International Journal of Nanoelectronics and Materials
Volume 14, No. 2, April 2021 [113-126]

Effect of Monomers for The Synthesis of Chitosan-Based Hydrogel

Ai Xin Lam[1], Yeit Haan Teow[1,2*], and Kah Chun Ho[1,2]

[1]Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia.
[2]Research Centre for Sustainable Process Technology (CESPRO), Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia.

Received 28 July 2020, Revised 3 December 2020, Accepted 7 December 2020

ABSTRACT

Artificial skin is introduced to treat thermal injuries and chronic skin wounds to prevent bacterial infection and water loss. It significantly reduced morbidity and mortality of skin wounds. However, it has limitations such as high cost, risk of infectious disease transmission, needs of multiple surgeries, a low tendency to adhere onto wound surface and can cause painfulness. Recently, chitosan-based hydrogel is explored in the development of artificial skin in biomedical application as it can overcome the drawbacks of the conventional artificial skin. In this study, chitosan-based hydrogel was synthesized using free radical emulsion polymerization and the effect of different formulation towards the biomedical application as artificial skin was studied. Different composition of monomers (chitosan (0.15-1.00 g), methacrylic acid (MAA) (0.25-1.50 g) and N-isopropylacrylamide (NIPAM) (0.25-1.50 g)) was studied to investigate their effects on characteristics of chitosan-based hydrogel including functional groups, degree of swelling, and porosity test to evaluate their potential as an artificial skin. The study showed that low chitosan composition and high NIPAM composition increased the degree of swelling and also porosity. Chitosan-based hydrogel with low amount of chitosan (0.15 g), MAA (0.88 g) and medium amount of NIPAM (0.88 g) was the optimized formulation as an artificial skin.

Keywords: Hydrogel, Chitosan, Artificial Skin, Biomedical Application

1. INTRODUCTION

Human skin is about 12-15% of body weight with 1-2 m^2 of surface area and considered as the largest organ in the body [1]. It plays an important immunity role which protecting body against pathogens and prevent excess water loss [2, 3]. A dressing is commonly required to cover the damaged skin instantly after skin injury. Artificial skin possess surface properties and tactile texture of humanoid robots and tactile sensors similar with real human skin had been introduced as wound dressing [4]. An artificial skin should maintain a moist condition and allow gaseous interchange at the wounded interface. It should also act as a barrier to microorganisms and eliminate excess exudates. The most vital properties of artificial skin is nontoxic and non-allergenic, a readily available biomaterial that requires minimal processing, having antimicrobial characteristic, and stimulus wound healing should be used [5]. However, high cost, risk of infectious disease transmission, needs of multiple surgeries and a low tendency to adhere onto wound surface are the main disadvantages of these artificial skin [6].

Hydrogel is first introduced in 1960 for biological application and developed by a natured three-dimensional cross-linked hydrophilic polymeric network. Hydrogel is insoluble at physiological temperature and pH but can undergo reversible swelling and retaining large amount of water compared to the dry weight of hydrogel in aqueous solution or biological fluids while maintaining

---

[*] Corresponding Author: yh_teow@ukm.edu.my

its shape until equilibrium stage achieved [7, 8]. Due to this swelling capacity, hydrogel possess the ability to maintain good blood compatibility, structural integrity, elasticity, and a degree of flexibility which similar to native tissues [9]. Chemical cross-linked hydrogel possess better gelation behaviour and mechanical strength, but limited to the toxicity of the cross-linker [10, 11]. In addition, most of the chemical cross-linked hydrogel may induce immune response, which make it unsuitable to be used as an artificial skin [12]. Thus, it is important to develop a chemical cross-linked hydrogels that have high degree of swelling, effective in drug delivery, biocompatibility and do not induce immune response.

Among the polymer, chitosan derived from chitin has gained much attention in biomedical application due to the lower potential risk of transmitting animal-originated pathogens and immunogenicity compared to other naturally derived materials [13]. Chitosan has excellent biocompatibility with animal or plant tissue due to the reactive hydroxyl and amino groups which make it easier to substitute by other groups or chelate with transitional metal ions [14]. Besides, these properties made chitosan easily to transformed into hydrogel and make it applicable for biomedical application [15]. Due to the non-toxicity, safe, stable, biodegradable and high antimicrobial activity of chitosan, it becomes an excellent excipient. It has been used in wound dressing application by the Food and Drug Administration in 2003 [16]. Additionally, drugs can be transported through the activation of stimuli-response hydrogel. Chhibber et al. [17] had developed chitosan-based hydrogel for topical delivery of moxifloxacin in order to treat *S. aureus*–infected burn wound. The drug loaded chitosan-based hydrogel showed better efficiency in eradicating bacteria in a delayed treatment of wound and it also possess better skin healing efficiency.

Methacrylic acid (MAA) and N-isopropylacrylamide (NIPAM) had been found to possess pH-responsive behavior and temperature-responsive behavior, respectively [18, 19, 20, 21]. Both of these materials can be incorporated into chitosan-based hydrogel by using chemical cross-linked to improve sensitivity of hydrogel towards pH and temperature [21]. This type of chitosan-based hydrogel smart material has potential in various applications including drug-delivery application for artificial skin [22]. The improvement in the sensitivity of pH and temperature can make chitosan-based hydrogel more responsive towards pH and temperature change when applied to human skin so it can have a better drug release system. Kwok et al. [23] reported that the high composition of MAA showed significant impact in pH responsive behaviour. It showed a high amount of MAA result a better responsive towards low pH which indicated by 0.18 mg/mL and 2.72 mg/mL of MAA with hydrodynamic radius of 320 nm and 250 nm, respectively at pH 3 at room temperature. The high amount of MAA used had proved to have a higher degree of deswelling and thus, it increased the amount of drug release from hydrogel. Besides, the incorporation of NIPAM into hydrogel also make it responsive to temperature change where deswelling occurs above 32 °C which was the lower critical solution temperature of NIPAM [19, 23]. Shekhar et al. [24] found that different amount of NIPAM had affected the degree of deswelling. High amount of NIPAM have a better responsive to temperature and thus, have a high rate of deswelling. It had indicated that a pure NIPAM hydrogel undergoes deswelling from 82% to 2%, while hydrogel which contained only 25% of NIPAM undergoes deswelling from 70% to 20% only. This result proved that the amount of NIPAM can also affect the amount of drug released from hydrogel. Thus, it was hypothesized that composition of MAA and NIPAM could affect the performance of artificial skin in term of swelling behavior.

