

Thermal and physical properties of CNF/glutaraldehyde-gelatin-based hydrogel

Roslina Lubis¹, Sri Wahyuna Saragih^{2*}, Wardatul Husna Irham², Ajmain³, Saisa⁴

¹Department of Biology, Faculty of Sains and Technology, Universitas Medan Area, Medan, Indonesia

²Department of Chemical Engineering, Institut Teknologi Sawit Indonesia, Medan, Indonesia

³Faculty of Health Science, Universitas Sains Cut Nyak Dhien, Kota Langsa, Aceh, Indonesia

⁴Department of Chemical Engineering, Faculty of Engineering, Universitas Serambi Mekkah, Banda Aceh 23245, Aceh, Indonesia

Abstract. The use of hydrogel as a wound dressing material is currently being massively developed. In addition to functioning to protect wounds, the use of hydrogel can also provide moisture in a measured manner and can be used as a drug delivery medium. In this study, hydrogel based on CNF and glutaraldehyde crosslinking agent and addition of gelatin were developed with various compositions 0.25; 0.5; 0.75 g to increase the ability of CNF and hydrogel to absorb water so that it is good to be applied as a wound dressing. The composting of the three materials aims to obtain a hydrogel with good thermal and physical properties. Based on physical character for a good ratio of swelling (666,62%) and degree of cross-linking (94%) on the hydrogel with a composition variation of 0.5 g of CNF addition. For the thermal stability of hydrogel var 2, T_{Max} 591°C provides better thermal stability than var 1 and var 3. The morphology of hydrogel shows very small and evenly distributed pores on the surface which can absorb more water.

Keywords: composite, degree of crosslinking, hydrogel, physical Properties, wound dressing,

INTRODUCTION

Damage to tissue on the skin caused by pressure trauma, sharp or blunt objects, animal bites, radiation explosions, contact with electrical chemicals, hot water, friction, and fire is usually referred to as a wound. The wound that occurs must be treated immediately correctly and appropriately. If there is inflammation in the wound, it can result in infection of the area around the wound if not treated immediately. Some of the stages that need to be done in wound management, one of which is using wound dressing [1].

In recent years, the application of natural polymers in biomedical materials has received widespread attention, including drug administration, wound dressings, tissue engineering scaffolding, etc. These natural polymers exist in a variety of natural materials, such as alginate, chitosan, collagen, starch, cellulose, etc [2]. Among these, cellulose is a major component in lignocellulose biomass and is the most abundant natural polymer on earth. Over the past two decades, cellulose nanomaterials, also known as nanocellulose,

have attracted rapidly evolving scientific and technological interest from both academic and industrial researchers [3].

Nanocellulose, CNC and CNF structure materials have a higher specific surface area and a more uniform particle size distribution so they tend to form more mechanically stable self-assembled structures (e.g. hydrogels) [4]. Recently, self-assembled CNC hydrogels [5] and CNF [6] through a cross-linking strategy are promising candidates for biomedical applications, due to their outstanding mechanical stability, controllable morphology, and high biocompatibility and biodegradability [7]. CNC (Cellulose Nanocrystal) and CNF (Cellulose Nanofiber) are pure cellulose derivatives, in contrast to CNC whose surface consists of crystals, the surface area of CNF consists of crystals and is amorphous [8]. CNF has wide areas of application due to its interesting properties, such as low weight, high aspect ratio, high reinforcing potential, and large surface area. CNF can also be applied in a variety of industrial areas or applications, such as nanofiller for polymeric nanocomposite, packaging and food packaging, paper printing, antibacterial paper, and conductive paper [9].

The use of hydrogel as a wound dressing is currently one of the interesting topics to develop. Hydrogels

*Corresponding Author:
yunasaragih@gmail.com

Received: 30 December 2022 | Revised: 24 January 2023
| Accepted: 16 february 2023

were first produced through crosslinking reactions and chemical polymerization reactions. Hydrogel has the purpose of being able to absorb and can even keep large amounts of water with a three-dimensional network structure consisting of natural synthesis materials. Therefore hydrogels are in great demand, especially in the field of biomedicine [10].

