

Acetylation of Nata de coco (bacterial cellulose) and membrane formation

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Abstract. Nata de coco (NDC), a bacterial cellulose formed by *Acetobacter xylinum*, was utilized to fabricate a membrane via acetylation and phase inversion methods. The NDC was activated and dissolved in N,N-Dimethylacetamide (DMAc) with lithium chloride (LiCl) at varying amounts of NDC, LiCl/DMAc ratio, activation temperature, and dissolution temperature. Acetylation was done by adding acetic anhydride (in a mass ratio of 1:12 NDC-anhydride) to NDC-DMAc/LiCl solution at a dissolution temperature of 110 °C for 3 hours. The modified-NDC was recovered via precipitation in methanol. The modified-NDC was washed with deionized water then freeze-dried. Modification was verified by determining the degree of substitution (DS) using titration and FTIR analysis. It was observed that the modification could be carried out at an NDC/DMAc (w/v) ratio of 1:75 at 120 °C for 1 hour, and addition of 8% (w/v) LiCl catalyst at 110 °C for 20 minutes. The DS of the modified-NDC was observed in the range of 2.84 – 3.69, which indicates a successful modification. This was further verified by the FTIR results. Membrane fabrication was carried out using the modified-NDC via immersion-precipitation and solvent evaporation methods. A successful membrane formation was observed using solvent evaporation.

1 Introduction

Nata de coco (NDC), a bacterial cellulose (BC), is a popular gelatinous delicacy in most Southeast Asian countries produced via fermentation of coconut milk assisted by a bacterium belonging to the genus *Acetobacter*. Although it is generally consumed as food, there has been a significant interest to NDC among the scientific community due to its purity and physio-mechanical properties.

Investigation on the potential of BC as a polymer composite greatly emerged in the recent years. In a study by Lima et al. [1], acetylation of bacterial cellulose was performed in the BC dissolve N,N-dimethylacetamide/ Lithium chloride (DMAc/LiCl) ionic solvent system. The degree of substitution (DS) was examined at various cellulose-acetic anhydride ratios of 1:50, 1:12, and 1:60. Furthermore, the reaction temperature was also varied. It was determined that the acetylation procedure done with 1:12 ratio at 110 °C would give the highest yield. The result of the degree of substitution for this system was 2.61. However, it was reported that the bacterial cellulose has lost its crystallinity, becoming amorphous.

There are other studies that dealt with the acetylation of dissolved cellulose in DMAc/LiCl system using acetic

anhydride. Ramos et al. [2] conducted the acetylation at 110 °C for 4 hours with varying acetic anhydride volume. They reported a DS ranged of 0.28-2.8. At higher acetic anhydride volume, the DS also increased the production of polysubstituted derivatives of cellulose. Ass [3] acetylated sisal and linter cellulose at 110 °C for 4 hours and 1 hour, respectively. The reported DS was 1.3 for sisal and 1.4-1.5 for linter cellulose. Lastly, El Seoud et al. [4] acetylated plant cellulosic materials at 60 °C for 18 hours. The acetic anhydride volume was also varied, thus resulting to a wide range of DS acquired. The reported values ranged from 1.5 to 4.5. These studies show that degree of acetylation increases with increasing anhydride concentration and increasing acetylation time.

As far as membrane fabrication is concerned, Dang et al. [5] synthesized forward osmosis (FO) composite membranes from *Acetobacter xylinum* modified by sodium alginate and cross-linked with calcium chloride. The study showed that the modified BC sheet material was suitable as FO membrane as the salt rejection was relatively high (98.57%). However, it has lower water flux (4.80 LMH) compared to 9.283 LMH of the pristine BC membrane.

In this study, cellulose acetate (CA) from NDC was synthesized through homogeneous acetylation. The

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effect of activation temperature, NDC/DMAc ratio, LiCl concentration, and dissolution temperature were observed. The DS was determined using titration, and the presence of acetyl groups in the acetylated NDC was verified via FTIR analysis. The acetylated NDC was then utilized in membrane fabrication via immersion-precipitation method and solvent evaporation method.

