

## Research Article

# Optimization of Preparation of Cellulose Nanocrystals from Peanut Shells Using Response Surface Methodology

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**Abstract:** The value of peanut shells as agricultural wastes can be increased by recycling and utilizing these waste shells for the production of nanomaterials. To achieve this purpose, this study prepared cellulose nanocrystals from peanut shells by sulfuric acid hydrolysis. The central composite design based on the response surface methodology was applied to study the effects of sulfuric acid concentration, reaction temperature and reaction time on the yield of cellulose nanocrystals and the regression model was established between the yield and three factors. The results showed that the yield of the cellulose nanocrystals was 44.94%, under the optimum conditions of 64.6% of sulfuric acid concentration, 49.5°C of reaction temperature and 28.5 min of reaction time. The morphology and crystallinity index of cellulose nanocrystals were examined by transmission electron microscopy and X-ray diffraction. Transmission electron microscopy showed that cellulose nanocrystals presented a rod-shaped nature with the diameter ranging from 5 to 25 nm. X-ray diffraction indicated that cellulose nanocrystals were the type of cellulose I pattern, with a crystallinity of 74.71%.

**Keywords:** Cellulose nanocrystals, optimum, peanut shells, response surface methodology, yield

## INTRODUCTION

Cellulose, the most abundant and accessible organic compound in nature, has been increasingly used as Cellulose Nanocrystals (CNCs) in the production of biodegradable materials. CNCs are typically rod-shaped cellulose particles, with diameters of 1 to 100 nm and lengths of tens to hundreds nanometers (Matos *et al.*, 2009). Their main features include large specific surface area (estimated to be several hundreds of m<sup>2</sup>/g), very high modulus of elasticity (approximately 150 GPa) and great aspect ratio (Flauzino Neto *et al.*, 2013), which determine their utilization as reinforcing agent in polymer composites (Favier *et al.*, 1995). For preparing CNCs, one of the most commonly used methods is acid hydrolysis, based on the fact that the crystalline regions are insoluble under acid conditions (Habibi *et al.*, 2010). Such a hydrolysis process can disrupt the disordered and amorphous parts of the cellulose and release ordered and definite crystals.

Over the past decade, several natural fibers such as flax, jute and sisal fibers have been studied as resources for preparing CNCs (Cao *et al.*, 2007; Wang *et al.*, 2009). Several cellulosic resources have been used to prepare CNCs. However, the use of peanut shells has not been reported. Peanut shells as agriculture wastes are mostly used as animal feed or wood fuel (Wilson *et al.*, 2006) but their component of 42.5% cellulose, 26.3% hemicellulose and 28.0% lignin (Guler *et al.*,

2008), indicating that they are suitable natural cellulose materials. Considering the performance of the CNCs as reinforcing particles depends on the source of the original cellulose (Beck-Candanedo *et al.*, 2005), preparation of CNCs from peanut shells can greatly increase the economic value of these agricultural wastes.

Response surface methodology as an important experimental design and analysis methodology is to optimize the preparation conditions. The objective of this study is to prepare CNCs from peanut shells by sulfuric acid hydrolysis and using response surface methodology to improve yield of CNCs. Meanwhile, their morphology and crystallinity index were examined by transmission electron microscopy and X-ray diffraction. Therefore, optimization of preparation of CNCs from peanut shells and analysis their structure can expand their application in the food industry.

## MATERIALS AND METHODS

**Materials:** Peanut shells were purchased from the local market. Sodium chlorite, sulfuric acid, sodium hydroxide and glacial acetic acid were purchased from Chemical Reagent Co. Ltd. (Tianjin, China).

**Preparation of peanut shells fibers:** The peanut shells were cut into about 1 cm length, washed with distill water and dried in the sun. The clean peanut shells were

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then treated with 2% NaOH for 4 h at 100°C with mechanical string (Jintan Zhongda Instrument Plant, Jiangsu, China), repeatedly filtered with distilled water to completely remove NaOH and then dried at 50°C for 24 h in an air-circulating oven. After this alkali treatment, the fibers were bleached with 1.7 w/v% NaClO<sub>2</sub> at pH 4.5 and 80°C for 4 h. The bleached fibers were washed repeatedly in distilled water until reaching a neutral pH and then dried at 50°C for 24 h in an air-circulating oven.

