Genipin Cross-linked Boron Doped Hydrogels: Evaluation of Biological Activities

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ABSTRACT

Genipin cross-linked/boron doped starch/polyvinily alcohol (PVA) based hydrogel (SH-GNP-B) was synthesized as a new material having antimicrobial and antioxidant activity. The prepared hydrogel was characterized by X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Thermal Gravimetric Analysis (TGA) and Scanning Electron Microscope (SEM) methods and evaluated for in vitro antimicrobial activities against selected organisms by disc diffusion tests. The antioxidant activity of the prepared hydrogels was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assays. Swelling behavior of the hydrogel was also investigated.

The synthesized hydrogel was thermally stable and showed pH independent swelling tendency. SH-GNP-B hydrogel was found to have antimicrobial activities against Staphylococcus aureus, Streptecocus pyogenes, Pseudomonas aeruginosa and Bacillus subtilis microorganisms and antioxidant activities. The antimicrobial activity was improved more effectively by doping boric acid (B) to hydrogel. The hydrogel exhibited weak antioxidant activity when compared to butylated hydroxytoluene (BHT).

KEYWORDS: *Genipin, Cross-linking, Antimicrobial activity, Antioxidant activity, Hydrogel.*

INTRODUCTION

There is increased interest in the synthesis of natural polymer-based hydrogels instead of synthetic polymers due to increasing demands for sustainability and environmental friendliness. Natural polymer-based hydrogels are important

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due to their unique properties like biocompatibility[1], biodegradability[2] and non-toxicity[3, 4]. Hence, these properties make these hydrogels ideal materials for pharmaceutical, medical, biomembrane and cosmetic applications. Hydrogels based on collagen[5], cellulose[6, 7], chitosan[8, 9], gelatin[10] and hyaluronic acid[11] are usually synthesized for these purposes.

Starch type hydrogels are currently receiving a lot of attention for medical and pharmaceutical applications, as starch is abundant, non-toxic, biocompatible and biodegradable[12, 13, 14]. Due to its biodegradable nature, starch is difficult to accumulate in nature and its use in various fields can play an important role in protecting the environment[15]. Up to now there are various starch based hydrogels reported in the literature. Chen et al., synthesized starch-based high-performance adsorptive hydrogels by grafting polyacrylic acid onto starch and then crosslinked with N,N'-methylene-bisacrylamide using a novel effective pretreatment for removal of organic cationic dye contaminants[16]. Hossain et al., developed oxidised cellulose nanofibrils and starch-based hydrogels in the presence of cationic surfactants dodecyl- and cetyltrimethyl ammonium bromides to investigate the rheological properties of these hydrogels[17]. Abdollahi et al. performed another study on bioactive carboxymethyl starch-based hydrogels containing CuO nanoparticles to evaluate the wound healing potential of the hydrogels. Swelling, antibacterial activities, antioxidant activities, cytotoxicity, and *in vivo* wound healing were investigated for the hydrogels and the study revealed the potency of the hydrogels as a skin wound dressing in tissue regeneration[18]. Nezami et al. obtained pH-sensitive and magnetic starch-based nanocomposite hydrogel as a drug carrier by the graft copolymerization of itaconic acid onto starch in the presence of Fe_3O_4 nanoparticles. The study demonstrated the hydrogel to be a promising material for magnetically targeted drug delivery as well as a dress for wound healing[19].

As seen in many studies, starch is generally not used alone because it cannot form a stable hydrogel. Hence, starch is to be blended with other hydrophilic materials to overcome this problem. PVA is a synthetic water soluble polymer suitable for blending with starch [20-23].

Sin et al., studied the interaction of PVA and cassava starch by differential scanning calorimetry method and proposed that blending of these raw materials is compatible[24]. Yu et al., prepared Laponite crosslinked starch/PVA hydrogels by freezing/thawing process and investigate the influence of crosslinker content on structure and properties of hydrogels[25]. In another study, Altaf et al. fabricated hydrogel membranes by esterification of PVA with starch and glutaraldehyde as a crosslinker, and incorporated essential oils in hydrogel membranes in order to achieve optimized anti-bacterial activity and mechanical strength[26].

In order to enhance the mechanical properties and chemical stability of hydrogels, crosslinkers such as glutaraldehyde, sodium hexametaphosphate and epichlorohydrin are used to chemically crosslink the polymer network[27-30].

Genipin is a superior cross-linking agent for especially biomedical applications due to its higher biocompatibility, less toxicity, and well cross-linking ability when compared to other

cross-linking agents. Genipin is generated through hydrolysis of geniposide which belongs to class of iridoid compounds and is a component of the Gardenia fruit[31-34]. Genipin has certain pharmacological properties such as antitumor[32], antioxidative[35], anti-diabetic[36], anti-inflammatory[37] and anti-infection[38]. There are some studies comparing genipin with commonly used crosslinkers for biological tissue fixation[39, 40] and also cross-linking of chitosan/gelatin[41, 42], chitosan/silica[43] and collagen[44] in which it has been revealed that genipin showed lower cytotoxicity and higher biocompatibility.

Some inorganic materials such as boron and boron compounds exhibiting high affinity to hydroxyl groups are suitable to form stable hydrogels with polyhydroxy polymers and further enhance the mechanical properties of the hydrogels [23, 45-48].

The evaluation of antimicrobial activities of biopolymer-based hydrogels has attracted more attention in recent years[49-52]. It is well known that bacteria, viruses, parasites, or fungi, plays a very important role in wound healing by affecting risk of infection, healing time, recovery time of patient, and costs to the health service[53]. Antioxidants can be used in order to improve the wound healing process and antioxidants are studied as a therapeutic strategy to treat chronic wounds. Infectious diseases caused by bacteria and viruses have become major public health threats. Hydrogels can be considered as effective antimicrobial formulations. It has been showed that hydrogels provided a moist environment around the wound site in order to improve the healing process by protecting damaged skin from cellular dehydration and angiogenesis[54].

In this study, we aimed to prepare boron doped cross-linked starch/PVA based hydrogels through a facile solution casting technique and investigate their potential as antimicrobial and antioxidant hydrogels. The key interest is to use a naturally occurring compound genipin as a superior cross-linker for the first time in starch/PVA based hydrogels due to the lower acute toxicity compared to many other commonly used synthetic cross-linking agents. The synthesized complexes were characterized by XRD, FTIR, TGA and SEM. Swelling behaviors of the hydrogels were also investigated. The *in vitro* antimicrobial activities of hydrogels were evaluated against selected organisms by disc diffusion tests. Antioxidant properties of genipin cross-linked and boron doped starch based hydrogels was confirmed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals.