2 Materials and methods

2.1. Materials and reagents

NDC was purchased from Malvar, Batangas (Philippines). DMAc ($\geq 99\%$) was purchased from Sigma-Aldrich Co. (USA). LiCl ($\geq 99\%$) was obtained from LOBA Chemie (India). Nitrogen (high purity) was purchased from Linde Philippines, Inc. (Philippines). Acetic anhydride and dichloromethane (DCM; $\geq 99.9\%$) were obtained from Ajax Finechem (Australia). Hydrochloric acid (HCl; 37%) and methanol ($\geq 99.9\%$) were obtained from RCI Labscan (Thailand). Acetone ($\geq 99.5\%$) was obtained from Tedia Company Inc. (USA). Sodium hydroxide pellets (NaOH; AR Grade) and potassium bromide (KBr; AR Grade) were obtained from UNIVAR (USA). Silicon Oil was obtained from QuintStar Petrol Chemical Services (Singapore).

2.2 Pretreatment of Nata de coco

The NDC was soaked in 1% w/v NaOH solution and was washed with distilled water until the pH of the material became neutral. Afterwards, NDC was shredded in a wet blender and was placed in a glass pan for drying in a rapid drying oven at 60 °C for 6 hours. It was kept in a dry container at room temperature for storage.

2.3 Dissolution of Nata de coco

Procedures by El Seoud et al. [4] and Lima et al. [1] were adopted for the dissolution of NDC. A mixture of dried powdered NDC and DMAc with mass to volume ratio ranged from 1:50 to 1:85 was agitated. To activate the dissolution, the mixture was heated to 120, 150, and 170 °C in an oil bath for 1 hour in a condensing system under nitrogen. Then the mixture was cooled to 110 °C. Five to 8% (w/v) LiCl was added to the mixture. The temperature at 110 °C was maintained for 20 minutes. The mixture was continuously stirred at room temperature for 12 hours. Complete dissolution is achieved when there is no powdered NDC that can be visibly detected in the solution and increase in viscosity becomes evident.

2.4 Acetylation of Nata de coco

The acetylation of dissolved NDC followed the technique of El Seoud et al. **Error! Reference source not found.** and Lima et al. [1] and the procedure of Jogunola et al. [6] was employed for the recovery of the acetylated NDC. The dissolved NDC in DMAc/LiCl

system was reheated to 110 °C under nitrogen flow and in a reflux condensing system. Acetic anhydride was then added to the mixture in NDC-to-anhydride proportion of 1:12 (w/v). The mixture was stirred at 110 °C for 3 hours. The system was cooled to room temperature. The resulting solution was dispersed in methanol to precipitate the acetylated NDC. The precipitates formed were filtered and were repeatedly washed with deionized water to remove any traces of DMAc, LiCl and other chemicals. Finally, the precipitates were freeze-dried for 2 days and ground into fine powders.

2.5 Characterization

The degree of substitution (DS) was determined using titration method [7] One gram of dried acetylated NDC was transferred to a 250 mL Erlenmeyer flask then 40mL of 75% ethanol was added. The flask was loosely stoppered and was heated to 55 °C for 1 hour. The same procedure was also done for 1 g dried NDC (blank). After heating, the flask was tightly stoppered and allowed to stand at room temperature for about 72 hours. The solutions were titrated with 0.5 M HCl until phenolphthalein end point. Afterwards, 1 mL of 0.5 M HCl was added as excess and NaOH was allowed to diffuse overnight. The disappearance of the faint pink color indicated complete neutralization. The small excess of HCl was then back titrated with 0.5 M NaOH solution to phenolphthalein end point. Percent acetyl content (% Ac) and DS were determined using equations (1) and (2), respectively.

$$\%Ac = \frac{[(V_{HCl,b} - V_{HCl,s})N_{HCl} + (V_{NaOH,s} - V_{NaOH,b})N_{NaOH}]}{m_s} \times 4.305 \quad (1)$$

$$DS = \frac{162 \times \%Ac}{(4305 - 43 \times \%Ac)} \quad (2)$$

where, $V_{NaOH,s/b}$ is the amount of required for titration of the sample and blank, respectively, in mL; N_{NaOH} is the normality of NaOH; $V_{HCl,s/b}$ is the amount of HCl required for titration of the sample and blank, respectively, in mL; N_{HCl} is the normality of HCl; and, m_s is the weight of the sample in g.

