PREPARATION OF NANOPARTICLES FROM CORN COBS BY CHEMICAL TREATMENT METHODS

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In order to prepare nanoparticles, corn cobs were treated with sodium hydroxide in the range 0-6 mole/litre (0-24% of sodium hydroxide on oven dry basis) at 165 $^{\circ}$C for 1.5 h at a liquor to solid ratio of 4.5:1. The sample obtained at the optimised condition (4.5 mole/litre) was washed with deionised water, disintegrated, and filtered through 80 mesh screens. Powder thus obtained was delignified by acidified sodium chlorite and dried in a vacuum oven to constant weight. Dried powder was further separated by 270 mesh screens. An average particle size approximately equal to 22 nm was observed by Transmission Electron Microscopy (TEM). Its crystallinity was determined by XRD analysis. The aggregated particle size was observed in the micron range by Scanning Electron Microscopy (SEM).

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INTRODUCTION

About 785 million tons maize is produced annually in the world. India is the seventh largest producer, and the corn cobs are either thrown out as waste (Hemant et al. 2006) or burnt, an application with low added value, causing environmental impact (Garrote et al. 1999). However, holocellulose (i.e. cellulose and hemicellulose) of corn cob can be used for chemical processing to obtain end products with added values worldwide at very low price.

Cellulose is the most abundant naturally occurring plant polysaccharide. It consists of long chains of anhydro-β-D-glucopyranose units (AGU) with each cellulose molecule having three hydroxyl groups per AGU, with the exception of the terminal ends, whereas hemicellulose is the second most abundant plant polysaccharide readily available, especially from annual plants and agriculture crop residues such as corn cobs, corn grain, wheat stems, seed coats, and sugar cane stalks. Polysaccharides associated with lignin constitute the cell wall of land plants; D-glucuronic acid, L-arabinose, and D-xylose are present in the cell wall of corn cobs in the approximate ratio of 2:7:19, respectively (Ebringerova et al. 1994). Both cellulose and hemicellulose are an interesting group of polysaccharides with potential properties for use in the biomedical area, as they have the ability to pass through the digestive tract unchanged. Owing to their resistance to digestion, they are eligible as potential excipients that could be used in the pharmaceutical industry (Olson et al. 1983).

Cellulose-based nanoparticles prepared from cellulose fibre (Zhang et al. 2007) and cotton linter (Hafraoui et al. 2008) contain more than 95% of α-cellulose, whereas in our investigations we observed that corn cob-based nanoparticles (treated with strong alkali) contain only around 72 % α-cellulose. However the difference has been reported in α-cellulose content owing to the sorption of xylan (McKinney 1946; Clayton and Phelps 1965). These nanoparticles can improve the pharmacokinetic properties of drugs and thereby reduce toxic side effects. In the recent past nanoscale particles of corn cob

xylan have been reported, ranging from 120 to 1790 nm (Garcia et al. 2001). Owing to their controlled release properties and biodegradability, corn cob-based nanoparticles can exhibit the desired fine-tuned drug delivery characteristics and achieve a broad range of usefulness and minimal toxicity of powerful drugs. In view of the growing demand and modernisation of pharmaceutical industries, nanoparticles of reduced dimension can bring substantial changes in therapeutic systems by developing improved pharmaceutical grade biopolymer. Therefore, the objective of present study is focused on evaluating the use of corn cobs to prepare nanoparticles in narrow size distribution ($\sim$< 30nm size) with an aim to explore possible pharmaceutical applications.

EXPERIMENTAL

Raw Material

Locally collected corn cob sample was milled into powder in a laboratory Wiley mill, and fractions passing through 40 mesh (400 μm) screens but retained on 80 mesh (177 μm) screens was collected. Sample was air-dried, homogenised in a single lot to avoid compositional differences among aliquots, and stored for compositional analysis.

The corn cob sample was chopped manually into 1.5 to 2.0 cm long and 0.25 to 0.5 cm thick pieces. This sample was also air-dried, homogenised in a single lot to avoid compositional differences among aliquots, and stored for chemical analysis.

Analysis of Raw Material

The compositional data of the corn cob samples used as a raw material (as the average of four replicate analyses) is shown in Table 1. The results are expressed as weight percent of holocellulose (α-, β- and γ- Cellulose), pentosans, Klason lignin, ethanol-benzene solubles, hot water solubles and ashes by using TAPPI Standard Test Methods T 249 cm-00 (T 203 cm-99), T 223 cm-01, T 222 om-06, T 204 cm-07, T 207 cm-08, and T 211 om-85, respectively. The other fractions, including uronic acids, soluble lignin, acetyl groups, etc., were not determined, owing to their minor importance for the purpose of this work.

