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## Preparation of Gelatin-Chitosan-Honey-Based Hydrogel for Potential Active Material of Wound Care Dressing Application

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**Abstract.** Hydrogel composed of gelatin, chitosan, and honey can be one of the right candidates for wound dressing application which provides both an antibacterial and a proper wound drainage management property to promote faster healing. Herein, preparation of hydrogel has been conducted by the physical blending of the solution of gelatin, chitosan, and honey at 40°C. Then, the mixture was cast to form hydrogel films by each 2-4 mm thickness and followed by drying at 37° C for 24 hours. The resulted hydrogels were characterized to confirm its potential as wound care dressing by measuring gel fraction, swelling index, and antibacterial activity. The gel fraction of the hydrogel composed of 10 and 20 grams of gelatin (each with 0.5 grams of chitosan and 20 grams of honey) was respectively 68.86 % and 65.68%. The hydrogel, composed of 20 g of gelatin and 7.5 g of chitosan, has shown the highest water retention capacity (swelling index) by 400 %. However, the presence of honey has slightly lowered both the gel fraction and swelling index of the resulted hydrogel. The antibacterial property of the resulting hydrogel in this work corresponds to the chitosan instead of honey and gelatin.

### Introduction

Hydrogels are soft moist or jelly-like materials as a result of their three-dimensional network, developed by many cross-linked polymers, which enable to retain a high quantity of adsorbed water[1]. Polymers are cross-linked through their functional groups either by chemical bonds or physical interactions such as hydrogen bond, ionic interaction, and hydrophobic forces[2]. Both synthetic polymers and natural polymers have been used to develop various hydrogels. Many hydrogels show their unique features in response to both chemical and physical stimuli[3]. Depending on its polymer structural, hydrogels also can resemble an extracellular matrix. These outstanding chemical, physical, and biological properties make their extensive applications as biomedical materials, for instance, tissue engineering, drug delivery system, and wound care dressing[1,4].

Hydrogel-based materials have been used as primary dressing materials, especially for managing moist wound[5]. Hydrogel-based materials absorb a high amount of wound moisture to form a viscous gel that allows the excess fluid removal[5]. In another side, hydrogels also capable of keeping the humid environment around the wound that important to enhance re-epithelialisation[5]. Their soft and non-adhesive properties give an advantage by preventing wound damage during their removing[1]. Moreover, the 3D network structure of hydrogel can facilitate tissue growth, macrophages activation, interleukin production to stimulate the cell adhesion and proliferation to complete wound healing process[6].

The ideal hydrogels for soft tissue regeneration scaffold in the wound healing process should be composed of protein and polysaccharide which mimic native extracellular matrices[7]. Natural polymers, such as chitosan and gelatin, are the most frequently used due to their biocompatibility[8]. Chitosan, a natural cationic polymer derived mainly from crustacean exoskeleton[9], is known for its antibacterial and antioxidant properties as well as its medical applications. Besides, chitosan has a potential hemostatic feature which helps natural blood clotting and block nerve ending to reduce pain[6]. Gelatin, which is a collagen hydrolysate, consists of single-strand molecules of triple-helix collagen structure fragment that rich with amino acid functional groups[7]. Gelatin has excellent

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filmogenic property, which allow the production of films[8]. Besides, its gelation property can act as a binding agent that constricts the vessel and stop the blood flow[6]. The chitosan and gelatin blending is usually conducted to improve the mechanical strength as well as to inhibit rapid biodegradation of the hydrogel [10].

Chitosan-gelatin-based wound dressing hydrogels can be modified further by chemical modification or altering their composition to enhance their bioactivity. For example, the addition of honey to the chitosan-gelatin hydrogel for wound care application has been previously studied [11,12]. Honey, a traditional wound care material, can be a right candidate as a bioactive agent in the gelatin-chitosan hydrogel to improve the healing effectiveness. This work aimed to prepare chitosan-gelatin-honey based hydrogel and observe the effect of each composition quantity toward the quality of resulting hydrogel.

### Methodology

**Material.** Chitosan pharmaceutical grade with 85-93% degree of acetylation was purchased from PT. Biotech Surindo. Fish-scales-based gelatin was obtained from the National Nuclear Energy Agency of Indonesia, BATAN. Honey "madu klengkeng" purchased from Perhutani, Indonesia. Acetic acid glacial was purchased from Sigma Aldrich. Distilled water was obtained from Chemical Engineering Laboratory, University of Sultan Ageng Tirtayasa.

