

## HYDROGELS BASED ON NATURAL POLYMERS FOR CARDIAC APPLICATIONS

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**Abstract.** In this work agar- and borax-based hydrogels with and without the addition of poly(vinyl alcohol) (PVA) at different concentrations were synthesized. Hydrogels were modified by the same amount of acetylsalicylic acid (ASA) which exhibits antithrombotic properties. The effect of modification by ASA on the properties of hydrogels was analyzed.

**Keywords:** agar, PVA, hydrogels, cardiac surgery, acetylsalicylic acid.

### 1. Introduction

The cardiac disease remains one of the leading causes of death globally, which warrants the need for novel treatment methods. Even though traditional pharmacotherapy and surgery improve the quality of the patient's life, they are invasive and expensive. Cardiac surgeons utilize several ways of surgically repairing various parts of the heart, e.g., the valves or the cardiac septum, which lead to prolongation of the patient's life. Biomaterials are often involved in such cases.<sup>1,2</sup> Their use constitutes a revolution in the world of cardiac surgery. One of the main advantages of using biomaterials is their multifunctionality – they can be used as scaffolding or carriers for the cells and growth factors, but they can also be used on their own. The introduction of so-called injectable biomaterials, placed by the injection, was a particularly revolutionary step. They often take the form of a modified hydrogel.<sup>3</sup> Their use may result in a reduction of the thickness of the wall of the left chamber of the heart. It can also preserve the function of the heart and increase angiogenesis, the process, in which capillaries are formed.<sup>1</sup>

The biomaterial is a vaguely defined term. Most often, it is understood as a material used for medical

applications, which interacts with biological systems. Biocompatibility is an ambiguous term since its definition is subject to frequent updates based on the latest research. Biocompatible material demonstrates properties that support the process of implantation and minimizes adverse reactions. To be feasible for use in cardiac implants, the biomaterials must be elastic, durable, and resistant. It is especially important as the implant should be able to endure around 2 billion heart cycles, which occur during the average lifespan. Furthermore, the implant should be not prone to calcification, support hemostasis, demonstrate antithrombotic properties, and should not induce immune reactions.<sup>1</sup> Recent research shows applications of biomaterials as carriers of drugs or stem cells used for regeneration of the damaged tissue.<sup>4</sup>

Due to their mechanical properties, hydrogels are used in multiple biomedical applications.<sup>5,6</sup> In recent years, significant focus has been placed on their ability to immobilize cells, proteins, or drugs, with the possibility of later controlled release.<sup>7</sup> Hydrogels are also often used as carriers, which may deliver an appropriate active substance to the intended locations in a controlled way. Hydrogels used in biomedical applications may be divided into two groups – natural and synthetic. When compared to the natural ones, synthetic hydrogels allow better control and repeatability of their physical and chemical properties, since the process may take place in a laboratory under strictly controlled parameters. However, the biocompatibility of hydrogels and their potential cell interactions must be taken into account.<sup>8,9</sup> Surface modification of the designed implants is often necessary for the process of connecting with the hydrogel active layers.<sup>10</sup> Our research is focused on polyurethane films, which may find applications in cardiological implants.

A lot of research is dedicated to the applications of polyurethanes for cardiac implants. They belong to a class of compounds called reactionary polymers. The mechanism in which they are formed involves the reaction between hydroxyl and isocyanate groups, which produces foam. These polymers may be also obtained in a thermoplastic form, which is harder and more suitable for medical applications. Thermoplastic polyurethanes are

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characterized by elasticity, high endurance to shearing stress and transparency.<sup>4</sup> Moreover, polyurethanes are highly resistant to microbes, which makes them a good choice of material for preventing infections. Currently, polyurethanes are most commonly found as insulators in heart stimulating electrodes.<sup>11</sup> Thermoplastic polyurethanes, which are to be used in the form of artificial blood vessels in the future, are also very popular. In cardiac surgery, the popular polyurethane elastomer (EPUR) is also used in the production of heart prostheses, among others due to its tensile strength. Another application of polyurethanes is, among others, surgical threads, drains, and urological coils.<sup>12</sup> A significant disadvantage of using long-term polyurethane implants in cardiac surgery is their sensitivity to oxidization and degradation *in vivo*, which may lead to significant problems after the implantation.<sup>1</sup> On the other hand, this property is highly beneficial in the case of biodegradable tissue scaffolds.<sup>13</sup> The physical and chemical properties of polyurethanes, *e.g.*, their sensitivity to degradation, depend on the compound used in the polymer chain.<sup>14</sup> It was demonstrated that antioxidant coating could greatly reduce the intensity of the oxidization.<sup>1</sup>

