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INVESTIGATION ON THE SWELLING KINETICS OF GELATIN BASED HYDROGELS

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Abstract. A series of gelatin-based hydrogels was prepared, and the effects of different crosslinking agents and agar content were studied in detail. Results indicate that borax and glutaraldehyde are good crosslinking agents. Moreover, all samples were described with a hindered Fickian water diffusion, making it an interesting choice for medical applications.

Keywords: gelatin, agar, swelling kinetics, crosslinking, hydrogel.

1. Introduction

Nowadays, the utilization of biopolymers has gained considerable significance for hydrogel fabrication. Due to their unique swelling properties and biocompatibility, biopolymer-based hydrogels have attracted significant attention in various fields, such as tissue engineering and drug delivery[1, 2]. Hydrogels possess supramolecular architecture, display complex viscoelastic properties, and can be synthesized utilizing different methods. Understanding the swelling kinetics of hydrogels is essential for optimizing their performance and tailoring their properties to specific requirements. Numerous factors, such as polymer concentration, crosslinking density, or average molecular weight between crosslinks influence the rate and extent of water uptake and induce changes in material performance and overall functionality[3]. Scientific evidence has also confirmed the influence of environmental variables like temperature or pH on the swelling process[4–7].

Among the diverse range of hydrogel-forming polymers, gelatin has emerged as a promising candidate for biomedical applications owing to its biodegradability, non-toxicity, low cost, and structure similar to the extracellular matrix of biological tissues[4, 5]. Gelatin is a biopolymer derived from collagen through partial hydrolysis. Its characteristics are influenced by factors such as molecular weight, collagen origin, and production process, which can include heat and enzymatic denaturation or extraction under alkaline (gelatin type B) or acidic (gelatin type A) conditions[8, 9]. Gelatin is soluble in water, easily accessible in the market, more cost-effective than collagen, and retains essential binding components crucial for cell adhesion[10]. The broad appeal of gelatin stems from its safety, biocompatibility, and ability to degrade naturally, making it non-toxic, non-carcinogenic, and environmentally friendly[10]. Gelatin's advantages make it widely used in fields such as the food industries and medicine. However, gelatin-based materials suffer from poor mechanical properties, thermal instability, and short degradation time[11].

Gelatin hydrogels can be readily formed with a low concentration of a few percent when the temperature is lowered below 30 °C. The physical gelation is attributed to the conformational transition of gelatin chains from coil to triple helix, making them desirable for food and pharmaceutical applications due to the absence of harmful substances[12]. Using an additional crosslinking agent can strengthen the structure of the material by providing additional intermolecular interactions between the polymer chains[4, 5]. Crosslinking of gelatin can be achieved through various physical and chemical techniques such as employing UV radiation, plasma, dehydrothermal treatment, or enzymes[11, 13].

Frequently, studies on hydrogels are focused on two or more polymers, which are combined to produce materials with specific properties. Research has demonstrated that the incorporation of alternative polymers, such as polyvinyl alcohol, chitosan, or agar, can impact the swelling capacity of gelatin-based hydrogels[14, 15]. Hydrogels composed of protein and polysaccharides have garnered much attention in the past decade and have been extensively studied[16, 17]. These systems form water-in-water type emulsions, leading to the formation of composite

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structures, which have been reported to enhance intramolecular hydrogen bonding between protein and polysaccharide chains compared to intermolecular hydrogen bonding[5].

This paper presents a comprehensive investigation into the rarely discussed swelling kinetics of gelatin-based hydrogels, focusing on key parameters that affect their swelling behavior. In this study, agar and gelatin were chosen as the representative polysaccharide and protein components. Apart from detailed swelling kinetics, measurements of contact angle and microscopic observations were conducted. The influence of different crosslinking agents and agar content in the material is discussed to observe and obtain an understanding of the relationship between its physical and chemical properties.

2. Experimental

2.1. Materials

The synthesis of hydrogel was carried out using: natural polymers: agar E406 (Biomus, Lublin, Poland), food grade gelatin 270 bloom (Mr Cook Corp., Katowice, Poland), crosslinking agents: calcium chloride $CaCl_2$ (Avantor Performance Materials Poland, Gliwice, Poland), sodium tetraborate (borax) $Na_2B_4O_7 \cdot 10\ H_2O$ (TechlandLab, Tarnobrzeg, Poland), glutaraldehyde 25 % solution pure (Chempur, Piekary Śląskie, Poland) and 1st class lab water as a solvent and swelling medium. For contact angle measurement, anhydrous glycerol (Gly) (Avantor Performance Materials Poland, Gliwice, Poland), ethylene glycol (EG) (Chempur, Piekary Śląskie, Poland), and diiodomethane (DJM) (LOBA CHEMIE, Mumbai, India) were used.

2.2. Methods

2.2.1. Synthesis of Hydrogels

Firstly, set amounts of gelatin and agar were dissolved in water at a temperature of around 100 °C under rigorous stirring. After that, crosslinking agents were added to the polymer solution and allowed to dissolve. Then, the mixture was left to cool down to around 60 °C to defoam and poured into petri dishes. To finish the crosslinking, freeze-thawing was performed: samples were first frozen at –20 °C for 24 hours, then thawed at room temperature for 1 hour, and, in the end, refrigerated at 4 °C for 24 hours. In the next step, hydrogels were dried using thermobalance MA 50.R (RADWAG, Radom, Poland) to constant mass at 40 °C (mass loss below 1 mg/120 s) and placed in distilled water for 24 hours to remove unreacted compounds. Finished hydrogels were then dried again, vacuum packed, and stored at 4 °C. Twelve types of hydrogel were prepared using this procedure (Table 1).

