Formulation and synthesis of hydrogels having lower critical solution temperature near body temperature

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Abstract. Copolymerization between bacterial cellulose nanocrystal (CN) and methyl cellulose (MC) was carried out using UV light to produce a biocompatible hydrogel at body temperature and liquid at room temperature. Viscosity and salt effect of the MC and copolymer solution at room temperature and its Lower Critical Solution Temperature (LCST) were evaluated. The analysis showed that the higher concentration of methyl cellulose and salt content in the solution produced lower LCST and higher solution viscosity. All samples of polymer solution with MC concentrations of 1 and 2% have a viscosity less than 5000 cP at room temperature. The solutions with MC concentration of 1, 2, and 3% have respectively LCST of 59, 58, and 57°C, while its copolymer solutions with CN concentration of 0.1, 0.3, and 0.5% have respectively LCST of 55, 51, and 41°C. The salt addition to the solution of MC-CN copolymer with concentrations of 1x and 1.5x Phosphat Buffered Saline (PBS) produces respectively LCST of 47 and 38°C. The results suggest that the copolymer solution of MC-CN could produce a lower LCST and the addition of salt could amplify the effect of LCST decrease that can be used to produce a biocompatible hydrogel with LCST as close as body temperature.

1. Introduction

Bone damaged such as bone fractures take a long time to recover. In fact it is possible that the patients will suffer permanent disability. Recent solution to overcome this problem is using polymer based biomaterial, a non-living material used in the medical field and is intended to interact with biological systems [1]. In 2005 the worldwide biomaterials market reached $300 billion with a projection to continuously grow at a rate of 20% per year [2]. New challenges in human health problems answered by the development of a revolutionary biotechnology, such as the development of artificial organs, tissue engineering, and controlled drug delivery system that cannot be separated from the role of biomaterials, especially biocompatible polymer hydrogel.

Hydrogel is an absorbive material made from polymers with a hydrophilic group bonded (crosslinked) physically or chemically so that it can absorb and hold water in three-dimensional structure without solute [3]. Some hydrogels show physical properties changing due to environmental condition changes and called as smart hydrogel [1]. The hydrogel type that is suitable for bone tissue engineering is called temperature responsive hydrogel [4]. Some polymer solutions have Lower Critical Solution Temperature (LCST), a temperature below which polymer solution remains in
solution form (one phase) and above which the polymer solution will separate into two phases, i.e.: liquid phase that contains no polymer and solid phase that riches with polymer [5]. These changes can take place reversibly. In the bone healing issue, LCST should be as close as possible to the physiological body temperature of about 37°C.

The common polymer used in smart hydrogel research is poly N-Isopropyl Acrylamide (P-NIPAAm) because it has LCST close to the physiological body temperature. Unfortunately, the N-Isopropyl Acrylamide as the monomer of P-NIPAAm is very expensive. Therefore, in the present research, other alternative polymeric materials which are cheaper and have a LCST as that of P-NIPAAm is used. The alternative material is modified methyl cellulose (MC) polymer. MC polymer has a LCST much higher than body temperature without any modification.

Many Researchers have worked in evaluating the temperature-sensitive properties of MC. Li (2002) conducted a study on the characteristics of methyl cellulose solution. This study revealed that methyl cellulose had LCST temperature of about 60°C [6]. Modification of MC thorough combination of MC with carboxy methyl cellulose, polyethylene glycol and chitosan had also been done and showed a potential to be an injectable material for post-surgery glue [7]. Methyl cellulose can also be combined with agarose for injectable property and can quickly turn into a solid gel at room temperature [8]. The combination of chitosan and methyl cellulose in various salt solutions were also made [9]. Another research on modifying properties of MC is by chemical crosslinking either with a cross linker glutaraldehyde or with gamma radiation [10].

In the previous works by the authors, the experiment have shown that the composite of bacterial cellulose and polyvinyl alcohol (PVA) showed potential uses in bone healing [11]. Cellulose is biocompatible [12] and has a fairly high tensile strength, the ability to store water in large quantities, high crystallinity, and easy to set up (high mouldability) [13]. The main issue in using this composite is that it is in solid form at room temperature and requires surgery to install. Our research here is focusing on formulation and synthesis of thermosensitive hydrogel from bacterial cellulose, that has liquid phase at room temperature so it is injectable and has solid phase at body temperature so the hydrogel solidifies.