Table 1. Composition of Corn Cob (as the average of four replicate determinations)

Fractions	Weight % on Oven Dry Basis
Holocellulose (α-, β- and γ- Cellulose)	73.04 (34.45,18.73 and 19.84 respectively)
Pentosan	28.23
Klason Lignin	14.01
Ethanol-Benzene Solubles	4.33
Hot Water soluble	6.04
Ash	2.21

Alkaline Treatment of Raw Material

The mannually chopped sample of corn cob was treated in a WEVERK electically heated rotary digester of 0.02 m^3 capacity having four bombs of 1 liter capacity each. The chips of corn cobs were treated with sodium hydroxide (i.e. autohydrolysis) in the range 0 to 6 mol/litre (0-24% of sodium hydroxide on oven dry basis) at a temperature 165°C for 1.5 h at liquor to solid ratio of 4.5:1. After completion of the treatment, the samples were washed on a 270 mesh (53μm) screen for the removal of residual chemicals. The samples were disintegrated and screened through 80 mesh screens

(177μm), and the screened product was washed, pressed, and crumbled. The samples were analysed for pentosan (T 223 cm-01), screening rejects, yield and lignin (T 22 om-88) by weight as per TAPPI Standard Test Methods (2007). The results are shown in Table 2.

Table 2. Effect of Chemical Treatment on Residual Lignin, Pentosan, Screened Yield, Rejects and Total Yield *

NaOH (mole/litre)	Reject (%)	Screened yield (%)	Total yield (%)	Lignin (%)	Pentosan (%)
0	33.20	26.23	59.43	22.236	12.61
0.5	43.32	23.70	67.02	18.276	25.69
1.0	53.22	19.70	72.92	14.022	26.12
1.5	43.10	24.72	67.82	11.377	28.89
2.0	26.67	29.03	55.70	7.660	32.93
2.5	19.68	33.41	53.09	7.248	35.25
3.0	14.54	38.32	52.86	5.073	34.91
3.5	10.67	42.17	52.84	4.080	34.77
4.0	7.85	43.27	51.12	3.069	34.63
4.5	4.20	45.41	49.61	1.626	34.46
5.0	1.99	41.79	43.78	1.557	30.33
5.5	1.52	37.66	39.18	1.371	26.81
6.0	0.26	37.04	37.30	1.311	23.62

*(results expressed as weight percent, oven dry basis)

Characterisation of Nanoparticles

High-resolution Transmission Electron Microscopy (TEM) was carried out with an FEI Technai G2 F20 microscope at 200 kV; the sample was air dried before using the TEM to characterise the size and morphology of the dried particles. For TEM observation, the samples were prepared in methanol at 100 μg/ml concentration and dispersed in an ultrasonicator for ten minutes. The samples for TEM analysis were obtained by placing a drop of the colloidal dispersion containing the corncob nanoparticles onto the carbon-coated copper grid. They were dried at room temperature and then examined using the TEM without any further modification. The particle sizes in morphology were measured using a scale bar in micrographs. X-ray diffraction (XRD) of samples was recorded on a Bruker AXS D8 Advance diffractometer with a scanning rate of $1^oC/min$ with CuKα radiation source (λ = 1.54060Å) operating at 40 kV and 30 mA. Scanning Electron Microscopy (SEM) was performed, using a FEI Quanta 200 F microscope. The samples were mounted on Au coated stubs and observed at 20 kV.

Table 3. Chemical Composition of Nanoparticles (NPs)

Fractions	Weight percent on oven dry basis
α- Cellulose	72.75
β- Cellulose	25.19
γ- Cellulose	02.06