Nicolet 6700 Fourier-transform Infrared (FTIR) spectrometer was utilized to determine the chemical functional groups of the unmodified dried NDC and acetylated NDC. The sample mixed with KBr was analyzed over a frequency range of 4000 to 500 cm^{-1} .

2.6 Membrane fabrication via phase inversion method

2.6.1 Immersion-precipitation method

The immersion-precipitation method [8] was employed for the fabrication CA membranes. First, CA in acetone solution in varying dope concentrations of 1% and 2% (w/v) were prepared. The solutions were agitated for 24 hours to ensure complete dissolution. Afterwards, the solutions were degassed for 3 hours in an ultrasonic bath.

An ample amount of dope solution was poured on a glass plate and was casted using a 30 μm casting knife. The film was allowed to evaporate for 20 seconds. Then, the film on the plate was immersed in a water coagulation bath at 20 $^{\circ}\text{C}$ until the membrane film was formed.

2.6.2 Solvent evaporation method

Synthesis of acetylated NDC membrane is in line with the procedure of Villalobos-Rodrigues et al. [9] via solvent evaporation method. Acetylated NDC solutions at 1% and 2% (w/v) in DCM were agitated for 72 hours. The solutions were then degassed for 3 hours in an ultrasonic bath. The resulting solutions were poured in to 150 mm petri dishes. The petri dishes were then placed in a fume hood with measured temperature of 25 $^{\circ}\text{C}$ and relative humidity of 60%. The membranes were allowed to form for 90 minutes. Twenty mL of water were then added to remove the membrane from the dish.

3 Results and discussion

3.1. Dissolution

3.1.1 Activation step

The dissolution of NDC in DMAc was activated by heating NDC in DMAc for 1 hour and shown in **Table 1**. It was observed that activation at 170 $^{\circ}\text{C}$ and 150 $^{\circ}\text{C}$ resulted to discoloration of the mixture. The best condition for activation that exhibited no discoloration was at 120 $^{\circ}\text{C}$.

Table 1. Qualitative results of the activation procedure.

Sample	NDC/DMAc (w/v)	Activation Temperature ($^{\circ}\text{C}$)	Observation
1	1:50	170	Dark Brown
2	1:50	150	Light brown
3	1:50	170	Dark Brown
4	1:50	150	Light Brown
5	1:80	150	Light Brown
6	1:80	150	Light Brown
7	1:75	120	White
8	1:75	120	White
9	1:75	120	White
10	1:75	120	White

Activation procedure prior to the actual dissolution step is necessary in order to accelerate the dissolution of NDC that has low solubility due to its high degree of polymerization [10] The activation step increases the diffusion kinetics during dissolution by allowing the polymer chains of NDC to unfold which allow the solvent to easily access the packed crystalline regions of the material. Normally, heat activation of NDC in DMAc is conducted at or near its boiling point [11] Ekmanis [12] explained that at this temperature, the solvent is able to penetrate and swell the cellulose fiber due to its high vapor pressure. However, at high temperatures cellulose

undergoes oxidative degradation causing discoloration of the solution. The degradation of NDC occurs when N,N-dimethylacetamide is formed when DMAc condenses [11] This highly reactive intermediate attacks the reducing ends of cellulose via endwise peeling reactions that result to formation of yellow-colored furan derivatives [13]

3.1.2 Dissolution step

Table 2 summarizes the effect of LiCl concentration and temperature in dissolution. The favorable result is a homogenous solution so as to efficiently acetylate NDC.

Table 2. Effect of LiCl concentration and temperature in the dissolution of NDC.

Sample	NDC/DMAc (w/v)	Percent LiCl (w/v)	Dissolution Temperature ($^{\circ}\text{C}$)	Observation
1	1:50	5	150	Undissolved
2	1:50	5	120	Undissolved
3	1:50	8	130	Dissolved
4	1:50	8	120	Dissolved
5	1:80	0.4	110	Undissolved
6	1:80	8	110	Undissolved
7	1:75	8	110	Dissolved
8	1:75	8	110	Undissolved
9	1:75	8	110	Dissolved
10	1:75	8	110	Dissolved

Samples 1 and 2 resulted to unsuccessful dissolution. The result was due to the size of the dried NDC being dissolved in the mixture. To aid the dissolution process, the material was further ground into fine powder to allow larger surface area in contact with the solvent. Finer material has a greater surface area thereby increasing collision happening between the fluid and the solid which leads to increase in the rate of reaction.