MATERIALS AND METHOD

Materials

Genipin (GNP) (Aldrich), potato starch (Fluka), polyvinyl alcohol (PVA) (Fluka), boric acid (B) (Merck), citric acid (Merck), disodium hydrogen phosphate (Merck), ethanol (Fluka) were used at analytical reagent grade and were not further purified. For pH adjustments, sodium hydroxide (Fluka) and hydrochloric acid (Riedel-de Haën) solutions were used.

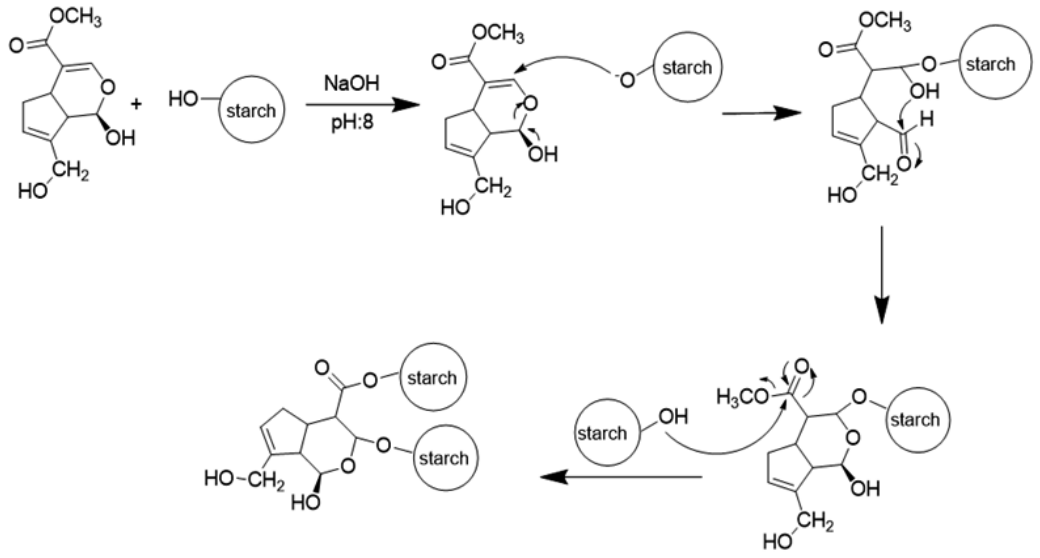
Heidolph MR standard magnetic stirrer, Polyscience 9006 refrigerating-heating circulator, Denver 215 pH meter were used for the preparation of the hydrogels. Millipore Milli-Q ultra-pure water system was used during the experiments.

Preparation and characterization of the hydrogels

Gelatinized form of the potato starch was used in the synthesis. For this purpose, the starch granules were dissolved in water and heated up to 90°C. Then 25 mL of 5% (w/v) gelatinized starch solution and 25 mL 10%

(w/v) aqueous PVA solution were mixed together at 1250 rpm and 70°C for one hour. This mixture was marked as SH. GNP or B or GNP-B blends were separately added to these mixtures. Before GNP was added the pH was altered to 8. Schematic illustration of cross-linking of starch with genipin was given in Scheme 1. The mixtures were refluxed at the same temperature for two hours and were poured into petri dishes, then dried at 25°C[28, 55]. The transparent hydrogels were named as SH-GNP, SH-B and SH-GNP-B, respectively and characterized with different techniques.

XRD patterns of the samples were obtained with Rigaku - Rint 2200/PC (Ultima 3) X-Ray diffractometer. Monochromatic Cu Kα radiation beam was used as X-ray source with 0.4 degree/minute scanning rate in the 2θ range of 2-10°. The FTIR analyses were realized by Perkin–Elmer Spectrum BX-II FTIR spectrophotometer in the range of 4000 and 400 cm^{-1}. TGA analysis of synthesized hydrogels and raw materials were conducted with Perkin Elmer Diamond TG/DTA Analyzer in porcelain pans under nitrogen atmosphere with 10°C/min heating rate. The surfaces of the samples were investigated with a FEI Quanta FEG 250 emission scanning electron microscope at 5 kV or 10 kV after coating with a thin gold layer.



Scheme 1 Hypothetically proposed reaction between starch and genipin

Swelling tests of hydrogels

Citric acid-disodium hydrogen phosphate buffer solutions (pH between 2-8) were used in the swelling tests. The equilibrium swelling times at water and these buffer solutions at different pHs were determined by immersing samples in these solutions and removing them at 5 min time intervals at room temperature. Until the equilibrium times the hydrogels were dried with filter paper, weighed and then taken back to the same solutions. In the determination of pH dependence of swelling action, the dry hydrogels were swelled up to equilibrium times in water and then were immersed in the buffer solutions having different pHs, respectively and stand up in each buffer solution for determined equilibrium times.

Evaluation of antimicrobial activity

Antimicrobial activity of the samples was evaluated according to guidelines of NCCLS (National Committee for Clinical Laboratory Standards) on disc diffusion susceptibility tests[56]. The tests were conducted as described in a previous study[28].

DPPH radical scavenging activity of hydrogels

Radical scavenging capacity of the SH-B, SH-GNP and SH-GNP-B was determined and compared to that of butylated hydroxytoluene (BHT) by using the DPPH radical scavenging method[57]. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability.

Briefly, 0.1 mM solution of DPPH radical was prepared in methanol and 1 mL of this solution was added to 1 mL of SH-B, SH-GNP and SH-GNP-B solution in dimethyl sulfoxide (DMSO) and positive control BHT solution in methanol at different concentrations (50-150 µg/mL). These solutions were vortexed thoroughly and incubated in dark for 30 min. The absorbance of the mixtures was measured at 517 nm using a UV-vis spectrophotometer.