**Preparation of hydrogel.** Beforehand, chitosan, gelatin, and honey were prepared as the solutions. Chitosan solutions were prepared by dissolving respectively 0.25 g, 0.5 g, 0.75 g, and 1 g of chitosan in 2.5 % v/v acetic acid solution by stirring using magnetic stirrer. Gelatin (10 g, 20 g, and 30 g respectively) were dissolved in warm water (60°C) and mixed by keeping the solution temperature minimum at 40°C to avoid gelation. Honey solutions were prepared by dissolving respectively 10 g and 20 g in 20 g of water. The chitosan solution, the gelatin solution, and the honey solution were mixed. Water was added to the mixture to give the final volume of 100 mL. Then the mixture was stirred thoroughly at 40°C. The resulting solution was cast on a glass plate by 2 – 4 mm in thickness. Finally, the hydrogel was dried in the oven at 37°C overnight. Some hydrogels have been prepared by various proportions of chitosan, honey, and gelatin, as shown in Table 1.

**Table 1.** Chitosan, gelatin, and honey composition for hydrogel preparation.

Entry	1	2	3	4	5	6	7	8	9	10	11
Chitosan (g)	0.5	0.5	0.5	0.5	0.5	0.5	0	0.25	0.5	0.75	1
Gelatin (g)	10	20	30	20	20	20	20	20	20	20	20
Honey (g)	20	20	20	0	10	20	20	20	20	20	20

**Gel fraction determination.** The determination of the gel fraction was conducted by a gravimetric method [12,13]. The hydrogel was cut into small pieces and dried in the oven at 60°C until it reached a constant weight ( $W_0$ ). Then, the dried hydrogel was immersed in water and shook for 24 hours at room temperature. Finally, the hydrogel was re-dried in the oven at 60°C to constant weight ( $W_1$ ). The gel fraction was calculated using Eq. 1.

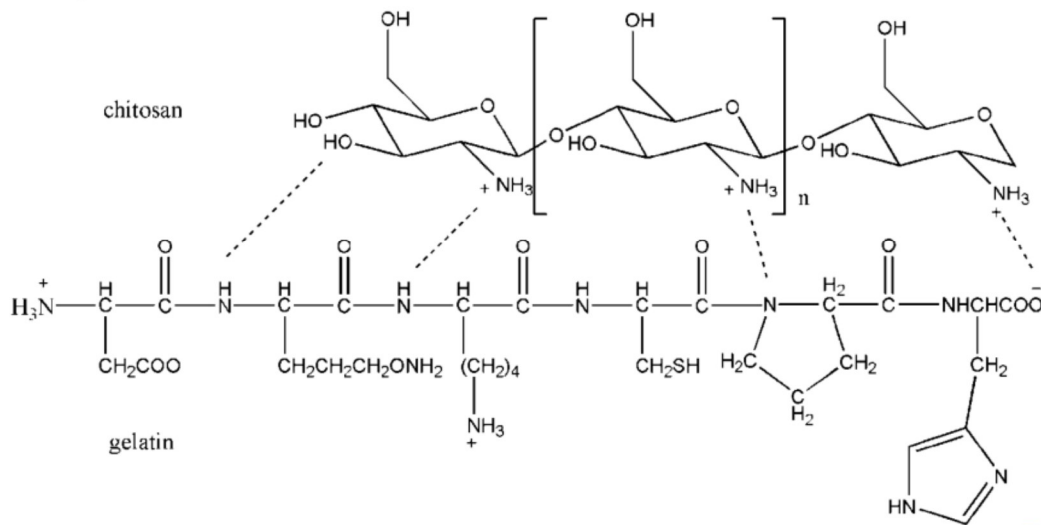
$$\text{Gel fraction} = \frac{(W_1 - W_0)}{W_0} \times 100\% \quad (1)$$

**Swelling index determination.** The hydrogel was dried in the oven at 37°C overnight. The dried hydrogel weight was recorded as  $W_0$ . Then the hydrogel was immersed in distilled water at room temperature. The hydrogel was weighed ( $W_t$ ) every 30 minutes during 120 minutes. Then, the swelling index was calculated using Eq. 2.

$$\text{Swelling index} = \frac{(W_t - W_0)}{W_0} \times 100\% \quad (2)$$



**Antibacterial property determination.** Antibacterial tests were conducted by using an inhibition zone method. The hydrogel sample was placed on the *E.coli* culture in a nutrient agar medium and incubated at 37°C for 24 hours. The developed clear zone around the hydrogel was notified as the potential antimicrobial activity. Then, the bacterial colonies in the clear zone layer were counted using a colony counter.