Table 1. Hydrogel samples and their compositions. The hydrogels were prepared using water, gelatin (GEL), agar (AG), sodium tetraborate (B), calcium chloride (C), and glutaraldehyde (G) used in the presented ratios

Sample	Composition, wt. %					
	Water	Agar	Gelatin	Sodium tetraborate	Calcium chloride	Glutaraldehyde
GEL	98	–	2	–	–	–
GEL/B	96	–	2	2	–	–
GEL/C	96	–	2	–	2	–
GEL/G	96	–	2	–	–	2
0.75GEL/0.25AG	98	0.5	1.5	–	–	–
0.75GEL/0.25AG/B	96	0.5	1.5	2	–	–
0.75GEL/0.25AG/C	96	0.5	1.5	–	2	–
0.75GEL/0.25AG/G	96	0.5	1.5	–	–	2
0.5GEL/0.5AG	98	1	1	–	–	–
0.5GEL/0.5AG/B	96	1	1	2	–	–
0.5GEL/0.5AG/C	96	1	1	–	2	–
0.5GEL/0.5AG/G	96	1	1	–	–	2

2.2.2. Swelling Kinetics

For the swelling examination, three rectangular samples with dimensions of 3x1.5 cm were cut out from each type of hydrogel and dried at 40 °C until reaching constant mass (mass loss below 1 mg/120 s), using the thermobalance MA 50.R (RADWAG, Radom, Poland). Next, each of the samples was placed in 20 mL of water at 20 °C, then removed from the medium in the set time intervals, placed on a piece of paper to remove the excess water and finally weighed using the analytical balance AS

110.R2 (RADWAG, Radom, Poland). After weighing, the sample was placed back in the container with water. The measurements were conducted after 5, 15, 30, 45, 60, 75, 90, 105, 120, 150 and 180 min of swelling. Swelling values were calculated using Eq. (1)[18]

$$W = \frac{m_t - m_0}{m_0} \times 100\%, \quad (1)$$

where W is swelling [%], m_t and m_0 are the weights [g] of swollen hydrogel after set time (t) and dried hydrogel, respectively.

Swelling curves for each hydrogel were presented using average swelling value with standard deviation calculated for each time interval (n = 4) using OriginPro 9.0 software (OriginLab Corporation, Northamption, USA).

To analyze the swelling control mechanism, several kinetic models were chosen to fit the experimental data (first order, second order, and Korsmeyer – Peppas models), using OriginPro 9.0 software (OriginLab Corporation, Northampton, USA).

In the case of the first-order kinetics (1OK), the swelling value at any time (t) is proportional to the uptake of a swelling agent, before reaching equilibrium swelling (W_{eq})[19]. The first-order kinetics is represented by Eq. (2)

$$\frac{dW}{dt} = K_1(W_{eq} - W_t), \quad (2)$$

where: W_{eq} is equilibrium swelling [%], W is the swelling at time (t) [%], and K_1 is the first–order swelling kinetics constant [min^{-1}].

Eq. (2) can be integrated to the nonlinear Eq. (3)

$$W = W_{eq}(1 - e^{-K_1 t}), \quad (3)$$

Swelling parameters were also calculated according to the second-order kinetics (2OK) using the Eq. (4)[20]

$$\frac{dW}{dt} = K_2(W_{eq} - W_t)^2, \quad (4)$$

where W_{eq} is equilibrium swelling [%], W is the swelling at set time (t) [%], and K_2 is the second-order swelling kinetics constant [%$^{-1}$ min^{-1}].

Eq. (4) integrates to the nonlinear Eq. (5)

$$W = \frac{K{W_{eq}}^2 t}{1 + K W_{eq} t}, \quad (5)$$

with the assumption that $W_0 = W = 0$ and $t_0 = t = 0$.

Korsmeyer – Peppas nonlinear diffusion (KPND) model can be applied to investigate the mechanism of water sorption into the hydrogel matrix (Eq. (6))[21]. The diffusion exponent (n) defines the sorption and/or release mechanism. According to the literature, for values $0 < n < 0.45$, the matrix is characterized by hindered Fickian diffusion, for n = 0.45 by Fickian diffusion, for $0.45 < n < 1$ anomalous transport, for n = 1 non-Fickian or case II transport and lastly for $n > 1$ super case II transport[22]

$$W = W_{eq} K_{KP} t^n, \quad (6)$$

where W is swelling [%], W_{eq} is equilibrium swelling [%], K_{KP} is swelling rate constant [min^{-1}], t represents the set time interval [min], and n is the diffusion exponent.

All models were fitted using Origin Pro 9.0 software using a nonlinear curve fit function with instrumental weighing, where the error of swelling measurement was taken into account.

2.2.3. Density, Average Molecular Weight between Crosslinks, and Crosslinking Density

The density of hydrogels was obtained using the analytical balance AS 110.R2 with Density Kit 85 (RADWAG, Radom, Poland) using hydrogel samples after reaching their equilibrium swelling. Density was estimated by weighing samples in air and in water. The measurements were carried out at a room temperature of 20° C. Based on the density values, the average molecular weight between crosslinks and crosslinking density were calculated according to Eq. (7)[23]

$$M_c = -\frac{d_p V_s \left({V_{2,s}}^{\frac{1}{3}} - \frac{V_{2,s}}{2} \right)}{ln(1 - V_{2,s}) + V_{2,s} + \chi {V_{2,s}}^2}, \quad (7)$$

where M_c is the average molecular weight between crosslinks [g/mol], d_p is the density of hydrogel [g/cm^3], V_s is a molar volume of water [–], $V_{2,s}$ is a volume fraction of hydrogel [–], which can be derived from Eq. (8)

$$V_{2,s} = [1 + \frac{d_p}{d_s}\left(\frac{M_a}{M_b} - 1\right)]^{-1}, \quad (8)$$

where d_p and d_s [g/cm^3] are densities of hydrogel and solvent, respectively, and M_a, M_b [g] are weights of swollen and dried hydrogel, respectively.