**Preparation of CNCs:** CNCs were prepared from the peanut shells fibers by acid hydrolysis. Five grams of peanut shells fibers were put into a 100 mL flask, then the sulfuric acid concentration (50-70%) was added into the flask, placed in a water bath at (35-55°C). They were fully mixed and reacted for the set time (20-40 min) with the stirring of the agitator. The resulted suspension was diluted 10-fold with cold water to stop the hydrolysis reaction and centrifuged for 10 min at 8000 rpm to remove the excessive sulfuric acid. The centrifugal process was repeated until the supernatant became turbid, resulted in a colloidal suspension. The colloidal suspension was collected and dialyzed against distilled water for several days until reaching a neutral pH. The suspension was quickly frozen by pouring liquid nitrogen into the sample container and freeze-dried overnight to remove water.

**Calculation of the yield of peanut shells CNCs:** The total peanut shells CNCs suspension was weighed after dialysis. Then the 20 mL CNCs suspension was placed into the weighing bottle and quickly frozen by pouring liquid nitrogen into the sample container and freeze-dried until constant weight. Finally, the freeze-dried sample was weighed with analytical balance. Yield was calculated as follows:

$$\text{Yield (\%)} = \frac{(m_1 - m_2)V_1}{m_3V_2} \times 100\%$$

In this equation, m<sub>1</sub> is the mass of freeze-dried sample and weighing bottle (g), m<sub>2</sub> is the mass of weighing bottle (g), m<sub>3</sub> is the mass of dry peanut shells cellulose (g), V<sub>1</sub> is the volume of the total peanut shells CNCs suspension (mL), V<sub>2</sub> is the volume of peanut shells CNCs suspension which was placed in the weighing bottle (20 mL).

**Experimental design:** To obtain the optimum preparation conditions of peanut shells CNCs, the response surface method was used in this study. The sulfuric acid concentration (X<sub>1</sub>), reaction temperature (X<sub>2</sub>) and reaction time (X<sub>3</sub>) were chosen as variables and yield (Y) was chosen as the response value. The parameters of the single factor tests are shown in

Table 1: Factors and levels of the response surface tests

Factors	Levels		
	-1	0	1
X <sub>1</sub> (%)	60	65	70
X <sub>2</sub> (°C)	45	50	55
X <sub>3</sub> (min)	25	30	35

Table 1. The data were analyzed using the Design Expert 8.0 (Stat-Ease, Inc., Minneapolis, MN).

**Transmission Electron Microscopy (TEM):** Transmission electron microscopy was performed with a Tecnai 20U-TWIN electron microscope (Philips, the Netherlands) operating at 100 KV accelerating voltage. A drop of diluted suspension of CNCs was deposited on the surface by physically interacted with the grid and powders. The nanoparticles were then stained with a 2 wt% solution of uranyl acetate for 1 min and dried at room temperature. Nano Measurer and Origin Pro software was used to measure the diameter and size distribution of CNCs.

**X-ray diffraction (XRD):** X-ray diffraction was carried out with a D8 Advance X-ray diffractometer (Bruker-AXS, Germany). The sample was scanned in the range of diffraction angle 2θ = 5~40° at a step of 0.02° in 1 sec. The crystallinity index (CrI) of the material was calculated according to the Segal method as shown in Equation (Segal *et al.*, 1959):

$$\text{CrI (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

In this equation, CrI is the crystallinity index, I<sub>002</sub> and I<sub>am</sub> are the peak intensities of crystalline and amorphous materials, respectively.

**Statistical analysis:** All tests were performed in triplicate and the results were given as means±standard deviations. Duncan's multiple range test was used to evaluate significant differences (p<0.05) between the result.

## RESULTS AND ANALYSIS

**Effect of sulfuric acid concentration on the yield of peanut shells CNCs:** The effect of sulfuric acid concentration on the yield of peanut shells CNCs was showed in Fig. 1. With sulfuric acid concentration ranging from 50 to 65%, the yield of peanut shells CNCs was increased with the increase of sulfuric acid concentration when reaction temperature was 40°C and reaction time was 25 min. The reason was that hydronium ions could penetrate into amorphous regions of cellulose and cause the hydrolytic cleavage of glycosidic bonds during the acid hydrolysis process (Li *et al.*, 2009). Whereas when the sulfuric acid