There are many methods of polyurethane surface modification. The modifications are usually carried out by radical polymerization of the monomers. They are also accompanied by such processes as, for example, silanization or immobilization of biological particles. Many of the modifications described in the literature are concerned with altering the properties of the polymer surface while preserving the mechanical properties of the base material. This effect can be obtained by covering the material with another polymer, or by placing biologically active compounds, which may inhibit the blood clot formation.<sup>15</sup> Examples of such a compound include aspirin (ASA) and heparin, which demonstrate significant antithrombotic properties. It must be noted though, that their biologically active layers are subject to fast degradation.<sup>1</sup>

## 2. Materials and Methods

### 2.1. Characteristics of Materials

To prepare the hydrogels, agar, polyvinyl alcohol (PVA), sodium tetraborate, and water were used. Acetylsalicylic acid (ASA) was used to modify the hydrogels. Agar is a natural gelling agent that is FDA-approved. Poly(vinyl alcohol) is a fully biodegradable polymer. Sodium tetraborate is a chemical compound with antibacterial, antiviral, and antifungal properties. Acetylsalicylic acid is an organic chemical compound with analgesic and anticoagulant properties.

### 2.2. Sample Preparation

Hydrogel samples were synthesized under reflux at the temperature ranging from 371 to 373 K. 50 g of each sample was prepared. The detailed composition of the samples is presented in Table 1. At first, agar, PVA, and sodium tetraborate were added to water and heated until complete dissolution under rigorous mixing. Sodium tetraborate acted as a cross-linking agent. Then, an appropriate amount of ASA was added to 50 g of each solution and dissolved. Then the mixtures were poured onto glass dishes and left until hardened. Samples were left in the freezer for 3 days. The remaining water was drained.

**Table 1.** Substrate ratios of ASA-modified hydrogels

Sample	Water [%]	Agar [%]	PVA [%]	Sodium tetraborate [%]	ASA [%]
0A	97.62	1.59	-	-	0.79
1A	96.09	1.56	-	1.56	0.78
2A	96.11	1.17	0.39	1.56	0.78
3A	96.12	0.78	0.78	1.55	0.78
4A	96.14	0.39	1.16	1.54	0.77

### 2.3. Swelling Kinetics

The swelling kinetics of the hydrogels was determined by measuring the water absorption to determine how long the hydrogels would reach equilibrium. Hydrogel samples were cut as disks with 10.9 mm diameter and then allowed to dry. After drying, their constant initial masses were measured (72 h, room temperature). Each sample was placed separately in a ceramic container, and then poured with 25 mL of distilled water. Analysis of the swelling kinetics was conducted in 5, 15, 30, 45, 60, 75, 90, 105, 120, 190, and 180 min. Swelling properties were calculated according to Eq. (1). Second-order kinetics equations (Eqs. 2 and 3) were used to determine the swelling kinetics and the equilibrium swelling.<sup>15</sup>

$$W = \frac{m_1 - m_0}{m_0} * 100 \% \quad (1)$$

where  $W$  is the swelling [%],  $m_1$  is a mass after time  $t$  [g],  $m_0$  is an initial mass [g].

$$K = \frac{1}{b * W_{eq}^2} \quad (2)$$

where  $K$  is the constant of the swelling kinetics [(%\*min)<sup>-1</sup>],  $b$  is the coefficient  $b$  of the straight line equation,  $W_{eq}$  is the equilibrium swelling [%].

$$\frac{t}{W} = \frac{1}{K * W_{eq}^2} + \frac{t}{W_{eq}} \quad (3)$$

where  $t$  is time [s].