Furthermore, χ is the solvent interaction parameter, which can be calculated knowing the value of $V_{2,s}$ by using Eq. (9)

$$\chi = \frac{ln(1 - V_{2,s}) + V_{2,s}}{{V_{2,s}}^2}, \quad (9)$$

To calculate the average density Eq. (10) was used[23]

$$q_c = \frac{d_p}{M_c}, \quad (10)$$

where q_c is the average crosslinking density [mol/dm^3], d_p is the density [g/cm^3] and M_c indicates the average molecular weight between crosslinks [g/mol].

2.2.4. Contact Angle

To measure the contact angle of dried hydrogel samples, a goniometer 90-U3-PRO (Rame-Hart, Succasunna, USA) with Drop Image Pro software (Media Cybernetics, Rockville, USA) was used with anhydrous glycerol (Gly), ethylene glycol (EG) and diiodomethane (DJM) as measuring liquids. Three measurements for each sample and each liquid were taken at room temperature. Collected data was then used for the calculation of the surface energy of solids using the Good- van Oss-Chaudhury model embedded in the above-mentioned software.

2.2.5. Optical Microscopy

The evaluation of changes in the microstructure of hydrogels was examined using the Delta Optical MET-1000-TRF optical microscope working in reflection mode using MTR3CMOS20000KPA camera and ToupView software.

3. Results and Discussion

3.1. Swelling Kinetics

Swelling kinetic parameters (W_{eq}, K_1, n, K_{KP}) for all analyzed samples are presented in Table 2, while swelling graphs are shown in Fig. 1. Values of equilibrium swelling were obtained from first- and second-order kinetic models. The highest value of equilibrium swelling was observed for GEL/C sample, which was equal to 1453 ± 29 % and 1617 ± 76 % for first and second order kinetic respectively. On the other hand, the lowest W_{eq} was noted for 0.5GEL/0.5AG/G sample with values of 174.3 ± 9.0 % for 1OK and 192.5 ± 9.8 % for 2OK. The addition of agar to hydrogels caused a decrease in swelling values compared to corresponding samples with gelatin only, regardless of the used crosslinking agent. Comparing samples with different agar amounts, the trend in swelling is not clear – for samples without a crosslinking agent and with glutaraldehyde, a significant drop in swelling was observed with higher agar amount in the composition. On the other hand, such effect was not observed for samples crosslinked with borax and calcium chloride. A similar relation was observed by P. M. Pandey *et al*. in their work. The researchers investigated hydrogels based on gelatin with an addition of different amounts of agar: from a pure 20 % gelatin solution to a 1:4 ratio (20 % gelatin solution: 2 % agar solution), crosslinked with glutaraldehyde. The obtained results were analogous to those described above, swelling values of prepared materials were decreasing with an increase of agar to gelatin ratio in the structure[5]. Even though hydrogel compositions with the addition of agar reached lower swelling values, they were also characterized by a more rapid swelling process, as they reached equilibrium swelling faster than samples based on pure gelatin.

Fig. 1 shows that most hydrogels with the addition of agar reached their final weight (and swelling as a result) after approximately 15 minutes of water sorption. Furthermore, comparing crosslinking agents, the equilibrium swelling was higher for samples with the addition of calcium chloride and borax, and, contrastingly, it was lower after crosslinking with glutaraldehyde. Both calcium chloride and borax cause physical crosslinking of the hydrogel structure, while glutaraldehyde leads to the creation of durable chemical bonds. The chemically crosslinked hydrogel network (with glutaraldehyde) was more densely packed than the network with weaker physical bonds (created by $CaCl_2$ and borax), which provides higher crosslinking density but, as a result, a lower ability to absorb water. Interestingly, in the case of hydrogel without agar but with the addition of $CaCl_2$, swelling results indicate the loosening of the hydrogel structure as opposed to the expected crosslinking. Compared to the GEL sample, GEL/C had both higher W_{eq} and K values regardless of the model. It is probably due to interactions between Ca^{2+} ions with the carboxylic acid groups within individual polypeptide chains[24]. Such interactions, also potentially resulting in the blocking of internal hydrogen bonding in gelatin chains, could also be justified by the decrease in swelling after 75 minutes of the experiment, suggesting that loosened hydrogel structure allowed unbound chains to be slowly dissolved in water. Such behavior was not observed for the sample with the addition of borax (GEL/B), probably because borax has a bulkier structure, allowing different parts of borax to interact with different gelatin chains, which is not possible for Ca^{2+} ions.

Obtained swelling kinetic constants were in the range from 0.0438 ± 0.0049 min^{-1} to 0.70 ± 0.11 min^{-1} and for 1OK and from (0.515 ± 0.048) · 10^{-4} $\%^{-1}$ min^{-1} to (39.4 ± 5.3) · 10^{-4} $\%^{-1}$ min^{-1} for 2OK respectively. The values of K_1 and K_2 were the highest for samples with 1:1 gelatin-to-agar ratio and the lowest for samples without the addition of agar. The more agar was added to the composition, the higher were the K_1 and K_2 values. An exception for this trend was observed for 0.75GEL/0.25AG/C sample, for which K_2 was equal to (260 ± 280) · 10^{-4} $\%^{-1}$ min^{-1}. This deviation is due to the decrease of swelling after 15 minutes of the experiment, which could indicate that although Ca^{2+} ions crosslinked the agar in the hydrogel structure, the gelatin was not bound and could be slowly rinsed from the structure, resulting in lowered sample mass at later experiment stages. The 2OK model is especially sensitive to such swelling changes in the first measurement points, thus for this sample, a rather sudden drop in its weight led to a standard deviation higher than the actual value.