Hydrogel is a three-dimensional structured polymer that can absorb water molecules. The unique property of this polymer is that it can undergo swelling and deswelling through drying. Hydrogels made from natural raw materials are superior to synthetic hydrogels because they are biodegradable and renewable [11]. One of the raw materials for cheap and abundant natural hydrogels in Indonesia is cellulose nano-fibers derived from abaka banana stems which have been cultivated in Indonesia [12].

Hydrogels can be formed through physical or chemical cross-link from homopolymers or copolymers so that a three-dimensional structure is formed. Crosslinking can be formed by covalent or non-covalent interactions. Covalently cross-link hydrogels are called chemical gels while non-covalently they are called physical gels. The way to physically form a crosslink is by hydrophobic interaction, charge interaction, or by forming hydrogen bonds. Charge interactions can occur between polymers and small molecules or between two different polymer charges [13].

CNF materials can be used as hydrogels through various techniques, including chemical reactions, reactions between functional groups, free radical polymerization, and irradiation [14]. Many CNF-based hydrogels have been developed for various biomedical fields, for example, poly (vinyl alcohol) hydrogels with nanocellulose fillers for ophthalmic applications: general characteristics and optical properties [15], CNF/poly(vinyl alcohol) hydrogels cross-linked by a freezing/thawing method and borax [16], and Gelatin hydrogel with CNF reinforcement and optimization as 3D printing for tissue repair applications [17].

Therefore, in this study, the combination of cross-linked CNF with glutaraldehyde and reinforced with gelatin control the mechanical properties of the hydrogel and the swelling ratio in the form of a composite as a hydrogel candidate. The composting of the three is expected to produce a hydrogel with good thermal and physical properties so that it can be used as a wound dressing material.

METHODOLOGY

Materials

Raw abaca fiber was obtained from Peunaron district, Aceh Timur – Indonesia. Gelatin, Glutaraldehyde, and aquades, obtained from Sigma-Aldrich. All chemicals were used without additional treatment.

Table 1. The hydrogel composition of CNF/glutaraldehyde and gelatin

Code	CNF (g)	Glutaraldehyde 2% (mL)	Gelatine (g)
Var 1	0.25	1	2
Var 2	0.5	1	2
Var 2	0.75	1	2

Synthesis of Hydrogel

Cellulose nanofibers were extracted from abacá pseudo-stem fibers using acid hydrolysis processed with a steam explosion and homogenization. The preparation and characterization of CNF were based on a previous study[18]. CNF based hydrogel was prepared based with several variations of CNF, each variation added gelatin 2 g and 2% glutaraldehyde (GA) was added as much as 1 mL. then each variation is also added with aquades as much as 50 mL. All mixtures are stirred at a temperature of 70°C for 2 hours until homogeneous. Then the hydrogel that has formed is poured on the mold and heated in an oven for 3 hours at a temperature of 50°C so that a hydrogel for wound dressing is obtained. The same treatment was performed for different CNF concentrations according to the experimental design presented in Table-1.

Swelling degree

Swelling testing was carried out by soaking each wound dressing variation of the same size, namely 1cm x 1cm into aqueous as much as 10 ml using time variations of 10 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes. During the testing process, measurements of the weight of the sample are carried out at any time. The weight gain of the sample is the amount of aquades absorbed by wound dressing.

Degree of crosslink

The percentage value of the degree of crosslink can be done by determining the percent degree of cross-link where the dry weight of the resulting hydrogel is weighed. Then the hydrogel is soaked with its solvent (chloroform) for 24 hours. After soaking, the hydrogel is put in the oven at a temperature of 60°C to dry for 3 hours. The dry weight of the hydrogel after immersion is determined by weighing using an analytical balance sheet.

Thermal and Morphological Analysis

The thermal stability of the material was determined by thermogravimetric analysis using DTG-60 Shimadzu in the temperature range of 30-600°C with a heating rate of 10°C / min. The combustion process is carried out under atmospheric conditions of nitrogen to