**Fig. 1.** The possible interaction between chitosan polycation and gelatin polyanion[16] in the formation of a three-dimensional network of the hydrogel.

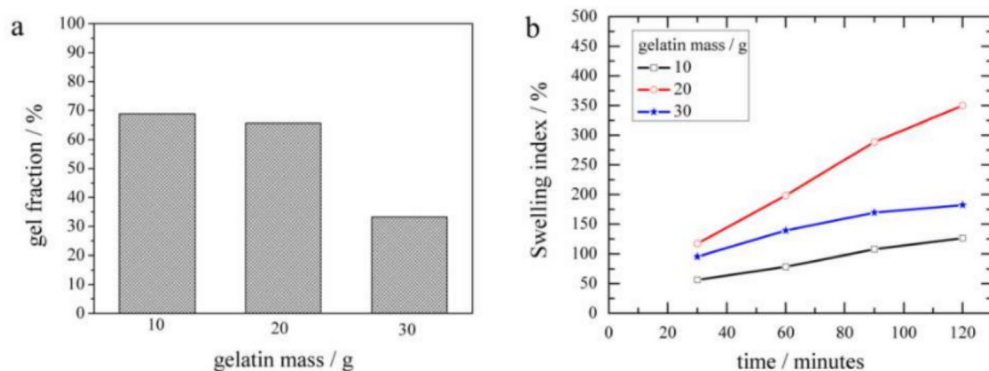
### Results and Discussion

Hydrogels have gained a great interest in biomedical research and applications[13,14]. They can retain water in its three-dimensional network which capable of deforming as soft wet material with the structure that resembles extracellular matrix [7,15]. Hydrogels can respond to various external stimuli which comprise pH, chemical composition, temperature, pressure, and electrical field [4]. These properties route the hydrogel application, especially for delivery drug system, wound dressing, and tissue engineering [4]. Herein, we prepared gelatin/chitosan/ honey hydrogel through a simple physical mixing process at low temperature. Chitosan was previously dissolved in 2.5 % of acetic acid to turn into chitosan polycation. Chitosan polycation and gelatin (which tend to form polyanion), were cross-linked by polyelectrolyte complexation process to form 3D networking hydrogel. The proposed chitosan-gelatin interaction to form hydrogel is illustrated in Fig. 1. The presence of ammonium ( $\text{NH}_3^+$ ) and hydroxyl (-OH) groups of chitosan results in hydrogen bonding with the amine (-NH-) and/ carboxyl (-COOH) groups of gelatin. The ionic bond between the ammonium groups ( $\text{NH}_3^+$ ) groups of chitosan and ionic carboxyl (-COO<sup>-</sup>) groups of gelatin. All those interactions result in a high order 3D structural bi-polymer system. Honey compositions, mainly sugar (glucose, fructose, sucrose, etc.) [10], are small enough that can be assumed to enter the 3D system, so it can be present throughout the resulting hydrogel. The resulting gelatin/chitosan/honey hydrogels have a yellowish (honey color) transperance soft film sheet with 2 - 4 mm thick, as shown in Fig. 2.



**Fig. 2.** The resulting gelatin/chitosan/honey hydrogel film sheet.

**Effect of gelatin mass.** Gelatin is commonly composited with chitosan to prepare a hydrogel [10] through a polyelectrolyte complexation (PEC) mechanism [13]. Herein, the effect of various gelatin quantities to the gel fraction of the as-resulted hydrogels was firstly evaluated. The gel fraction implies the cross-linked degree of the polymeric chain [5] of bi-polymer gelatin and chitosan. Fig. 3a shows that the hydrogels with a better gel fraction have been resulted in by the gelatin/chitosan ratio of 20:1 and 40:1. However, the hydrogel with higher gelatin/chitosan ratio shows a lower gel fraction. According to the illustration in Fig. 1, the possible most potent interaction between chitosan and gelatin could be the ionic bond between the  $\text{NH}_3^+$  of chitosan and  $-\text{COO}^-$  of gelatin. A higher gelatin/chitosan ratio may hide the chitosan as the polycation source, hence lowers ionic bond and reduces crosslink order.