The fitting of the Korsmeyer – Peppas model did not lead to obtaining equilibrium swelling values but allowed to calculate diffusion exponent (n) instead. All compositions were characterized by an n value lower than 0.45, which indicates a hindered Fickian water diffusion through the hydrogel matrix. The highest n was observed for the GEL sample (0.233 ± 0.025), which was the closest to reaching the Fickian diffusion mechanism (n = 0.45)[22], while the lowest n, equal to 0.065 ± 0.012, was noted for 0.75GEL/0.25AG. Overall, higher n values were obtained for samples based on pure gelatin and were slightly lower for samples with the addition of agar. An exception was 0.5GEL/0.5AG/G sample which was characterized by a higher value (n = 0.203 ± 0.017) than other gelatin-agar compositions.

Table 2. Equilibrium swelling and swelling kinetics constant values of the gelatin-based hydrogels

Sample	First order (non-linear)			Second order (non-linear)			Korsmeyer – Peppas		
	W_{eq}, %	K_1, min^{-1}	R^2	W_{eq}, %	$K_2 \times 10^{-4}$, $\%^{-1}\ min^{-1}$	R^2	n	K_{KP}, min^{-1}	R^2
GEL	1135 ± 20	0.0438 ± 0.0049	0.997	1267 ± 16	0.515 ± 0.048	0.999	0.233 ± 0.025	364 ± 44	0.996
GEL/B	521.1 ± 9.0	0.095 ± 0.021	0.982	547.4 ± 7.6	2.53 ± 0.49	0.993	0.1729 ± 0.0092	217 ± 11	0.998
GEL/C	1453 ± 29	0.0618 ± 0.0076	0.997	1617 ± 76	0.54 ± 0.18	0.991	0.230 ± 0.061	500 ± 140	0.975
GEL/G	436.6 ± 6.3	0.1180 ± 0.0063	0.998	472 ± 13	4.615 ± 0.650	0.996	0.168 ± 0.029	213 ± 23	0.980
0.75GEL/0.25AG	404.9 ± 2.5	0.190 ± 0.012	0.986	413.5 ± 1.2	17.8 ± 1.1	0.998	0.065 ± 0.012	308 ± 15	0.911
0.75GEL/0.25AG/B	449.68 ± 0.99	0.3730 ± 0.0090	0.999	456.5 ± 1.8	23.5 ± 2.1	0.999	0.115 ± 0.020	272 ± 25	0.974
0.75GEL/0.25AG/C	418.5 ± 2.5	0.70 ± 0.11	0.999	418.4 ± 3.1	260 ± 280	0.999	0.118 ± 0.025	253 ± 28	0.960
0.75GEL/0.25AG/G	296.2 ± 6.9	0.171 ± 0.031	0.975	312.5 ± 4.0	11.0 ± 1.5	0.995	0.113 ± 0.012	182.8 ± 9.0	0.987
0.5GEL/0.5AG	270.8 ± 3.2	0.428 ± 0.077	0.989	277.7 ± 2.5	37.0 ± 7.4	0.996	0.095 ± 0.017	185 ± 13	0.962
0.5GEL/0.5AG/B	429.0 ± 2.2	0.469 ± 0.031	0.999	433.0 ± 2.0	39.4 ± 5.3	0.999	0.104 ± 0.019	274 ± 23	0.966
0.5GEL/0.5AG/C	427.2 ± 4.0	0.286 ± 0.045	0.995	437.0 ± 3.5	14.8 ± 2.6	0.998	0.112 ± 0.020	259 ± 23	0.977
0.5GEL/0.5AG/G	174.3 ± 9.0	0.172 ± 0.054	0.907	192.5 ± 9.8	9.7 ± 3.6	0.946	0.203 ± 0.017	73.9 ± 5.3	0.990