Herein, study of formulation of chitosan-based hydrogel should be done in order to have an optimized performance for this artificial skin. However, there have hitherto been few studies on the chitosan-based hydrogel formulation towards its biomedical application as artificial skin. In order to study the effect of formulation of chitosan-based hydrogel towards its biomedical application as artificial skin, this study investigates different composition of monomer (chitosan, MAA and NIPAM) for the synthesis of chitosan-based hydrogel using central composite design

(CCD). Several characterizations including functional groups, degree of swelling, and porosity test were done to evaluate the properties of chitosan-based hydrogel as an artificial skin.

2. MATERIAL AND METHODS

2.1 Materials

Chitosan powder (75-85 % deacetylated) and MAA (purity: 99 %) purchased from Sigma Aldrich, US, and NIPAM (purity > 98 %) supplied from TCI chemical, Japan were used as the polymers for the synthesis of chitosan-based hydrogel. Acetic acid bought from R&M Chemicals, Malaysia was used as the solvent for chitosan dissolution. In addition, N, N'-methylenebisacrylamide (MBA) (purity ≥ 99 %) supplied by Solarbio, China was used as cross-linker while gelatin provided by R&M Chemicals, Malaysia was used as gelling agent for the synthesis of chitosan-based hydrogel.

2.2 Synthesis of Chitosan-based Hydrogel

The synthesis of chitosan-based hydrogel was modified from Rasib et al. [21]. Firstly, 1 v/v% of glacial acetic acid in 50 mL of deionized water was prepared. Pre-determined weight of chitosan powder was gradually added into acetic acid solution and stirred at 400 rpm for 2 h until it completely dissolved at room temperature. Next, pre-determined weight of MAA and NIPAM were added into the mixture under 400 rpm of constant stirring for 20 min at 30 °C in a three-neck round-bottom flask. Following, 0.03 g of MBA was added to the three-neck round-bottom flask with continuous stirring at 400 rpm for 1 h until milky solution was observed. The temperature of the reaction mixture was gradually increased to 70 °C and maintained for 30 min. Then, 3 g of gelatin was added into the reaction mixture as a gelling agent. The reaction was continued at 70 °C with continuous stirring at 400 rpm for 1 h under nitrogen gas purging. After that, the reaction mixture was cooled to room temperature for 1 day to form hydrogel. The semi-dried chitosan-based hydrogel was further dried in an oven at 40 °C for 1 day to completely remove the residual solvent until constant weight was obtained [25].

Design Expert software version 7.0.0 (Stat-Ease Inc, USA) was employed to optimize chitosan-based hydrogel formulation. 3 numeric factors - chitosan powder weight (0.15-1.00 g) [21, 26, 27], MAA weight (0.25-1.50 g) [26], and NIPAM weight (0.25-1.50 g) [26] were manipulated in total volume of 50 mL of the synthesized of chitosan-based hydrogel. Each numeric factor was varied over 5 levels, including axial points (±1.68), factorial points (±1) and center-point (0). The composition of chitosan-based hydrogel is summarized in Table 1 with 6 center-point and total experiment of 20.

Table 1 Composition of chitosan-based hydrogel

Sample	Chitosan (g)	MAA (g)	NIPAM (g)
1	0.15	0.88	0.88
2	0.32	0.50	0.50
3	0.32	0.50	1.25
4	0.32	1.25	0.50
5	0.32	1.25	1.25
6	0.58	0.25	0.88
7	0.58	0.88	0.25
8	0.58	0.88	0.88

9	0.58	0.88	0.88
10	0.58	0.88	0.88
11	0.58	0.88	0.88
12	0.58	0.88	0.88
13	0.58	0.88	0.88
14	0.58	0.88	1.50
15	0.58	1.50	0.88
16	0.83	0.50	0.50
17	0.83	0.50	1.25
18	0.83	1.25	0.50
19	0.83	1.25	1.25
20	1.00	0.88	0.88

2.3 Characterization of Chitosan-based Hydrogel

Several characterizations of chitosan-based hydrogel had been done to study the effect of formulation towards the properties of chitosan-based hydrogel.

2.3.1 Functional groups

Fourier transform infrared (FTIR) spectroscopy, Nicolet 6700 (Thermo Fisher, US) was used to determine the functional groups present on chitosan-based hydrogel. Chitosan-based hydrogel was subjected to FTIR spectroscopy under attenuated total reflectance (ATR) mode at wavenumber ranging from 4000-400 cm^{-1}.

2.3.2 Degree of swelling

5 mg of chitosan-based hydrogel was weighted and immersed in 50 mL of distilled water at room temperature for 24 h [25, 28]. Swollen chitosan-based hydrogel was then taken out from the distilled water, blotted with filter paper, and weighted. The degree of swelling (SW) was calculated using equation (1):

$$Degree\ of\ swelling, SW = \frac{W_1 - W_0}{W_0} \times\ 100\% \qquad (1)$$

where W_0 is the initial weight of chitosan-based hydrogel (g) and W_1 is the weight of chitosan-based hydrogel after swelling (g).

2.3.3 Porosity

5 mg of chitosan-based hydrogel was weighted and immersed in 50 mL of distilled water at room temperature for 24 h [29]. The chitosan-based hydrogel was then taken out from the distilled water, superficially dried with filter paper, and weighted. The porosity (ε) of chitosan-based hydrogel was calculated using equation (2):

$$Porosity, \varepsilon = \frac{W_1 - W_0}{\rho V_1} \times\ 100\% \qquad (2)$$

where V_1 is the initial volume of distilled water (cm^3), and ρ is the density of distilled water (0.997 g cm^{-3}).