## 2.4. Cross-Link Density

Round samples having a diameter of 10.9 mm were cut from each hydrogel. In order to compute the cross-link density, it was necessary to estimate the mean molecular mass ( $M_c$ ) between the nodes of the network. This mass was determined in accordance with the Flory-Rehner theory (Eq. 4). Later, Eq. (5) was applied.<sup>17</sup>

$$M_c = \frac{d_p V_S \frac{V_{2,S}^{\frac{1}{3}} - V_{2,S}}{2}}{\ln 1 - V_{2,S} + V_{2,S} + \chi V_{2,S}^2} \quad (4)$$

where  $M_c$  is an average molecular weight of polymer chains between network nodes [g/mol],  $d_p$  is a hydrogel density in the hydrated state [g/cm<sup>3</sup>],  $V_S$  is a specific volume of the solvent, in this case, distilled water, amounting to 18.1 [cm<sup>3</sup>/g],  $\chi$  is the Kappa coefficient, also known as the Flory-Huggins coefficient,  $V_{2,S}$  is a coefficient indicating the capacity of the hydrogel allowing diffusion of the solvent to its structure [mL/mol].

$$\rho_c = \frac{d_p}{M_c} \quad (5)$$

where  $\rho_c$  is a hydrogel cross-link density [mol/cm<sup>3</sup>].

## 2.5. Water Content

To determine the water content ( $H$ ) of the hydrogels, three samples of each hydrogel were prepared. Round samples having a diameter of 10.9 mm were cut from each hydrogel. Their initial mass was measured. Then, the samples were dried for 72 h at room temperature. The final mass was measured. Water content was calculated by applying Eq. (6).<sup>18</sup>

$$H = \frac{m_2 - m_1}{m_1} * 100\% \quad (6)$$

where  $H$  is the water content in hydrogels [%],  $m_2$  is a dry sample mass [g],  $m_1$  is a mass of the hydrated sample [g].

## 2.6. Contact Angle, Work of Adhesion and Surface Energy

Diiodomethane contact angle was measured to determine the hydrophilic properties of the analyzed hydrogels. Ramé-Hart Instrument goniometer was used with DROPimage Pro software. Three small pieces were cut out from each sample. A drop of polar diiodomethane ( $\mu = 1.15$  D) was then placed by micro-syringe on the surface of each sample. Diiodomethane was used because the contact angles of water were close to 0 for all samples, which made the comparative analysis of the samples infeasible. Ten measurements of contact angle were performed for each piece of analyzed samples, giving in total of 30 measurements per sample, which were used

then to determine the mean contact angle. Measurements were performed for both the hydrated and dehydrated samples. Photographs of contact angles were taken. The software was also used to determine the surface energy and adhesion work for analyzed hydrogels. DROPimage Pro did not specify the method, which was used to compute the adhesion work. Measurements were performed using the “One Liquid Tool” mode, which allows us to determine both these values using one measuring liquid. Each result was a mean of three measurements. The software in the mentioned mode uses Eq. (7):

$$\cos\theta + 1 = 2 \frac{\gamma_S}{\gamma_L} e^{-\beta \gamma_L - \gamma_S^2} \quad (7)$$

where  $\beta$  is an experimentally determined constant [0.0001247 (mN/m)<sup>-2</sup>],  $\gamma_S$  is the surface tension of the solid phase [mJ/m<sup>2</sup>],  $\gamma_L$  is the surface tension of the liquid [mJ/m<sup>2</sup>].

## 2.7. Microscopy

Microscopic photographs of the structure of hydrated and dehydrated hydrogels were taken using a metallographic microscope DELTA<sup>®</sup> Optical MET-1000-TRF with 200  $\mu$ m resolution (5x lens) for each sample. Samples were placed on a plate and then Toup View software was used to take photos in transmission mode.