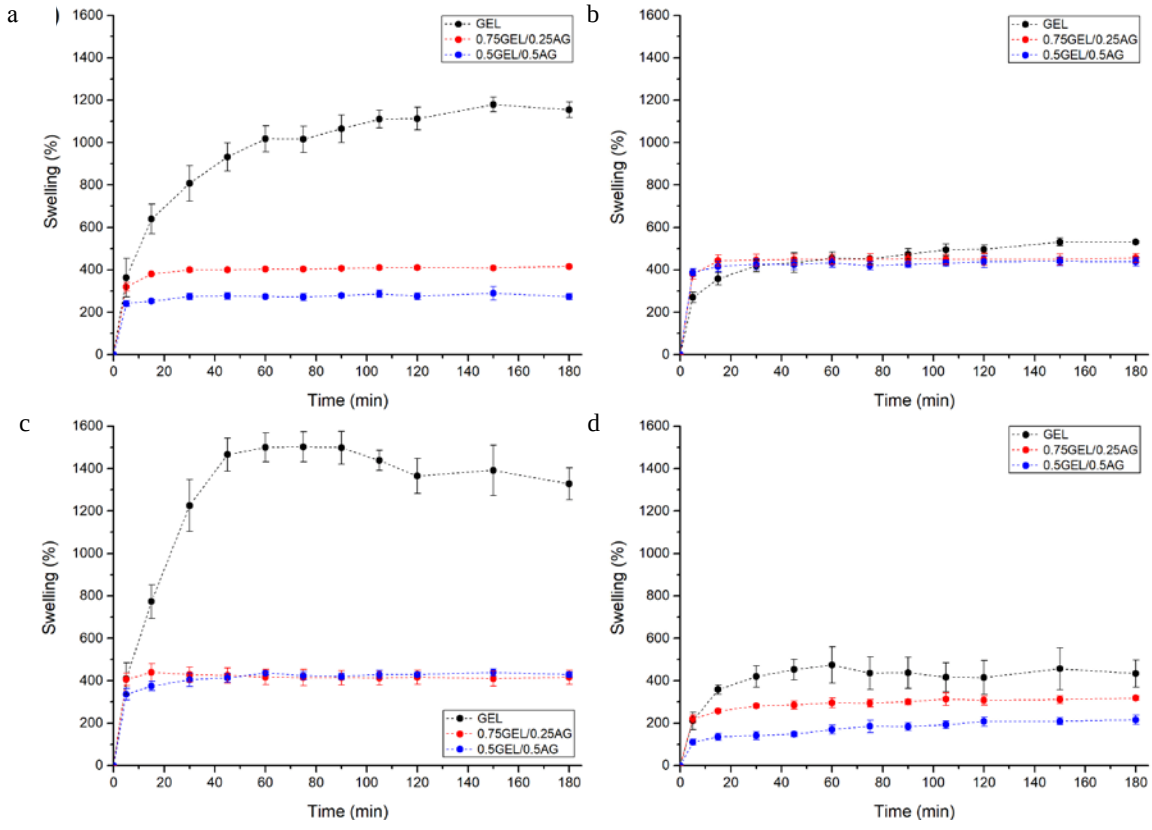

Fig. 1. Swelling behaviour of gelatin-based hydrogels. Samples: a – without crosslinking agent; b – with sodium tetraborate; c – with calcium chloride; d – with glutaraldehyde

3.2. Density, Average Molecular Weight between Crosslinks and Crosslinking Density

The density of obtained materials was in a range from 1.0096 ± 0.0013 g/cm^3 to 1.0338 ± 0.0064 g/cm^3, which was only slightly higher than the value of water density (equal approximately to 1 g/cm^3) because most (96–98 wt. %) of all hydrogels' compositions was water. Measurements of density and swelling allowed us to further calculate the average molecular weight between crosslinks (M_c) and crosslinking density (q_c) of hydrogels', presented in Table 3.

The lowest M_c (79 ± 12 g/mol) and the highest q_c (13.1 ± 2.3 mol/dm^3) were obtained for 0.5GEL/0.5AG/G sample, which was characterized by the lowest equilibrium swelling. Respectively, the highest M_c (1418 ± 72 g/mol) and the lowest q_c (0.714 ± 0.034 mol/dm^3) were noted for the sample with the highest swelling – GEL/C. High q_c values indicated a high crosslinking level of the polymer network, which led to high crosslinking density, low average molecular weight between crosslinks, and, as a result, lower ability to absorb high amounts of medium. Analogously to swelling results, the addition of agar to gelatin composition caused a decrease in average molecular weight between crosslinks and an increase in crosslinking density. The hydrogels with 1:1 gelatin-to-agar ratio were characterized by the lowest M_c and the highest q_c out of non-crosslinked compositions. Furthermore, samples with glutaraldehyde that are strongly, chemically crosslinked were characterized by the highest q_c and lowest M_c, while compositions crosslinked physically with $CaCl_2$ and borax reached much lower q_c and higher M_c values.

The dependence between M_c and swelling values was observed in many experimental works by others, but one of the first mentions was found in an article from 1976 by N. Peppas and E. W. Merrill, where the authors determined M_c based on swelling results of PVA-based hydrogels[25]. The relation between swelling behavior, average molecular weight between crosslinks, and crosslinking density was also described by G. Hoti *et al.*, where researchers were investigating ester-bridged β-cyclodextrin nanosponges. G. Hoti et al. observed higher swelling ability in aquatic media for samples with higher M_c and lower q_c values, similar to the obtained experimental results[26].

3.3. Contact Angle

Measurements of contact angle, surface energy, and work of adhesion for the obtained hydrogels in their dehydrated state were investigated in contact with three measuring liquids: glycerol, ethylene glycol, and diiodomethane. Results of measurements with glycerol were presented in Table 4, while a comparison of contact angles for 0.75GEL/0.25AG-based compositions measured with three liquids, as well as calculated polar and dispersive components of surface energy, were shown in Table 5. All the results were also presented in the form of graphs in Fig. 2.

Glycerol contact angle (CA) values were in a range from 41.5 ± 7.1° for the GEL/G sample to 63.7 ± 3.3° for the GEL sample. Most of the samples were characterized by comparable CA values, reaching approximately 60°, while crosslinking with glutaraldehyde caused a visible decrease of contact angle for all gelatin-agar compositions, falling under 50°. The observations may indicate an increase of hydrophilicity for chemically crosslinked samples, while for other samples the differences were within measuring error. The direct measurement of the water contact angle was not possible due to the rapid absorption of the liquid. Surface free energy (SFE) of hydrogels was in range from 38.3 ± 1.9 mJ/m^2 (GEL) to 50.2 ± 3.6 mJ/m^2 (GEL/G). The values of SFE were opposite to CA, the highest SFE value was observed for the lowest CA. Contrastingly to SFE, work of adhesion (WA) (range from 91.0 ± 3.3 mJ/m^2 to 110.1 ± 5.2 mJ/m^2) was higher for the samples characterized by higher CA and lower SFE.