The DPPH free radical was measured using equation 1:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

$A_{control}$ = absorbance of DPPH alone

A_{sample} = absorbance of DPPH along with different concentrations of hydrogels

RESULTS AND DISCUSSION

XRD results

X-ray diffraction patterns of the hydrogels (Figure 1) and their raw materials were investigated. Due to its amylopectin component, starch is a B-type semi-crystalline and shows typical peaks at 17.0° and 22.0° [58]. PVA yields peaks at 2θ =19.0° and 40.0°. The most intense of them is observed at 19.0° [55]. The single crystal peaks of boric acid are at 14.5° and 28.0° [59]. The XRD peaks of SH-B hydrogel are observed at 2θ =17.3° and 19.6°. SH-GNP hydrogel shows peaks at 2θ =17.4°, 19.9°, 21.6°, 23.9° and 26.8° where the peaks of SH-

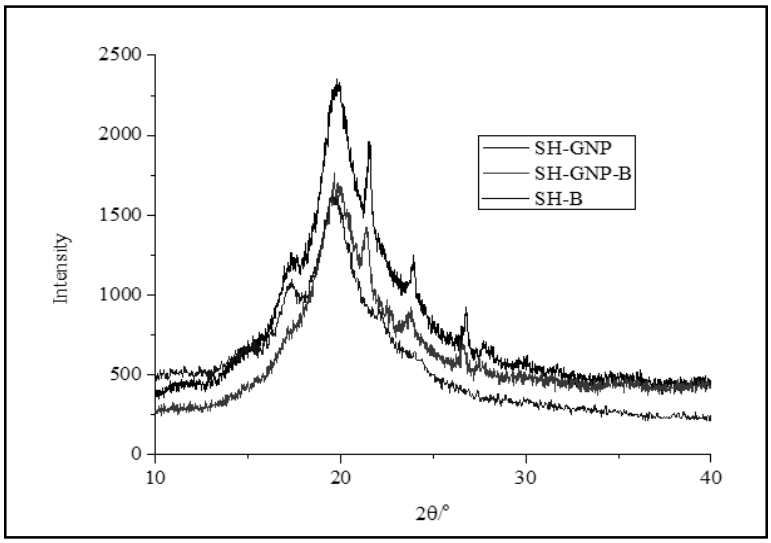


Figure 1. XRD patterns for the hydrogels

GNP-B are seen at 2θ =19.9°, 21.3°, 23.9° and 26.5°. According to the X-ray diffraction results of the samples, all of the hydrogels are amorphous compounds with a strong peak around 2θ = 20.0° and their crystallinity are mostly stem from PVA[55]. The results of XRD analysis showed that in synthesized hydrogels the morphology of the starch completely changed after activation with PVA. It was found that the intensities of all peaks were increased by cross-linking, and the crystalline peak of PVA at 2θ = 40.0° exactly disappeared. In SH-GNP-B hydrogel, peak intensities decreased compared to SH-GNP. It could be stated that the hydrogel containing B was less crystalline. The reason why single crystal B peaks couldn't be seen in SH-B and SH-GNP-B was that there was no or little B left in the micro particles of hydrogels[60]. Raw genipin shows peaks at approximately 2θ =12.0°, 19.0°, 20.0°, 22.5° 24.0° and 26.5°[61]. Some of the peaks of genipin were overlapped with the peaks of starch or PVA however the peaks observed approximately at around 2θ = 24° and 27° in SH-GNP and SH-GNP-B hydrogels were due to the genipin in the structure.

FTIR results

In the FTIR spectra of starch (Figure 2) a broad band observed at around 3400 cm^{-1} belongs to hydroxyl groups. Aliphatic C-H stretchings of = CH- in the ring were seen at 2927 cm^{-1}. The band about the water adsorbed by starch was located at 1651 cm^{-1} and the band which was probably caused by C-H bending vibrations is located at 1372 cm^{-1}. Characteristic absorption bands of starch originating from C-O and C-C stretchings were seen between 1016-1159 cm^{-1}. Depending on the high hydrophilic forces, intermolecular and intermolecular hydrogen bonds were expected to occur between the PVA chains. For this reason, a wide band about hydrogen bonded -OH groups were observed at 3428 cm^{-1} in the spectrum of PVA. Symmetric and asymmetric C-H stretching bands were also noticed at 2924 and 2857 cm^{-1}. C-H bending vibrations of aliphatic groups were observed at 1443 cm^{-1}. The band at about 1639 cm^{-1} belongs to carbonyl groups of residual acetate which remains during the PVA formation from poly (vinyl acetate) by hydrolysis [62]. C-O stretching of the secondary alcohol was seen at 1090 cm^{-1}.

FTIR spectra of hydrogels were given in Figure 3. In the spectrum of SH-GNP, the band observed between 3450-3000 cm^{-1} and in addition 2878 and 1342 cm^{-1} bands showed the hydroxyl groups having hydrogen bonds and the hydrocarbon chromophore, respectively. C-O stretching bands were observed in the range of 1150-800 cm^{-1}. The stretching vibration of C=O bond was observed at 1709 cm^{-1}. When the FTIR spectra of SH-B and SH-GNP-B hydrogels were compared with the spectrum of SH-GNP, it was seen that there were significant changes between the shapes, intensities and frequencies of the bands.

TGA results

The TG curves of starch, PVA and hydrogels were shown in Figure 4. By examining the thermograms, it was found that starch has two; PVA and hydrogels have three thermal degradation steps.

The shoulder observed in the second degradation peak of starch may be caused by amylose and amylopectin fractions of starch having different rate of degradations where amylose in linear structure was more rapidly degraded[21].