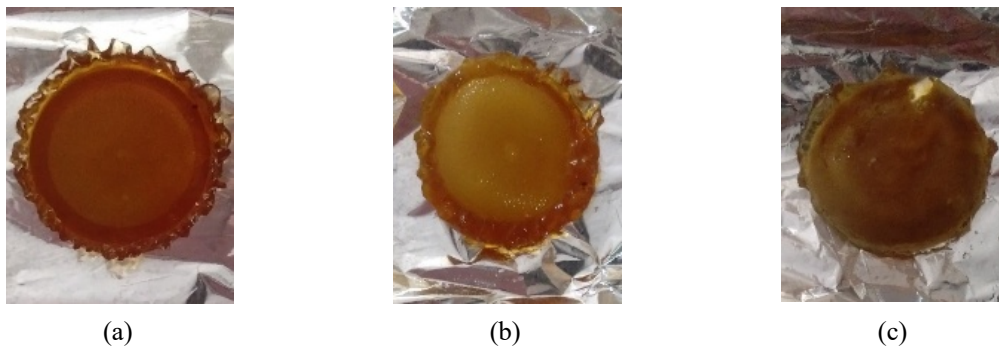


Figure 1. Hydrogel synthesis after drying: a) Var 1, b) Var 2, dan c) Var 3.

Table 2. CNF/glutaraldehyde and gelatin-based Swelling Hydrogel Ratio.

Time (minutes)	Weight (g)			Swelling Ratio (%)		
	Var 1	Var 2	Var 3	Var 1	Var 2	Var 3
0	0.75	0.78	1.00	0	0	0
10	1.68	2.29	2.10	123 ± 1	193.52 ± 0.5	110.33 ± 0.58
30	2.30	3.42	2.88	206.39 ± 0.34	338.19 ± 0.24	187 ± 1
60	3.83	4.78	3.59	410.62 ± 0.4	512.61 ± 0.53	258.67 ± 0.58
90	4.17	5.46	4.17	455 ± 1	601 ± 1	316.67 ± 0.58
120	4.23	5.98	4.67	465 ± 1	666.62 ± 0.4	369 ± 1

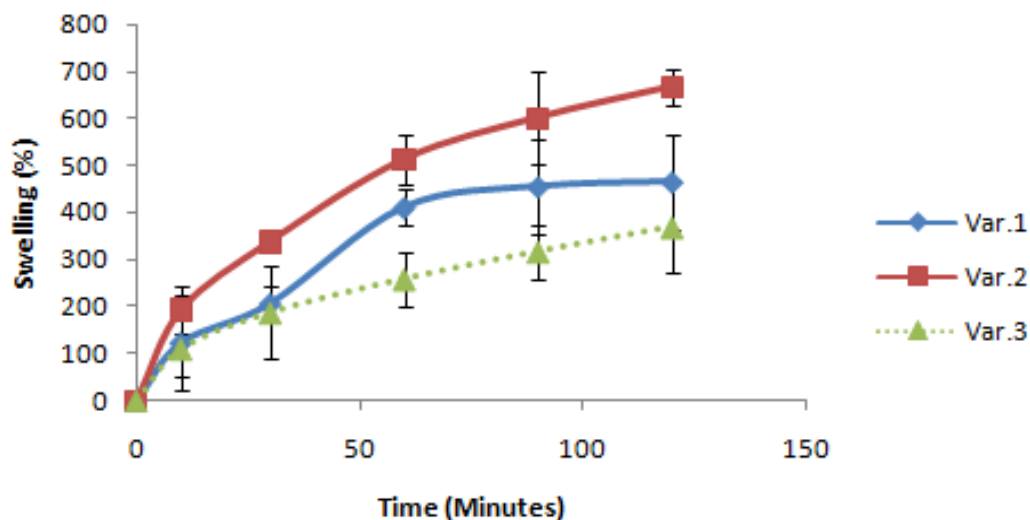


Figure 2. Swelling Hydrogel Ratio curves of CNF/Glutaraldehyde and Gelatin.

prevent the degradation process due to thermo-oxidative. The residual period obtained at a temperature of 600°C is expressed as ash content. The fault surface morphology of the test specimen was determined using Scanning Electron Microscopy (SEM) JEOL JSM-6510LA. The dimensions of the test sample are 1 mm x 1mm, to improve the image quality obtained the sample needs to be coated with gold to improve the conductivity of the material.

RESULTS AND DISCUSSION

CNF/Glutaraldehyde-Gelatin-Based Hydrogel

Hydrogel synthesis carried out according to the predetermined variations in composition produces hydrogels as shown in Figure 1. In each CNF variation, 0.25, 0.5, and 0.75 g have different thicknesses.