RESULTS AND DISCUSSION

Effect of Autohydrolysis on Pentosan Content

Figure 1 shows that the percent of pentosan (12.61%) was less in auto hydrolysed corn cob samples than that of the raw material (28.23%). Since the hemicellulose fraction of corncobs had a relatively high content of acetylated xylan (a polymer made up of

xylose units with acetyl substituent), when corn cobs were treated with water at 165°C, the hydronium ions (H_3O^+) generated from the water, i.e. auto ionisation, caused both xylan depolymerisation (to give xylooligomers and xylose) and cleavage of acetyl groups (to give acetic acid, which in turn increases the hydronium concentration in the reaction medium) (Garrote et al. 2002), leading to liquors with pH in the range 3-4 after treatment (Heitz et al. 1986). Thus in acidic environment, selective solubilisation of hemicelluloses and extractives takes place, which results, due to autohydrolysis, in less pentosan content than in the raw material and a solid phase enriched in cellulose and lignin.
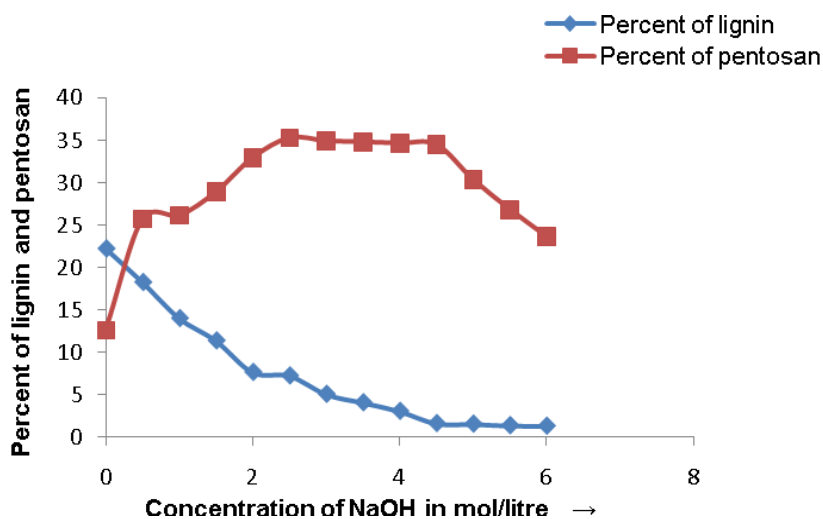

Fig. 1. Percent of lignin and pentosan at different concentrations of sodium hydroxide

Effect of Autohydrolysis on Lignin Content

Figure 1 also shows that the percent of lignin (22.24%) in an auto-hydrolysed sample was higher than in the raw material (14.01%) owing to selective removal of hemicelluloses and extractives (Garrote et al. 2002) and carbonium ion initiated repolymerisation takes place more or less simultaneously with lignin depolymerisation reaction (Jiebing and Goran 2008). An increase in hydronium ion concentrations (a large reduction in pH) during the autohydrolysis process (Garrote et al. 2002), and a large increase in phenolic hydroxyl groups due to degradation of lignin lead to the formation of carbonium ions from the benzylic position (Jiebing and Goran 2008).

In the presence of other electron-rich carbon atoms such as the C-2/C-6 present in guaiacyl and syringyl rings, condensation reactions may, however, compete with the acidolysis, leading to repolymerisation (Lora and Wayman 1979; Robert et al. 1986). The presence of repolymerised/condensed structures is also indicated by the colour change from almost colourless to brownish-yellow (Jiebing and Goran 2008). On the basis of the above explanation it was confirmed that lignin removal becomes almost negligible, whereas hemicelluloses and extractives removal becomes very large, leading to an increase the percent of lignin during autohydrolysis.

Effect of Alkali Charge on Pentosan Content

From Fig. 1 it is observed that percent of pentosans increased first sharply with increase in alkali concentration from 0 (i.e. autohydrolysis) to 2.5 mole/litre (owing to hinderance of the solubilisation of hemicelluloses), then remain almost practically constant up to 4.5 mole/litre, and afterwards it decreased. The resistance of the residual

pentosans in alkaline treatment is explained by McKinney (1946) by stating that some of the wood hemicelluloses were originally chemically bound to lignin by a glycoside linkage and that during alkaline treatment a trans-glycosidation reaction takes place in which the hemicellulose is transferred to form a glycosidic link with the cellulose. Clayton and Phelps have also explained that the chemical bonds are formed between the cellulose and the sorbed hemicellulose because the activation energies for the sorption of both galactoglucomanan and xylan with a low uronic acid content was found to be less than 10 Kcal/mol (Clayton and Phelps 1965). But after 4.5 mole/litre of sodium hydroxide the pentosan content decreased sharply, owing to hemicellulose degradation at the higher concentration of alkali. This explains the greater retention of pentosans when treatment was carried out in the presence of reagents that stabilize the polysaccharide against alkaline degradation. Hence 4.5 mole/litre of sodium hydroxide concentration may be considered as an optimum dose of alkali for further processing.