Dissolution was achieved after pulverizing the sample. Furthermore, increasing the LiCl concentration to 8% as suggested by Dupont [11] further aided the dissolution of NDC in DMAc. This was supported by the study of Zhang et al. [14] which pointed out that LiCl causes breakage of the intermolecular hydrogen bonding networks of cellulose via formation of strong hydrogen bonds of hydroxyl protons of cellulose and Cl^- anion and through solvation of Li^+ cation and free DMAc molecules.

Gelatinous solutions were obtained at NDC/DMAc ratio of 1:50. To resolve this problem, the NDC/DMAc ratio was change to 1:80 and 1:75. The results show that homogenous NDC solution can be attained at an NDC to DMAc ratio of 1:75 with 8% (w/v) LiCl at a dissolution temperature of 110 $^{\circ}\text{C}$.

A partial dissolution was observed in samples 5 and 6 due to large amount of solution and improper heating. For sample 8, LiCl was first dissolved in a small amount of DMAc before mixing it in the solution. Nonetheless, this method was found ineffective and direct addition of LiCl in the mixture is recommended.

3.2. Acetylation

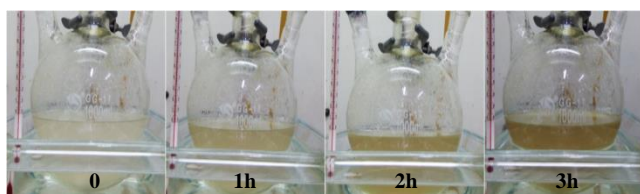


Figure 1. Discoloration of NDC solution through time.

Five successful samples (3, 4, 7, 9, and 10) from the dissolution step were acetylated. The acetylation was done in NDC-anhydride ratio of 1:12 at 110 °C for 3 hours. As shown in **Fig. 1**, it was observed that the discoloration persisted throughout the procedure. The discoloration can be attributed to oxidative degradation of NDC whose rate might have increased via the formation of N,N-dimethylketeniminium cations in the presence of LiCl added during the dissolution step. Since the solution was reheated to 110 °C, it is suspected that the furan formation via peeling reaction of NDC with N,N-dimethylacetoacetamide resumed to occur. N,N-dimethylketeniminium cations, that caused the glycosidic bonds cleavage, allowed more active sites at which the peeling reaction could occur; hence producing more furan derivatives.

3.2.1 Degree of substitution

Percent acetyl content was determined by following the titration method. Based from literatures, cellulose acetate has 39.8% acetyl content while cellulose triacetate ranges from 43 - 49%. **Table 3** shows the percent acetyl content of batches 7, 9, and 10 which indicate that the acetylated NDC were cellulose triacetate form.

Table 3. Calculated percent acetyl groups and degree of substitution of successfully acetylated solutions.

Sample	Acetylation Temperature (°C)	% Ac	DS
7	110	49.51	3.69
9	110	45.20	3.10
10	110	43.05	2.84

3.2.2 Chemical functional groups

The FTIR analysis of NDC and the acetylated NDC is shown in **Fig. 2**. For the unmodified dried NDC, the peaks at 3420 cm⁻¹ and 1077 cm⁻¹ as shown in **Fig. 2(a)** represent the hydroxyl group and the -CO stretching from the hydroxyl group, respectively. These peaks were also particularly present in the FTIR analysis of NDC conducted by Radiman & Yuliani [15]. On the other hand, **Fig. 2(b)** shows stronger transmittance peaks at 1742 cm⁻¹ and 1069 cm⁻¹ which are attributed to ester carbonyl and -CO stretching from the acetyl groups, respectively.