Swelling Degree of Hydrogel

The results of the swelling test in this study can be seen in Table 1 and Figure 2 using time variations of 10 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes, it can be concluded that the addition of CNF causes percent swelling to increase. This is because CNF has a hydrophilic group [19] which triggers a synergistic effect with other polymer groups that form hydrogen bonds between molecular chains so that water molecules easily penetrate the pores of the hydrogel [20]. The hydrophilic group of other polymers that affects percent swelling is increasing, namely the amine group (NH₂) in gelatin [21]. In the addition of CNF 0.5 g the value of the swelling ratio increases and decreases again the value of the swelling ratio at the addition of 0.75 g CNF, it is likely that at the addition of 0.5 g CNF, there is an optimum point in var 2 hydrogel. So that in var 3 with the addition of an excessive amount of CNF causes the swelling percent to decrease. This demonstrates that the hydrogel structure's density has increased such that the entry of water molecules into the resulting hydrogel network is prevented [22].

From the table and figure it is shown that the value of the swelling ratio decreases when the added CNF increases. As a result, the hydrogel's ability to absorb the liquid medium will be affected. From the table, it can be seen that there is a decrease in the ratio value of cross-link hydrogels as the CNF is added.

Degree of Cross-link

Measurement of percent degree of cross-link by soaking the hydrogel with chloroform solvent for 24 hours. Before soaking weighed the initial weight of the dry hydrogel. After muffle it is dried in the oven and weighed the final weight of the hydrogel is. After drying, the weight of the maker is smaller than the initial weight, there is a fraction dissolved in chloroform which is likely not to occur crosslink in the hydrogel. The dissolved fraction constitutes the entire speciation that can dissolve in chloroform. A hydrogel with a certain mass is immersed in chloroform until equilibrium is obtained. A certain amount of the missing hydrogel mass is expressed as a dissolved fraction [23].

Testing the percentage degree of crosslinking of a hydrogel is a measure for the formation of a hydrogel which is characterized by the presence of tissues that bind polymer chains so that it can be used for various applications (e.g. cosmetic fields). The percentage of crosslinking formation is closely related to the ability or how much potential the hydrogel responds to certain circumstances or conditions given by the environment, for example in a water environment, the hydrogel is inflated or dissolved [24]. This is to previous research that biomedical hydrogels for facial mask applications need to be examined for their ability to swell, cross-link, irritate the skin and be antimicrobial [25]. This

statement is supported by Saragih *et al* [18], MBA-CNF hydrogel exhibits a swelling and crosslinking degree of 3582 and 85%, indicating the capability of the compounds to adsorb the water molecules due to higher hydroxyl groups. As a comparison, chitosan-CNF hydrogels exhibit a lower swelling degree of 137% but a higher crosslinking degree up to 90%, due to the decrease in the number of functional groups that formed a hydrogen bond. Besides, the percentage value of the crosslinking degree correlates with its ability for responding to the surrounding condition [26]

Based on table 3, it can be seen that var 2 the hydrogel decreases its cross-linking degree with the addition of CNF. The greater the amount of CNF used, the denser and less elastic the hydrogel will be. The chemical bond occurring in the var 2 the hydrogel has likely occurred optimally in the addition of 0.5 gr CNF.

Based on the cross-binding degree data above, shows that the CNF-based hydrogel cross-linking degree is inversely proportional to the swelling ratio of the hydrogel. The degree of cross-linking is high then the water absorption in the hydrogel is low.

Thermal and Morphological Analysis of Hydrogel

Thermal analysis was performed to investigate the degradation characteristics and thermal stability of CNF-based hydrogel samples with the presence of glutaraldehyde and gelatin crosslinking agents for hydrogel mechanism controllers shown in Figure 3a) and 3b) The thermal stability of CNF-based hydrogels was determined by TGA/DTG analysis.