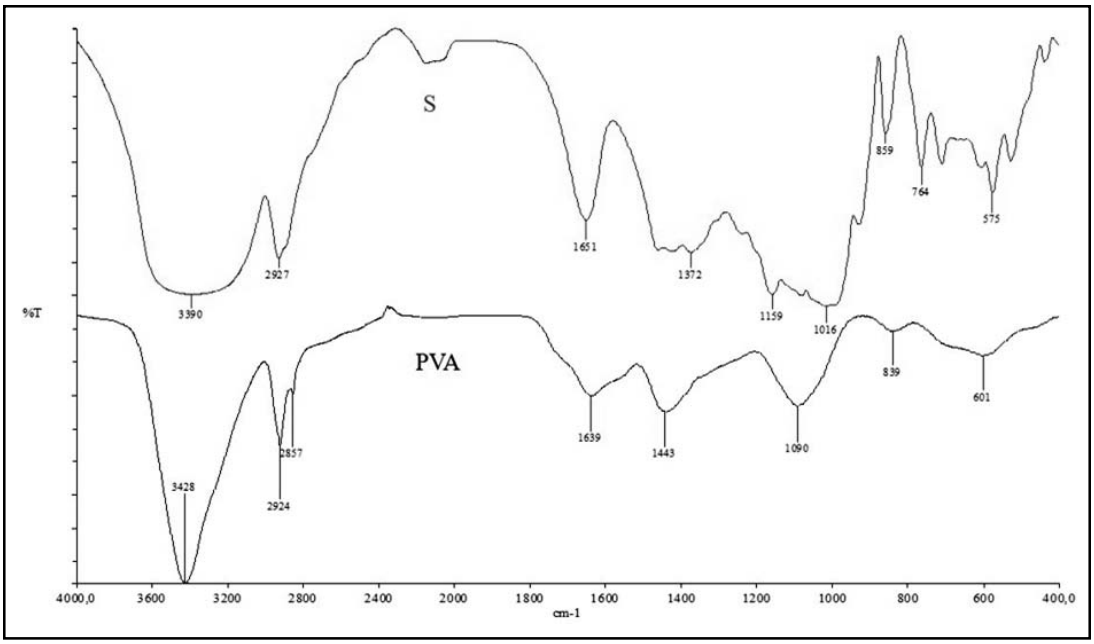


Figure 2. FTIR spectrum of PVA and starch

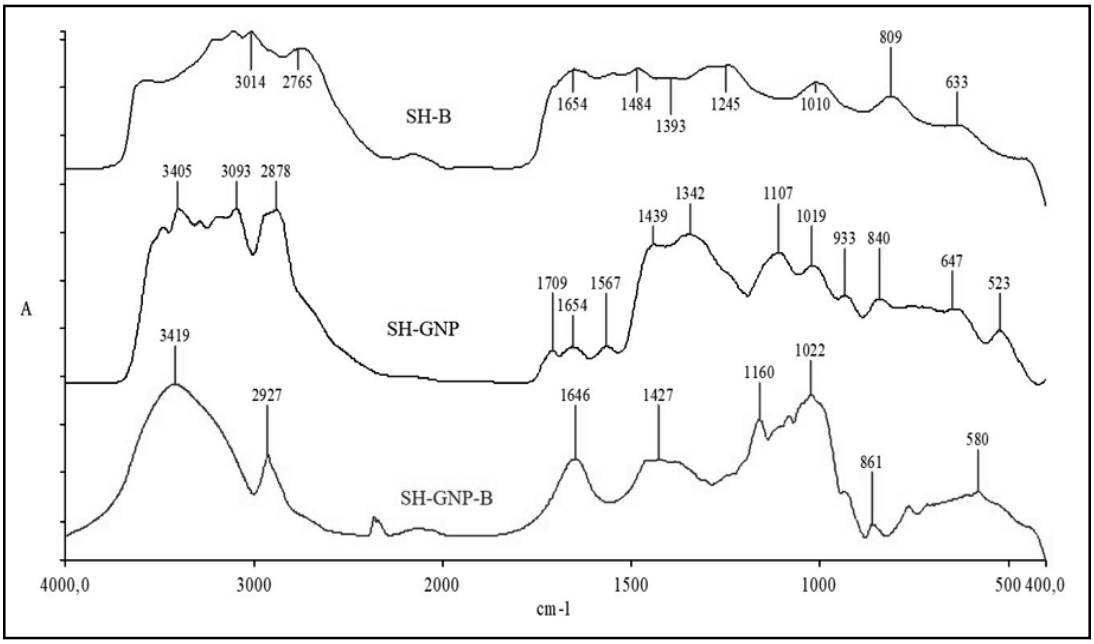


Figure 3. FTIR spectrum of the hydrogels

The weight loss corresponds to the evaporation of the water adsorbed in the samples in the first step at 33-114°C. Other steps were associated with thermal degradation of hydrogels. The major weight loss was realized in the hydrogels in the second step at around 300°C with average of 67% which was ascribed to the decomposition of starch and elimination of bound water from PVA. The third step decomposition was observed at approximately 500°C with an average weight loss of 13% mainly due to the complete pyrolisation of PVA[25].

Synthesized hydrogels were observed to be more thermally stable than their raw materials by the effect of cross-linking with GNP. The use of B in SH-GNP-B hydrogel further improved the stability compared to SH-GNP.

SEM results

The SEM images of the raw materials and the hydrogels at x300 magnification were shown in Figures 5 and 6. When the surfaces of the raw materials were examined, the potato starch exhibited smooth oval-shaped spheres (Figure 5a), PVA had a rough surface (Figure 5b) and

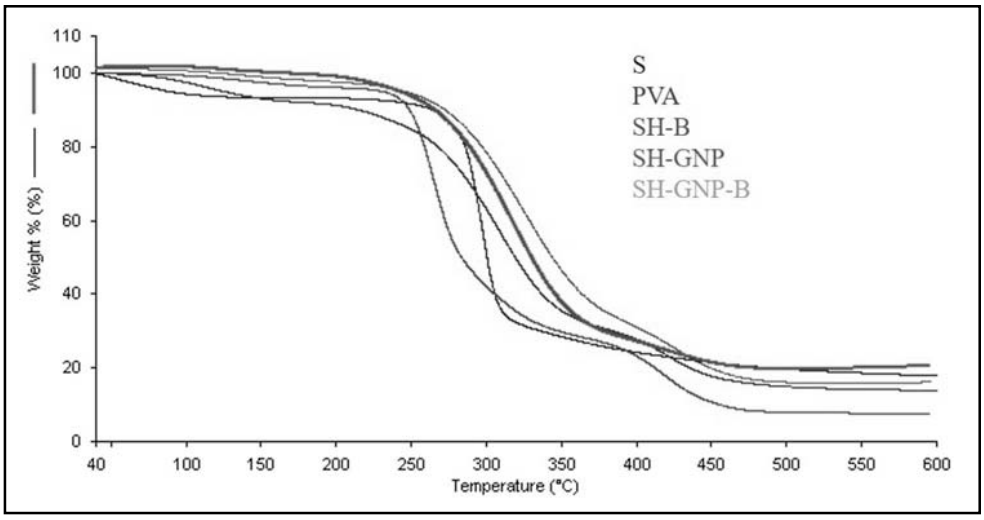


Figure 4. TG curves of starch, PVA and hydrogels

B was noticed as big spheres having a diameter of ~ 450 μm (Figure 5c). According to SEM images after interaction with PVA the morphological structure of starch was altered by the hydrogel formation as it was supported by XRD analysis results. SH-B had a non-uniform irregular structure (Figure 6a), but as a result of cross-linking with genipin the structure becomes regular and the particles were seen in the form of bubbles (Figure 6b). In SH-GNP-B, the surface was completely smooth (Figure 6c). The irregular and non-constricted view observed in SH-B shows that B is not as effective as genipin alone in the cross-linking of starch and PVA.

