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Title of the article : Optimization Of Acid Concentration And Hydrolysis Time In The Isolation Of Microcrystalline Cellulose From Water hyacinth (*Eichornia crassipes solm)*

ABSTRACT

Water hyacinth (*Eichornia crassipes*) is aquatic weed being able to disrupt the aquatic ecosystem. As this plant contains high cellulose, it has a potential source of microcrystalline cellulose (MCC). Therefore, it has high economic value. This study determined optimal hydrolysis conditions to isolate MCC from the water hyacinth. The optimum conditions were obtained by a 3^2 factorial design with acid concentration and hydrolysis time as independent variables and physical properties of MCC as dependent variables. Based on One Way ANOVA analysis, the acid concentration had an effect on all MCC characteristics ($p<0,05$). In optimum condition, hydrolysis process produced MCC of 91.70%, angle of repose 20.695, and moisture absorption capacity of 3.24%. Furthermore, the FTIR spectra of MCC have the same peaks as those of Avicel® PH101 and its crystallinity observed from the XRD spectrum was 78.23%. In addition, based on DX®10 analysis we concluded that the optimal hydrolysis condition was at concentration of HCl 1.5 M for 30 minutes.

Keywords : water hyacinth, microcrystalline cellulose, optimum condition hydrolysis, avicel,

INTRODUCTION

Water hyacinth (*Eichornia crassipes*) is one of the fastest growing aquatic plants and is easily spreading and is generally considered as weed. The existence of water hyacinth (WH) in the water surface blocks sunlight cause imbalance of aquatic life. However, WH has the potential as an adsorbent for water pollutants. It's ability as an adsorbent for water pollutants has been reported such as Cadmium, lead and cupper, and dyes [1–4]. Aside from being an adsorbent, WH also shows pharmacological activities such as antioxidant, antimicrobial, antifungal and anticancer [5–7].

WH can be an economic problem because it has a negative impact on fishies ecosystem, slowing or preventing water flow, inhibiting irrigation and slowing hydroelectric power generation[8]. Various strategies have been adapted to control this weed, and most promising approach is the effective utilization of the plant for commercial purpose. In this area, we explore the process of extraction very important fibre that will be economic benefit and put a stop to the environmental challenge pose by WH [9].

Study have shown that, chemical content of WH fibers are around 60% cellulose. 8% hemicellulose and 17% lignin [10–12]. Based on its cellulose content, WH has the potential as the source of microcrystalline cellulose (MCC) which has high economic value and is able to reduce the population of WH[13]. Microcrystalline cellulose is widely used in the pharmaceutical, cosmetic and food industries.[14] MCC is widely used as an excipient because of its neutrality, non toxic and hygroscopicity[15]. MCC is generally used in the tablet manufacturing industry, it can produce tablets with a high level of hardness but short disintegration time.[16] Apart from having economic benefits to produce of MCC, WH also reduces water pollutants. Previous researchers has successfully produced nanocrystalline cellulose (NCC) from WH and the isolation was carried out under one hydrolysis condition with 5 M HCl[12]. Hydrolysis is a suitable method for producing MCC. The time and acid

concentration factors can be optimized [17]. Acid hydrolysis is preferred because the reaction conditions are easier to control for the production process per batch and the time is shorter [14]. Different conditions of the hydrolysis process will produce MCC with different properties [18]. Therefore, the aim of this study was to determine the optimum of acid concentration and hydrolysis time in isolation of MCC from WH. The optimal conditions were determined by using a 3^2 factorial design with hydrolysis time and acid concentration as independent variables. The physicochemical properties of microcrystalline cellulose yield as dependent variable were compared to Avicel® PH101.

METHODOLOGY

Material

Water hyacinth were collected at January 2019 from the Indralaya area, South Sumatra, Indonesia. The plant was identified in Indonesian Institute of Sciences. The voucher specimen (Voucher number : HBSU/1009/18) was deposited at Biology Department of Sriwijaya University. Avicel® PH 101 purchase from Sigma Aldrich. All chemicals were analytical grade.

Determination Percentages of Cellulose, Hemicellulose and Lignin

Determination of the cellulose, hemicellulose and lignin percentages in WH were carried out following Chesson-Datta method [19]. Lignin removal and alpha cellulose were carried out isolation according to Ohwoavworhua *et al* procedure[20].