2. Materials and Methods

2.1 Materials

Materials used in this study are commercial nata de coco as bacterial cellulose source, technical NaOH, Methyl cellulose SM4000, NaCl, KCl, Na₂HPO₄, KH₂PO₄, HCl 95%, Phosphat buffered saline
(PBS) solution, benzophenone, citric acid, and ethanol 95%. Home-made UV chamber was used for copolymerization process. The chamber has four 9W UV lamps.

2.2 Method

2.2.1. Synthesis of Cellulose Nanocrystals

The synthesis of cellulose nanocrystals consist of 2 steps: First pretreatment, nata de coco was soaked in NaOH 1 M for 2 h at 80°C then washed several times. Next, hydrolysis of cellulose was performed with 65% sulfuric acid for 1 h at 45°C. Then filtered using Whatman 42 filter paper several times until the solution became clear that indicated that most cellulose nanocrystals are on the filter paper and then dried.

2.2.2. Making MC Solution

The solvent used to make MC solution here were distilled water, NaCl solution, and PBS solution. MC solution was made by 2 step dissolution: The first used hot dissolution at 80°C and then continued with cold dissolution at room temperature.

2.2.3. Preparation of MC-CNCopolymer

The copolymer was made from MC and CN monomers. The CN monomer was prepared by sonication of CN in 30 mL of distilled water for 2 h. CN suspensions then stirred with a magnetic stirrer at a speed of 1500 rpm for 15 min. Benzophenone (0.01% g/v solution) was dissolved in 95% ethanol in 1 mL. Benzophenone solution and citric acid (at 0.05% g/v solution) was added to CN solution. The solution then combined with MC solution to achieve desired composition and stored in refrigerator for minimal 4 h. The mixture solution of MC and CN was poured into a petri dish and placed into UV-Chamber. UV exposure had been given for 30 min and then the copolymer product was stored in polyethylene plastic vials.

2.2.4. Characterization

CN size was characterized using a Particle Size Analyzer (PSA) machine. The viscosity of the polymer solution was measured using Brookfield RV viscometer at room temperature. FTIR characterization was conducted to analyze the copolymerization result by comparing the FTIR spectra of precursors with that of copolymer product. There are two methods of LCST measurement used in this study. The first is spectrophotometric method, used for polymer solutions with salinity variations without CN. For each polymer solution, transmittance of wavelength 500 nm was measured at temperatures varied from 30 to 70°C with 2°C interval. Then its LCST was determined at transmittance of 0.5. Spectrophotometric method could not be applied to copolymer samples because the samples were cloudy so the measurement would not be accurate. Instead the LCST was determined using Differential Scanning Calorimetry (DSC) tests by adding water as solvent and varying temperature from 30 to 58°C, then the results verified by visual observation.
2.2.5. Experimental Variation

Sample variation in the experiment shown in Table 1. Each sample is given a special name to make easy the analysis.