3. RESULTS AND DISCUSSION

3.1 Functional groups

Figure 1 shows the FTIR spectroscopy of chitosan and Sample 18 of chitosan-based hydrogel. Chitosan spectrum was used as the benchmark to observe the change of functional groups through cross-linking process during hydrogel formation. Teow et. al. [28] had synthesized cellulose hydrogel using different composition of cellulose and gelatin, FTIR spectroscopy showed similar spectrum for different composition cellulose hydrogel. Hydrogel synthesized using same material but different composition will be having similar spectrum. Thus, sample 18 spectroscopy was chosen as the comparison because it contains high amount of chitosan, hence the peaks are more pronounced [30]. As depicted by chitosan spectrum in Figure 1, broad band located between 2800 and 3600 cm^{-1} is attributed by hydrogen bonds (O-H). It is overlapping with the stretching vibration of primary amines bonds (N-H) at the wavelength of 2870 cm^{-1}. Whereas, the medium peak located between 1570 and 1660 cm^{-1} is assigned to the carbonyl group (C=O) of the acetyl groups, while the peaks fall between 1310 and 1380 cm^{-1} referred to the alkane bending (C-H). On the other hand, ether group (C-O-C) symmetric and anti-symmetric group is indicated in the range of 1000 to 1100 cm^{-1} [21]. Heterogeneous chemical structure of chitosan which consist from both 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose had gave all these bonds to chitosan [31].

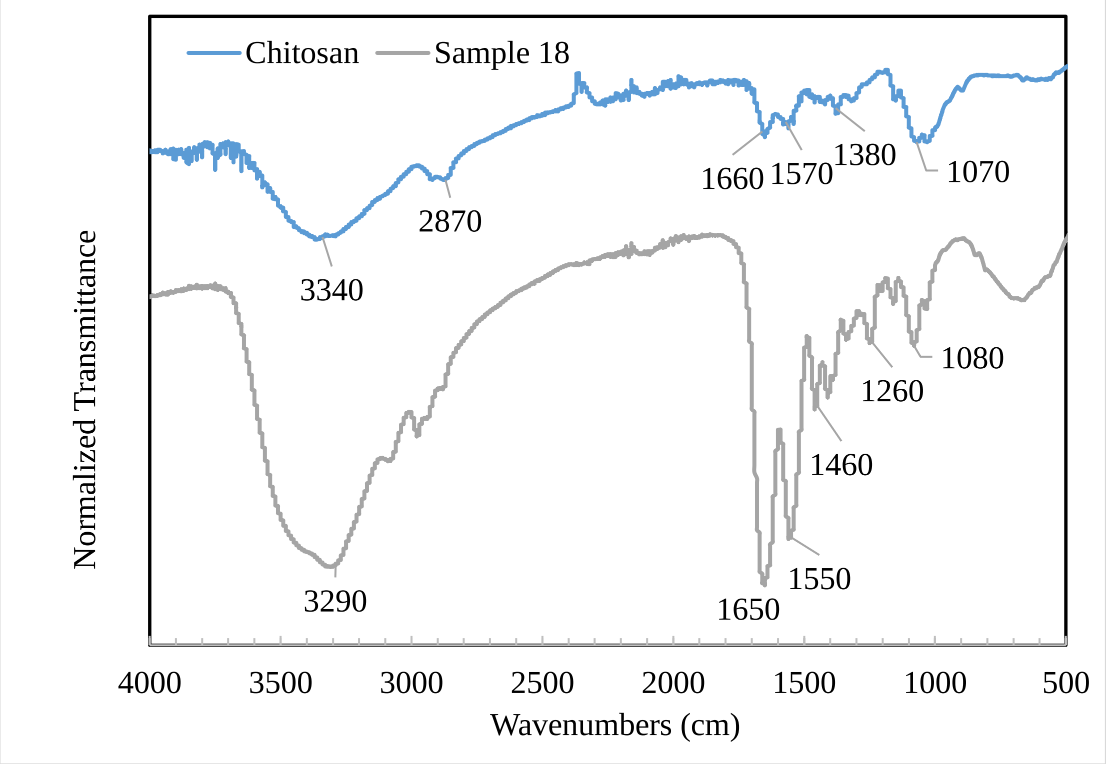
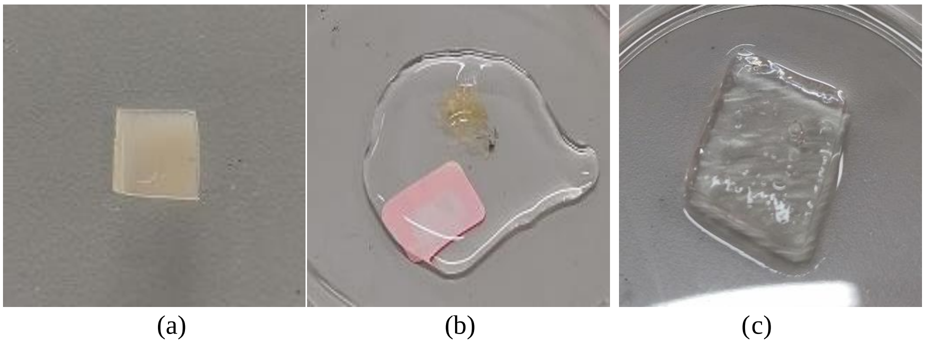


Figure 1. FTIR spectroscopy of chitosan and Sample 18 of chitosan-based hydrogel

In the case of Sample 18 of chitosan-based hydrogel, the significant functional groups difference with the chitosan spectroscopy was peaks at 1550 cm^{-1} which indicated the carboxylic carbonyl group (-COOH) of MAA and the double bond (C=C) within the MAA and NIPAM monomers were shown at the peaks of 1460 cm^{-1} [21]. Besides, another significant peak in chitosan-based hydrogels spectrum at the region of 1600 to 1650 cm^{-1} referred to carbonyl group (C=O) of MAA which involved in electrostatic interaction with ammonium cation of chitosan. In addition, the peak at 1260 cm^{-1} corresponds to the stretching of methyl groups (-CH_3) of NIPAM. The primary amines bond (N-H) of chitosan spectroscopy at wavelength of 2870 cm^{-1} had disappeared in chitosan-based hydrogel spectroscopy could be due to the influence of ionic interactions on the inter-molecular structure of polymer chain [32]. This primary amines peak decreased due to the cross-linked activity of primary amines groups of chitosan with functional groups in MAA, NIPAM and MBA [33]. In short, most of the functional group of both of the monomers and chitosan can be found in the chitosan-based hydrogel spectroscopy, hence the hydrogel network is formed within both of the monomer and chitosan cross-linked. The similar peaks between chitosan spectrum and chitosan-based hydrogel spectrum also confirmed that the chitosan-based hydrogel had been successfully synthesized.

