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Proposed Reaction Mechanism of Chitosan-graft-Maleic from Chitosan and Maleic Anhydride

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Abstract. Chitosan (Ch), a natural polysaccharide derived from chitin is a widely used raw material in biomaterial research. Modification of chitosan using maleic anhydride (MA) have been done by several researchers with dimethyl sulfoxide (DMSO) and acetic acid as a common solvent. In the present work the chitosan was grafted with maleic anhydride using acetic acid and water as a solvent toward ecofriendly functionalization process. In addition, proposed mechanism of this grafting was further studied. The total acidic content and conversion of grafted films were calculated with varying concentration of MA:Ch ratio and temperature. The success of chitosan grafting with maleic was verified by Fourier Transform Infrared Spectroscopy (FTIR) analysis. FTIR analysis showed the appearance of new ester group and shifting of amide group from FTIR spectra which indicates that the esterification and amidation occurred in this grafting process.

1. Introduction

In the last few decades, research about biomaterials has become an interesting topic. In order to meet the requirements for biomedical applications, suitable materials with good biocompatibility and excellent properties is needed. One of prospective material for biomaterial is chitosan. Chitosan, (1-4)-2-amino-2-deoxy-β-D-glucan, is the second most abundant polysaccharide after cellulose [1]. It can be obtained by deacetylation of chitin [2]. Chitosan is indicated by the amount of degree of deacetylation (DD). DD is a parameter that indicates the presence of amino groups inside polymers. It can be called as chitosan if the DD is greater than 50%, while chitin is indicated by the amount of DD less than 50%. Higher DD means that chitosan has better solubility than chitin in aqueous acid solution [3].

Chitosan is commonly used as biomaterial because it has good antibacterial properties [4] and swelling ability [5]. It also has a good film forming ability [6] which is useful to form a thin film. In addition, It has many active functional groups (amine and hydroxyl groups) along the chains which could be easily modified to form better film properties. Dilution of chitosan in acid solution (below pH 6.3) can lead to protonation of amine group and convert it into ammonium groups [7].

Chitosan can be modified with maleic anhydride (MA) to add carboxyl group. Hasipoglu *et al* (2005) [8] already researched on chitosan-graft-maleic acid in acetic acid solution using ceric ammonium nitrate (CAN) as the redox initiator. Similar procedure to synthesize chitosan-graft-maleic was also carried out by other researchers [5]. They investigated the swelling behaviour of chitosan-graft-maleic for several pH values and also conducted the antibacterial activity test for the obtained samples. The result showed that chitosan-graft-maleic have a pH sensitive swelling behavior and antibacterial activity.

There was also an investigation on maleilated chitosan (MCS) [9]. A modified chitosan using MA was carried out and stirred in DMSO for 24 hours. The obtained maleilated chitosan then further crosslinked with methacrylated poly(vinyl alcohol) (MPVA) by UV irradiation to promote photo-crosslinking between vinyl group from MCS and MPV in the form of nanofibers. The nanofibers could be used as wound dressing materials.

The use of dimethyl sulfoxide (DMSO) and acetic acid as solvent to graft maleic anhydride into chitosan has done in the past by researchers. In this work, chitosan was grafted with maleic anhydride in acetic acid and water as solvent toward ecofriendly functionalization. Then, the grafted chitosan would be analyzed with Fourier Transform Infrared Sprectroscopy (FTIR). Total acidic content of films both in acetic acid and water as a solvent were measured. Further, study on grafting reaction mechanism was done.

2. Methods

2.1. Materials

Low molecular wight chitosan was obtained from Sigma Aldrich, USA (75 – 85% DD). Maleic Anhydride (MA) (≥99%) was purchased from Nacalai Tesque Inc, Japan. Acetic acid was supplied by Merck, Germany (100%).