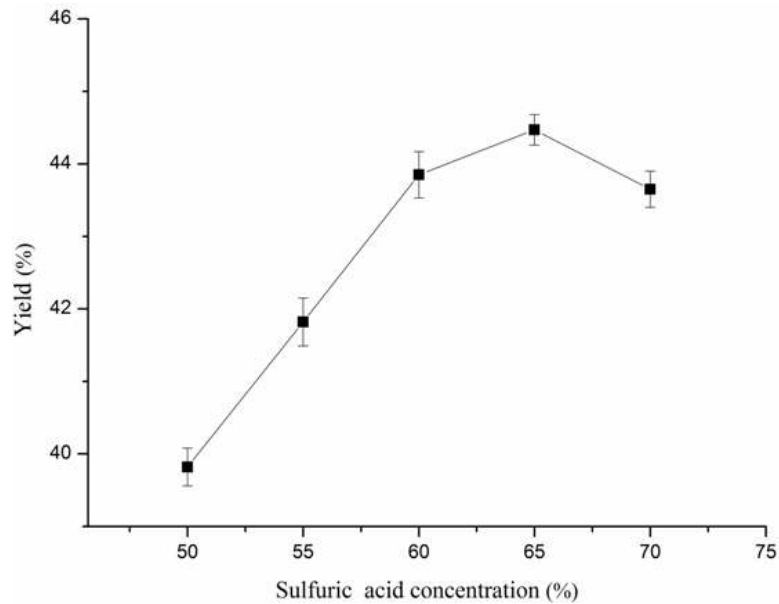


Fig. 1: Effect of sulfuric acid concentration on the yield of peanut shells CNCs

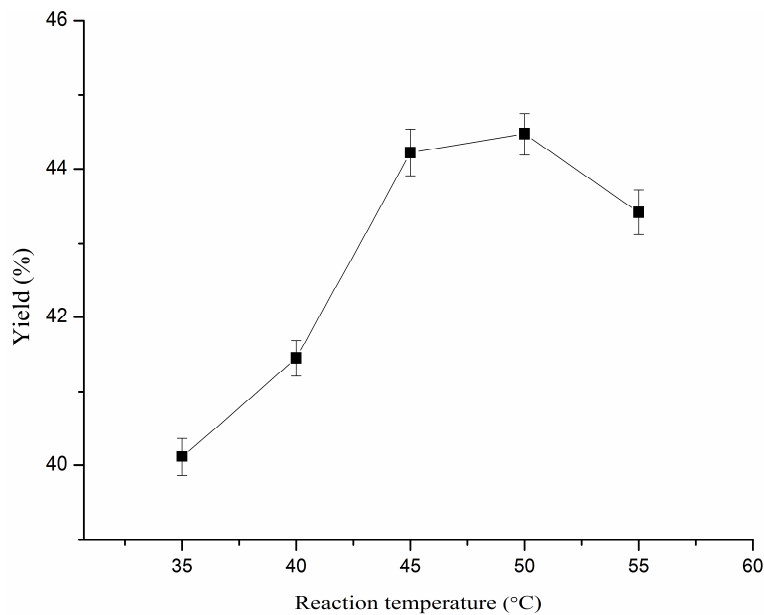


Fig. 2: Effect of reaction temperature on the yield of peanut shells CNCs

concentration exceeded 65%, the yield was decreased because too high (above 65%) sulfuric acid concentration would degrade the cellulose to yield sugar molecules.

**Effect of reaction temperature on the yield of peanut shells CNCs:** The effect of reaction temperature on the yield of peanut shells CNCs was showed in Fig. 2. From 35 to 50°C of reaction temperature, the yield of peanut shells CNCs was increased and then decreased after 50°C of reaction time when the sulfuric acid concentration was 65% and reaction time was 25 min. With the reaction temperature increased ranging from

35 to 50°C, glycosidic bond of cellulose was broken, so the length and diameter of peanut shells fibers became nano particles to increase the yield of CNCs. When the reaction temperature exceeded 50°C, cellulose molecule would be further broken down into glucose at higher temperature. Meanwhile, the suspension became darkened when the temperature rose to 55°C, which leaded to decline of the yield.