| No | Variables                          | Variations                     | Sample Name | No | Variables                          | Variations      | Sample Name |
|----|-----------------------------------|--------------------------------|-------------|----|-----------------------------------|-----------------|-------------|
| 1  | Methyl Cellulose and NaCl Concentrations (12 samples) | MC 1% - NaCl 0% | MC1             | 3  | Methyl Cellulose and PBS Concentration (9 samples) | MC 1% - PBS 0.5x | MCa         |
|    |                                   | MC 1% - NaCl 0.5%            | MC2         |    |                                   | MC 1% - PBS 1x  | MCb         |
|    |                                   | MC 1% - NaCl 0.7%            | MC3         |    |                                   | MC 1% - PBS 1.5x| MCc         |
|    |                                   | MC 1% - NaCl 0.9%            | MC4         |    |                                   | MC 2% - PBS 0.5x| MCd         |
|    |                                   | MC 2% - NaCl 0%              | MC5/A       |    |                                   | MC 2% - PBS 1x  | MCe         |
|    |                                   | MC 2% - NaCl 0.5%            | MC6         |    |                                   | MC 2% - PBS 1.5x| MCh         |
|    |                                   | MC 2% - NaCl 0.7%            | MC7         |    |                                   | MC 3% - PBS 0.5x| MCf         |
|    |                                   | MC 2% - NaCl 0.9%            | MC8         |    |                                   | MC 3% - PBS 1x  | MCI         |
|    |                                   | MC 3% - NaCl 0%              | MC9         |    |                                   | MC 3% - PBS 1.5x| MCG         |
|    |                                   | MC 3% - NaCl 0.5%            | MC10        |    |                                   |                 |             |
|    |                                   | MC 3% - NaCl 0.7%            | MC11        |    |                                   |                 |             |
|    |                                   | MC 3% - NaCl 0.9%            | MC12        |    |                                   |                 |             |
| 2  | Nanocrystal Cellulose (NC) Concentration (4 samples) | NC 0% | B               |    |                                   |                 |             |
|    |                                   | NC 0.1%                      | C           |    |                                   |                 |             |
|    |                                   | NC 0.3%                      | D           |    |                                   |                 |             |
|    |                                   | NC 0.5%                      | E           |    |                                   |                 |             |

3. Results and Discussion

3.1. Cellulose Nanocrystal

Cellulose particle size distribution is shown in Figure 1. Assuming all particle size are homogeneous, the smallest size of cellulose nanocrystal is 127 nm and the average size is 171 nm. Based on this distribution it can be stated that the cellulose nanocrystal has the right size distribution.
3.2. Copolymerisation

To evaluate the copolymerization of methyl cellulose and cellulose nanocrystal, the IR spectrum of the copolymer is compared to the spectrum of methyl cellulose (Figure 2). The copolymerization is indicated by weakening the -OH peak at wave number around 1315 cm⁻¹ that found in all samples. The peak weakening is caused by some -OH groups of CN react to form –C-O-C- groups. Another peak representing-OH groups with hydrogen bonds is indicated by the wave number 3500-3200 cm⁻¹, which was found in all samples, including the reference sample (A). The peak is weaker in all samples containing CN (C, D, E, F, and G), though the different may not be significant.
3.3. Effect of Salt Concentration

Viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. It is an important factor to understand the durability of the polymer solution. The results of viscosity measurements of polymer solution samples MC1-MC4 are presented in Figure 3. At concentration of 1% MC, the viscosity decreases when the RPM increases. The RPM increment is proportional to shear rate increment in the polymer solution. This means the MC solution has tension thinning properties. It is interesting to note here that increasing concentration of NaCl makes solution viscosity going up.

Viscosity measurements of polymer solution samples MC5-MC8 and MC9-MC12 is presented in Figure 4 and Figure 5. The addition of NaCl has almost no effect on the viscosity of the solution. As in concentrations of 1% and 2%, the 3% MC is also thinning tension. The higher the concentration of NaCl, the more viscous the solution will be.
3.4. Effect of PBS Concentration

Polymer solution viscosity of samples MCa-MCc, MCd-MCf, and MCg-MCi are presented in Figure 6, Figure 7, and Figure 8. Viscosity of 1% MC solution in PBS is lower compared to that of solution with the same concentration in NaCl. 1x PBS is equivalent to the concentration of NaCl 0.9%. The same phenomenon was also observed at MC concentration of 2 and 3%. The PBS concentration gives significant impact in sample MCd-MCf dan MCg-MCi, but not in sample MCa dan MCc.
3.5. Effect of CN Concentration

Samples B-E viscosities are presented in Figure 9 and Figure 10. Higher CN concentration produces more viscous copolymer solution. However, the viscosity values are lower than the viscosity of 2% MC solution. This lower viscosity is desirable and an advantage that makes the fluid injected easier.