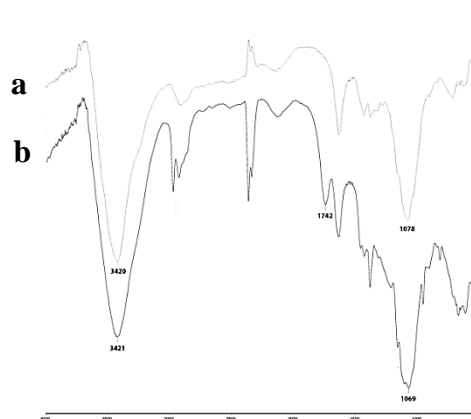


Figure 2. FTIR spectrum of (a) unmodified nata de coco and (b) acetylated of nata de coco.

The FTIR result of the acetylated NDC was akin to the FTIR analysis of cellulose acetate in the studies of Radiman & Yuliani [15] and Lindu et al. [16]. Significantly, the presence of 1742 cm⁻¹ peak in the acetylated NDC and its absence in NDC denotes conversion of hydroxyl groups to acetyl groups and thus, suggests successful acetylation of cellulose. The formation reaction of cellulose to cellulose triacetate is illustrated in **Fig. 3**.

3.3. Membrane formation

Table 4 summarizes the solubility of cellulose acetate and acetylated NDC in acetone and DCM. It can be seen from the data that CA and acetylated NDC samples were soluble in DCM. While only CA was dissolved in acetone. The solubility of cellulose acetate is dependent on its acetyl content. CA with DS of 2 - 2.5 (%Ac = 39.9%) are soluble in acetone, dioxane, and DCM while higher degree of substitution types are only soluble in DCM.

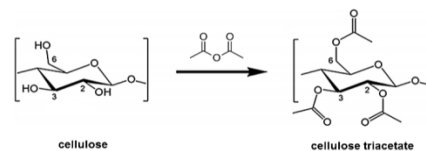


Figure 3. Acetylation reaction of cellulose to cellulose triacetate with acetic anhydride.

Table 1. Solubility data of CA and acetylated NDC in solvents utilized in membrane formation.

Solvent	% Acetyl Content			
	CA	Acetylated NDC		
	39.8%	43.05%	45.20%	49.51%
Acetone	Dissolved	Undissolved	Undissolved	Undissolved
DCM	Dissolved	Dissolved	Dissolved	Dissolved

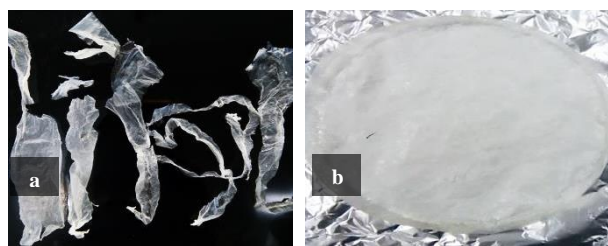


Figure 4. Acetylated NDC membranes formed via (a) immersion-precipitation and (b) solvent evaporation

Although acetylated NDC was found soluble in DCM, it was also noted that the dissolution of acetylated NDC in DCM was at most 2% w/v. The acetylated NDC in DCM were both casted through immersion-precipitation and solvent evaporation methods. **Fig. 4** presents the membranes formed after performing these methods. The membranes formed via immersion precipitation were not durable and very thin as seen in **Fig. 4(a)**. Whereas, the membranes synthesized via solvent evaporation as shown in **Fig. 4(b)** was denser, thicker and has a paper-like texture. Due to this, solvent evaporation method was preferred for the formation of acetylated NDC membranes.

4 Conclusion

Acetylated cellulose was produced from the acetylation of NDC dissolved in DMAc/LiCl solvent system. NDC, activated at the optimal NDC/DMAc (w/v) ratio of 1:75 at 120 °C for 1 hour, was dissolved by adding 8% (w/v) LiCl catalyst at 110 °C for 20 minutes. Acetylation of the dissolved samples by acetic anhydride was performed at NDC/acetic anhydride (w/v) ratio of 1:12 for 3 hours. The degree of substitution of the acetylated NDC ranged from 2.84 – 3.69 which means that the acetylated material derived was cellulose triacetate. Membrane fabrication was carried out using the modified-NDC via immersion-precipitation and solvent evaporation methods. A successful membrane formation was observed using solvent evaporation.

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