**Effect of reaction time on the yield of peanut shells CNCs:** The effect of reaction time on the yield of peanut shells CNCs was showed (Fig. 3). When the sulfuric acid concentration was 65% and reaction

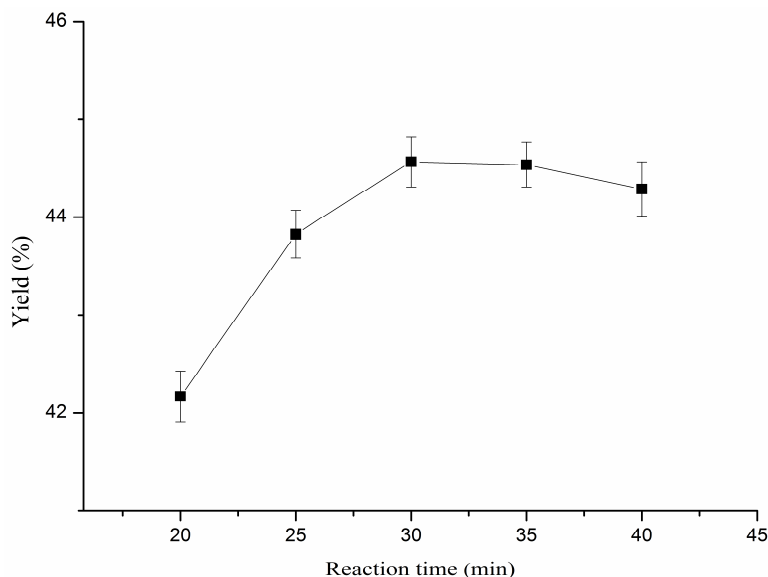


Fig. 3: Effect of reaction time on the yield of peanut shells CNCs

Table 2: Design and results of Box-Behnken test

No.	X <sub>1</sub> (%)	X <sub>2</sub> (°C)	X <sub>3</sub> (min)	Y (%)
1	60	50	35	44.11
2	70	50	35	43.54
3	70	45	30	43.26
4	60	55	30	43.28
5	70	50	25	44.06
6	60	45	30	43.67
7	65	50	30	44.76
8	65	55	25	43.42
9	65	50	30	44.84
10	65	50	30	44.93
11	65	45	35	42.91
12	60	50	25	44.08
13	65	55	35	43.58
14	65	45	25	44.22
15	65	50	30	44.92
16	65	50	30	44.89
17	70	55	30	43.37

temperature was 40°C, the yield of peanut shells CNCs was increased with the increase of reaction time, when the reaction time was 30 min, the yield of CNCs reached 44.57%, which reached the maximum values. The reason was that acid solution diffused into peanut shells fibers during preparation process, which promoted the fracture of glycosidic bond, so the fibers were broken down into nano particles. When the reaction time exceeded 30 min, the yield tended to decrease moderately, because the concentration of sulfuric acid was gradually reduced as the reaction proceeds and the reaction between the fibers and the sulfuric acid then slowed down (Fan and Li, 2012).

**Optimum preparation conditions of peanut shells CNCs:** To obtain the optimum preparation conditions of peanut shells CNCs, sulfuric acid concentration (60-70%), reaction temperature (45-55°C) and reaction time (25-35 min) were used to Box-Behnken design and

obtain the regression model. Design and results of the Box-Behnken tests were shown in Table 2.

Based on the design and results of Box-Behnken tests in Table 2, the Design Expert software was used to fit the regression model and the obtained regression equation was expressed as follows:

$$Y = 44.87 - 0.01X_1 - 0.051X_2 - 0.21X_3 + 0.12X_1X_2 - 0.14X_1X_3 + 0.37X_2X_3 - 0.53X_1^2 - 0.94X_2^2 - 0.39X_3^2$$

The significant analysis was showed in Table 3. The significance level of the model was less than 0.0001, which showed that the model and method were reliable. The model determination coefficient R<sup>2</sup> (0.9883) suggested the fitted model could explain 98.83% of the total variation, which further confirmed that the model could fit well the experimental results.

The factors X<sub>3</sub>, X<sub>2</sub>X<sub>3</sub>, X<sub>1</sub><sup>2</sup>, X<sub>2</sub><sup>2</sup> and X<sub>3</sub><sup>2</sup> significantly affected the yield of CNCs (p<0.01) and the factors X<sub>1</sub>, X<sub>1</sub>X<sub>3</sub> significantly affected the yield of CNCs (p<0.05). So it was not the linear relationship between the effects of different factors on the response values. The response surface figures were shown in Fig. 4.

Taking the maximum yield as the objective, the 64.6% of sulfuric acid concentration, 49.5°C of reaction temperature and 28.5 min of reaction time Under these conditions, the predicted yield of CNCs was 44.90%.