Microcrystalline Cellulose Preparation

MCC preparation was carried out in nine conditions based on variations in acid concentration and hydrolysis time determined by using a 3^2 factorial design. Variations in acid concentration and hydrolysis time showed in Table 1.

The 50 g α-cellulose mass hydrolyzed under the conditions according to Table 1. The hot mixture was poured into cold water while stirring vigorously with a spatula and allowed to

stand overnight. The microcrystalline cellulose obtained was washed with distilled water until neutral, filtered with a Buchner funnel then dried in an oven at 57-60ºC for 60 minutes and then milled and sieved using a mesh sieve no. 25 [20].

Characterization of Microcrystalline Cellulose (MCC)

The physical and chemical properties of microcrystalline cellulose were determined referring to Ohwoavworhua's.[20] The properties included: pH, true density, relative density, Car's index, Hausner ratio, angel of repose, solubility, hydration capacity, swelling capacity, moisture sorption capacity and percent of yield.

Data Analysis

DX® 10 software (Stat-Ease Inc. USA) was used for data analysis on the nine formula conditions. Minitab®17 and SPSS®17 (one sample t-test) were used for the analysis of the optimum formula and percentage calculation of residual standard error (RSE%).

Optimum Condition Analysis

The optimum conditions of MCC hydrolysis from WH were determined based on the highest *desirability* value of the specified parameters. Comparative tests were carried out by using the Minitab® 17 program between predictive data (from the DX® 10 program) and actual data (from the research). Calculation of *residual standard error* (RSE) or the percentage of error was done to ensure the accuracy of predictive data using equation (1).

$$\% RSE = \frac{research\ data - prediction\ data}{prediction\ data} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

The optimum conditions of MCC from WH suggested by the DX® 10 program were compared to Avicel® PH101. Furthermore, MCC obtained from the optimum conditions were characterized for crystallinity index and IR spectra.

Crystallinity Index Determination

The crystallinity index determination was performed with Xray Diffractometer (Rigaku® Mini Flex 600. USA) at a voltage of 40 kV and a current of 35 mA. Samples were measured in solid form of and the crystallinity index was calculated by the following formula: The crystallinity index was determined by Segal's equation[21].

$$\% \, Cristallinity \, Index = \left(\frac{I_{22} - I_{am}}{I_{22}} \right) \times 100 \quad \ldots\ldots(2)$$

I_{22} = the maximum intensity of the 002 lattice diffraction (2θ ~ 22 °)

I_{am} = the maximum intensity diffraction of amorphous part (2θ ~ 18 °)

Analysis of Functional Groups

Microcrystalline cellulose from optimum conditions were analyzed by using IR spectrophotometer (Thermo Scientific®) to determine the functional groups and compared to the spectrum Avicel® PH101.

RESULTS AND DISCUSSION

Determination of Cellulose, Hemicellulose and Lignin Percentages

Water hyacinth contain biomass components such as cellulose, hemicellulose and lignin. The analysis showed that the content of cellulose hemicellulose and lignin in the WH sample were 46.15%; 13.46% and 23.07%. The percentages of cellulose hemicellulose and lignin in our research were different from those reported by previous research [10,12].

Preparation of Microcrystalline Cellulose

The isolation of microcrystalline cellulose was performed by using the acid hydrolysis method at a certain temperature and acid concentration. The acid hydrolysis can damage the amorphous regions of cellulose and produce crystalline.[22] Microcrystalline cellulose produced through this process were in the form of white powder. The transformation from alpha cellulose to microcrystalline cellulose occurred due to termination of the amorphous chain to produce cellulose with a micro size that has a high crystalline index [23].

Characteristics of Microcrystalline Cellulose

Microcrystalline cellulose resulted from nine hydrolysis conditions showed organoleptic properties as specified in British Pharmacopeia (2004) in the form of fine white powder and odorless (Figure 1).

The characteristics of MCC powder from the nine treatment for variation in acid concentration and hydrolysis time can be seen in table 2 . Table 2 showed that the Carr's index MCC in each sample were in the range of 24−36 %. The resulting Carr's index value indicates that the sample has poor flow properties. This was due to greater the amorphous nature of the sample which causes the particle size to be smaller. The smaller particle size resulting in the greater compressibility, consequently the more difficult the powder to flow[24]. ANOVA analysis on 9 hydrolysis conditions showed that the acid concentration had an effect on all MCC characteristics ($p<0,05$). However, the hydrolysis time and the interaction between the two factors had no significant effect on the Car's index response ($p>0,05$). DX®10 analysis shows that the acid concentration and hydrolysis time could increased the response of Carr's index (Equation 3). The Hausner ratio has a value that is directly proportional to the Carr's index. MCC from hydrolysis at conditions 1-3 has poor flow properties because the value was more than 1.5[25]. DX®10 analysis shows that the acid concentration could improved the response of Hausner ratio and hydrolysis time decreases the Hausner ratio (Equation 4).