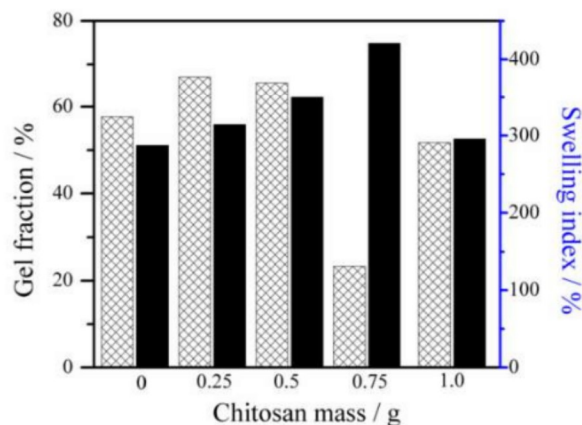


**Fig. 3.** (a) Histogram shows the gel fractions correspond to gelatin mass composition in hydrogel (b) swelling index of various hydrogels as the time function.

The effect of gelatin composition toward the swelling index of the gelatin-chitosan-based hydrogels was also investigated. Swelling index implies the capability of the hydrogel to absorb and retain water in its 3D structure. Water is bound inside by hydrogen bonding. Therefore, swelling index correlates with the presence and the orientation of many functional groups that responsible for hydrogen bonding such as carboxyl ( $-\text{COOH}$ ), amine ( $-\text{NH}-$ ), and hydroxyl ( $-\text{OH}$ ), which present both in chitosan and gelatin. High swelling index may represent that many hydrogen bonds occurred. Fig. 3b shows the swelling index of the hydrogels as resulted from the different gelatin mass composition as the time function. Herein, the best swelling index behavior was resulted in by 20 g of gelatin in composition. This result also comparable with the other work [10]. The swelling index at higher gelatin content was getting lower, which probably correlates with the decrease of gel fraction, as explained above.

**Effect of chitosan mass.** The effect of chitosan mass on the hydrogel quality is presented in Fig. 4. It is well known that gelatin has an excellent filmogenic [17] as well as gelation properties [16]. Therefore, the hydrogel, composed of gelatin and honey without chitosan, shows a gel fraction of almost 60 % and a swelling index of 300 %. Gelatin has a molecular structure which enables to form

a zwitterionic system that stimulates cross-link. The high swelling index of gelatin hydrogel corresponds to the abundance of functional groups for hydrogen bond interaction. The addition of chitosan has improved the hydrogel quality by increasing the gel fraction up to almost 70 %. The interaction polycation chitosan and polyanion gelatin can lead to the development of high order cross-link structure as previously explained. The swelling index also increases from 300 % to 400 % progressively with the mass of chitosan added. This improvement can be generated due to the presence of chitosan automatically increases the hydroxyl groups (-OH) that retain more water by hydrogen bonding in the hydrogel structure. These obtained swelling indexes were also comparable with many other gelatin-chitosan-based hydrogel results[8,11,18].



**Fig. 4.** The gel fraction (y-axis left) and swelling index (y-axis right) correspond to the mass of chitosan on hydrogel composition.

The antibacterial properties have been tested by culturing *E.coli* bacteria on the hydrogels. Table 2 shows the bacterial colonies grew almost four times higher on the hydrogel composed of gelatin and honey without chitosan. The presence of chitosan significantly reduces the growth of bacteria *E.coli* by around 75 %. Thus, chitosan is the crucial part that responsible for the antibacterial property of the resulting hydrogel. Chitosan, as polycation, has a high positive charge density, which leads to strong electrostatic interaction [13] hence generates its antibacterial activity. Fig. 5 also shows the comparison of the hydrogel product with and without chitosan. We observed mold growth massively on the hydrogel without chitosan (Fig. 5a). Nevertheless, almost no observed mold grows on the hydrogel containing chitosan (Fig. 5b). Chitosan has antifungal properties by suppressing the sporulation and spore germination [14].