Table 3. Density, average molecular weight between crosslinks and crosslinking density of the gelatin-based hydrogels

Sample	$d_p\left[\frac{g}{cm^3}\right]$	$M_c\left[\frac{g}{mol}\right]$	$\rho_c\left[\frac{mol}{dm^3}\right]$
GEL	1.024 ± 0.024	1101 ± 59	0.931 ± 0.043
GEL/B	1.0174 ± 0.0045	303 ± 19	3.33 ± 0.15
GEL/C	1.0178 ± 0.0042	1418 ± 72	0.714 ± 0.034
GEL/G	1.0096 ± 0.0013	209 ± 50	5.0 ± 1.2
0.75GEL/0.25AG	1.023 ± 0.015	214.8 ± 9.1	4.77 ± 0.15
0.75GEL/0.25AG/B	1.0289 ± 0.0058	225 ± 27	4.63 ± 0.60
0.75GEL/0.25AG/C	1.019 ± 0.012	244 ± 24	4.21 ± 0.41
0.75GEL/0.25AG/G	1.031 ± 0.015	140.7 ± 9.7	7.35 ± 0.42
0.5GEL/0.5AG	1.019 ± 0.017	111 ± 15	9.3 ± 1.1
0.5GEL/0.5AG/B	1.0338 ± 0.0064	230 ± 15	4.41 ± 0.27
0.5GEL/0.5AG/C	1.012 ± 0.018	229 ± 22	4.54 ± 0.37
0.5GEL/0.5AG/G	1.0184 ± 0.0084	79 ± 12	13.1 ± 2.3

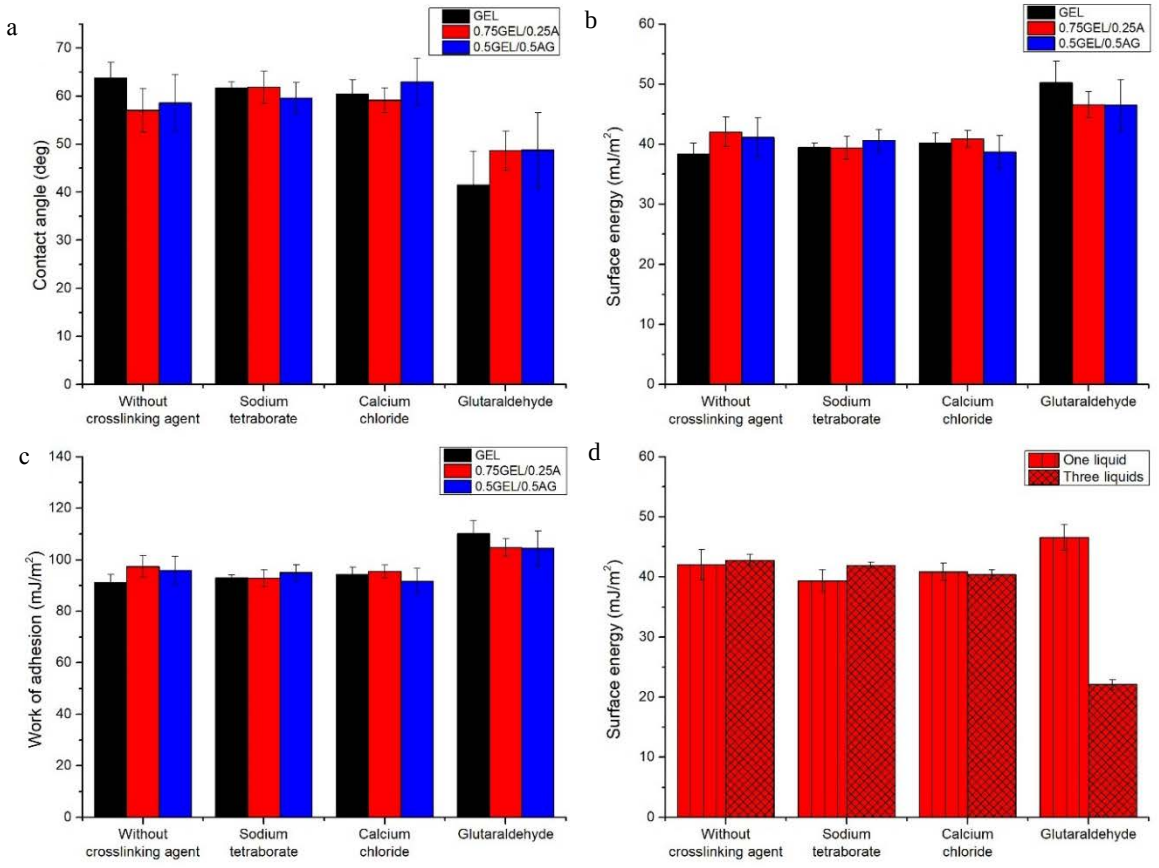

Fig. 2. Contact angle investigation on gelatin-based hydrogels. a – glycerol contact angle; b – one liquid surface free energy; c – one liquid work of adhesion; d – comparison of one and three liquids total free surface energies for 0.75GEL/0.25AG based samples

Table 4. Contact angle, surface energy and work of adhesion in contact with glycerol of the gelatin-based hydrogels