CNF/glutaraldehyde and gelatin-based hydrogels undergo three stages of change during the heating process in the TGA device shown in figure 3a). The first stage is the process of releasing water molecules (evaporation) in the temperature range of 30-110°C. The evaporation process leads to a mass loss of 3-7% of the initial mass. the second stage of change occurs in the range of 110-355°C is a process of dehydroxylation and devolatilization and undergoes a mass reduction of 55%. This mass reduction can be caused by the structural degradation of the CNF. The carbonization and decomposition of organic compounds is the third stage that occurs during the thermal testing process in the range of 355-600°C and experiences a mass reduction above 75% [27],[28]

Figure 3b) shows the existence of a decomposition process of CNF/glutaraldehyde and gelatin-based hydrogels there are 3 stages characterized by the presence of three peaks on the DTG curve. T_{Max} in the decomposition process of var 1 the hydrogel can be seen at a temperature of 565 °C, var 2 at 591 °C, and var 3 at 578 °C, this shows that var 2 the hydrogel provides better thermal stability than var 1 and var 3.

The SEM micrograph generated in Figure 3c) from var 2 which is a various with good swelling shows a slightly rough flat surface, indicating there are some

particles indicating the presence of non-interacting CNF. CNF/glutaraldehyde and gelatin-based hydrogels have very small, evenly surfaced fine pore pores that can absorb more water. This can be seen from the highest percentage of CNF/glutaraldehyde and gelatin-based hydrogel swelling ratios reaching 666.62%.

Surface analysis with SEM can provide an overview of the surface topology of the hydrogel. The ability of a hydrogel to absorb water is related to the presence of a porous space in the hydrogel [29]. In general, in addition to the gel surface, porous space is also contained in the body (bulk) of tissue polymers. The porous space allows the process of diffusion of water into the polymer network as free water and enters first through capillary forces, then interacts with the functional groups in the polymer network to form intermediate water and bound water. Bonded water is water that binds hydrogen to polymeric tissues and exhibits high mobility within polymeric tissues [30].

CONCLUSION

The combination of CNF/glutaraldehyde with gelatin in composite form is effective for improving the swelling ability of hydrogels. In this study, the variation of CNF 0.5 gr is a hydrogel that has good thermal stability at a temperature of 591 °C and has smooth and even pores on the surface that can absorb more water so that it has the highest swelling, which reaches 666.62%.

ACKNOWLEDGMENT

The author would like to thank Universitas Sains Cut Nyak Dhien and Universitas Medan Area for the facilities used in this research.