2.2. Grafting Preparation

The solution was prepared by dissolving MA (1; 2; 4 g) in distilled water (100 mL) followed by chitosan (2 g). The reaction was then carried at 30; 50; 70 oC and stirred at constant speed for 5 hours. Then about 10 mL of the solution was casted on petridish (Ø10 cm) and dried in oven for 24 hours at 50 oC. The dried film was washed by large amount of acetone (25% v/v) to remove the unreacted MA. To compare the result between films that using distilled water as solvent and acetic acid as solvent, the same method was carried out by dissolving MA in acetic acid (1% v/v) instead of distilled water. For standardization purpose, chitosan (2 g) was dissolved in acetic acid (1% v/v) and stirred for 5 hours at 30 oC. Each sample was encoded as Ch for standard, Ac2170 for acetic acid solution, and W1230 for distilled water. The first number indicates weight of MA, while the second shows the weight of chitosan, and the last two numbers represents the temperature. Therefore the samples code would be Ch; Ac4270; W1230; W2230; W4230; W4250; and W4270.

2.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed to investigate the change of functional groups in chitosan and chitosan-graft-maleic. In this research SHIMADZU IRPrestige-21 was used in the range of 4000-400 cm-1. Each of the samples were scanned using KBr method.

2.4. Total Acidic Measurement

The amount of total acidic content was measured using back titration method [10]. About 10 mg of sample was dissolved in 20 mL of NaOH solution (0.01 M), then titrated by HCl (0.01 M). The obtained data was calculated using Eq. (1) to get the total acidic content (AC, mmol/mg).

$$AC = \frac{C_{NaOH} \times V_{NaOH} - C_{HCl} \times V_{HCl}}{m_{sample}} \tag{1}$$

Where C_{NaOH} represents the concentration of NaOH (M); while V_{NaOH} is the volume of NaOH (mL); C_{HCl} is the concentration of HCl (M); V_{HCl} is the volume of HCl (mL); and m_{sample} is the mass of sample (mg).

Assuming that the amount of acidic contents describes the amount of MA attached in chitosan backbone as explained in Fig 3, the conversion of MA reacted can be calculated (X_{MA}) as:

$$X_{MA} = \frac{AC_{Washed}}{AC_{Unwashed}} \tag{2}$$

3. Results and Discussions

3.1. Physical Appearance

The physical appearance of unwashed and washed film is shown on Fig 1a and 1b respectively. The result shows that the unwashed W4270 has a white color while the washed W4270 has a transparent appearance. The white color in unwashed W4270 is suspected to be unreacted MA. The films then washed with large amount of acetone (25% v/v) to remove the excess of MA. From the investigation, the appearance of white color only occurs when the ratio of MA:Chitosan is 2:1. However, the washing is done for all samples to ensure that unreacted MA is removed.

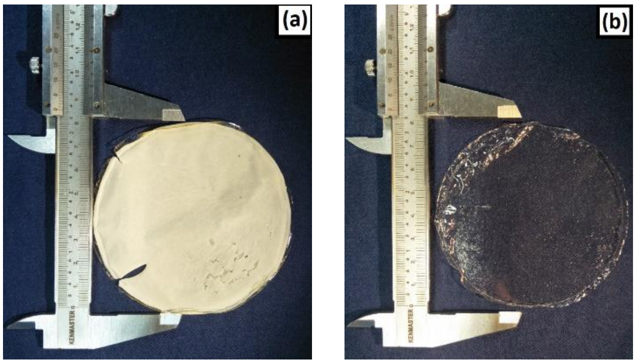

Figure 1. Physical Appearance of Unwashed (a) and Washed (b) W4270.

3.2. FTIR Study

The possible functional groups from the samples can be obtained from FTIR. The FTIR spectra of maleic anhydride is shown in Fig. 2a. The obtained peaks are compared with several studies [5], [8], [11]–[13]. The broad band around 3000 cm^{-1} appeared due to the stretching of carboxylic acid (hydrogen bonding). Strong absorption at 1890.24 and 1705.07 cm^{-1} belongs to asymmetrical and symmetrical C=O stretching of cyclic anhydride molecules. Meanwhile, the peak at 1589.34 cm could be assigned as C=C stretching of maleic anhydride. The stretch of C–O–C for maleic anhydride appears at 1265.30 cm^{-1}. Chitosan spectra on FTIR (Fig. 2b) shows that a broad absorption around 3500 that belongs to O–H and N–H stretching (hydrogen bonding). The presence of amide I and amide II are appeared on 1635.64 and 1558.48 cm^{-1} respectively, while the C–N peak present at 1373.32 cm^{-1} below. The ether stretching of chitosan show at 1080.14 cm^{-1}.