Sample	Contact angle, °	Surface free energy, mJ/m^2	Work of adhesion, mJ/m^2
GEL	63.7 ± 3.3	38.3 ± 1.9	91.0 ± 3.3
GEL/B	61.7 ± 1.3	39.41 ± 0.68	93.0 ± 1.2
GEL/C	60.4 ± 3.0	40.2 ± 1.7	94.3 ± 2.9
GEL/G	41.5 ± 7.1	50.2 ± 3.6	110.1 ± 5.2
0.75GEL/0.25AG	57.0 ± 4.6	42.0 ± 2.5	97.4 ± 4.2
0.75GEL/0.25AG/B	61.8 ± 3.4	39.4 ± 1.9	92.9 ± 3.3
0.75GEL/0.25AG/C	59.1 ± 2.6	40.9 ± 1.5	95.4 ± 2.5
0.75GEL/0.25AG/G	48.6 ± 4.1	46.6 ± 2.2	104.7 ± 3.4
0.5GEL/0.5AG	58.6 ± 5.9	41.1 ± 3.3	95.9 ± 5.6
0.5GEL/0.5AG/B	59.6 ± 3.2	40.6 ± 1.8	95.0 ± 3.1
0.5GEL/0.5AG/C	63.0 ± 4.9	38.7 ± 2.8	91.7 ± 4.9
0.5GEL/0.5AG/G	48.7 ± 7.9	46.5 ± 4.2	104.5 ± 6.7

Total surface energy, as well as its polar and dispersive components, were calculated based on the results of measurements with three (polar and nonpolar) measuring liquids. Total SFE gives us information about the wettability of the measured materials' surfaces. With an increase of SFE values, the wettability of the surface also increases. Similarly to the measurements with glycerol, only samples crosslinked with glutaraldehyde exhibited different results (22.10 ± 0.83 mJ/m^2), while for other samples obtained SFEs were around 41 mJ/m^2. Interestingly, concerning samples crosslinked with borax and $CaCl_2$, despite having similar W_{eq} and total SFE, the polar component was largely different.

Table 5. Contact angle and surface free energy values for selected compositions, calculated based on the results of measurements with 3 measuring liquids for 0.75GEL/0.25AG-based hydrogels

Sample	Contact angle, °			Surface free energy of solid, mJ/m^2				
	Glycerol	Ethylene glycol	Diiodomethane	Polar	Dispersive	Total	Polar [+]	Polar [–]
0.75GEL/0.25AG	57.0 ± 4.6	37.4 ± 4.3	37.5 ± 5.4	1.90 ± 0.99	40.90 ± 0.37	42.80 ± 0.97	0.03 ± 0.04	28.7 ± 4.2
0.75GEL/0.25AG/B	61.8 ± 3.4	36.9 ± 2.5	39.7 ± 5.2	1.5 ± 0.4	40.40 ± 0.33	41.90 ± 0.51	1.30 ± 0.13	0.46 ± 0.29
0.75GEL/0.25AG/C	59.1 ± 2.6	30.1 ± 2.5	31.8 ± 3.2	-3.10 ± 0.71	43.50 ± 0.21	40.40 ± 0.75	2.40 ± 0.16	0.97 ± 0.39
0.75GEL/0.25AG/G	48.6 ± 4.1	33.0 ± 1.2	31.5 ± 4.9	-21.50 ± 0.94	43.6 ± 0.3	22.10 ± 0.83	0.95 ± 0.07	121.0 ± 2.3

3.3. Optical Microscopy

The surfaces of the obtained hydrogel samples were examined using optical microscopy. The micrographs were presented in Fig. 3 for dried 0.75GEL/0.25AG hydrogels and in Fig. 4–6 for swollen GEL, 0.75GEL/0.25AG and 0.5GEL/0.5AG hydrogels, respectively. Main differences were observed between samples containing various crosslinking agents. Hydrogel samples without crosslinking agents were characterized by significantly smoother surfaces. Materials modified with glutaraldehyde had the most uneven, rough surface in comparison to other hydrogel compositions. On GEL, GEL/B, and GEL/C surfaces several cracks could be observed. Single bubbles that could be seen on some of the surfaces were probably a result of transferring the hydrogel solution into a Petri dish to solidify. The observed pattern, strongly visible mainly in Fig. 3, *a* and Fig. 4, *b* and *c* is a result of dehydrating the samples on a Teflon mat to avoid adhesion to the container.

4. Conclusions

In this work, the effects of the type of crosslinking agent and agar content on the swelling kinetics, contact angle, and microstructure of gelatin-based hydrogels were presented. Results indicate the pronounced effect of glutaraldehyde on the hydrogels affecting all measured properties. The most optimal properties were observed for gels with the addition of agar and crosslinked with borax (0.75GEL/0.25AG/B and 0.5GEL/0.5AG/B). These two gel types had uniform structures and were characterized by moderate equilibrium swelling values, which were rapidly achieved just after 15 minutes of immersion in water. Furthermore, these gels were characterized by contact angles and SFEs in the optimal range for cell growth. Therefore, these composite hydrogel structures may be an interesting option for use, *e. g.*, wound dressings, bioinks, or cell scaffolds. Future studies in this regard should focus on cell viability, rheological properties, and swelling in physiological mediums.

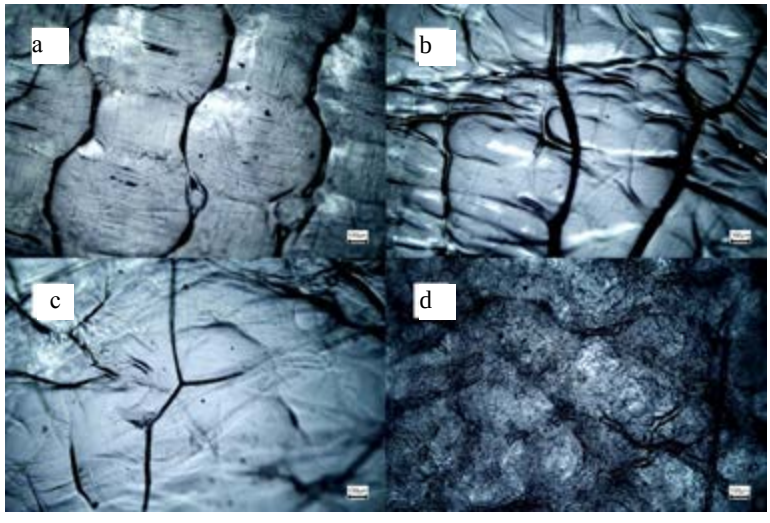

Fig. 3. Micrographs of the dried 0.75GEL/0.25AG hydrogels: a – without crosslinking agent; b – with sodium tetraborate; c – with calcium chloride; d – with glutaraldehyde