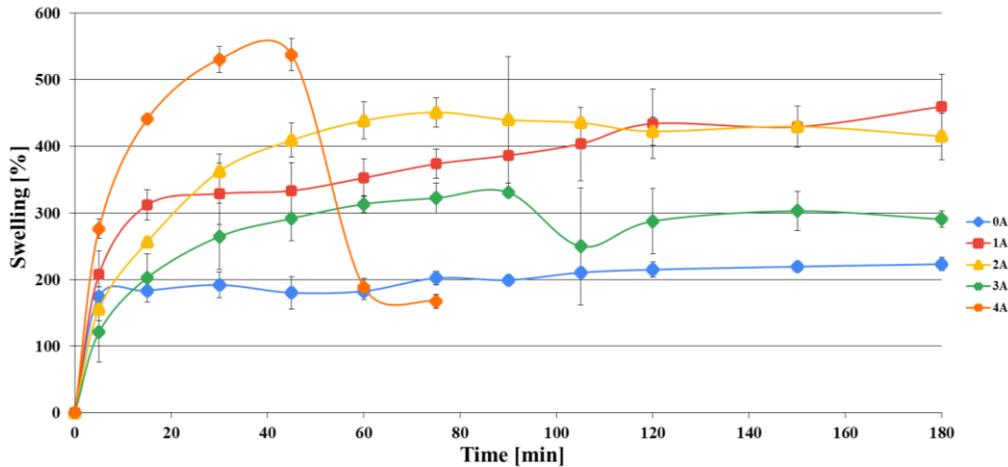
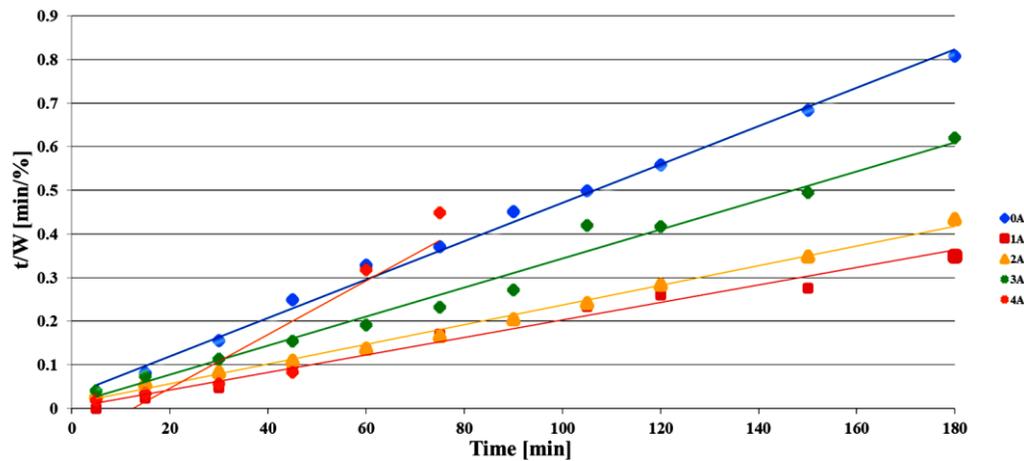
## 3. Results and Discussion

### 3.1. Swelling Kinetics

The most rapid increase in swelling behavior was observed for samples 0A, 3A and 2A. Samples modified by ASA addition demonstrated slower swelling. The introduction of ASA may reduce the water absorption of the hydrogels. The shape of curves in Fig. 1 may be also affected by the sample structure. Lower cross-link levels lead to better water penetration. Sample 4A was the most difficult to analyze since its degradation was the fastest as a result of high PVA content and led to increased hygroscopicity. Swelling kinetic parameters ( $K$ ,  $W_{eq}$ ) for all analyzed samples are represented in Table 2. Analysis of curves depicted in Fig. 2 provided the  $R^2$  estimate of the accuracy of the second-order kinetics model for the constant  $K$  (Table 3). The results show that this method should not be used for the hydrogel 4A, due to the value of  $R^2$ :  $R^2(4A) = 0.84$ ,  $R^2(3) = 0.50$ ,  $R^2(4) = 0.52$ . Furthermore, the value of the  $b$  coefficient in the regression equation would be negative, which would result in an infeasible solution with the negative value of  $K$ . Hence the equation was used to determine  $K$  and  $W_{eq}$  constants only for the samples 0A, 1A, 2A, and 3A.

**Table 2.** Derived  $K$  constants and equilibrium swelling values of the analyzed hydrogels

Sample	0A	1A	2A	3A	4A
$K$ [ $1/\% \cdot \text{min}$ ]	$1.88 \cdot 10^{-3}$	$0.58 \cdot 10^{-3}$	$0.18 \cdot 10^{-3}$	$4.04 \cdot 10^{-3}$	-
$W_{eq}$ [%]	227.32	452.24	443.47	300.36	-

**Fig. 1.** Relation between swelling and time**Fig. 2.** The plot of  $t/W=f(t)$  relation for the analyzed hydrogels**Table 3.** Regression equations and  $R^2$  values for the analyzed samples