3.6. LCST (Sol-Gel Temperature)

Results of LCST measurement using spectrophotometer are presented in Figure 11. While results of LCST measurement using DSC and visual observations are shown in Figure 12. From Figure 11 we can see that sample G compared to the other samples has LCST closest to body temperature (38°C).
Addition and grafting process in copolymerisation of CN and MC give effects on decreasing LCST. The higher CN concentration results in lower LCST as shown by data of sample C-E. This is due to MC and NC that merged into the copolymer. The existence of CN which is not soluble in water increases hydrophobic effect that appears when the temperature raises above the sol-gel temperature. The presence of salt will amplify the effect of CN on lowering LCST. The lowest LCST observed in sample G which contain 2% MC and 0.3% CN in 1.5x PBS solution. Ions from dissociation of the salt is the main factor affecting the decrease of LCST. Cations contained in the PBS is Na⁺ and K⁺, while the anion-anion is Cl⁻, H₂PO₄⁻ and H₃PO₄⁻. Anion plays a major role in lowering the sol-gel
temperature. Hofmeister series gives an idea of how strong anion can make the process of sol-gel phase changes. Hofmeister series ions actually shows ability to precipitate proteins but the same phenomenon is also observed in the solution of MC[14].

Anions of Hofmeister series are: citric$^->$ tartaric$^=#$ SO4$^{2-}$ > HPO4$^{2-}$ > CrO4$^{2-}$ > acetate $>$ HCO$^{3-}$ > Cl$->$ NO3$->$ CLO3$[14]$. Anions are located on the right called cosmotrof and have hygroscopic nature. In PBS solution, H$_2$PO$_4$ has most dominant effect in lowering the LCST either MC or MC-CN copolymer solution. The effects of these ions are also observed in the results of viscosity measurements. Increasing the salt concentration increases the viscosity. Ions from salt will disturb hydrogen bonding in water and raises solvation. Ion solvation phenomenon is shown in Figure 12 [15].

![Figure 13. Solvation Process. Anion (left) and Kation (right)](image-url)

4. Conclusion

Formulation and synthesis of a hydrogel having LCST near body temperature had been made from copolymers of methyl cellulose (MC) and cellulose nanocrystals (CN) that were prepared by the grafting method using UV light. Higher concentration of MC and CN in the solution will be lowering LCST and increasing solution viscosity. Higher concentration of NaCl and PBS in the solution will also be lowering LCST and increasing solution viscosity. All samples of the polymer solution with concentrations of 1% and 2% MC have viscosities less than 5000 cp at room temperature. Copolymer solution with CN concentration of 0.1, 0.3, and 0.5% have LCST of 55°C, 51°C, and 41°C, respectively. Copolymer solution of MC-CN with PBS concentration 1x and 1.5x have LCST of 47°C and 38°C, respectively. Best formulation obtained in this study to produce LCST as close as possible to body temperature is at 2% MC, 0.3% CN, and 1.5x PBS solution. It would be possible to get a better formula having LCST below body temperature by further optimization and modification of the present result.

References

[1] Jun L X 2009 Smart Biomaterials Dissertation National University of Singapore.
[2] Chu P K and Liu X (Ed) 2008 Biomaterials Fabrication and Processing HANDBOOK (CRC Press, Boca Raton).
[3] Amin M et al 2012 Carbohydrate Polym. 88 465-473.
[4] Liao H T et al 2011 Tissue Engineering: Part C17 (11) 1139–1149.
[5] Kopeczek J and Yang J 2007 Polymer Int. 56 1078–1098.
[6] Li L et al 2002 Langmuir 18 7291-7298.
[7] Zhang Y et al 2014 Carbohydr. Polym. 101 171–178.
[8] Martin B C et al 2008 J. Neural Eng. 5 221–231.
[9] Tang Y et al 2010 Carbohydr. Polym. 82 833–841.
[10] Rimdusit S et al 2012 Engineering J. 16(4).
[11] Abidin A Z and Graha H P R 2014 Adv. Mater. Res. 950 24-28.
[12] Esposito A et al 2005 J. Biomater. 26 4101–4110.
[13] Svensson A et al 2005 Biomaterials 26(4) 419-31.
[14] Bain M K et al 2012 Int. J. Biological Macromol. 51 831–836.
[15] Rosler E 2013 The Hofmeister series a review of monatomic ions near the hydrophobic/water interface Thesis. University of Amsterdam.