Effect of Alkali Charge on Lignin Content

The objective of alkaline treatment is to degrade and dissolve away the lignin and leave behind most of the cellulose and hemicellulose in the form of particles. According to Jiebing and Goran (2008) sodium hydroxide suppresses the repolymerisation reactions by inhibiting the acid catalyzed reactions. On increasing the concentration of sodium hydroxide, the hydronium ions will be neutralised, and the medium shifts from acidic to alkaline. Hence, carbonium ion formation becomes less, and lignin depolymerisation dominates over lignin repolymerisation. From Fig. 1 it is seen that the percentage of lignin decreased sharply as the concentration of sodium hydroxide increased up to 4.5mole/litre, and afterward the percentage of lignin became almost constant. Therefore, on the basis of the above studies, 4.5 mole/litre of sodium hydroxide concentration may be considered as the optimum level of alkali.

Effect of Alkali Charge on Yield

Figure 2 shows that the screened yield first decreased and reached a minimum (19.7%) and then increased up to a maximum (45.41%) and again decreased. Auto-hydrolysis of the raw material provided the hydronium ions to bring about the acidic condition. But alkali charge was given to the raw material, consuming the hydronium ions, leading to neutralization. Hence, screened yield was greater during autohydrolysis in comparison to treatment given at 0.5 to 1.5 mole/litre of sodium hydroxide concentration. After that, the screened yield increased and rejects decreased with increased alkali charge, and the maximum yield was found at 4.5 mole/litre sodium hydroxide concentration. Therefore, an alkali dose of 4.5mole/litre sodium hydroxide concentration may again be considered as the optimum dose of alkali.

Figure 2 also shows that the rejects increased as sodium hydroxide concentration increased from 0 to 1 mole/litre concentration (owing to neutralization of medium) and then decreased continuously owing to alkaline treatment.

Treatment Selectivity

On the basis of the above studies, the process conditions were optimised to obtain maximum yield and minimum chemical consumption. Based on experimental studies an alkali dose of 4.5 mole/litre of NaOH concentration was found to be optimum for chemical treatment of corn cobs raw material.
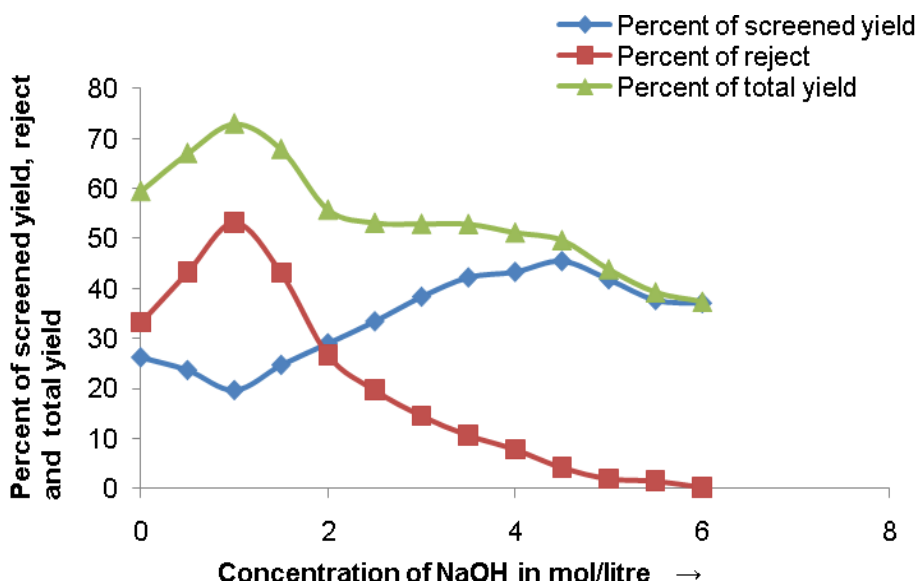
Fig. 2. Percent of screened yield, reject and total yield at different concentrations of sodium hydroxide

The samples obtained at the optimised condition were delignified with acidified sodium chlorite solutions and dried in a vacuum oven. The obtained powder after delignification and drying was further screened from 270 (53μm) mesh size for the determination of chemical composition. By using TAPPI Standard Test Methods T 203 cm-99 α-, β-, and γ-cellulose were determined. The results are shown in Table 3.When 4.5 mole/litre of NaOH chemical treatment was given to corn cob raw materials at a temperature 165°C for 1.5 h at liquor to solid ratio of 4.5:1, most of the γ-cellulose was removed except for retaining γ-cellulose (2.06 %), α-cellulose (72.75 %), and β-cellulose (25.19 %), as a result of which crystallinity values were found to increase from 37% to 48% as confirmed by XRD analysis. The obtained powder was also characterised by TEM, XRD, and SEM analyses.