The results about determination of the equilibrium swelling times were given in Figure 7. The equilibrium times at water and at each pH solution were obtained to be ~30, 75 and 50 min for SH-B, SH-GNP and SH-GNP-B, respectively. For boron based complexes shorter equilibrium times were observed. Swelling values ranging from 200 to 400% were found for all hydrogels. The cross-linked starch granules reach a compact structure than natural starch, so absorb less water and therefore the swelling ratio decreases. In this sense, cross-linking of SH-GNP-B is higher than that of SH-GNP.

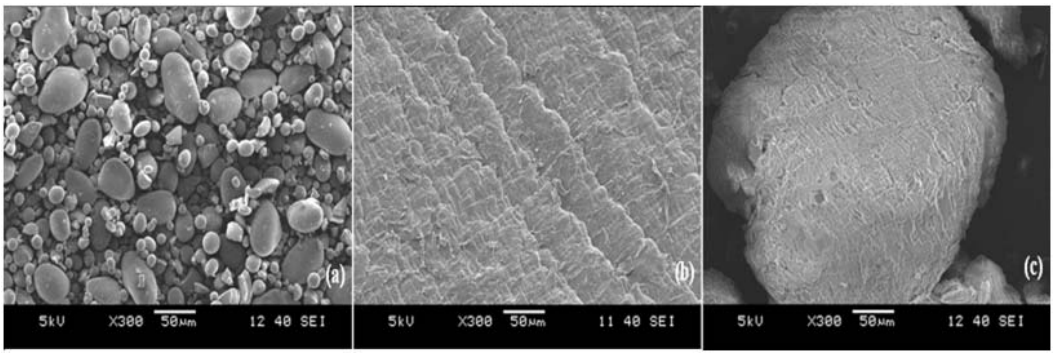


Figure 5. SEM images of (a) S, (b) PVA, (c) B

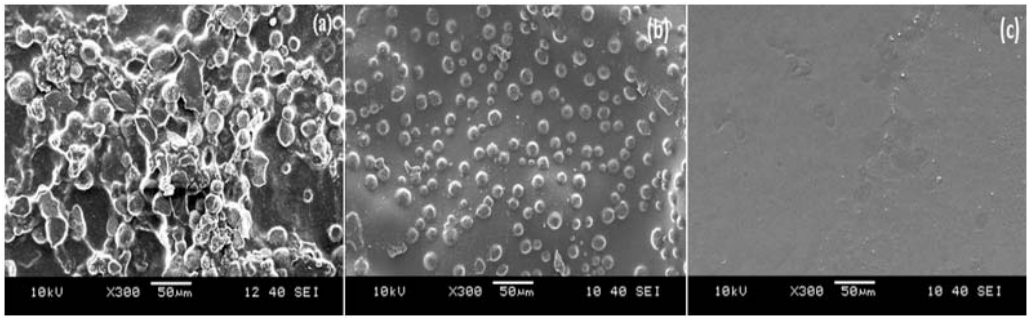


Figure 6. SEM images of (a) SH-B, (b) SH-GNP, (c) SH-GNP-B

The swelling characteristics of hydrogels at different pH were examined at pH 2-8. Because the maximum swelling time was determined as 75 min for one of the hydrogel (SH-GNP), all hydrogels were stored in water and then in all solutions at specific pHs for two hours during the investigation of pH dependent swelling characteristics. The swelling percentages of SH-B, SH-GNP and SH-GNP-B in water were obtained as 350%, 300% and 265%, respectively. The swelling characteristics does not depends on pH as starch and PVA do not contain any ionizable groups[63].

In vitro antimicrobial activity test results of hydrogels

Antimicrobial activity results of the hydrogels against Gram-negative and Gram-positive bacteria by the inhibition zone method were shown in Table 1. Inhibition zone formations confirmed that SH-GNP-B has the antimicrobial activity, while SH-GNP and SH-B did not have any antimicrobial activities against tested six microorganisms. None of the hydrogels showed antifungal activity against *Candida albicans*. It was found that SH-GNP and SH-B was not able to inhibit bacterial growth. However, SH-GNP with B was