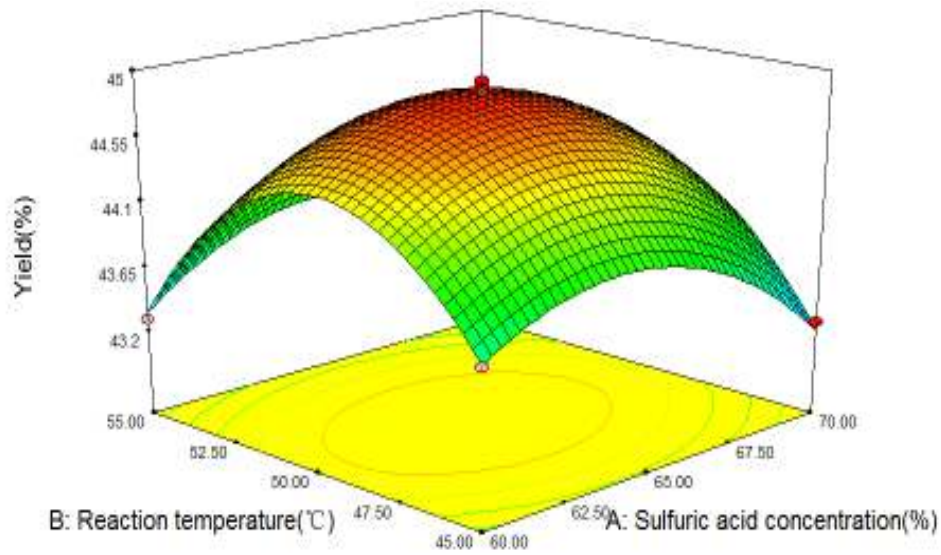
To further verify the predicted values, duplicate experiments were performed under the optimum preparation conditions of CNCs. The mean yield of CNCs measured was 44.94%, which was consistent with the predicted one (44.90%). The results showed that the response surface methodology can be used to optimize the preparation conditions of CNCs to increase the yield.

Table 3: Regression analysis of variance

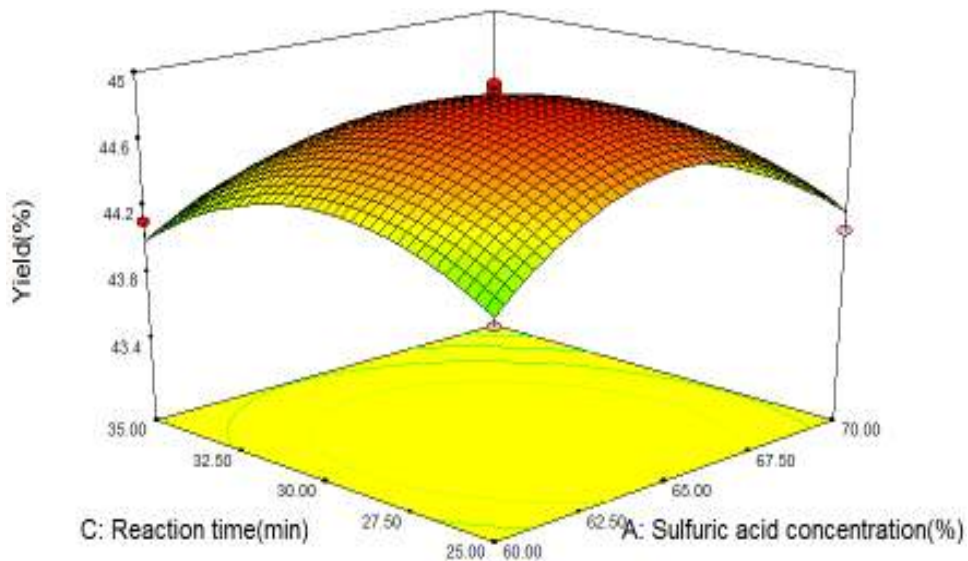
Source	SS	df	Mean square	F	P	Significance
Model	7.254039	9	0.806004	65.7158	<0.0001	**
X <sub>1</sub>	0.103513	1	0.103513	8.439666	0.0228	*
X <sub>2</sub>	0.021012	1	0.021012	1.713208	0.2319	
X <sub>3</sub>	0.3362	1	0.3362	27.41133	0.0012	**
X <sub>1</sub> X <sub>2</sub>	0.0625	1	0.0625	5.095801	0.0586	
X <sub>1</sub> X <sub>3</sub>	0.075625	1	0.075625	6.165919	0.0420	*
X <sub>2</sub> X <sub>3</sub>	0.540225	1	0.540225	44.04607	0.0003	**
X <sub>1</sub> <sup>2</sup>	1.178278	1	1.178278	96.06832	<0.0001	**
X <sub>2</sub> <sup>2</sup>	3.752152	1	3.752152	305.9235	<0.0001	**
X <sub>3</sub> <sup>2</sup>	0.645357	1	0.645357	52.61776	0.0002	**
Residual	0.085855	7	0.012265			
Lack of fit	0.066375	3	0.022125	4.543121	0.0889	
Pure error	0.01948	4	0.00487			
Cor total	7.339894	16				