3.2 Degree of Swelling

The images of chitosan-based hydrogel immersed into distilled water are shown in Figure 2. It can be seen that the chitosan-based hydrogel became swollen due to the water diffusion into hydrogels. The degree of swelling of chitosan-based hydrogels with different composition after immersing in distilled water for 24 h are shown in Figure 3. As presented in Figure 3, all chitosan-based hydrogels were having high degree of swelling in which all of them were able to take up to 2,000 times of its dry weight. High swelling degree of chitosan-based hydrogels was attributed to the hydrophilic nature of chitosan in hydrogel's network due to the presence of hydroxyl group (-OH) and amine groups (-NH_2) as indicated by FTIR spectroscopy of chitosan-based hydrogel in Figure 1 [34]. Both of these groups interact with hydrogen bond of water molecules and increased the degree of swelling by inducing the translational entropy of counter ions and elevated osmotic pressure and swelling dynamics.



(a) (b) (c)

Figure 2. Diagram of (a) initial, (b) immersed at distilled water, (c) swollen of chitosan-based hydrogel

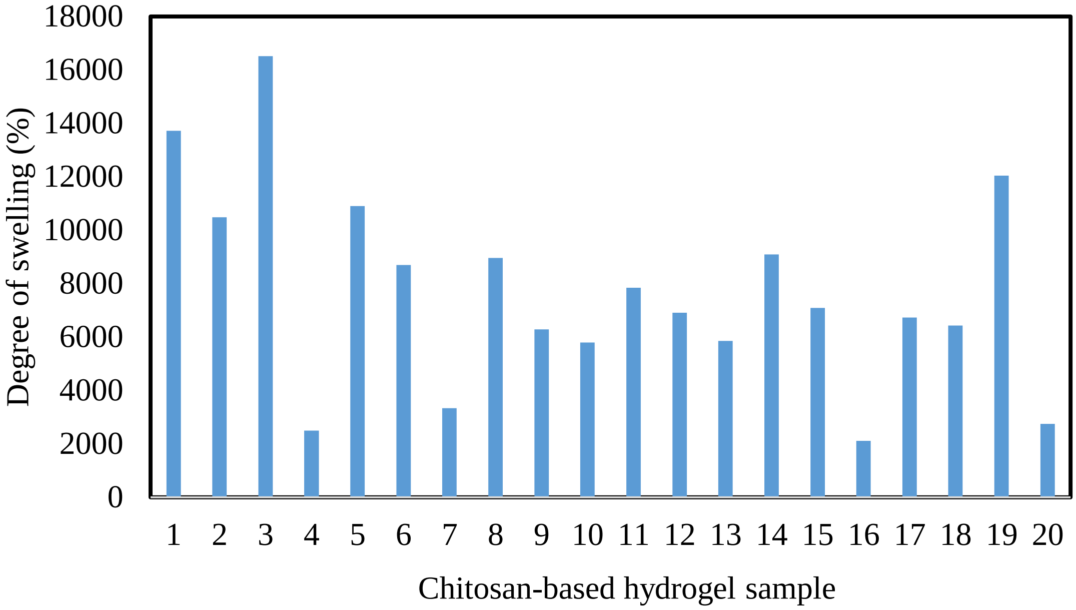


Figure 3. Degree of swelling of chitosan-based hydrogels with different composition

The highest degree of swelling is achieved by chitosan-based hydrogel Sample 3 at about 16,520% while the lowest degree of swelling was presented by chitosan-based hydrogel Sample 16 at about 2,120%. This main difference between Sample 3 and Sample 16 is the amount of chitosan and NIPAM which Sample 3 contained 0.32 g of chitosan and 1.25 g of NIPAM, while Sample 16 contained 0.83 g of chitosan and 0.50 g of NIPAM. This showed an increasing in amount of chitosan and decreasing of amount of NIPAM had decreased the degree of swelling of chitosan-based hydrogel.

Figure 4 presented the degree of swelling of chitosan-based hydrogels categorized based on its composition to investigate the effect of chitosan, MAA, and NIPAM towards hydrogel swelling. The degree of chitosan-based hydrogel swelling decreased from 13,720% to 2,750% as the chitosan composition increased from 0.15 g to 1.00 g while maintaining the weights of MAA (0.88 g) and NIPAM (0.88 g) (Sample 1, 8, 20). Cross-linking degree of the hydrogel is among the dominant factor that will affect the degree of swelling [28]. At higher chitosan composition, there are more junction zones formed among chitosan, MAA, and NIPAM [28]. Thus, the cross-linking degree of high chitosan composition chitosan-based hydrogel is higher than that of low chitosan composition chitosan-based hydrogel. The high cross-linking degree chitosan-based hydrogel has more compact and rigid structure that restrict the repulsion within chitosan and thus, it cannot be relaxed to sustain large quantity of water molecules and lowered the water holding space within hydrogel and its degree of swelling [21, 28, 34]. Additionally, the stronger intermolecular and intramolecular hydrogen bonds within chitosan chain also restricted the swelling degree of chitosan-based hydrogel [21, 35]. Besides, at low composition of chitosan (0.15 g), Sample 1 possess an extremely high degree of swelling due to the presence of hydroxyl group (-OH) and amine group (-NH) inducing the translational entropy of counter ions and elevated osmotic pressure and swelling dynamics [34]. The increasing in the composition of chitosan had increased the viscosity of chitosan-based hydrogel and thus provide a steric effect of chitosan outweighs ionic effect of ionic groups [34]. The unionized amino groups of chitosan restricted the movement of water molecules and resulting in the elimination of osmotic pressure and electrostatic repulsion between protonated amino groups leads to decrease in swelling of chitosan-based hydrogel in distilled water [34].