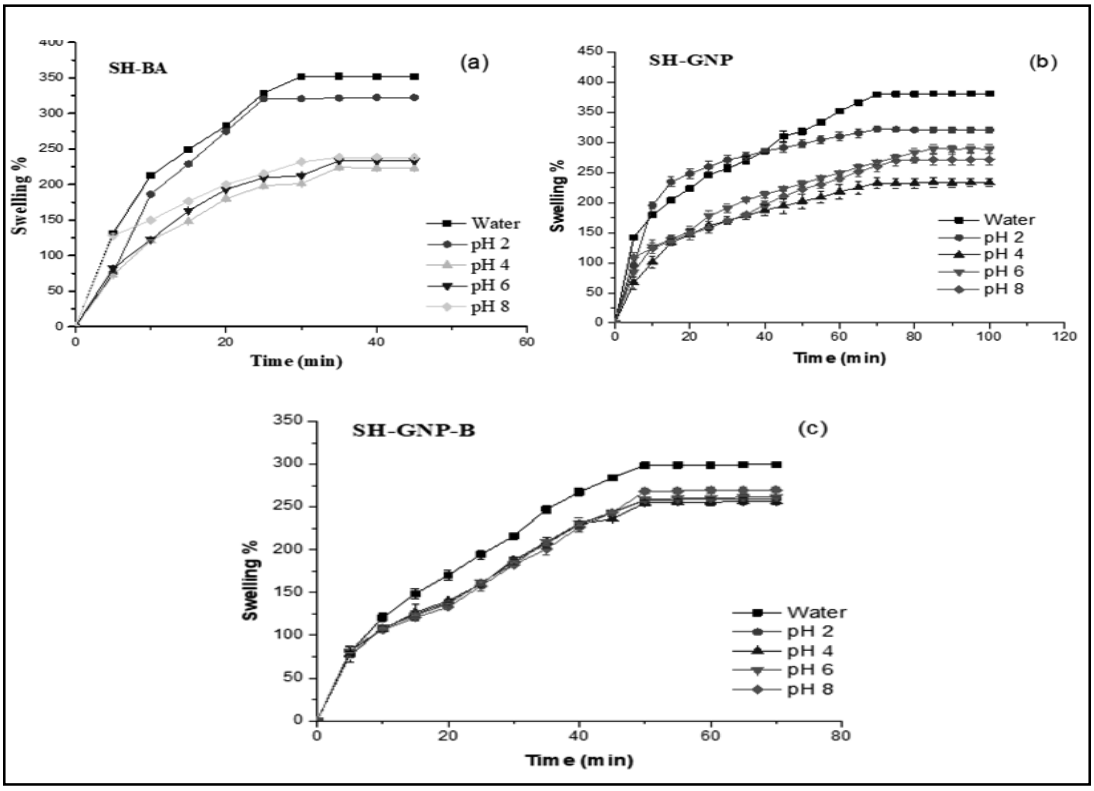


Figure 7. Swelling times for (a) SH-B, (b) SH-GNP, (c) SH-GNP-B

able to inhibit the bacterial growth to some degree with 10-mm inhibition against *S. aureus, S. pyogenes, P. aeruginosa* and *B. subtilis* microrganisms. Therefore, the presence of GNP and B enhanced the antimicrobial ability of hydrogel significantly. These results are comparable with standard antibiotics, and denoting SH-GNP-B showed moderate antimicrobial activity. The antibacterial activity of SH-GNP-B is more evident on Gram-positive bacteria. None of the tested hydrogels have antibacterial activity against *E. coli.* The synergistic antimicrobial activity of hydrogel could be obtained by

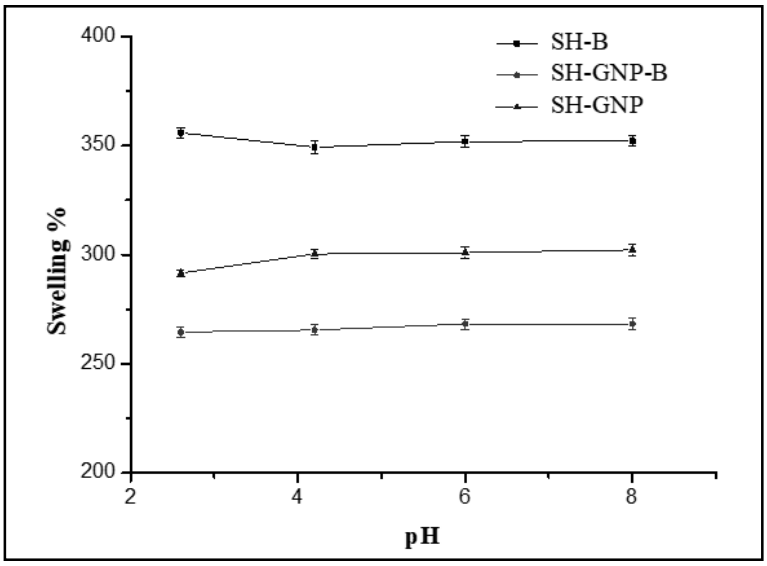


Figure 8. Swelling ratios of hydrogels as a function of pH

TABLE 1. Antimicrobial activity results for SH-GNP-B, SH-GNP, SH-B and standard antibiotics

Tested organisms	**Zones of inhibition (mm)**							
	Hydrogels (12 mg/9 mm disc)			**Standard antibiotics (µg/6 mm paper disc)**				
	SH-GNP-B	**SH-B**	**SH-GNP**	**OFX5**	**NET30**	**ER15**	**AMC30**	**AFB30**
Escherichia coli ATCC 25922	-	-	-	28	26	14	20	NT
Staphylococcus aureus ATCC 25923	10	-	-	20	18	18	>30	NT
Streptecocus pyogenes ATCC 19615	10	-	-	22	>30	>30	28	NTA
Pseudomonas aeruginosa ATCC 27853	10	-	-	16	22	R	R	NT
Bacillus subtilis ATCC 11774	10	-	-	24	>30	>30	24	NT
Candida albicans ATCC 10231	-	-	-	NT	NT	NT	NT	13

Note: Ofloxacin (OFX); Netilmycin (NET); Erythromycin (ER); Amoxycillin /clavulanic acid (AMC); Amphotericin (AFB); Not tested (NT); Resistance (R); Not active (-)

combining B and SH-GNP. Yoon et al., found that *B. subtilis* was more sensitive than *E. coli* to the nanoparticles[64]. They concluded that the lower sensitivities of *E. coli* as compared to *B. subtilis* was due to the outer membrane of Gram-negative bacteria such as *E. coli*, which was containing lipopolysaccharide molecules resulting in the effective resistive barrier against nanoparticles. Hydrogel prepared in this work may be used as wound and burn dressings.

Antioxidant activity

The antioxidant activities of the genipin cross-linked boron doped hydrogels were investigated with that of butylated hydroxytoluene as positive control. In view of this, the antioxidant activity of hydrogels was *conducted* by DPPH radical-scavenging assays. In the DPPH assay, the H-transfer reactions were monitored by UV-vis spectroscopy, whereby the decay of the DPPH visible band (max at 517 nm in methanol) was recorded. This reflects the conversion of the DPPH radical into the corresponding colorless hydrazine (DPPH-H), by the antioxidant. SH-GNP-B exhibited a similar radical scavenging activity with that of SH-GNP at varying concentrations tested (50, 75, 100, 125, 150 µg/mL). SH-B showed less DPPH scavenging activity than these two samples at the same concentrations. Positive control BHT showed better DPPH radical scavenging activity than three hydrogels samples at all concentrations.

As shown in Figure 9, SH-GNP-B and SH-GNP showed an inhibitory effect of 47.66% and 48.57% on the DPPH radical at 150 (µg/mL). There is no statistically significant difference between SH-GNP-B and SH-GNP. SH-B showed 36.00% radical quenching activity at 150 (µg/mL) concentrations. The positive control BHT at the concentration of 150 (µg/mL) radical quenching activity is 91.00%. Statistically significant ($p<0.05$) correlation was found among the studied hydrogels and the control BHT.