$$Y_1 = 30.29 + 4.51A[1] - 0.57A[2] + 1.27B[1] - 1.71B[2] + 0.13A[1]B[1] - 0.071A[2]B[1] - 0.20A[1]B[2] - 0.21A[2]B[2] \quad (3)$$

$$Y_2 = 11.99 + 0.39A[1] - 0.06A[2] + 0.11B[1] - 3.10B[2] + 0.024A[1]B[1] - 8.35A[2]B[1] - 0.01A[1]B[2] - 0.01A[2]B[2] \quad (4)$$

$$Y_3 = 31.16 - 1.80A[1] + 0.24A[2] - 0.75B[1] + 0.21B[2] - 0.85A[1]B[1] + 0.47A[2]B[1] + 0.33A[1]B[2] - 0.20A[2]B[2] \quad (5)$$

$$Y_4 = 3.22 + 0.16A[1] + 1.90A[2] + 0.08B[1] - 0.01B[2] + 0.02A[1]B[1] - 0.07A[2]B[1] - 0.02A[1]B[2] + 0.02A[2]B[2] \quad (6)$$

$$Y_5 = 20.02 + 4.42A[1] - 0.46A[2] + 1.48B[1] + 0.02B[2] + 0.67A[1]B[1] - 0.34A[2]B[1] - 0.18A[1]B[2] + 0.05A[2]B[2] \quad (7)$$

$$Y_6 = 87.06 + 5.85A[1] + 2.17A[2] + 3.66B[1] + 0.33B[2] - 2.46A[1]B[1] - 1.19A[2]B[1] - 0.84A[1]B[2] - 1.131A[2]B[2] \quad (8)$$

Information:

Y_1 = Carr's index response; Y_2 = Hausner ratio response ; Y_3 = Hydration capacity response; Y_4 = Moisture sorption capacity response; Y_5 = Angle of repose response; Y_6 = Yield percentage response
A = Acid concentration; B = Hydrolysis time

The hydration capacity value increased with the increasing acid concentration and hydrolysis time (Equation 5). The lowest hydration capacity value was at 27.757% caused in that condition the sample was still amorphous. Amorphous properties could attract ions from the water so that it becomes more soluble, as a result, the weight of the sediment produced is less. The hydration capacity of condition 4 was 31.117% had similar value with Avicel® PH101 result of 31.146%.

The value of moisture absorption capacity and the angle of repose decreased with increasing acid concentration and hydrolysis time (equation 6 and 7). The increasing of two factors would increase the crystalline form, making it difficult for water to penetrate into the powder structure, consequently the moisture absorption capacity is reduced. The angle of repose of MCC on the condition 4 showed a similarity to Avicel® PH 101. Based on the relationship between the angle of repose and flow properties, both of them have excellent flow properties because they had an angle of repose less than 25°.

The yield measurement aims to find out the total of samples obtained under certain hydrolysis conditions. The yield of MCC was calculated by comparing the of weight hydrolyzed microcrystalline cellulose to the dry weight of α-cellulose. The increasing in acid concentration and hydrolysis time process resulted in more glucose monomers being dissolved during washing so that the yield of microcrystalline yield decreased. The high acid

concentrations and long reaction times lead to excessive degradation of cellulose so that the acid penetrates rapidly into the tissue layer to hydrolyze cellulose and then hydrolyzes the amorphous regions of the cellulose crystals. The result was a decrease in yield [26].

Determination of Optimal Conditions

The optimum conditions were determined based on the angle of repose, the yield percentage and the water sorption capacity. The parameters were considered the most important priority responses in the MCC manufacturing process. The percentage of yield has importance (+++++) because it affects the number of MCC generated. The angle of repose relates to the flow properties of the MCC using as an excipient in tablet manufacturing, whereas the moisture sorption contributed to the stability of MCC and also reflects the physical stability of cellulose-based tablets when stored in humid conditions[20], so that two parameters had importance (+++).