Transmission Electron Microscopy (TEM) Analysis

TEM photographs (Fig. 3) show that the average particle size was 22 nm, and the corresponding electron diffraction pattern showed only diffused signals, as expected for amorphous materials.

X-Ray Diffraction (XRD) Analysis

From Fig. 5 it was determined that the crystalline content of nanoparticles increased from 37% (delignified raw materials) to 48% (nanoparticles). The crystallinity was found to increase owing to smaller particle size (Zhang et al. 2007) and removal of amorphous substances such as lignin, hemicelluloses, and extractives.

The XRD pattern of corn cob-based nanoparticles showed several relatively strong reflection peaks. The main diffraction peaks were assigned: $2\theta = 16.230^o$ (d = 5.60778), 22.221^o (d = 3.99738), and 34.787^o (d = 2.57686). The average particle size may be estimated by using Scherrer's equation,

$$D = K\lambda / \beta \cos\theta \qquad (1)$$

where K is the shape factor, λ is the X-ray wavelength (1.54060 Å), β is the line broadening at half the maximum intensity (FWHM) in radians, θ is the Bragg angle, and

D is the mean size of the ordered (crystalline) domains. The dimensionless shape factor has a typical value of about 0.93, but varies with the actual shape of the crystallite. The reflecting peaks at $2\theta = 22.221^{o}$ and 34.787^{o} are used to estimate the average size (~6 nm and ~13 nm respectively) of the corn cob-based nanoparticles.

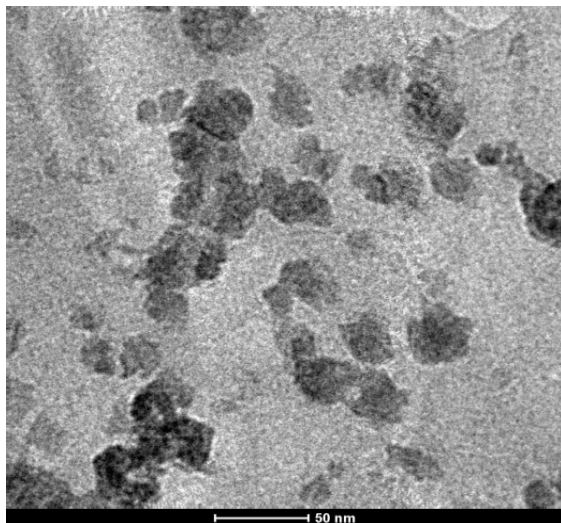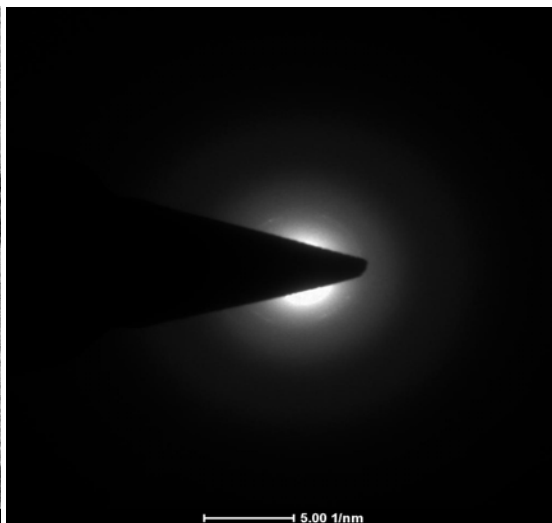**Fig. 3 (a)**   **Fig. 3(b)**

