Preparation and Characterization of Local Indonesian Chitosan-graft-Maleic Anhydride as Drug Carrier

Daniel Timotius¹, Rochmadi¹, Yuni Kusumastuti¹,⋆
¹Department of Chemical Engineering, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

⋆yuni_kusumastuti@ugm.ac.id

Abstract. The modification of local Indonesian chitosan as a drug carrier is conducted by grafting with maleic anhydride (MA). The grafted film resulting in a negative charge of carboxyl group attach on chitosan backbone. Several ratio of MA/chitosan is conducted to evaluate the effect of MA on the drug release behavior. Chitosan and MA was dissolved and stirred in 1% (v/v) acetic acid for 24 hours. Tween 80 and curcumin are added to the solution as surfactant and drug model respectively. The homogeneous solution is casted into polyethylene petri dish and dried under room temperature. Chitosan-graft-MA is characterized by Fourier Transform Infrared (FTIR) in addition to gravimetric titration to characterize the existing of carboxyl groups of the films. The swelling and degradation ability in phosphate buffered saline (PBS) solution is showed an increasing trend as the addition of MA. The kinetic study of drug release is performed in Peppas and first order model. An optimum condition of grafting is achieved at 150% (w/w).

1. Introduction
Chitosan, (1-4)-2-amino-2-deoxy-β-D-glucan, is the second abundant polysaccharide after cellulose [1] and can be obtained by deacetylation of chitin from exoskeleton of crustacean [2]. The properties of chitosan such as biodegradation, biocompatible and non-toxic make it to be a good materials in biomedical application [3], [4]. Chitosan (≥50 % degree of deacetylation) is soluble in dilute acid solutions [5]. The solubility occur due to protonation of –NH₂ which lead to formation of polycationic polymer [6]. It is also known that chitosan has a good film forming properties [5], [7]–[11].

In order to improve on some properties of chitosan film (e.g. mechanical properties, swell-ability, pH sensitivity) it is commonly use crosslinking method [12]. There are at least 2 classification of crosslinking method on chitosan, chemical crosslinking and physical crosslinking [6]. Chemical crosslinking provide better mechanical strength than physical crosslinking [13]. However, some chemical crosslinker are tended to be toxic [12]. Some researchers have begun to find a cross-linker with low toxicity [7], [8], [10], [14]. MA becomes one of the candidates as crosslinker. A group of researchers [15] have reported that modification hyaluronic acid by MA tend to be non-toxic and showing a good drug release behavior on pilocarpine hydrochloride drug model. In another study [16], addition of MA on chitosan shows a good swelling behavior and antimicrobial properties.

The grafting of chitosan by MA will lead to appearance of carboxyl group attached on chitosan backbone. The carboxyl group will donate negative charges on the film. The double bond from MA also can be used for further modification. In this research, we develop a local Indonesian chitosan film grafted by MA as a potential patch drug delivery system and wound dressing application.

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2. Materials and Methods

2.1. Materials

A local Indonesian chitosan (>90% degree of deacetylation, 10-500 cps viscosity, <1.5% ash content, 0.5% protein content) was obtained from PT Biotech Surindo (Cirebon, Indonesia). Acetic acid (100%) was purchased from Merck, Germany. Tween 80 was supplied by Merck, France. Curcumin was obtained from Bio Basic Inc, Canada.

2.2. Film preparation

Preparation of chitosan grafted MA solution was similar to a study [17] with some modification. Chitosan and MA were weighed about 0.6 g and 0.3 g respectively, then dissolved and stirred in 30 mL of acetic acid (1% v/v) for 24 hours. Tween 80 and curcumin were added to the solution followed by stirring to form homogeneous solution. About 5 mL of homogeneous solution was casted on petri dish and dried under ambient temperature to form a thin film. This method was repeated with several ratios of MA as presented in Table 1. All ratios in Table 1 were based on chitosan weight.

Table 1. Composition of each sample

| Samples | MA (% w/w) | Tween 80 (% w/w) | Curcumin (% w/w) |
|---------|------------|------------------|-------------------|
| CM0     | 0          | 70               | 20                |
| CM50    | 50         | 70               | 20                |
| CM100   | 100        | 70               | 20                |
| CM150   | 150        | 70               | 20                |

2.3. Fourier Transform Infrared (FTIR) Spectroscopy

The functional groups of samples was measured by FTIR spectroscopy. The FTIR study was performed by SHIMADZU IRPrestige-21 in the range of 400 – 4000 cm⁻¹. All the samples were scanned with a resolution of 4 cm⁻¹ and 10 number of scans.