REFERENCES

- [1] Ramadhani, F.; Miratsi, L.; Humaeroh, Z.; Afriani, F. 2021. Kemampuan swelling hidrogel berbasis PVA/alginat. *Proc. Natl. Colloq. Res. Commun. Serv.* 149–151. DOI: 10.33019/snppm.v5i0.2727.
- [2] Khan, M. I. H.; An, X.; Dai, L.; Li, H.; Khan, A.; Ni, Y. 2019. Chitosan-based polymer matrix for pharmaceutical excipients and drug delivery. *Curr. Med. Chem.* **26** (14) 2502–2513. DOI: 10.2174/0929867325666180927100817
- [3] Zhu, H.; Luo, W.; Ciesielski, P. N.; Fang, Z.; Zhu, J. Y.; Henriksson, G.; Himmel, M. E.; Hu, L. 2016. Wood-derived materials for green electronics, biological devices, and energy applications. *Chem. Rev.* **116** (16) 9305–9374. DOI: 10.1021/acs.chemrev.6b00225.
- [4] Gu, F.; Wang, W.; Cai, Z.; Xue, F.; Jin, Y.; Zhu, J. Y. 2018. Water retention value for characterizing fibrillation degree of cellulosic fibers at micro and nanometer scales. *Cellulose* **25** (5) 2861–2871. DOI: 10.1007/s10570-018-1765-8.
- [5] Chau, M.; Sriskandha, S. E.; Pichugin, D.; Thérien-Aubin, H.; Nykypanchuk, D.; Chauve, G.; Methot, M.; Bouchard, J.; Gang, O.; Kumacheva, E. 2015. Ion-Mediated Gelation of Aqueous Suspensions of Cellulose Nanocrystals. *Biomacromolecules* **16** (8) 2455–2462. DOI:10.1021/acs.biomac.5b00701.
- [6] Xu, C.; Zhang, B.; Wang, X.; Cheng, F.; Xu, W.; Molino, P.; Bhacer, M.; Su, D.; Rosenau, T.; Willfor, S.; Wallace, G. 2018. 3D printing of nanocellulose hydrogel scaffolds with tunable mechanical strength towards wound healing application. *J. Mater. Chem. B* **6** (43) 7066–7075. DOI:10.1039/c8tb01757c.
- [7] Xu, W.; Wang, X.; Sandler, N.; Willför, S.; Xu, C. 2018. Three-dimensional printing of wood-derived biopolymers: a review focused on biomedical applications. *ACS Sustainable Chem. Eng.* **6** (5) 5663–5680. DOI: 10.1021/acssuschemeng.7b03924.
- [8] Abitbol, T.; Rivkin, A.; Cao, Y.; Nevo, Y.; Abraham, E.; Ben-Shalom, T.; Lapidot, S.; Shoseyov, E. 2016. Nanocellulose, a tiny fiber with huge applications. *Curr. Opin. Biotechnol.* **39** 76–88. DOI: 10.1016/j.copbio.2016.01.002.
- [9] Mahendra, I. P.; Wirjosentono, B.; Tamrin; Ismail, H.; Mendez, J. A. 2019. Thermal and morphology properties of cellulose nanofiber from TEMPO-oxidized lower part of empty fruit bunches (LEFB). *Open Chem.* **17** (1) 526–536. DOI: 10.1515/chem-2019-0063.
- [10] Erizal, E.; Pratiwi, E. W.; Perkasa, D. P.; Noviyantih, N.; Abbas, B.; S. Sudirman, S. 2018. Imobilisasi propanolol hcl pada hidrogel poli(vinil alkohol)-natrium alginat dengan teknik radiasi. *Jurnal Kimia dan Kemasan* **40** (1) 47–56. DOI:10.24817/jkk.v40i1.2755.
- [11] Rocha-García, D.; Betancourt-Mendiola, M. L.; Wong-Arce, A.; Rosales-Mendoza, S.; Reyes-Hernandez, J.; Gonzalez-Ortega, O.; Palestino, G. 2018. Gelatin-based porous silicon hydrogel composites for the controlled release of tramadol. *Eur. Polym. J.* **108** 485–497. DOI: 10.1016/j.eurpolymj.2018.09.033.
- [12] Saragih, S. W.; Hardiyanti, R.; Mahendra, I. P. 2021. Antimicrobial activity of cellulose nanofiberbased hydrogels from abacá banana pseudo-stem fibre. *Rasayan J. Chem.* **14** (1) 578–583. DOI: 10.31788/RJC.2021.1415883.
- [13] Liu, W. C.; Wang, H. Y.; Lee, T. H.; Chung, R. J. 2019. Gamma-poly glutamate/gelatin composite hydrogels crosslinked by proanthocyanidins for wound healing. *Mater. Sci. Eng. C* **101** 630–639. DOI: 10.1016/j.msec.2019.04.018.
- [14] Jayaramudu, T.; Ko, H. U.; Kim, H. C.; Kim, J. W.; Muthoka, R. M.; Kim, J. 2018. Electroactive hydrogels made with polyvinyl alcohol/cellulose nanocrystals. *Mater. (Basel)* **11** (9) 1615. DOI: 10.3390/ma11091615.