Design Expert® 10 analysis using a factorial design showed that the condition had the desired specifications of 0.986. A desirability value indicates that the acid concentration and hydrolysis time predicted from the program will produce an MCC with the desired criteria. The results of the optimal conditions from the DX® 10 program were acid concentration of 1.5 N and hydrolysis time of 30 minutes. This condition is predicted to produce MCC with a yield of 91.71%, the moisture sorption capacity of 3. 24 and angle of repose 20.69.

Comparative analysis of Microcrystalline Cellulose

The comparative analysis of the research data with the predicted data was done by one sample t-test. The results of the analysis are shown in the table 3.

Table 3 showed that the *p-value* on the yield, the angle of repose and the moisture sorption capacity had $p > 0.05$ which means that there was no significant difference between the research data and the predicted data from DX® 10. Residual Standard Error analysis was performed to assess the accuracy of the data. The smaller the % RSE value, the more accurate

it is because it is getting closer to the predicted value[27]. The % RSE value indicated by all responses were less than 1%. These results indicate that the prediction data with the research data are compatible. It can be concluded that the research can prove the predictions by the DX® 10 program.

FTIR Spectrophotometry

Measurement by FTIR (Thermo Scientific®) spectrophotometry was carried out on MCC produced under optimal conditions compared with commercial microcrystalline cellulose Avicel ® PH-101. The results of the analysis can be seen in Figure 2.

Based on Figure 2, the infrared spectrum showed the same absorption pattern in the functional group region between WH microcrystalline cellulose and Avicel PH 101. The FTIR spectroscopy of both microcrystalline cellulose peak at 2800-2900 cm^{-1} and 3300−3350 cm^{-1} which came from the stretching of the CH bonds of aliphatic and stretching O-H. The two peaks were derived from hydroxyl (OH) groups which attached to the alpha carbon chain. There are peaks in fields 1300-1400 cm^{-1}, indicating intramolecular hydrogen bond and O-H bending. The peak at 1000 cm^{-1} is associated with stretching of C-O-C. The FTIR spectrum shows that there are no peaks around 1700 cm^{-1}. This indicates that after the chemical process of the cellulose, the non-cellulose fiber content were lost because they had been dissolved by the solvent.[28]

Crystallinity Index

XRD analysis (Rigaku® Mini Flex 600) aims to analyze the crystal structure of the MCC from these optimal conditions. The results of sample analysis can be seen in Figure 3.

Figure 3 shows that the MCC of the optimal conditions had a higher peak at the value of $2\Theta = 22.17^{o}$. Meanwhile. the lattice (002) and amorphous were at the value of $2\Theta = 22.17^{o}$ and $2\Theta = 15.83^{o}$ with intensities of 2637 and 574 respectively. The peaks in the MCC spectrum showed more clearly that the acid hydrolysis process was able to remove some

amorphous material from cellulose alpha[29]. Calculation of the crystallinity index was found that crystalline index of MCC 78.23%. This value indicates that the sample belongs to microcrystalline cellulose because the value of the normal crystallinity index ranges from 55 to 80 % depending on the source of cellulose and reaction conditions[30,31]. Avicel® PH101 has a crystallinity index of 77.27% [21]. Some studies show the percentage value of MCC crystallinity are different from various sources: kenaf is 70% [32]; peanut shell is 74%[33], bagasse is 76%, rice straw 78%, cotton stalks 77% [34] and sisal fiber (*Agavae sisalana Perrine*) 60%[35]. Empty Fruit Bunch Palm Oil 73%.[36] The crystalline index value of MCC from WH was not much different than the Avicel® PH101 and some of the reported MCC thus it can be concluded that the samples produced were the form of microcrystalline cellulose.

Comparison of optimal MCC with Avicel® PH 101

The optimized microcrystalline cellulose of WH samples were compared with Avicel ® PH 101 to ensure that the MCC produced was in accordance with the standard of commercially available microcrystalline cellulose such as Avicel ® PH 101. The results of the evaluation of the two microcrystalline cellulose can be seen in table 4 .

Table 4 shows that MCC from WH produced with optimization did not have significant difference with Avicel® PH101. This research also proved that the samples generated from the hydrolysis of acid concentration with 1.5 N HCl and hydrolysis time for 30 minutes was the optimal condition to produce microcrystalline cellulose that had characteristics similar to the commercially available Avicel ® pH101.