2.4. Carboxyl Content Measurement

In order to measure the amount of carboxyl group in modified chitosan film, back titration method was performed [15]. About 20 mg of each sample was dissolved in 20mL of NaOH solution (0.005 N) and followed by titration using HCl (0.005 N). The data were calculated by Equation (1) to obtain the acidic contents for each sample.

\[
\text{Acidic content} = \frac{C_{NaOH} \times V_{NaOH} - C_{HCl} \times V_{HCl}}{m_{sample}}
\]  

(1)

2.5. Swelling Ratio and Degradation Study

The swelling and degradation properties were measured by immersing the sample in PBS solution. The swelling method was similar to a study [7]. Swelling ratio was done by immersing Ø15mm of film for 30 minutes, while for degradation study was continued for 24 hours. The immersing of film in PBS was done under normal physiological temperature (about 37 °C). The film was weighed before and after immersing (wet weight for swelling ratio and dried weight for degradation study). The obtained data were calculated by Equations (2) and (3) for swelling ratio and degradation study respectively.

\[
\text{Swelling Ratio}(\%) = \frac{W_t}{W_0} \times 100\%
\]  

(2)

\[
\text{Degradation}(\%) = \frac{W_D - W_t}{W_0} \times 100\%
\]  

(3)

2.6. Drug Release Kinetics

The drug release kinetics was measured by immersing Ø10 mm of film in 20 mL PBS solution under constant stirring rate. Concentration of curcumin was analysed at periodic interval with
spectrophotometer VIS. Optimum wave length for curcumin was attained at 420 nm. The concentration was calculated by calibration curve. The drug release model was followed first-order and Peppas model. It was commonly used to express non-constant release from any reservoir [18]. The model was presented in Equation (4) and (5) for first order and Peppas model respectively:

\[
\frac{M_t}{M_\infty} = 100(1 - e^{-kt})
\]

(4)

\[
\frac{M_t}{M_\infty} = Kt^n
\]

(5)

3. Result and discussion

3.1. FTIR Spectroscopy

A study on FTIR spectroscopy was conducted to analyze the functional group of modified chitosan. The result of FTIR spectroscopy study is presented in figure 1. This result is similar from other study [16]. The scanning result for chitosan show peaks at 1651.07 cm\(^{-1}\) and 1381.03 are belong to amide I and amide II functional group. While the broad band around 3425.58 cm\(^{-1}\) is signed as –NH and –OH stretching.

The appearance peak around 1712.79 cm\(^{-1}\) in CM50 is belong to carboxyl group that attach to the chitosan backbone. This result was similar to other study that use MA to modify hyaluronic acid [15]. In another study, the researcher modify cellulose nanocrystals by MA [19] and show the appearance of carboxyl group around 1724 cm\(^{-1}\).

![Figure 1. FTIR Spectrum for MA, Cts, and CM50.](image)

3.2. Carboxyl Content Measurement

Back titration method was effective to be used in measuring the acidic content of the films. The result is presented in figure 2. An increasing trend of the acidic content in each sample confirm that the grafting method was successfully done. The carboxyl content can be obtained by calculating the difference on acidic content of CM50, CM100, and CM150 compared CM0. The amount of carboxyl group in CM50, CM100, and CM150 are 2.15±0.08; 3.70±0.02; and 4.35±0.41 respectively. The increasing values of acidic contents occur due to the variation of ratio used in this study. This result is similar to a study that using MA to modify hyaluronic acid in form of gels [15].
3.3. Swelling Ratio and Degradation Study

The swelling ability of film is important, since the drugs have to diffuse through film matrix. It also can demonstrate the ability of exudates absorption for some specific wound cases. The result for swelling behavior for each samples are shown in figure 3. The swelling of samples tend to increase as the addition of MA. This may occur because of the appearance of new hydrophilic carboxyl group on the chitosan backbone [7]. The value of swelling ratio for CM0, CM50, CM100, and CM 150 are 176.67±2.89%; 221.93±16.32%; 309.65±17.48%; and 239±25.63% respectively.

While the degradation study was done to determine the degradation ability of each sample. Figure 4 shows the degradation of film after 24 hours of immersing. As it is shown in figure 4, the addition of MA lead to increase the degradation of film.
3.4. Drug Release Kinetics

Curcumin concentration along the time for each samples are presented in figure 5 for First Order Model and figure 6 for Peppas Model. It can be seen that a burst release occur at the beginning (around the first 30 min). It may be caused by the dissolving of drugs at the surface of film. The concentration of drugs are not significantly increasing after 30 minutes. It means that the release rate is getting slower as time goes by. The concentration properties of each samples also confirm that chitosan grafted with MA will decrease the drug release rate. The constant values of CM0, CM50, CM100, and CM150 are presented in Table 2.

The values of n in Peppas Model indicate the diffusion mechanism of drug. The results show that the release of curcumin in film is following the Fickian diffusion mechanism (n ≤ 0.45) [20]. While first order kinetic model explain that the release of drug is following first order reaction kinetic. The reaction is assumed as the change of drug phase from solid to liquid. The values of k are the kinetic reaction constant of each samples.

![Figure 4. Degradation of CM0, CM50, CM100, and CM150.](image)

![Figure 5. Concentration Profile of Curcumin for First Order Model.](image)
Figure 6. Concentration Profile of Curcumin for Peppas model.