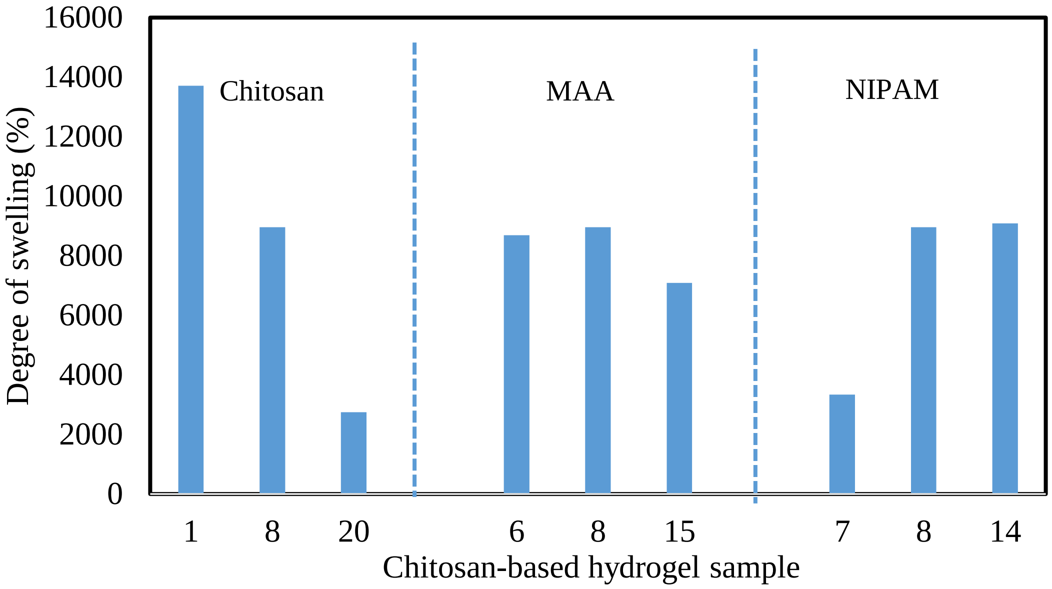


Figure 4. Degree of swelling of chitosan-based hydrogels categorized based on its composition

In contrast, MAA and NIPAM affect the degree of swelling of chitosan-based hydrogels by its particle size. Sample 7, 8 and 14 indicated the degree of swelling for NIPAM range from 0.25 g to 1.50 g at constant chitosan (0.58 g) and MAA (0.88 g), showed an increasing from 3,340% to 9,090%. Higher composition of NIPAM (Sample 14 with 1.50 g NIPAM) increased cross-linking degree of chitosan-based hydrogel, thus lower the particle size of chitosan-based hydrogel. Therefore, more water can be entrapped into the chitosan-based hydrogel [26, 34]. It shows higher ability of water holding and thus, increase the degree of swelling. However, it shows minimum difference when the amount of NIPAM is 0.88 g and 1.50 g which was Sample 8 and 14, respectively. This is because the cross-linking degree is too high, thus limits the water holding space. Besides, NIPAM is a thermal-responsive polymer; it will order itself and form hydrogen bond with the water molecules due to osmotic pressure driven by its hydrophilic nature at low temperature [19]. On the other hand, the degree of swelling showed in Sample 6, 8 and 15 for MAA were in the range of 0.25 g to 1.50 g at constant chitosan (0.58 g) and NIPAM (0.88 g); and it is almost same which is in the range of 8,500% to 9,000%. This is supported by Kwok et al. [23]'s study where a similar degree of swelling in term of hydrodynamic radius of 400 nm at room temperature and neutral pH as the amount of MAA increased from 10 mg to 150 mg. The increased number of carboxylic groups due to the higher amount of MAA had increased its hydrogen bonding, thus it is denser and degree of swelling decreased. However, it is observed that the effect of composition of MAA on degree of swelling is much smaller than NIPAM and may due of its ability in affecting particle size. MAA affected the particle size of chitosan-based hydrogel are less significant compared to NIPAM. This is in accordance to the interaction plot for particle size reported by Rasib et al. [26] where MAA composition affect the particle size for about 250 μm while NIPAM composition affect the particle size about 750 μm . This may due to MAA have a smaller molecular structure compared to NIPAM as shown in Figure 5, thus had less significant effect on the interaction in affecting particle size.

(a) (b)

Figure 5. Molecular structure of (a) MAA (b) NIPAM [21]

3.3 Porosity

The porosity of chitosan-based hydrogels was presented in Figure 6. All formulation of chitosan-based hydrogels possesses a porosity which were range in between 1-8%. By comparing the trend of degree of swelling showing in Figure 3, degree of swelling of chitosan-based hydrogel is observed to be highly affected by porosity where the degree of swelling is directly proportional to its porosity. This is because the primary mechanism of swelling is diffusion of water into the pores of hydrogel [36]. Pourjavadi et al. [37] also indicated that the porous structure of the hydrogel increases the rate and amount of swelling.

The highest porosity is achieved by chitosan-based hydrogel Sample 3 at about 8% while the lowest porosity was presented by chitosan-based hydrogel Sample 16 at about 1%. The main difference between Sample 3 and Sample 16 is the amount of chitosan and NIPAM which Sample 3 contained 0.32 g of chitosan and 1.25 g of NIPAM, while Sample 16 contained 0.83 g of chitosan and 0.50 g of NIPAM. This showed an increasing in amount of chitosan and decreasing of amount of NIPAM had decreased the porosity of chitosan-based hydrogel similar to the degree of swelling as discussed in previous section.