**Table 2.** Bacterial colonies counted after culturing on the hydrogel for the antibacterial test.

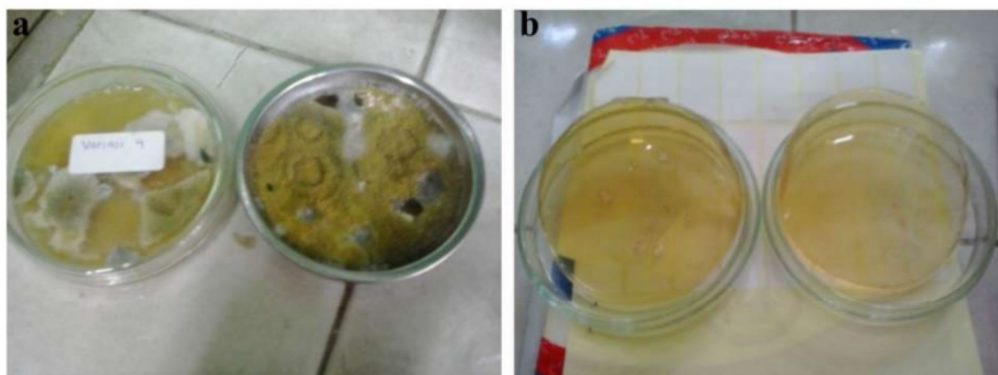
Hydrogel*	1	2	3	4
Chitosan mass (g)	0	0.25	0.75	1.0
Bacterial (colonies)	44	11	15	11

\*Composed of 20 g of gelatin, 20 g of honey, and varied of chitosan mass.

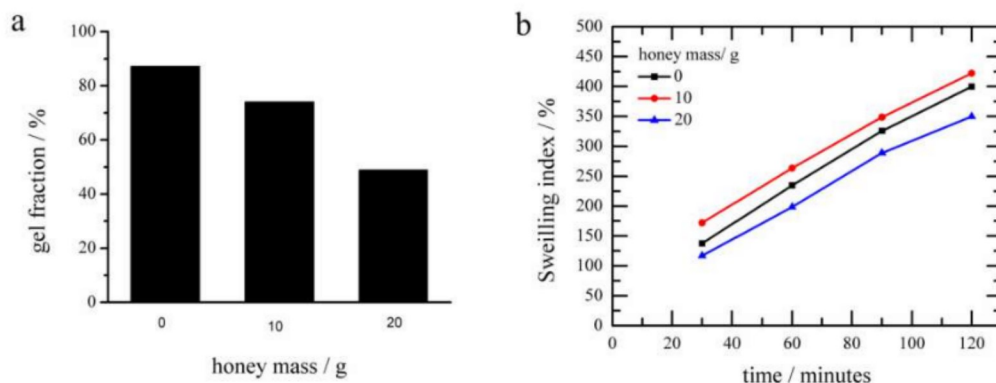
**Effect of honey mass.** Honey, added to the gelatin-chitosan hydrogel dressing, was aimed to improve the effectiveness of wound healing. The effect of honey composition on the hydrogel properties was investigated. Fig. 6a shows that the presence of honey seems lowering the gel fraction, which probably due to honey sugar molecules interfere with the chitosan-gelatin interaction, hence destroys the crosslink order. The swelling index of the resulting hydrogel also tends to decrease as the increase of honey content (Fig. 6b). The addition of honey may also change the orientation of the functional groups of the chitosan-gelatin network, which alters the hydrogen bond strength. The presence of honey in chitosan-gelatin hydrogel also has led to unexpected higher bacterial growth (not shown here). In the hydrogel preparation, honey was previously diluted by water. According to the work of



Basualdo, et al., 2007, diluted honey showed negative result toward antibacterial properties test [15]. It is suggested to use of pure honey without any dilution for wound dressing application. However, not all honey types are useful to inhibit bacterial growth [15].



**Fig. 5.** (a) The massive mold colonies grew visibly observed on hydrogels composed of gelatin and honey (b) no colonies grow on the hydrogels composed of gelatin, chitosan, and honey.



**Fig. 6.** (a) Histogram of gel fraction of the hydrogels (composed of 20 g of gelatin, and 0.5 g of chitosan) corresponds to various honey mass composition (b) swelling index of the hydrogels as the function of time.

### Summary

The hydrogels, composed of gelatin, chitosan, and honey, have been successfully prepared. The composition, especially chitosan and gelatin proportion, determines the resulted hydrogel quality in the term of gel fraction and swelling index. Chitosan is an essential part of obtaining a high degree of cross-link structure of hydrogel that has improved the swelling index as well as the gel fraction. Chitosan has also effectively inhibited both bacterial and fungal growth on the hydrogel. The effectiveness of honey composition toward hydrogel qualities and antibacterial property still need to be further evaluated.