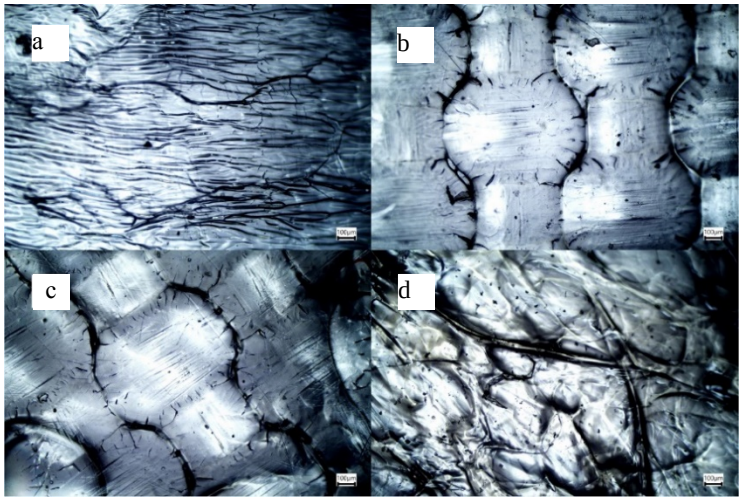

Fig. 4. Micrographs of the swollen GEL hydrogels: a – without crosslinking agent; b – with sodium tetraborate; c – with calcium chloride; d – with glutaraldehyde

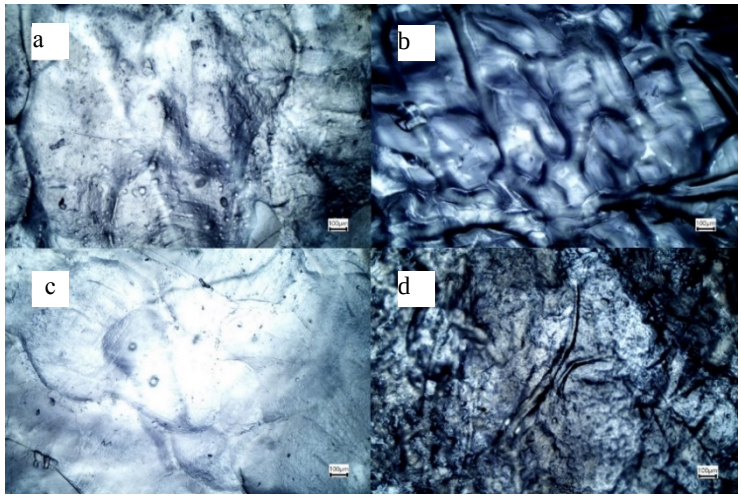

Fig. 5. Micrographs of the swollen 0.75GEL/0.25AG hydrogels: a – without crosslinking agent; b – with sodium tetraborate; c – with calcium chloride; d – with glutaraldehyde

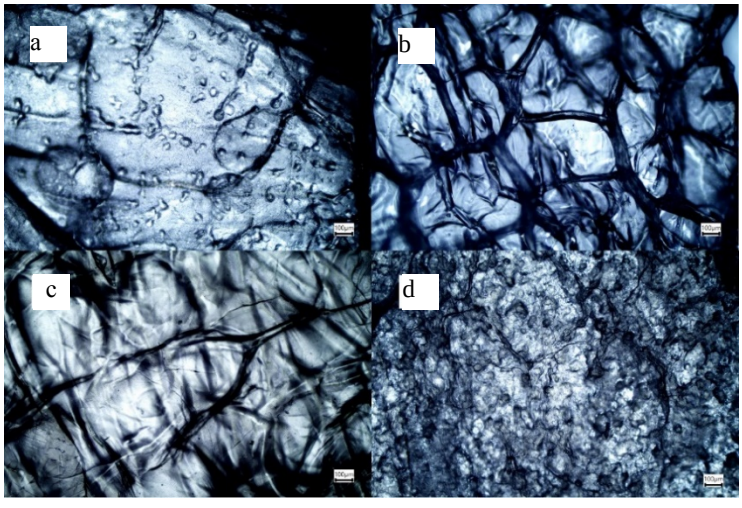

Fig. 6. Micrographs of the swollen 0.5GEL/0.5AG hydrogels: a – without crosslinking agent; b – with sodium tetraborate; c – with calcium chloride; d – with glutaraldehyde

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ДОСЛІДЖЕННЯ КІНЕТИКИ НАБУХАННЯ ГІДРОГЕЛІВ НА ОСНОВІ ЖЕЛАТИНУ

Анотація. *Одержано низку гідрогелів на основі желатину та детально вивчено вплив різних зшиваючих агентів і вмісту агару. Результати показують, що бура і глутаральдегід є хорошими зшиваючими агентами. Крім того, усі зразки описано утрудненою фіківською дифузією води, що робить їх цікавим вибором для медичних застосувань.*

Ключові слова: *желатин, агар, кінетика набухання, зшивання, гідрогель.*