Sample	Regression equation	$R^2$
0A	$y = 0.0033293x + 0.0107749$	0.9802938
1A	$y = 0.0022112x + 0.0251285$	0.9904859
2A	$y = 0.0022549x + 0.0114215$	0.9937221
3A	$y = 0.0033293x + 0.0107749$	0.9802938
4A	$y = 0.0061x - 0.0758$	0.8423

Comparing the reference sample 0A (with no sodium tetraborate added) to sample 1A (with sodium tetraborate), it can be concluded that the addition of borax increases the value of the equilibrium swelling ( $W_{eq}$ ) by about 215 %. On the contrary, the increasing content of PVA in hydrogels 2A and 3A causes a slight decrease in the  $W_{eq}$  values and prolongs

the time taken for the hydrogel samples to reach equilibrium with the swelling medium. This property may allow the prolonged absorption of body fluids at the site of application of the implant coated with the hydrogel. Additionally, it may also enable a longer sustained release of the anticoagulant substance at the application site.

### 3.2. Cross-Link Density

The highest value of the cross-link density (Table 4) was achieved by sample 4A ( $291.42 \text{ mol/cm}^3$ ). The high cross-link density indicates a low molecular weight between the nodes of the network. There is a possibility that ASA was involved in cross-linking as it breaks down into salicylic and acetic acid in the aquatic environment. Salicylic acid has  $-\text{OH}$  and  $-\text{COOH}$  groups. Due to this, it can create additional bonds between sodium tetraborate and PVA or agar chains. The high cross-link density of these samples can complicate the reactions with solvents and the interference of other substances in their structure may be difficult. Cross-link density correlates with the equilibrium swelling. The higher the cross-link density, the smaller the interference of the solvent in the hydrogel and its swelling. Hence, the low equilibrium swelling  $W_{eq}$  values for the modified samples were observed. The increasing value of the cross-link density is also noticeable in the case of samples containing PVA in the composition because it also influences the cross-linking of the hydrogel structure. The high cross-link density causes the decrease of swelling  $W$ , while the low density increases the value.

### 3.3. Water Content

The water content (Fig. 3) was found to be between  $67.17 \pm 2.14 \%$  and  $97.07 \pm 0.74 \%$ . As shown in Fig. 3, the

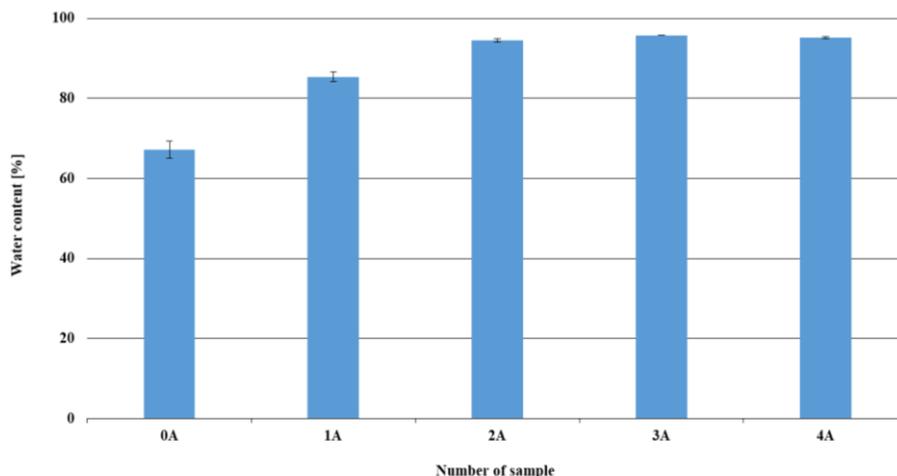
highest water content was exhibited by sample 4A ( $97.07 \pm 0.74 \%$ ), with the highest content of PVA (1.2 g). This polymer is characterized by high hydrophilic properties; hence each sample with PVA content demonstrated high water content. The PVA content thus affected the hygroscopicity of the analyzed samples.

### 3.4. Contact Angle, Work of Adhesion and Surface Energy

Fig. 4 depicts the mean contact angles for all analyzed samples of hydrogels modified by ASA. None of the contact angles exceeds  $90^\circ$ , so each hydrogel will be hydrophilic in case of contact with water. Such behavior means that hydrogel probably will not cause adverse reactions after contact with the physiologic fluid. Among the hydrated samples, the highest contact angle value was measured for hydrated sample 1A. The highest contact angle among the dehydrated samples was measured for 4A. These samples would be the worst candidates for applications, where contact with fluids is expected since the contact angle should be as low as possible. The smallest values were obtained for the dehydrated sample 2A and the hydrated sample 3A. These samples contain a different content of PVA. There was a visible difference in contact angles between samples with- and without PVA. Contact angles of hydrated and dehydrated samples of the same hydrogel differ significantly. The difference can be caused by the variation in the quantitative composition of the analyzed hydrogels.