Conclusion

The optimal conditions for the isolation of microcrystalline cellulose from WH were at 1.5 N of acid concentration and hydrolysis time 30 minutes. The characteristics of microcrystalline cellulose from WH produced under those conditions had similar

characteristics to Avicel PH 101. The XRD analysis showed a crystalline index value of 78.23% similar to Avicel® PH 101 which had crystalline index of 77.27 %.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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Table 1 . Variation in Acid Concentration and Hydrolysis Time

Treatment	HCl (N)	Time (minutes)
F1	1	30
F2	1	45
F3	1	60
F4	1.5	30
F5	1.5	45
F6	1.5	60
F7	2	30
F8	2	45
F9	2	60

Table 3. Comparative Analysis of Prediction Data and Research Data

The response	Prediction	Research ± SD	% RSE	*p-value*
Yield	91.71	91.77 ± 0.26	0.063	0.99
Angle of repose	20.69	20.69 ± 0.07	0.002	0.99

Moisture absorption capacity	3.24	3.22 ± 0.01	0.002	0.97

Table 2 . Characteristics of MCC from water hyacinth

No	pH	True Density (g / ml)	Relative Density (g/ml)	Carr's Index (%)	Hausner Ratio (%)	Hydration Capacity (%)	Swelling Capacity (%)	Moisture sorption capacity	Angle of repose	Yield (%)
1	6.81±0.03	0.26±0.00	0.42±0.00	36.20±0.34	1.57±0.01	27.76±0.02	51.07±1.86	3.50 ± 0.01	26.60±0.82	94.24±0.43
2	6.75±0.03	0.28±0.00	0.46±0.00	34.60±0.58	1.53±0.01	29.90±0.01	50.40±0.70	3.35 ± 0.01	24.28±0.75	92.63±0.53
3	7.16±0.02	0.31±0.00	0.46±0.01	33.60±0.47	1.50±0.01	30.43±0.03	50.79±1.37	3.32 ± 0.00	22.46±1.03	92.51±0.82
4	7.26±0.03	0.36±0.00	0.52±0.00	30.91±0.49	1.44±0.01	31.12±0.02	21.66±2.88	3.22 ± 0.01	20.69±0.08	91.770.26
5	6.86±0.03	0.42±0.02	0.60±0.02	29.50±1.05	1.41±0.02	31.42±0.02	26.66±2.88	3.22 ± 0.02	19.63±0.29	88.54±0.36
6	7.22±0.04	0.47±0.01	0.65±0.02	28.73±1.66	1.40±0.03	31.67±0.06	25.00±0.00	3.21 ± 0.01	18.34±0.21	87.80±0.26
7	7.11±0.02	0.53±0.03	0.73±0.02	27.57±2.84	1.38±0.05	32.35±0.03	25.00±0.00	3.19 ± 0.01	17.21±0.13	86.64±0.36
8	6.95±0.02	0.65±0.07	0.89±0.06	26.77±2.81	1.36±0.05	32.79±0.02	12.50±0.00	3.04 ± 0.00	16.22±0.63	81.81±0.40
9	6.94±0.02	0.75±0.07	1.00±0.04	24.75±4.16	1.33±0.07	33.00±0.01	16.66±0.00	2.93 ± 0.00	14.74±	69.43±0.17
Avicel PH101	7.35±0.03	0.35±0.00	0.48±0.00	27.91±0.22	1.38±0.00	31.14±0.01	20.00±0.00	3.22 ± 0.08	20.16±0.44	-

Table 4 . Comparison of the Characteristics of MCC of WH and Avicel®PH 101

No.	Parameter	Water hyacinth	Avicel® PH101
1	Organoleptic		
	a. Form	Fine powder	Fine powder
	b. Color	White	White
	c. Smell	Odorless	Odorless
	d. Taste	Tasteless	Tasteless
2	Identification using iodized zinc chloride reagent	Had blue-violet color	Had blue-violet color
3	pH	7.26 ± 0.02	7.35 ± 0.02
4	Solubility (%)	0.19 ± 0.01	0.11 ± 0.01
5	Loss of drying	5.9426 ± 0.026	5.99 ± 0.03
6	Starch test	Did not have blue color	Did not have blue color
7	True Density (g / mL)	0.36 ± 0.01	0.35 ± 0.00
8	Relative Density (g/mL)	0.52 ± 0.01	0.48 ± 0.01
9	Flow properties		
	• Carr's index	30.91 ± 0.50	27.91 ± 0.23
	• Hausner Ratio	1.45 ± 0.01	1.39 ± 0.00
	• Angle of repose	20.69 ± 0.08	20.22 ± 0.41
10	Hydration Capacity	31.12 ± 0.02	31.15 ± 0.01
11	Swelling Capacity	21.67 ± 2.89	20.00 ± 0.00
12	Moisture sorption Capacity	3.24 ± 0.01	3.22 ± 0.01
13	Yield (%)	91.77 ± 0.26	--