R<sup>2</sup> = 0.9883

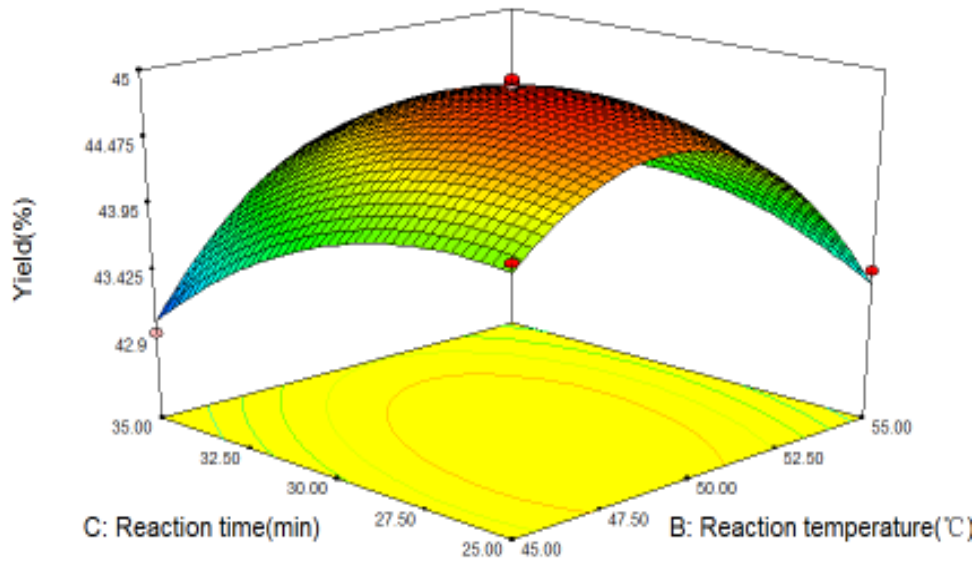
\*: Significant (p<0.05); \*\*: Very significant (p<0.01); df: degrees of freedom; SS: Sum of squares



(a)



(b)



(c)

Fig. 4: Response surface plot for yield of peanut shells CNCs by sulfuric acid hydrolysis