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**References**

- [1] G.F. El Fawal, M.M. Abu-Serie, M. A. Hassan and M. S. Elnouby, Hydroxyethyl cellulose hydrogel for wound dressing: Fabrication, characterization and in vitro evaluation, *Int. J. Biol. Macromol.* 111 (2018) 649–59
- [2] A.S. Hoffman, Hydrogels for biomedical applications, *Adv. Drug Deliv. Rev.* 64 (2012) 18–23
- [3] S. Chatterjee and P. C. Hui, Stimuli-Responsive Hydrogels: An Interdisciplinary Overview, in L. Popa, M. V. Ghica and C. E. Dinu-Pîrvu (Eds) *Hydrogels-Smart Materials for Biomedical Applications* London, IntechOpen, 2018, Chapter 2
- [4] Z. Shariatinia and A. M. Jalali, Chitosan-based hydrogels: Preparation, properties and applications, *Int. J. Biol. Macromol.* 115 (2018) 194–220
- [5] D. Mukherjee, S. Santhosh, G. Dath and A. Ghosh, Development and characterization of chitosan-based hydrogels as wound dressing materials, *J. Drug Deliv. Sci. Technol.* 46 (2018) 498–510
- [6] P. T. S. Kumar, G. Praveen, M. Raj, K. P. Chennazhi and R. Jayakumar, Flexible, micro-porous chitosan–gelatin hydrogel/nanofibrin composite bandages for treating burn wounds, *RSC Adv.*, 4 (2014) 65081–7
- [7] C. Yang, L. Xu, Y. Zhou, X. Zhang, X. Huang, M. Wang, Y. Han, M. Zhai, S. Wei and J. Li, A green fabrication approach of gelatin/CM-chitosan hybrid hydrogel for wound healing, *Carbohydr. Polym.*, 82 (2010) 1297–305
- [8] B. Lu, T. Wang, Z. Li, F. Dai, L. Lv, F. Tang, K. Yu, J. Liu and G. Lan, Healing of skin wounds with a chitosan-gelatin sponge loaded with tannins and platelet-rich plasma, *Int. J. Biol. Macromol.*, 82 (2016) 884–91
- [9] R.S.C.M.D.Q. Antonino, B.R.P.L. Fook, V.A.D.O. Lima, R.Í.D.F. Rached, E.P.N. Lima, R.J.D.S. Lima, C.A.P. Covas and M.V.L. Fook, Preparation and characterization of chitosan obtained from shells of shrimp (*Litopenaeus vannamei* Boone), *Mar. Drugs* 15 (2017)1-12
- [10] Z. Peng, Z. Peng and Y. Shen, Fabrication and Properties of Gelatin/Chitosan Composite Hydrogel, *Polym. Plast. Technol. Eng.*, 50 (2011) 1160–4
- [11] T. Wang, X. K. Zhu, X. T. Xue and D. Y. Wu, Hydrogel sheets of chitosan, honey and gelatin as burn wound dressings, *Carbohydr. Polym.*, 88 (2012) 75–83
- [12] N. Yusof, A. H. Ainul Hafiza, R. M. Zohdi and M. Z. A. Bakar, Development of honey hydrogel dressing for enhanced wound healing, *Radiat. Phys. Chem.* 76 (2007) 1767–70
- [13] E. Caló and V. V. Khutoryanskiy, Biomedical applications of hydrogels: A review of patents and commercial products, *Eur. Polym. J.* 65 (2015) 252–67
- [14] A. S. Hoffman, Hydrogels for biomedical applications, *Adv. Drug Deliv. Rev.* 54 (2002) 3–12
- [15] M. A. Haque, T. Kurokawa and J. P. Gong, Super tough double network hydrogels and their application as biomaterials, *Polymer (Guildf)*, 53 (2012) 1805–22
- [16] N. Devi, C. Deka, T. K. Maji and D. K. Kakati, Gelatin and Gelatin–Polyelectrolyte Complexes: Drug Delivery, *Encycl. Biomed. Polym. Polym. Biomater.*, 2016 3557–69
- [17] I.C.F. Moraes, R.A. Carvalho, A.M.Q.B. Bittante, J. Solorza-Feria and P.J.A. Sobral, Film forming solutions based on gelatin and poly(vinyl alcohol) blends: Thermal and rheological characterizations, *J. Food Eng.*, 95 (2009) 588–96
- [18] D.R. Barleany, R.S. Dhamar Lestari, M. Yulvianti, T.R. Susanto, Shalina and Erizal, Acrylic Acid Neutralization for Enhancing the Production of Grafted Chitosan Superabsorbent Hydrogel, *Int. J. Adv. Sci. Eng. Inf. Technol.*, 7 (2017) 702



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