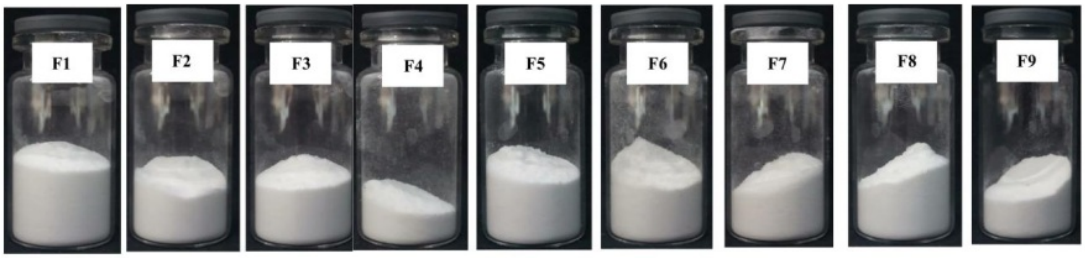

Figure 1. Microcrystalline cellulose from water hyacinth isolated in nine hydrolysis conditions

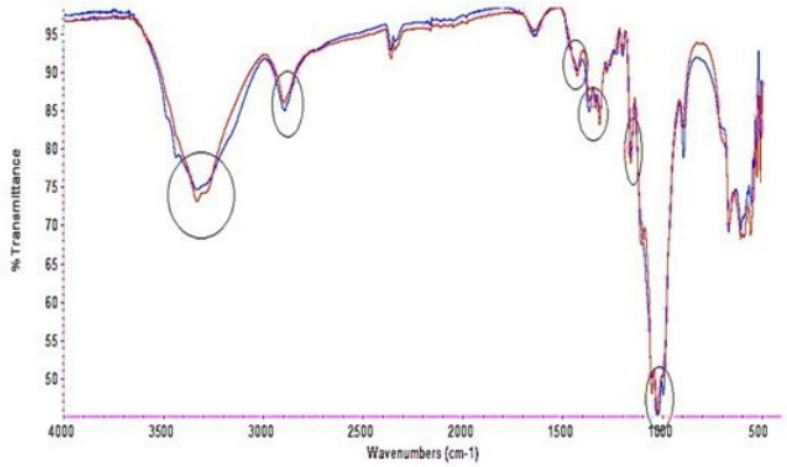

Figure 2 . FTIR spectroscopy microcrystalline cellulose of WH and Avicel ® PH-101

Information :

Microcrystalline cellulose of WH

Avicel ® PH-101

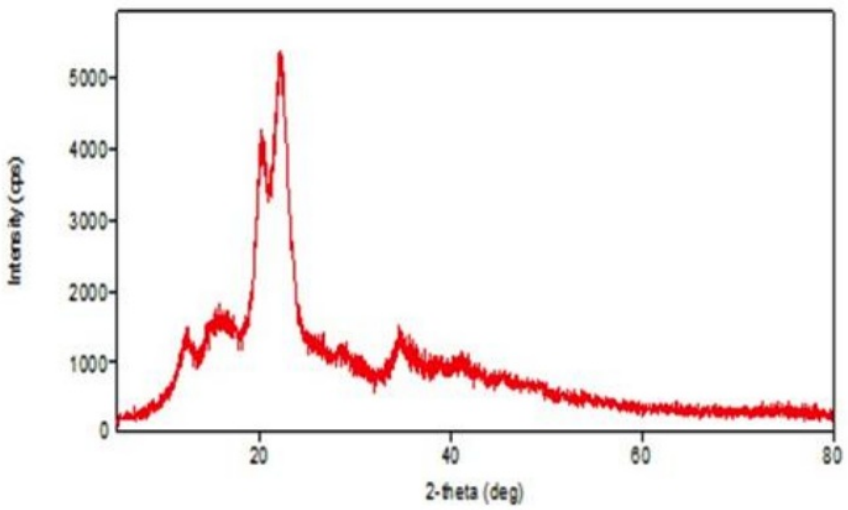

Figure 3 . XRD microcrystalline cellulose of WH

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