**Table 4.** Density,  $M_c$ , Kappa-coefficient, and cross-link density of the hydrogels modified with ASA

Sample	Density [ $\text{g/cm}^3$ ]	$M_c$ [ $\text{g/mol}$ ]	Kappa	Cross-link density [ $\text{mol/cm}^3$ ]
0A	1427.17	132.11	-0.6213	10.80
1A	912.37	221.05	-0.56783	4.13
2A	998.37	161.43	-0.58649	6.18
3A	978.07	48.29	-0.67879	20.25
4A	1012.80	3.47	-1.69834	291.42



**Fig. 3.** The water content of the hydrogel samples

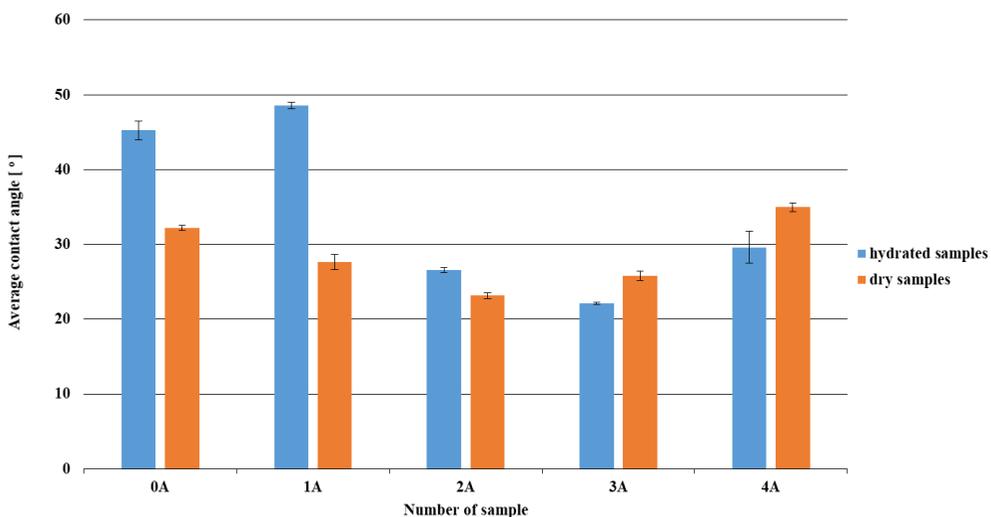


Fig. 4. The average contact angle of the hydrogel samples

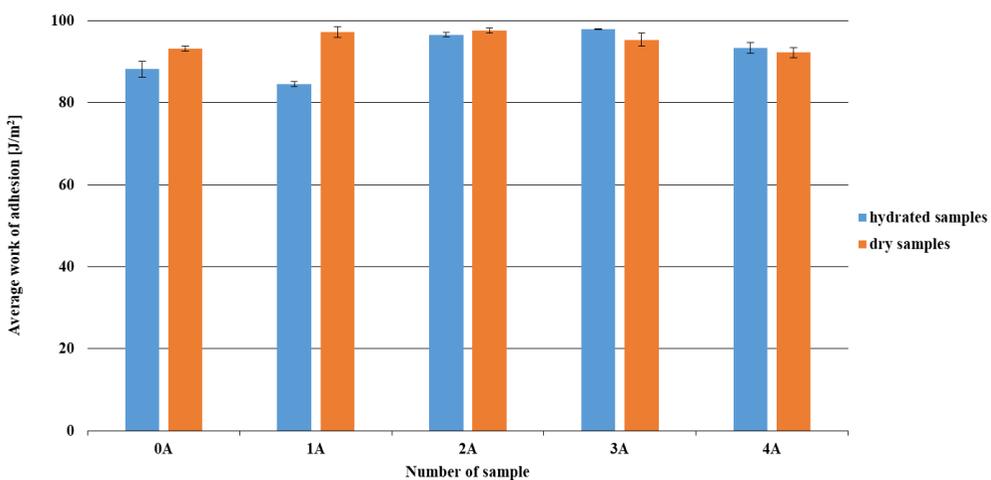


Fig. 5. Average work of adhesion of the hydrogel samples

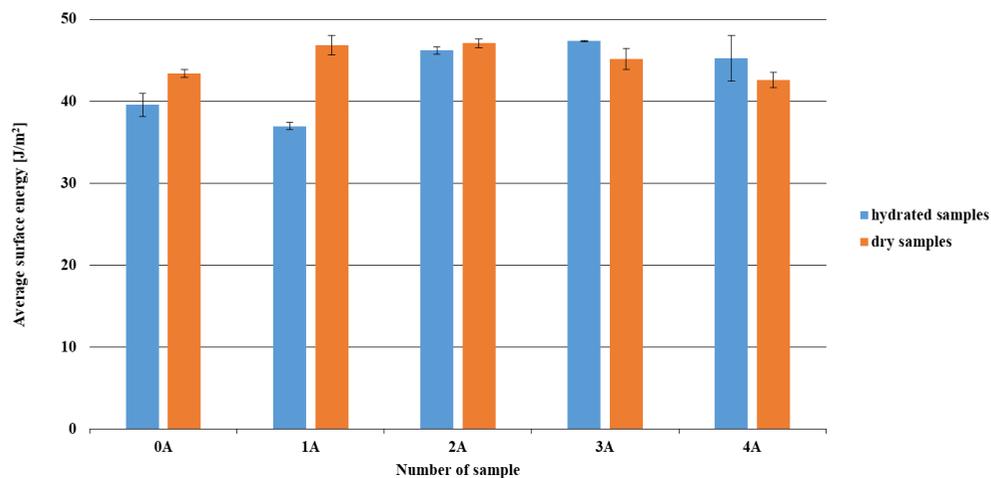
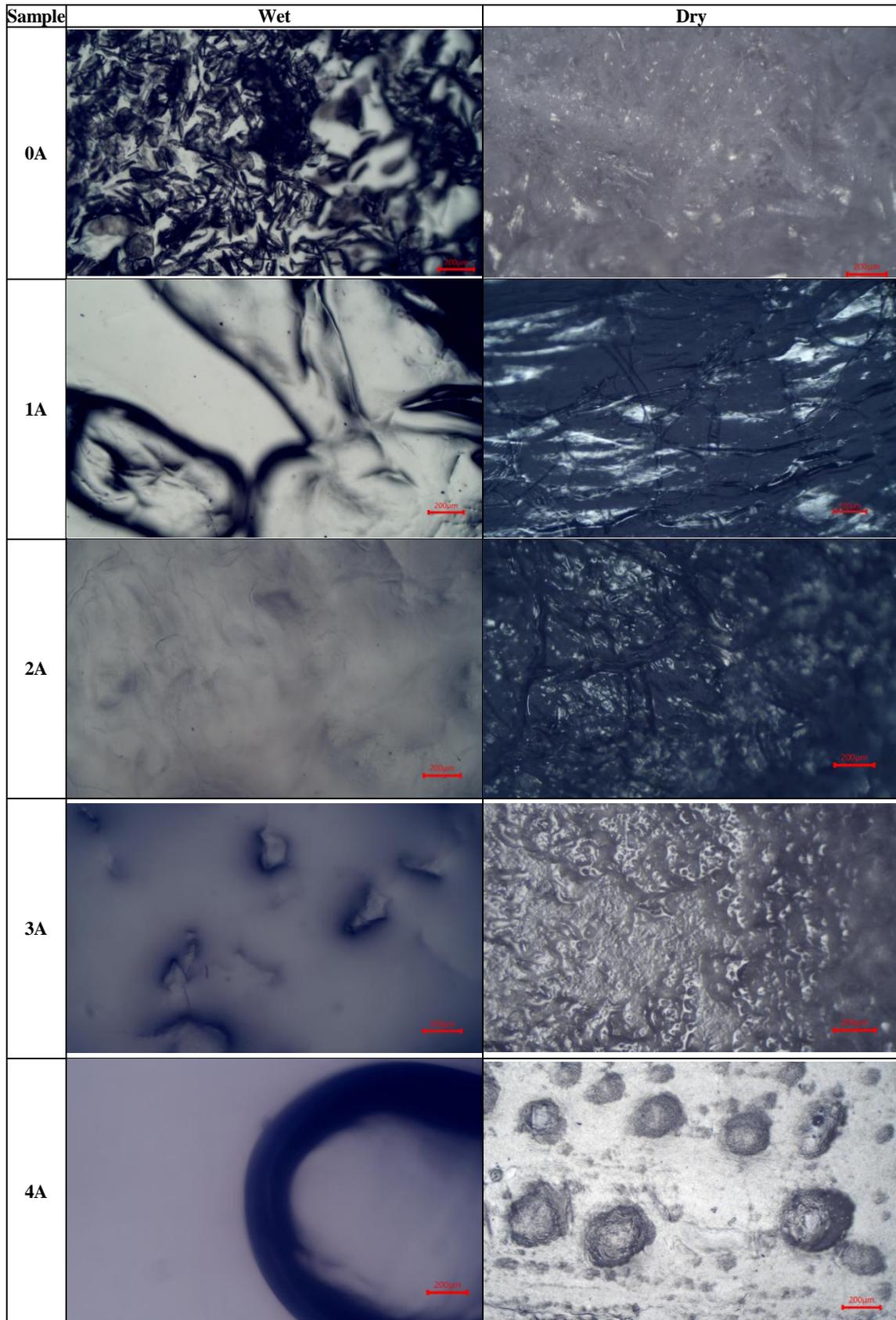


