lebedeva, A. Cherkashina, T. Tykhomyrova, V. Moiseev, V. Lebedev, “Research of biologically active polymeric hydrogel transdermal materials”. In: Ivanov, V., Pavlenko, I., Liaposhchenko, O., Machado, J., Edl, M. (eds) Advances in design, simulation and manufacturing VI. DSMIE 2023. Lecture Notes in Mechanical Engineering. Springer, Cham. 2023.

[12] S. R. Derkach, N. G. Voron’ko, N. I. Sokolan, D. S. Kolotova, Y. A. Kuchina, “Interactions between gelatin and sodium alginate: UV and FTIR studies”, Journal of Dispersion Science and Technology, vol. 5, pp. 690-698, April 2019.

[13] V. Venezia, P.R. Avallone, G. Vitiello, B. Silvestri, N. Grizzuti, R. Pasquino, G. Luciani, “Adding humic acids to gelatin hydrogels: a way to tune gelation”, Biomacromolecule, vol. 23(1), pp. 443–453, January 2022.

[14] L. Cao, W. Lu, A. Mata, K. Nishinari, Y. Fang, “Egg-box Model-based Gelation of Alginate and Pectin: A Review”, Carbohydr. Polym., vol. 242, pp. 116389, August 2020.

[15] M. Mecwan, J. Li, N. Falcone, M. Ermis, E. Torres, R. Morales, A. Hassani, R. Haghniaz, K. Mandal, S. Sharma, S. Maity, F. Zehtabi, B. Zamanian, R. Herculano, M. Akbari, J.V. John, and A. Khademhosseini, “Recent advances in biopolymer-based hemostatic materials”, Regenerative Biomaterials, vol. 9, pp. rbac063-26, September 2022.

[16] D. Pribadi Perkasa, E. Erizal, T. Purwanti, A.E. Tontowi, “Characterization of semi-interpenetrated network alginate/gelatin wound dressing crosslinked at sol phase”, Indones. J. Chem., vol. 18, pp. 367 – 375, 2018.

[17] V. Lebedev, D. Miroshnichenko, Z. Xiaobin, S. Pyshyev, D. Savchenko, and Y. Nikolaichuk, “Use of humic acids from low-grade metamorphism coal for the modification of biofilms based on polyvinyl alcohol,” Petroleum and Coal, vol. № 63 (4), pp. 953-962, December 2021.

[18] N.P. Klochko, V.A. Barbash, S.I. Petrushenko, V.R. Kopach, K.S. Klepikova, D.O. Zhadan, O.V. Yashchenko, S.V. Dukarov, V.M. Sukhov, A.L. Khrypunova, “Thermoelectric textile devices with thin films of nanocellulose and copper iodide”, Journal of Materials

Science: Materials in Electronics, vol. 32, pp. 23246–23265, August 2021.

[19] P. Sundarrajan, P, Eswaran, A. Marimuthu, L.B. Subhadra, P. Kannaiyan, “One pot synthesis and characterization of alginate stabilized semiconductor nanoparticles”, Bulletin of the Korean Chemical Society, vol. 33(10), pp. 3218–3224, 2012.

[20] D. Ghosh, A. Pramanik, N. Sikdar, P. Pramanik, **“**Synthesis of low molecular weight alginic acid nanoparticles through persulfate treatment as effective drug delivery system to manage drug resistant bacteria”, Biotechnology and Bioprocess Engineering, vol. 16, pp. 383-392, April 2011.

[21] L. Radev, M. Fernandes, I. Salvado, D.Kovacheva, "Organic/Inorganic bioactive materials Part III: in vitro bioactivity of gelatin/silicocarnotite hybrids", *Open Chemistry*, vol. 7(4), pp. 721-730, October 2009.