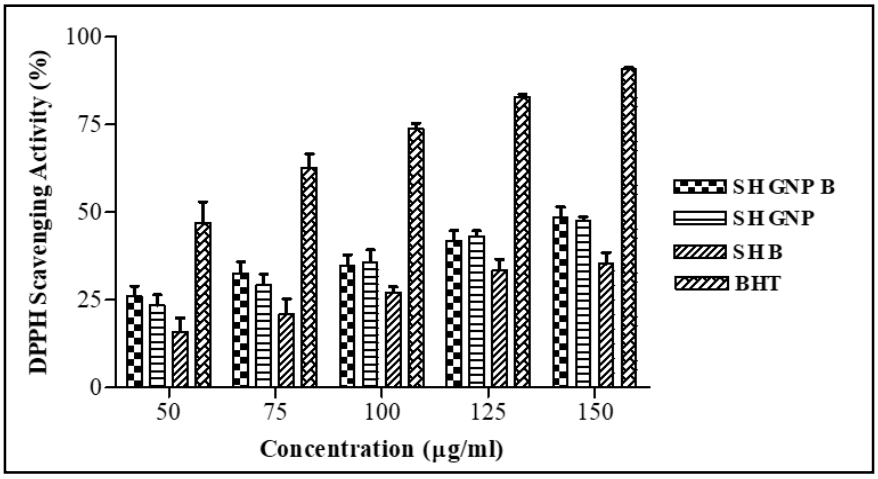


Figure 9. Antioxidant activity (%) of SH-B, SH-GNP, SH-GNP-B and BHT. Each value means ± SD of three experiments.

CONCLUSIONS

In this study, a new genipin cross-linked and boron doped starch/PVA based hydrogel, was synthesized and characterized as a potential material to be used in biomedical applications.

This hydrogel showed moderate antimicrobial activity against *S. aureus, S. pyogenes, P. aeruginosa* and *B. subtilis*. The hydrogel exhibited weak antioxidant activity when compared to BHT.

Therefore, the results demonstrated that the prepared SH-GNP-B hydrogel could have the potential to be used in biomedical applications due to its antimicrobial characteristics.

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REFERENCES

1. N.S. El-Sayed, H. Awad, G.M. El-Sayed, Z.A Nagieb and S. Kamel, *Polym. Bull.* 77(12) (2020): 6333-6347.
2. R.R. Mohamed, M.H.A. Elella and M.W. Sabaa, *Int. J. Biol. Macromol.* 98 (2017): 302-313.
3. S. Sudarsan, D.S. Franklin, M. Sakthivel and S. Guhanathan, *Carbohydr. Polym.* 148 (2016): 206-215.
4. H. Liu, C. Wang, C. Li, Y. Qin, Z. Wang, F. Yang, Z. Li and J. Wang, *RSC Adv.* 8(14) (2018): 7533-7549.
5. Y. Gao, W. Kong, B. Li, Y. Ni, T. Yuan, L. Guo, H. Lin, H. Fan and X. Zhang, *Colloids Surf B Biointerfaces* 167 (2018): 448-456.
6. D. Kundu and T. Banerjee, *Heliyon* 6(1) (2020): e03027.
7. J. Nath and S.K. Dolui, *Appl. Clay Sci.* 155 (2018): 65-73.
8. Z. Shariatinia and A.M. Jalali, *Int. J. Biol. Macromol*. 115 (2018): 194-220.
9. S.W. Huang, F.C. Yeh, Y.R. Ji, Y.F. Su, Y.S. Su, M.H. Chiang, S.C. Tzeng, C.Y. Fu, A. Cheng, Y.C. Wang and Y.T. Lee, *Drug Deliv. Transl. Res.* (2021): 1-13.
10. J. Nath, A. Chowdhury, I. Ali and S.K. Dolui, *J. Appl. Polym. Sci.* 136(22) (2019): 47596.
11. E. Larrañeta, M. Henry, N.J. Irwin, J. Trotter, A.A. Perminova and R.F. Donnelly, *Carbohydr. Polym.* 181 (2018): 1194-1205.
12. N.L. Vanier, J.P. Oliveira, G.P. Bruni, S.L.M.E. Halal, F.A. Villanova, E.R. Zavareze, A.R.G. Dias and P.Z. Bassinello, *J. Sci. Food Agric.* 99 (2019): 1207-1214.
13. F. Tao, C. Shi and Y. Cui, *J. Sci. Food Agric.* 98 (2018): 5470-5478.
14. S. Zeng, J. Zhang, G. Zu and J. Huang, *Carbohydr. Polym.* 267 (2021): 118198.
15. I. Gonçalves, D. Hernández, C. Cruz, J. Lopes, A. Barra, C. Nunes, J.A.L. Silva, P. Ferreira and M.A. Coimbra, *Carbohydr.Polym.* 254 (2021): 117236.
16. L. Chen, Y. Zhu, Y. Cui, R. Dai, Z. Shan and H. Chen, *Chem. Eng. J.* 405 (2021): 126953.
17. K.M.Z. Hossain, V. Calabrese, M. A. da Silva, S.J. Bryant, J. Schmitt, J.L Scott and K.J. Edler, *Carbohydr. Polym.* 233 (2020): 115816.
18. Z. Abdollahi, E.N. Zare, F. Salimi, I. Goudarzi, F.R. Tay and P. Makvandi, *Int. J. Mol. Sci.* 22(5) (2021): 2531.
19. S. Nezami, M. Sadeghi and H. Mohajerani, *Polym. Degrad. Stab*. 179 (2020): 109255.

20. R. Jayasekara, I. Harding, I. Bowater, G.B.Y. Christie and G.T. Lonergan, *J. Polym. Environ.* 11 (2003): 49-56.

21. B. Sreedhar, M. Sairam, D.K. Chattopadhyay, P.S. Rathnam and D.M. Rao, *J. Appl. Polym. Sci.* 96 (2005): 1313-1322.

22. H. Chattopadhyay, A.K. De and S. Datta, *Int. Sch. Res. Notices* 2015 (2015): 1-8.

23. R. Li, J. Lin, Y. Fang, C. Yu, J. Zhang, Y. Xue, Z. Liu, J. Zhang, C. Tang and Y. Huang, *Ceram. Int.* 44 (2018): 22439-22444.

24. L.T. Sin, W.A.W.A. Rahman, A.R. Rahmat and M.I. Khan, *Carbohydr. Polym*. 79 (1) (2010) 224-226.

25. C. Yu, X. Tang, S. Liu, Y. Yang, X. Shen and C. Gao, *Int. J. Biol. Macromol.* 117 (2018): 1-6.

26. F. Altaf, M.B.K. Niazi, Z. Jahan, T. Ahmad, M.A. Akram, A. Safdar, M.S. Butt, T. Noor and F. Sher, *J. Polym. Environ.* 29(1) (2021): 156-174.