Table 2. Constant Values of Each Samples and Models.

| Samples  | k       | n   | K (1/min) |
|----------|---------|-----|-----------|
| CM0      | 38.6037 | 0.1896 | 0.0553    |
| CM50     | 88.7728 | 0.0339 | 2.0732    |
| CM100    | 11.0325 | 0.4042 | 0.0159    |
| CM150    | 13.1170 | 0.3739 | 0.0178    |

4. Conclusions
Indonesian chitosan film grafted by MA has been successfully produced. The FTIR analysis showed an appearance of carboxyl functional group in modified chitosan film. The increasing of acidic content also confirm that carboxyl groups are found in the modified film. Swelling ratio and degradation ability of film also increased as the ratio of MA was increased. The addition of MA lead to decrease the release rate of drug in PBS solution. The drug release mechanism for curcumin in film is following Fickian diffusion mechanism. While the optimum ratio of MA/chitosan is 150% (w/w).

5. References
[1] Ali A and Ahmed S 2018 A review on chitosan and its nanocomposites in drug delivery Int. J. Biol. Macromol. 109 273–286
[2] Sinha V R, Singla A K, Wadhawan S, Kaushik R, Kumria R, Bansal K and Dhawan S 2004 Chitosan microspheres as a potential carrier for drugs Int. J. Pharm. 274 1–33
[3] Kean T and Thanou M 2010 Biodegradation, biodistribution and toxicity of chitosan Adv. Drug Deliv. Rev. 62 3–11
[4] Mengatto L, Helbling I and Luna J 2012 Recent advances in chitosan films for controlled release of drugs Recent patents drug Deliv. formulation 6 156–170
[5] Liu Y, Shen X, Zhou H, Wang Y and Deng L 2016 Chemical modification of chitosan film via surface grafting of citric acid molecular to promote the biomineralization Appl. Surf. Sci. 370 270–278
[6] Lawrie G, Keen I, Drew B, Chandler-Temple A, Rintoul L, Fredericks P and Grøndahl L 2007 Interactions between alginate and chitosan biopolymers characterized using FTIR and XPS Biomacromolecules 8 2533–41
[7] Escárcega-Galaz A A, Sánchez-Machado D I, López-Cervantes J, Sanches-Silva A, Madera-
Santana T J and Paseiro-Losada P 2018 Mechanical, structural and physical aspects of chitosan-based films as antimicrobial dressings *Int. J. Biol. Macromol.* **116** 472–481

[8] Costa-Júnior E S, Barbosa-Stancioli E F, Mansur A A P, Vasconcelos W L and Mansur H S 2009 Preparation and characterization of chitosan/poly(vinyl alcohol) chemically crosslinked blends for biomedical applications *Carbohydr. Polym.* **76** 472–481

[9] Pratt D Y, Wilson L D and Kozinski J A 2013 Preparation and sorption studies of glutaraldehyde cross-linked chitosan copolymers *J. Colloid Interface Sci.* **395** 205–211

[10] Hamedi H, Moradi S, Hudson S M and Tonelli A E 2018 Chitosan based hydrogels and their applications for drug delivery in wound dressings: A review *Carbohydr. Polym.* **199** 445–460

[11] Pellá M C G, Lima-Tenório M K, Tenório-Neto E T, Guilherme M R, Muniz E C and Rubira A F 2018 Chitosan-based hydrogels: From preparation to biomedical applications *Carbohydr. Polym.* **196** 233–245

[12] Berger J, Reist M, Mayer J M, Felt O, Peppas N A and Gurny R 2004 Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications *Eur. J. Pharm. Biopharm.* **57** 19–34

[13] Hasipoglu H N, Yilmaz E, Yilmaz O and Caner H 2005 Preparation and characterization of maleic acid grafted chitosan *Int. J. Polym. Anal. Charact.* **10** 313–327

[14] Yucel S, Ozdemir Z O, Kesgin C, Terzioglu P, Unlu S and Erdogan Y 2013 Swelling Behavior and Cytotoxicity of Maleic Acid Grafted Chitosan *Int. J.* **7** 232–235

[15] Hernández-Moreno D, de la Casa Resino I and Soler-Rodríguez F 2014 Maleic Anhydride *Encycl. Toxicol. Third Ed.* **3** 138–141

[16] Vasi A M, Popa M I, Butnaru M, Dodi G and Verestiuc L 2014 Chemical functionalization of hyaluronic acid for drug delivery applications *Mater. Sci. Eng. Mater. Biol. Appl.* **C 38** 177–185

[17] Lavanya R, Gomathi T, Vijayalakshmi K, Saranya M, Sudha P N and Anil A 2017 Adsorptive removal of copper (II) and lead (II) using chitosan-g-maleic anhydride-g-methacrylic acid copolymer *Int. J. Biol. Macromol.* **104** 1495–1508

[18] Coutts-Lendon C A, Wright N A, Mieso E V and Koenig J L 2003 The use of FT-IR imaging as an analytical tool for the characterization of drug delivery systems *J. Control. Release* **93** 223–248

[19] Zhou L, He H, Li M, Huang S, Mei C and Wu Q 2018 Enhancing mechanical properties of poly(lactic acid) through its in-situ crosslinking with maleic anhydride-modified cellulose nanocrystals from cottonseed hulls *Ind. Crop. Prod.* **112** 449–459

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