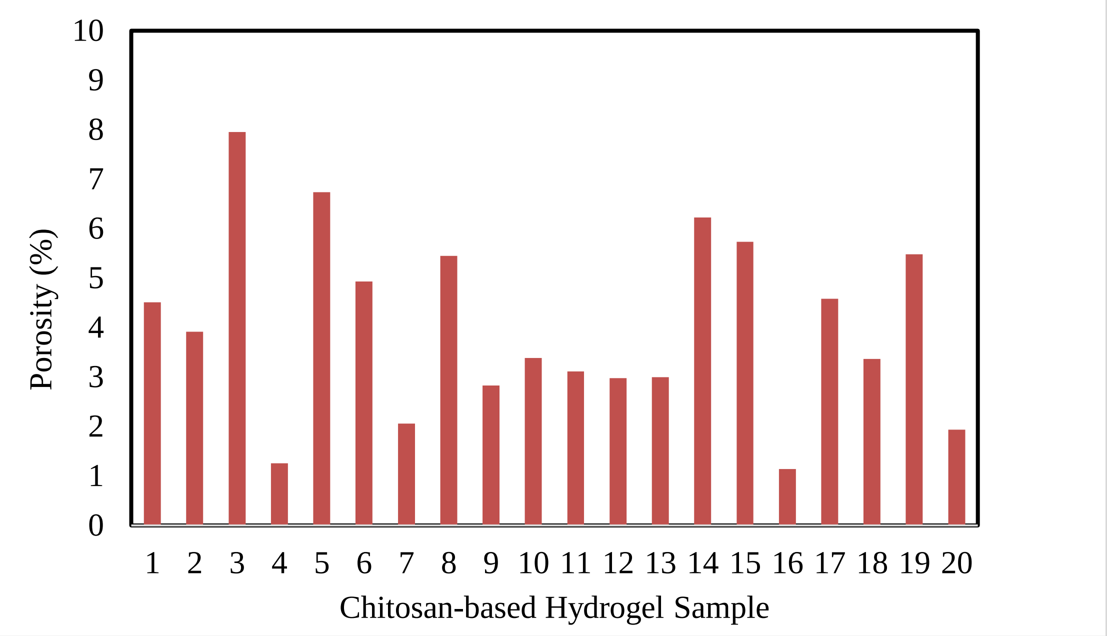


Figure 6. Porosity of chitosan-based hydrogels with different composition

Figure 7 represents the porosity of chitosan-based hydrogels categorized based on its composition to study the effect of different formulation. Generally, the porosity of chitosan-based hydrogel was increased from 4.5% to 5.5%, then decreased from 5.5% to 1.9% as the chitosan composition was increased from 0.15 g to 1.00 g at constant MAA (0.88 g) and NIPAM (0.88 g)

(Sample 1, 8, 20). The concentration of chitosan increased from 0.15 g to 0.58 g (Sample 1, 8), which efficiently prevent the bubbles in hydrogel solution from escaping during drying process and caused the formation of interconnected channels [38]. However, the structure of chitosan-based hydrogel will become more rigid as the concentration of chitosan further increased from 0.58 g to 1.00 g (Sample 8, 20), and thus, porosity decreased. In contrast, MAA showed very little effect towards the porosity of chitosan-based hydrogel. The porosity increased slightly from 5% to 6% for Sample 6, 8, 15 which represent 0.25 g, 0.88 g and 1.5 g of MAA respectively, at constant chitosan (0.58 g) and NIPAM (0.88 g). The increased in amount of MAA had increased its carboxylic groups to formed hydrogen bonding in chitosan-based hydrogel which increased cross-linked density and thus, increased porosity. This increasing is relatively small due to the smaller effect on particles size as discussed in Section 3.2. While NIPAM increases from 0.25 g to 1.5 g (Sample 7, 8, 14) at constant chitosan (0.58 g) and MAA (0.88 g), it was observed a drastic increased in porosity from 2.1% to 5.5% and a slightly increased from 5.5% to 6.2%. The increased in amount of NIPAM from 0.25 g to 0.88 g (Sample 7, 8) had increased the cross-linking degree of chitosan-based hydrogel and thus, it is denser and have a more porous structure. Whereas, for the increasing in amount of NIPAM from 0.88 g to 1.5 g (Sample 8, 14) had only caused a slightly increased in porosity because NIPAM had reached its maximum cross-linking degree; further increasing in the amount of NIPAM would not contribute much in the porosity [28]. The porosity of hydrogel are easily to be altered by the cross-linked density in the gel structure [39].

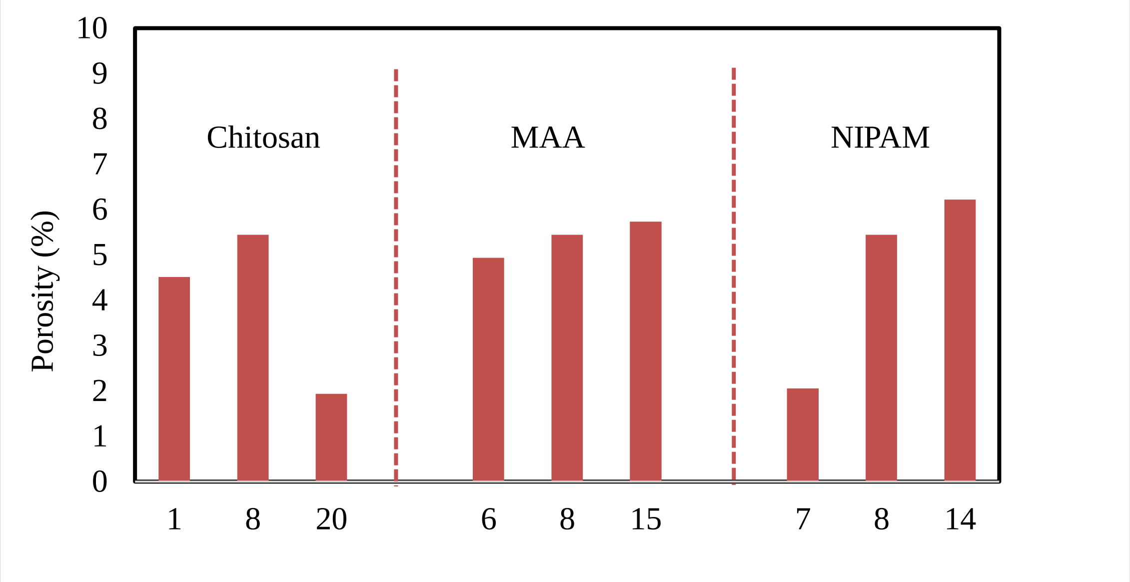


Figure 7. Porosity of chitosan-based hydrogels categorized based on its composition

3.4 Comparison of Degree of Swelling of Chitosan-based hydrogel

The degree of hydrogel swelling is the most important parameters which control the release patterns of solvents and drugs from these polymeric networks. Chitosan-based hydrogel synthesized in this study are among the hydrogels that possess the highest degree of swelling (16520%) as compared with other studies tabulated in

Table 2. The result showed that the degree of swelling of chitosan have a low degree of swelling at about 120%. When chitosan is incorporated with others material such as yeast, the degree of swelling can be increased up to 3400%. According to Rasib et al. [21], addition of MAA and NIPAM into chitosan-based hydrogel increased the degree of swelling up to 1100%. This had proved that incorporated of additives into chitosan-based hydrogel had improved its degree of swelling. In this study, the high degree of swelling could due to incorporation of NIPAM, MAA, and gelatin in the composition of chitosan-based hydrogel. Incorporation of NIPAM, MAA and gelatin in the gel

network improved the hydrophilicity of network as well as increased the surface area for water diffusion [40]. In addition, NIPAM and MAA are categorized as responsive polymeric materials which possess temperature-responsive behavior and pH-responsive behavior, respectively [19, 20, 21, 41]. Thus, it is expected that the degree of swelling is could be enhanced by manipulating temperature and pH.