27. K. Das, D. Ray, N.R. Bandyopadhyay, A. Gupta, S. Sengupta, S. Sahoo, A. Mohanty and M. Misra, *Ind. Eng. Chem. Res.* 49 (2010): 2176-2185.

28. E.A. Bursali, S. Coskun, M. Kizil and M. Yurdakoc, *Carbohydr. Polym.* 83 (2011): 1377–1383.

29. Z. Liu, Y. Dong, H. Men, M. Jiang, J. Tong and J. Zhou, Carbohydr. Polym. 89 (2012): 473-477.

30. J. Maitra and V.K. Shukla, *Am. J. Polym. Sci.* 4 (2014): 25-31.

31. B. Manickam, R. Sreedharan and M. Elumalai, *Curr. Drug Deliv.* 11 (2014) 139-145.

32. Y.S. Shin, M. Cho, G.H. Park, H. Cho and H. Kang, *J. Bacteriol. Virol.* 46 (2016): 10-113.

33. S. Dimida, A. Barca, N. Cancelli, V. De Benedictis, M.G. Raucci and C. Demitri, *Int. J. Polym. Sci.* 2017 (2017): 1-8.

34. M.A.K. Zanjani, M.R. Ehsani, B.G. Tarzi and A. Sharifan, *Iran. J. Pharm. Res.* 17 (2018): 753-766.

35. Y. Koriyama, K. Chiba, M. Yamazaki, H. Suzuki, K. Muramoto and S. Kato, *J. Neurochem.* 115 (2010): 79-9.

36. H.B. Vaidya, A.A. Ahmed, R.K. Goyal and S.K. Cheema, *J. Pharm. Pharm. Sci.* 16 (2013): 530-540.

37. H.J. Koo, Y.S. Song, H.J. Kim, Y.H. Lee, S.M. Hong, S.J. Kim, B.C. Kim, C. Jin, C.J. Lim and E.H. Park, *Eur. J. Pharmacol.* 495 (2004): 201-208.

38. C.H. Chang, J.B. Wu, J.S. Yang, Y.J. Lai, C.H. Su, C.C. Lu and Y.M. Hsu, *J. Food Sci.* 82 (2017): 3021-3028.

39. H.W. Sung, R.N. Huang, L.L. Huang and C.C Tsai, *J. Biomater. Sci. Polym. Ed.* 10(1) (1999): 63-78.

40. C.C Tsai, R.N. Huang, H.W Sung and H.C Liang, *J. Biomed. Mater. Res.* 52(1) (2000): 58-65.

41. L. Cui, J. Jia, Y. Guo, Y. Liu and P. Zhu, *Carbohydr. Polym.* 99 (2014): 31-38.

42. V. Chiono, E. Pulieri, G. Vozzi, G. Ciardelli, A. Ahluwalia and P. Giusti, *J. Mater. Sci.: Mater. Med.* 19(2) (2008): 889-898.

43. Y. Liu, Z. Cai, L. Sheng, M. Ma, Q. Xu and Y. Jin, *Carbohydr. Polym.* 215 (2019): 348-357.

44. K. Adamiak and A. Sionkowska, *Int. J. Biol. Macromol.* 161 (2020): 550-560.

45. C. Song, Y. Lv, K. Qian, Y. Chen and X. Qian, *J. Polym. Res.* 26 (2019): 52.

46. S. Gao, J. Guo, L. Wu and S. Wang, *Carbohydr. Polym.* 73 (2008): 498-505.

47. Z.Q. Duan, Y.T. Liu, X.M. Xie and X.Y. Ye, *Chinese Chem. Lett.* 24 (2013): 17-19.

48. Y.P. Wu, Y. Xue, S. Qin, D. Liu and W.W. Lei, *J. Mater. Chem. C* 6 (2018): 1363-1369.

49. D.N. Iqbal, S. Shafiq, S.M. Khan, S.M. Ibrahim, S.A. Abubshait, A. Nazir, M. Abbas and M. Iqbal, *Int. J. Biol. Macromol.* 164 (2020): 499-509.

50. R. Eivazzadeh-Keihan, F. Radinekiyan, H.A.M. Aliabadi, S. Sukhtezari, B. Tahmasebi, A. Maleki and H. Madanchi, *Sci. Rep.* 11(1) (2021): 1-13.

51. S. Heedy, M.E. Marshall, J.J. Pineda, E. Pearlman

and A.F. Yee, *ACS Appl. Bio Mater.* 3(11) (2020): 8040-8048.

52. S. Sivakanthan, S. Rajendran, A. Gamage, T. Madhujith and S. Mani, *Food Res. Int.* 136 (2020): 109327.
53. S. Finnegan and S.L. Percival, *Adv. Wound Care* 4(7) (2015): 398-406.
54. C. Ghobril and M.W. Grinstaff, *Chemi. Soc. Rev.* 44 (2015): 1820-1835.
55. K. Pal, A.K. Banthia and D.K. Majumdar, *Mater. Lett.* 62 (2008): 215-218.
56. C.L. Clark, M.R.J. Jacobs and P.C. Appelbaum, *J. Clin. Microbiol.* 36 (1998): 3579–3584.
57. V. Roginsky and E.A. Lissi, *Food Chem.* 92 (2005): 235-254.
58. P. Cairns, L. Sun, V.J. Morris and S.G. Ring, *J. Cereal Sci.* 21 (1995): 37-47.
59. M. S. Bingöl and M. Çopur, *J. CO_2 Util.* 29 (2019): 29-35.
60. B. Li, L. Wang, D. Li, Y.L. Chiu, Z. Zhang and J. Shi, *J. Food Eng.* 92 (2009): 255–260.
61. S.L. Trevor, M.F. Butler, S. Adams, P.R. Laity, J.C. Burley and R.E. Cameron, *Cryst. Growth Des.* 8 (2008): 1748–1753.
62. R. Jayasekara, I. Harding, I. Bowater, G.B.Y. Christie and G.T. Lonergan, *Polym. Test.* 23 (2004): 17–27.
63. B. Kim and N.A. Peppas, *Macromolecules* 35 (2002): 9545–9550.
64. K.Y. Yoon, J.H. Byeon, J.H. Park and J. Hwang, *Sci. Total Environ.* 373 (2007): 572–575.

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