In order to prove that the reaction is success, the FTIR spectra of unwashed and washed Ac2170 were studied. Fig 2c shows several strong bands from maleic anhydride that still appears on the spectra, especially around bandwidths of 3000 cm-1, 1897.95 cm-1, 1589.34 cm-1, 1465.90 cm-1 and 1265.30 cm-1. As it washed with acetone 25% (v/v), the unreacted maleic anhydride then disappeared from the film. It is proved by the disappearance of those bands from maleic anhydride. A little shifting from amide I and amide II on washed Ac2170 spectra indicates that the amidation has taken place. The appearance of strong peak at 1705.01 cm-1 for washed Ac2170 (Fig 2d) proves that the grafting process also occurred in the hydroxyl groups (C=O from ester group of grafted maleic) [14]. The disappeared peak at 1265.30 cm-1 (Fig. 2d and 2e) shows that the ring opening mechanism of chitosan graft maleic is success Those results above show that maleic anhydride is successfully grafted to chitosan through ring opening mechanism. Further discussion about reaction mechanism is explained below.

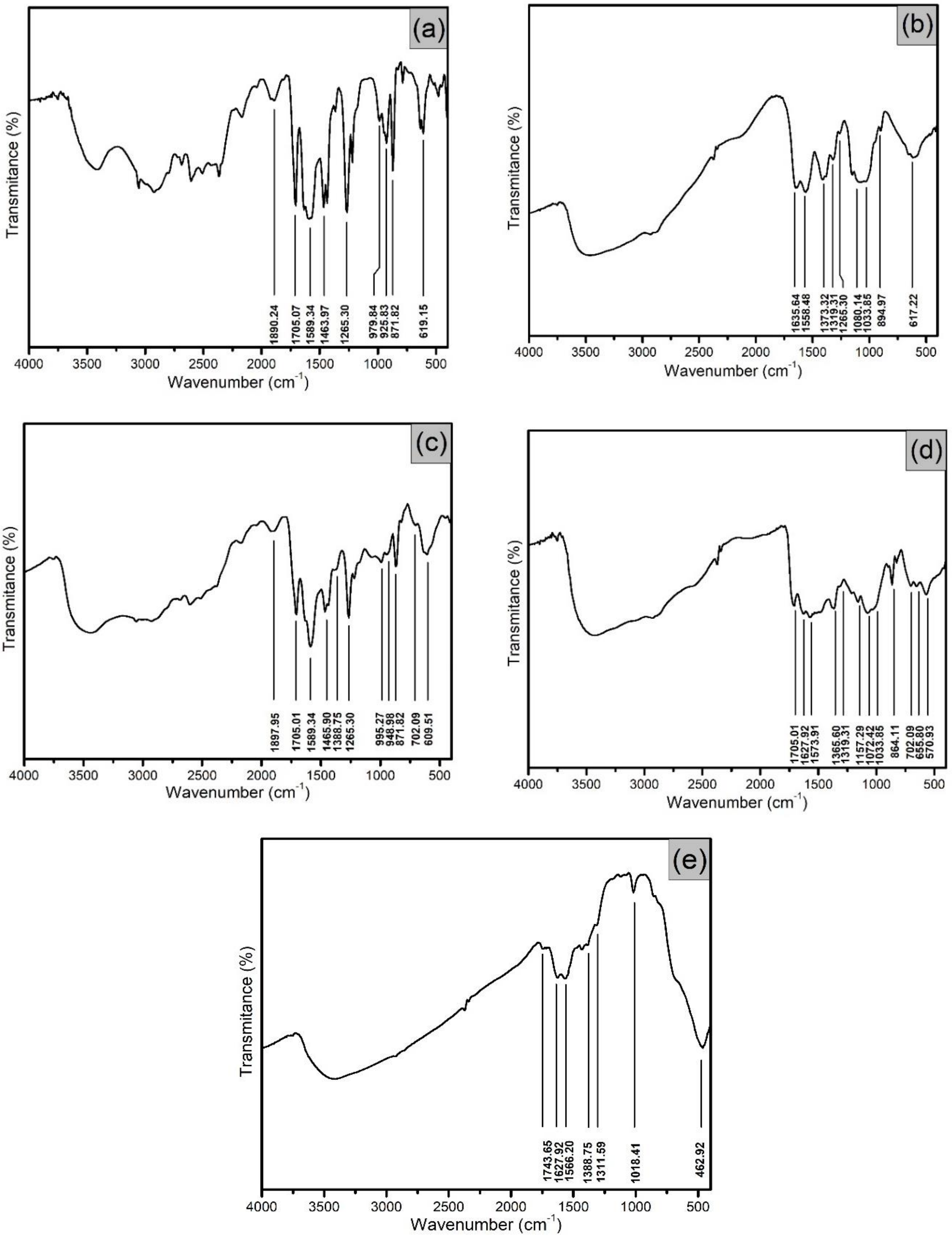

Figure 2. FTIR Spectra for Maleic Anhydride (a), Ch (b), Unwashed Ac2170 (c), Washed Ac2170 (d), and W4230 (e).

3.3. Reaction Mechanism

From the study of FTIR it can be concluded that reaction of chitosan with maleic anhydride follows two kind of reactions, specifically amidation of primary amine group and esterification of hydroxyl group [15]. The general mechanism is presented on Fig. 3. Free electron pairs of chitosan (both from hydroxyl and primary amine group) will attack the carbon atom on carbonyl group of maleic anhydride which results in transition product of tetrahedral. The next step is ring opening of maleic anhydride, followed by the attack of water molecules on the hydrogen so that they become hydronium ions. The hydronium ions will have an equilibrium with carboxyl and amine group from chitosan-graft-maleic. This equilibrium is influenced by several factors, such as initial acid concentration and type of acid which requires further study [16].