Fig. 6. Mean surface energy of the hydrogel samples

**Fig. 7.** ASA modified hydrogels

### 3.5. Microscopy

Optical microscopy was used to characterize the structure and appearance of hydrogen samples (Fig. 7). The analysis was focused on the impact of ASA presence. Analysis of photographs of hydrated samples showed the increasing smoothness of the surface. Most likely, it is caused by the presence of PVA, since the samples without PVA were characterized by the presence of streaks and blisters. Furthermore, the cross-linking of sample 0A was clearly visible. The ASA present in the sample 0A caused the strong folding of the surface. In the case of hydrated samples with PVA, the visible folding was minimal. Other hydrated samples were characterized by the absence of any specific structures. On the contrary, the structural elements were clearly visible in the dehydrated samples. The presence of PVA caused the dehydrated samples to be increasingly matte with a lack of clarity. Sample 4A had visible remnants of the air bubbles, but most likely they did not significantly affect the hydrogel structure.

### 4. Conclusions

Natural-based hydrogels could be a great material for potential application in medicine, especially for cardiac implants. Due to their natural origin, they show high biocompatibility, hemocompatibility, and nontoxicity. Presented agar- and PVA-based hydrogels crosslinked with sodium tetraborate and modified with ASA showed promising physical and swelling properties to be used as a bioactive, antithrombotic layer covering cardiac implants. During the literature search, other examples of studies, in which hydrogels containing both PVA and agar were used as the carrier for the drug release system, were not found.

Hydrogel samples, which contained simultaneously the PVA and the agar base, were successfully synthesized. Modification of hydrogels with ASA leads to significant changes in structure, in particular, the modified samples became strongly folded, which confirms the strongly cross-linked structure of the hydrogels. Sample 2A seems to be the most suitable for the intended application since it demonstrates the best properties in terms of equilibrium swelling. It shows the slightly decreased value of  $W_{eq}$  compared to sample 1A, but it also demonstrates a more appropriate  $K$  value, resulting in prolonged swelling medium absorption. This hydrogel in combination with polyurethane could be potentially used in cardiac surgery. This hydrogel is highly hydrophilic, which is desirable for hydrogels used in this field of medicine.

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### ГІДРОГЕЛІ КАРДІОЛОГІЧНОГО ПРИЗНАЧЕННЯ НА ОСНОВІ ПРИРОДНИХ ПОЛІМЕРІВ

**Анотація.** У цій роботі описано синтез гідрогелів на основі агару та бури з додаванням і без додавання полівінілового спирту за різних концентрацій. Гідрогелі були модифіковані однаковою кількістю ацетилсаліцилової кислоти (АСК), яка має антитромботичні властивості. Проаналізовано вплив модифікації гідрогелів АСК на їхні властивості.

**Ключові слова:** агар, ПВС, гідрогелі, кардіохірургія, ацетилсаліцилова кислота.