Table 2 Comparison of different type of hydrogels in degree of swelling

Types	Concentration	Degree of swelling	References
Chitosan hydrogel	7.5 w/v% chitosan	120%	Ou et al. [42]
Chitosan/yeast hybrid hydrogel	3 w/v% chitosan	3400%	Feng et al. [35]
	40 w/v% yeast		
		850%	
		430%	
		570%	
Chitosan-p(MAA-co-NIPAM) hydrogel	0.5 w/v% chitosan	1100%	Rasib et al. [21]
	1 w/v% MAA		
	0.5 w/v% NIPAM	220%	
		400%	
		240%	
		450%	
Chitosan-based hydrogel	0.65 w/v% chitosan	16520%	This study
	1 w/v% MAA		
	2.5 w/v% NIPAM		
	6 w/v% gelatin		

4. CONCLUSION

Chitosan-based hydrogel was successfully synthesized using free radical emulsion polymerization method where the method was easily to be achieved. The incorporation of MAA and NIPAM had increased the pH and temperature responsive of chitosan-based hydrogel respectively which make it more suitable to control the release system of drug delivery. From the characterization result, FTIR analysis indicated that chitosan-based hydrogel was successfully produced which indicated by the similar spectrum between chitosan-based hydrogel and chitosan. Besides, the degree of swelling and porosity were highly affected by chitosan and NIPAM, while not significantly affected by MAA. Low chitosan composition and high NIPAM composition increased the degree of swelling and also porosity. Hence, Sample 1 with low amount of chitosan (0.15 g), MAA (0.88 g) and medium amount of NIPAM (0.88 g) was the optimized formulation where it possesses 13,800% degree of swelling and porosity of 4.5%. Future prospect of chitosan-based hydrogel in drug delivery system, wound dressing and healing, tissue engineering and skin care product had been recommended in this study. Drug delivery system are the main recommendation in this study where the drug absorption and desorption kinetic study should be carried out in order to make this chitosan-based hydrogel more useful in treating different kinds of wounds.

ACKNOWLEDGEMENTS

The authors would like to thank to the funding Geran Universiti Penyelidikan (GUP-2017-098) and Dana Modal Insan (MI-2019-017) that provide to this study.

REFERENCES

[1] Elert, G., “Surface Area Of Human Skin”, *The Physics Factbook*, (2019), 1–2. https://hypertextbook.com/facts/2001/IgorFridman.shtml
[2] Kabashima, K., T. Honda, F. Ginhoux, & G. Egawa, “The immunological anatomy of the skin”, Nature Reviews Immunology. **19** (2019) 19–30.
[3] Collier, M., “Protecting vulnerable skin from moisture-associated skin damage”, British Journal of Nursing. **25** (2016) S26–S32.
[4] Shimizu, R., & Y. Nonomura, “Preparation of artificial skin that mimics human skin surface and mechanical properties”, Journal of Oleo Science. **67** (2018) 47–54.
[5] Bianchera, A., O. Catanzano, J. Boateng, & L. Elviri, “The Place of Biomaterials in Wound Healing”, Therapeutic Dressings and Wound Healing Applications. (2020) 337–366.
[6] Nicholas, M.N., M.G. Jeschke, & S. Amini-Nik, “Methodologies in creating skin substitutes”, Cellular and Molecular Life Sciences. **73** (2016) 3453–3472.
[7] Haan, T.Y., & K. Rames, “Hydrogel with Thermo-Responsive Behavior for Drug Adsorption and Release”, Malaysian Journal of Analyical Sciences. **23** (2019) 390–400.
[8] Zamani Alavijeh, R., P. Shokrollahi, & J. Barzin, “A thermally and water activated shape memory gelatin physical hydrogel, with a gel point above the physiological temperature, for biomedical applications”, Journal of Materials Chemistry B. **5** (2017) 2302–2314.
[9] Morteza, B., M. Naimeh, & M. Mehdi, *Emerging Concepts in Analysis and Applications of Hydrogels*, (2016).
[10] Parhi, R., “Cross-linked hydrogel for pharmaceutical applications: A review”, Advanced Pharmaceutical Bulletin. **7** (2017) 515–530.
[11] Palantöken, S., K. Bethke, V. Zivanovic, G. Kalinka, J. Kneipp, & K. Rademann, “Cellulose hydrogels physically crosslinked by glycine: Synthesis, characterization, thermal and mechanical properties”, Journal of Applied Polymer Science. **137** (2020) 23–25.
[12] Mir, M., M.N. Ali, A. Barakullah, et al., “Synthetic polymeric biomaterials for wound healing: a review”, Progress in Biomaterials. **7** (2018) 1–21.
[13] Sari, K., A.B.S. Utomo, K. Abraha, et al., “Effect of milling time on the microstructure and dielectric properties of chitosan nanopowder”, International Journal of Nanoelectronics and Materials. **13** (2020) 1–8.
[14] Prasanthi, N.L., H. Roy, N. Jyothi, & V.S. Vajrapriya, “A Brief Review on Chitosan and Application in Biomedical Field”, American Journal of PharmTech Research. **6** (2016).
[15] Osman, N., A. Idris, P. Abu Bakar, M.A. Mohd Abdull Majid, R. Radzali, & J.Y.C. Liew, “Detection of copper (II) ion on chitosan film using microstrip ring resonator”, International Journal of Nanoelectronics and Materials. **11** (2018) 179–186.
[16] Hamedi, H., S. Moradi, S.M. Hudson, & A.E. Tonelli, “Chitosan based hydrogels and their applications for drug delivery in wound dressings: A review”, Carbohydrate Polymers. **199** (2018) 445–460.
[17] Chhibber, T., V.S. Gondil, & V.R. Sinha, “Development of Chitosan-Based Hydrogel Containing Antibiofilm Agents for the Treatment of Staphylococcus aureus–Infected Burn Wound in Mice”, AAPS PharmSciTech. **21** (2020) 2–13.
[18] Teow, Y.H., M.S.H. Ghani, W.N.A.W.M. Hamdan, N.A. Rosnan, N.I.M. Mazuki, & K.C. Ho, “Application of Membrane Technology towards The Reusability of Lake Water, Mine Water, and