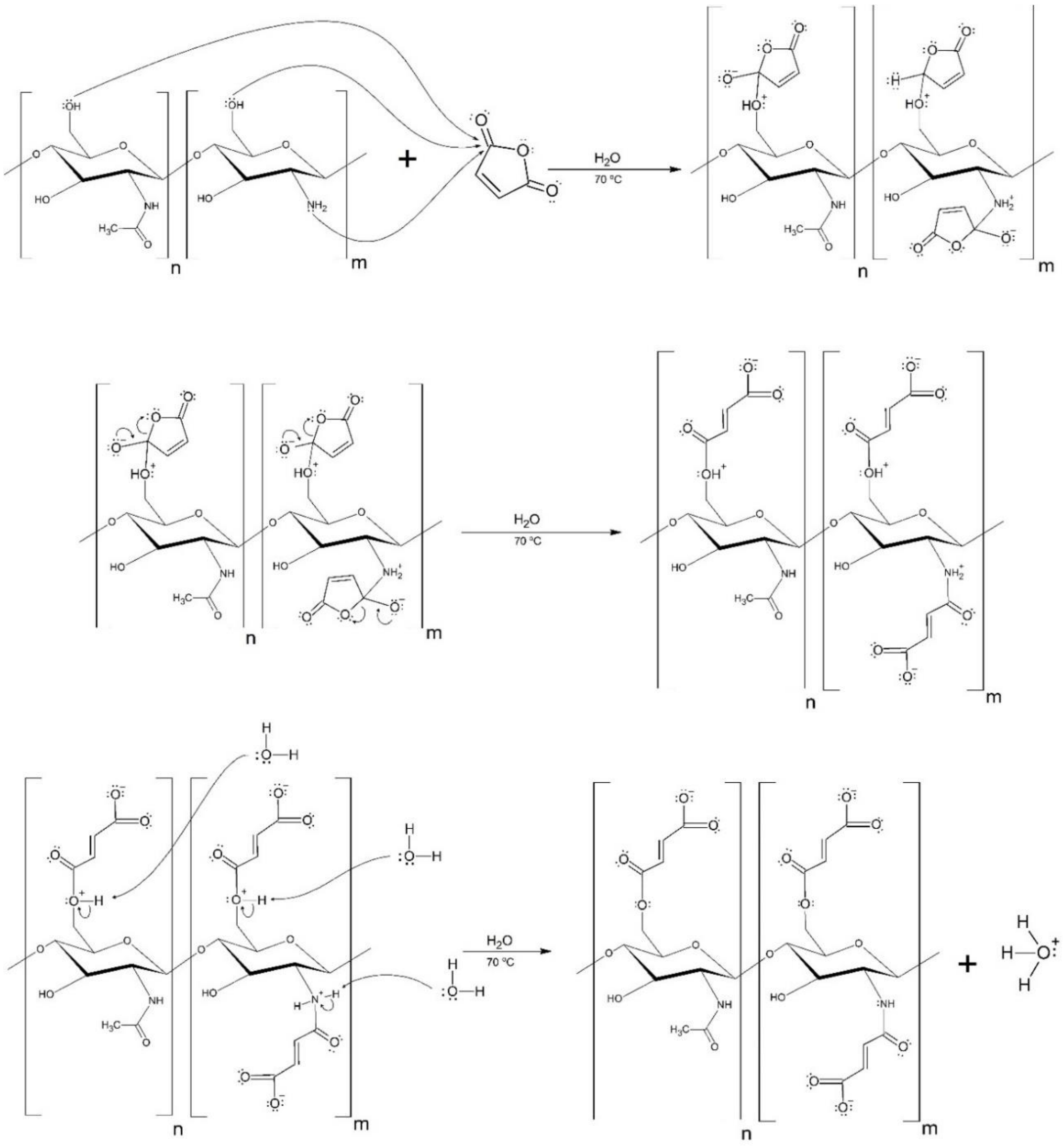

Figure 3. Reaction Mechanism of Chitosan-graft-Maleic in Water

The presence of carboxyl groups on the chitosan backbone give contribution on the appearance of negative charges in chitosan. Since chitosan becomes natural cationic polymers after being dissolved in acid solution, the appearance of anion molecules would turn chitosan into a polyampholyte [14], [17], a pH sensitive material with an ability to adsorb protein molecules. This material could be useful for medical applications such as membrane, drug delivery, and tissue engineering.

3.4. Total Acidic Content

Total acidic content of all the samples are calculated with equation (1). The comparison among samples are presented on Fig 4, which show that increasing the weight ratio of MA would increase total acidic content of the product. However, it also raise the amount of unreacted MA. Total acidic content is already utilized by other researcher to measure the amount of carboxyl group from modified hyaluronic acid [11]. Furthermore, increasing the temperature would also lead to raise the total acidic content.