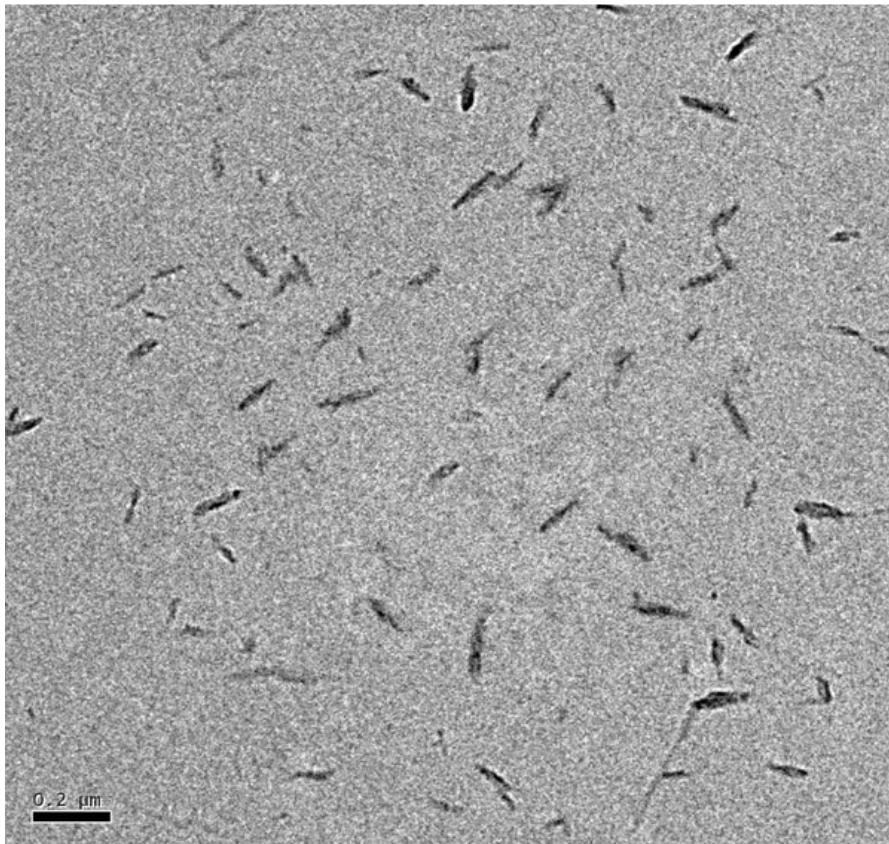


Fig. 5: TEM image of peanut shells CNCs

**The Morphology of CNCs:** Figure 5 shows TEM image of CNCs. Rod-like nanoparticles can be observed, which indicated that the preparation of CNCs from peanut shells was successful. The diameter of

CNCs ranged from 5 to 25 nm, smaller than CNCs from sisal fibers (Morán *et al.*, 2008), but similar to CNCs extracted from rice husks. In addition, most of these CNCs (around 74%) have aspect ratio ranged from 10

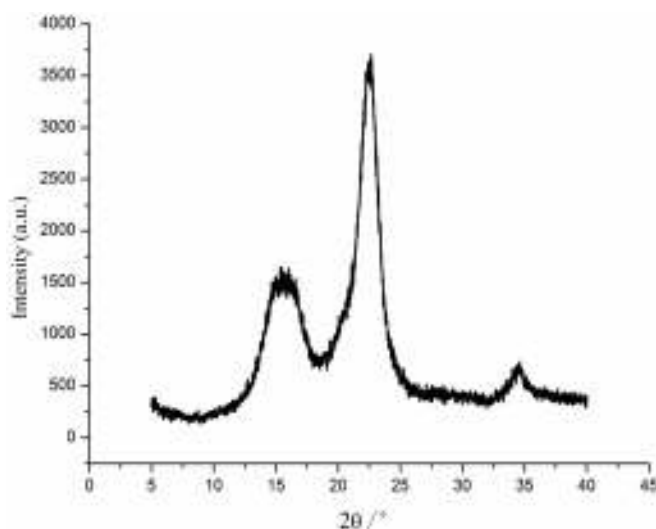


Fig. 6: XRD pattern of peanut shells CNCs

to 25. It is worth noting that the aspect ratio was kept above 10, which is considered as the minimum aspect ratio for a good strength transmission for any reinforcement (Jiang *et al.*, 2007), so the CNCs from peanut shells have great potential application to be used as reinforcing capability on composites.

**XRD analysis:** Figure 6 shows the XRD pattern of CNCs. In the diffraction pattern, there were crystalline peaks around  $2\theta = 16^\circ$ ,  $22^\circ$  and  $35^\circ$ , which presented the type of cellulose I pattern (Klemm *et al.*, 2005). CrI was found to be about 74.71% for the peanut shells CNCs, which indicating good crystallinity. This good increased crystallinity was also expected to increase their stiffness, rigidity and strength.

### CONCLUSION

This study investigated the optimization of preparation of cellulose nanocrystals from peanut shells using response surface methodology. It was found that the sulfuric acid concentration, reaction temperature and reaction time all affected the yield of CNCs. The yield of CNCs was first increased and then decreased with the increase of the above-mentioned three factors. In the three factors, the sulfuric acid concentration, reaction temperature and reaction time significantly affected the yield of CNCs, whereas the reaction temperature was not significant. The central composite design based on the response surface methodology can be used to establish the regression model of yield of CNCs. Based on the variance analysis, the regression equation can fit well the changes in the emulsifying properties. According to the regression equation, the optimum preparation conditions of the CNCs were 64.6% of sulfuric acid concentration,  $49.5^\circ\text{C}$  of reaction temperature and 28.5 min of reaction time. Under these conditions, the yield of CNCs was 44.94%. The CNCs

presented a rod-shaped nature with the diameter ranging from 5 to 25 nm. The crystal type of CNCs was cellulose I pattern, with a crystallinity of 74.71%.

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