Tube Well Water", Jurnal Kejuruteraan. **29** (2017) 131–137.
[19] Saad, A., R. Mills, H. Wan, M.A. Mottaleb, L. Ormsbee, & D. Bhattacharyya, "Thermo-responsive adsorption-desorption of perfluoroorganics from water using PNIPAm hydrogels and pore functionalized membranes", Journal of Membrane Science. **599** (2020) 117821.
[20] Md Rasib, S.Z., H. Md Akil, A. Khan, & Z.A. Abdul Hamid, "Controlled release studies through chitosan-based hydrogel synthesized at different polymerization stages", International Journal of Biological Macromolecules. **128** (2019) 531–536.
[21] Rasib, S.Z.M., Z. Ahmad, A. Khan, et al., "Synthesis and Evaluation on pH- and Temperature-Responsive Chitosan-p(MAA-co-NIPAM) Hydrogels", International Journal of Biological Macromolecules. (2017).
[22] Pandey, M., N. Mohamad, W.L. Low, C. Martin, & M.C.I. Mohd Amin, "Microwaved bacterial cellulose-based hydrogel microparticles for the healing of partial thickness burn wounds", Drug Delivery and Translational Research. **7** (2017) 89–99.
[23] Kwok, M. hin, J. Ambreen, & T. Ngai, "Correlating the effect of co-monomer content with responsiveness and interfacial activity of soft particles with stability of corresponding smart emulsions", Journal of Colloid and Interface Science. **546** (2019) 293–302.
[24] Shekhar, S., M. Mukherjee, & A.K. Sen, "Studies on thermal and swelling properties of Poly (NIPAM-co-2-HEA) based hydrogels", Advances in materials Research. **1** (2012) 269–284.
[25] Ashri, A., N. Amalina, A. Kamil, et al., "Modified Dioscorea hispida starch-based hydrogels and their in-vitro cytotoxicity study on small intestine cell line (FHS-74 Int)", International Journal of Biological Macromolecules. (2017).
[26] Rasib, S.Z.M., H.M. Akil, & A.S. Yahya, "Effect of Different Composition on Particle Size Chitosan-PMAA-PNIPAM Hydrogel", Procedia Chemistry. **19** (2016) 388–393.
[27] Chandrasekharan, A., Y.J. Hwang, K. Seong, & S. Park, "Acid-Treated Water-Soluble Chitosan Suitable for Microneedle-Assisted Intracutaneous Drug Delivery", (2019) 1–14.
[28] Teow, Y.H., L.M. Kam, & A.W. Mohammad, "Synthesis of cellulose hydrogel for copper (II) ions adsorption", Journal of Environmental Chemical Engineering. **6** (2018) 4588–4597.
[29] Pandey, M., M.C.I. Mohd Amin, N. Ahmad, & M.M. Abeer, "Rapid synthesis of superabsorbent smart-swelling bacterial cellulose/acrylamide-based hydrogels for drug delivery", International Journal of Polymer Science. **2013** (2013).
[30] Ren, L., X. Yan, J. Zhou, J. Tong, & X. Su, "Influence of chitosan concentration on mechanical and barrier properties of corn starch/chitosan films", International Journal of Biological Macromolecules. **105** (2017) 1636–1643.
[31] Abdullah, J., "Immobilization of Tyrosinase in Nanocrystalline Cellulose/Chitosan Composite Film for Amperometric Detection of Phenol", Malaysian Journal of Analytical Science. **20** (2016) 978–985.
[32] Nasri-Nasrabadi, B., A. Kaynak, C. Wong, P. Heidarian, K. Badii, & A.Z. Kouzani, "Uniform Micellization: The Key to Enhanced Mechanical Strength and Swelling Efficiency of Chitosan Hydrogel", Fibers and Polymers. **20** (2019) 11–18.
[33] Rahman, N.A., S.A. Hanifah, A.M.N. Zani, & A. Ahmad, "Modification of chitosan for preparation of poly(N-isopropylacrylamide/O-nitrochitosan) Interpenetrating polymer network", Sains Malaysiana. **44** (2015) 995–1001.
[34] NS, M., A. M, M. MU, et al., "Chitosan/Xanthan Gum Based Hydrogels as Potential Carrier for an Antiviral Drug: Fabrication, Characterization, and Safety Evaluation", Front. Chem. **8** (2020).
[35] Feng, D., B. Bai, H. Wang, & Y. Suo, "Enhanced mechanical stability and sensitive swelling performance of chitosan/yeast hybrid hydrogel beads", New Journal of Chemistry. **40** (2016) 3350–3362.
[36] Gan, S., S. Zakaria, C.H. Chia, R.S. Chen, A. V. Ellis, & H. Kaco, "Highly porous regenerated cellulose hydrogel and aerogel prepared from hydrothermal synthesized cellulose carbamate", PLoS ONE. **12** (2017) 1–13.
[37] Pourjavadi, A., Z. Bassampour, H. Ghasemzadeh, M. Nazari, L. Zolghadr, & S.H. Hosseini, "Porous Carrageenan-g-polyacrylamide/bentonite superabsorbent composites: swelling and dye adsorption behavior", Journal of Polymer Research. **23** (2016) 1–10.

[38] Majid, S., H. Bukhari, S. Khan, M. Rehanullah, & N.M. Ranjha, “Synthesis and Characterization of Chemically Cross-Linked Acrylic Acid / Gelatin Hydrogels : Effect of pH and Composition on Swelling and Drug Release”, **2015** (2015).
[39] Sharma, S., & S. Batra, *Chapter 5. Recent advances of chitosan composites in artificial skin: the next era for potential biomedical application*, Elsevier Inc., (2019).
[40] Das, D., P. Ghosh, & A. Ghosh, “Stimulus-responsive, Biodegradable, Biocompatible, Covalently Crosslinked Hydrogel Based on Dextrin and Poly (N-isopropyl acrylamide) for In-vitro / In-vivo Controlled Drug Release”, Applied Materials & Interfaces. (2015).
[41] Pelton, R., “Poly(N-isopropylacrylamide) (PNIPAM) is never hydrophobic”, Journal of Colloid and Interface Science. **348** (2010) 673–674.
[42] Ou, A., & I. Bo, “Chitosan Hydrogels and their Glutaraldehyde-Crosslinked Counterparts as Potential Drug Release and Tissue Engineering Systems - Synthesis , Characterization , Swelling Kinetics and Mechanism”, Journal of Physical Chemistry & Biophysics. **7** (2017).