Assuming that the amount of acid represents the amount grafted MA, the conversion of MA could be calculated with equation (2) as presented on Table 1. It shows that the conversion of W1230 and W2230 are similar, meanwhile the conversion of W4230; 4250; 4270 are less than W1230 and W2230. Even though they have smaller conversion than those with smaller ratio of MA, the amount of total acidic content raised as the ratio increased, just as another studies' result [8].

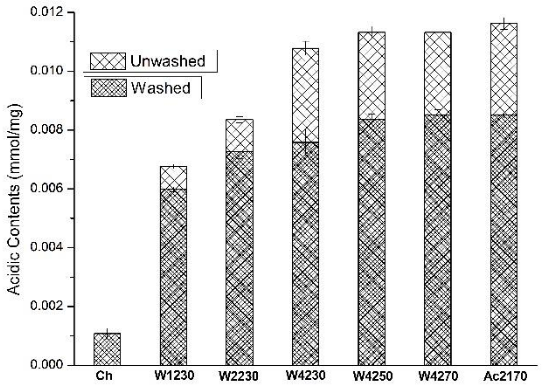

Figure 4. Total Acidic Content of Each Sample

Table 1. Conversion of Each Samples

Samples	Conversion, %
W1230	88.46 ± 1.22
W2230	88.98 ± 2.76
W4230	63.06 ± 3.86
W4250	73.15 ± 1.61
W4270	75.00 ± 1.62

4. Conclusions

Maleic anhydride was successfully grafted to chitosan by using acetic acid solution and distilled water as solvent. The grafting mechanism consists of esterification and amidation of chitosan as confirmed by FTIR. The result shows that increasing the temperature will increase the conversion of chitosan. Further study about the interaction between chitosan and maleic anhydride are still necessary to understand the behaviour and characteristic of chitosan-graft-maleic. Another study on correlation to measure the conversion is needed, since the actual interaction between hydronium, carboxyl, and amine group is an equilibrium.

Acknowledgments

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