- [15] Tummala, G. P.; Rojas, R.; Mihranyan, A. 2016. Poly(vinyl alcohol) hydrogels reinforced with nanocellulose for ophthalmic applications: general characteristics and optical properties. *J. Phys. Chem. B* **120** (51) 13094–13101. DOI: 10.1021/acs.jpcc.6b10650
- [16] Takeno, H.; Inoguchi, H.; Hsieh, W. C. 2020. Mechanical and structural properties of cellulose nanofiber/poly(vinyl alcohol) hydrogels cross-linked by a freezing/thawing method and borax. *Cellulose* **27** (8) 4373–4387. DOI: 10.1007/s10570-020-03083-z.
- [17] Jiang, Y.; Xu, X.; Liu, D.; Yang, Z.; Zhang, Q.; Shi, H.; Zhao, G.; Zhou, J. 2019. Preparation of cellulose nanofiber-reinforced gelatin hydrogel and optimization for 3D printing applications. *BioRes.* **13** (3) 5909–5924. DOI: 10.15376/biores.13.3.5909-5924.
- [18] Saragih, S. W.; Wirjosentono, B.; Eddiyanto.; Meliana, Y. 2020. Influence of crosslinking agent on the morphology, chemical, crystallinity and thermal properties of cellulose nanofiber using steam explosion. *Case Stud. Therm. Eng.* **22** 100740. DOI: 10.1016/j.csite.2020.100740.
- [19] Du, H.; Liu, W.; Zhang, M.; Si, C.; Zhang, X.; Li, B. 2019. Cellulose nanocrystals and cellulose nanofibrils based hydrogels for biomedical applications. *Carbohydr. Polym.* **209** 130–144. DOI: 10.1016/j.carbpol.2019.01.020.
- [20] Gritsch, L.; Motta, F. L.; Contessi Negrini, N.; Yahia, L. H.; Farè, S. 2018. Crosslinked gelatin hydrogels as carriers for controlled heparin release. *Mater. Lett.* **228** 375–378. DOI: 10.1016/j.matlet.2018.06.047.
- [21] Mu, S.; Liu, W.; Zhao, L.; Long, Y.; Gu, H. 2019. Antimicrobial AgNPs composites of gelatin hydrogels crosslinked by ferrocene-containing tetrablock terpolymer. *Polym.* **169** 80–94. DOI: 10.1016/j.polymer.2019.02.047.
- [22] Li, T.; Lei, Y.; Guo, M and Yan, H. 2018. Crosslinked poly(vinyl alcohol) hydrogel microspheres containing dispersed fenofibrate nanocrystals as an oral sustained delivery system. *Eur. Polym. J.* **101** 77–82. DOI: 10.1016/j.eurpolymj.2018.02.003.
- [23] Bai, H.; Li, Z.; Zhang, S.; Wang, W.; Dong, W. 2018. Interpenetrating polymer networks in polyvinyl alcohol/cellulose nanocrystals hydrogels to develop absorbent materials. *Carbohydr. Polym.* **200** 468–476. DOI: 10.1016/j.carbpol.2018.08.041.
- [24] Nishino, T. 2017. Cellulose fiber/nanofiber from natural sources including waste-based sources. In: Baillie C, Jayasinghe R, editors. *Green Compos. (2nd Ed.)*. Sawston: Woodhead Publishing. p. 19-38. DOI: 10.1016/B978-0-08-100783-9.00010-1.
- [25] Curvello, R.; Raghuwanshi, V. S.; Garnier, G. 2019. Engineering nanocellulose hydrogels for biomedical applications. *Adv. Colloid Interface Sci.* **267** 47–61. DOI: 10.1016/j.cis.2019.03.002.
- [26] Patel, Z. J.; Patel, M. C.; Chatrabhuji, P. M.; Patel, V. A.; Patel, D. R. 2020. Synthesis and characterization of cross-linked tri-polymers of poly acrylic acid as water thickening agents. *Rasayan J. Chem.* **13** (1) 333–338. DOI: 10.31788/RJC.2020.1315526.
- [27] Singh, S. K.; Kulkarni, S.; Kumar, V.; Vashistha, P. 2018. Sustainable utilization of deinking paper mill sludge for the manufacture of building bricks. *J. Cleaner Prod.* **204** 321–333. DOI: 10.1016/j.jclepro.2018.09.028.
- [28] Sutay Kocabaş, D.; Erkoç Akçelik, M.; Bahçegül, E.; Özbek, H. N. 2021. Bulgur bran as a biopolymer source: Production and characterization of nanocellulose-reinforced hemicellulose-based biodegradable films with decreased water solubility. *Ind. Crops Prod.* **171** 113847. DOI: 10.1016/j.indcrop.2021.113847.
- [29] Guo, J.; Guo, X.; Wang, S.; Yin, Y. 2016. Effects of ultrasonic treatment during acid hydrolysis on the yield, particle size and structure of cellulose nanocrystals. *Carbohydr. Polym.* **135** 248–255. DOI: 10.1016/j.carbpol.2015.08.068.
- [30] De France, K. J.; D'Emilio, E.; Cranston, E. D.; Geiger, T.; Nyström, G. 2020. Dual physically and chemically crosslinked regenerated cellulose-gelatin composite hydrogels towards art restoration. *Carbohydr. Polym.* **234** 115885. DOI: 10.1016/j.carbpol.2020.115885.