Fig.3. (a) Representative TEM image of corn cob based nanoparticles (scale bar represents 50 nm) and (b) the corresponding diffraction pattern.
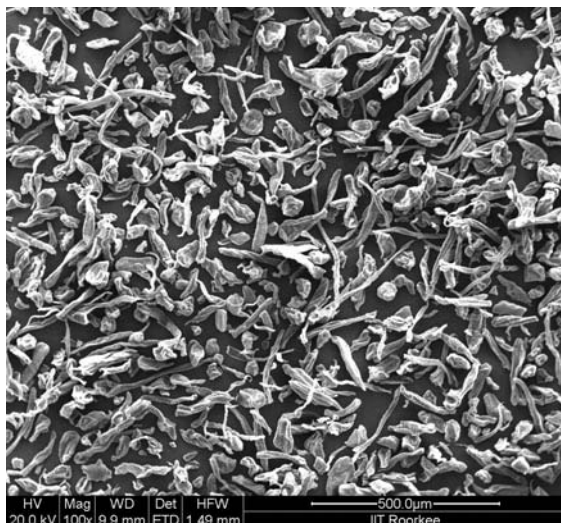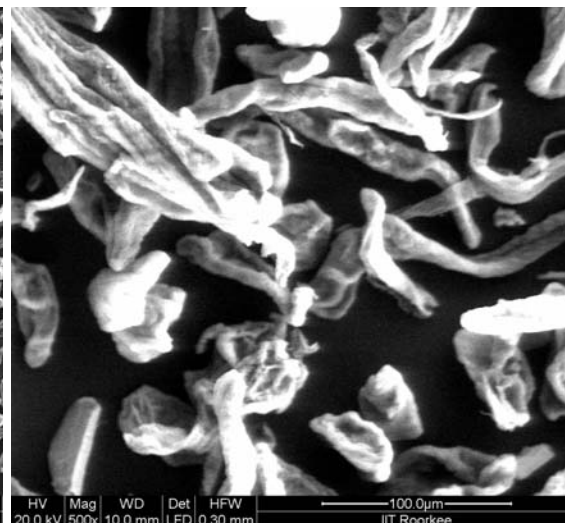

Fig. 4 (a)   **Fig. 4 (b)**

Fig. 4. SEM image (a) Aggregated corn cob based nanoparticles (scale bar represents 500 μm) and SEM image (b) Same sample at lower magnification (scale bar represents 100 μm).
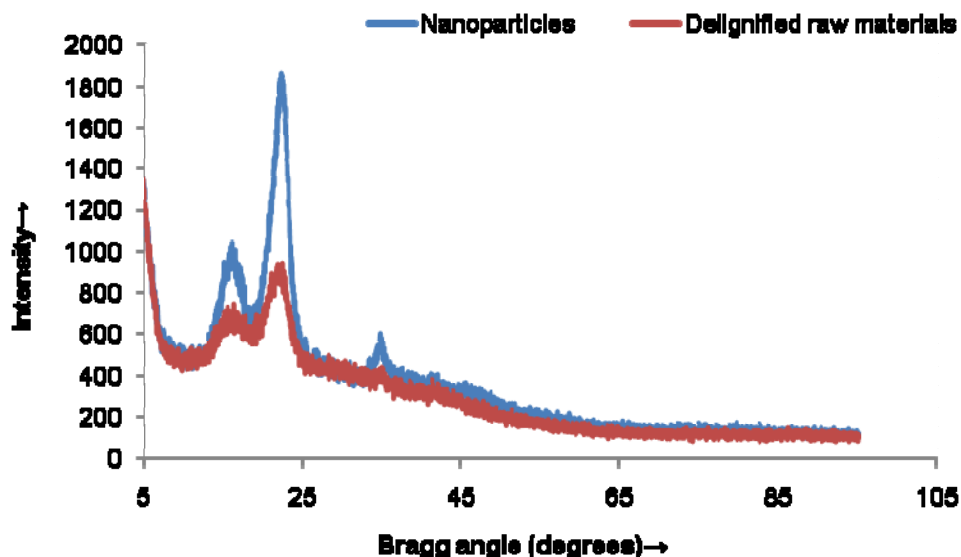
Fig. 5. X-ray diffraction curve of delignified raw material and nanoparticles

Scanning Electron Microscopy (SEM) Analysis

Figure 4 shows clusters of particles, with varying structures, having an average diameter ranging from 24 μm to 200 μm, which is attributed to aggregation of particles through a solid bridging mechanism. In order to keep free-flowing nanoparticles separate, then they must be prepared and stored in a liquid medium, designed to facilitate sufficient interparticle repulsion forces to prevent aggregation.

CONCLUSION

The present work demonstrates that the nano-scale particles of various structure can be synthesised from corn cob by 4.5 (18% on oven dry) mole/litre of sodium hydroxide concentration for 1.5 h at 165°C. The particle size obtained in the present work varied in the range of 10 to 40 nm (i.e. narrow size distribution). There was also a tendency for the crystallinity to increase as the particle sizes became smaller. These nanoparticles under study can have possible use in drug delivery systems.

ACKNOWLEDGEMENT

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