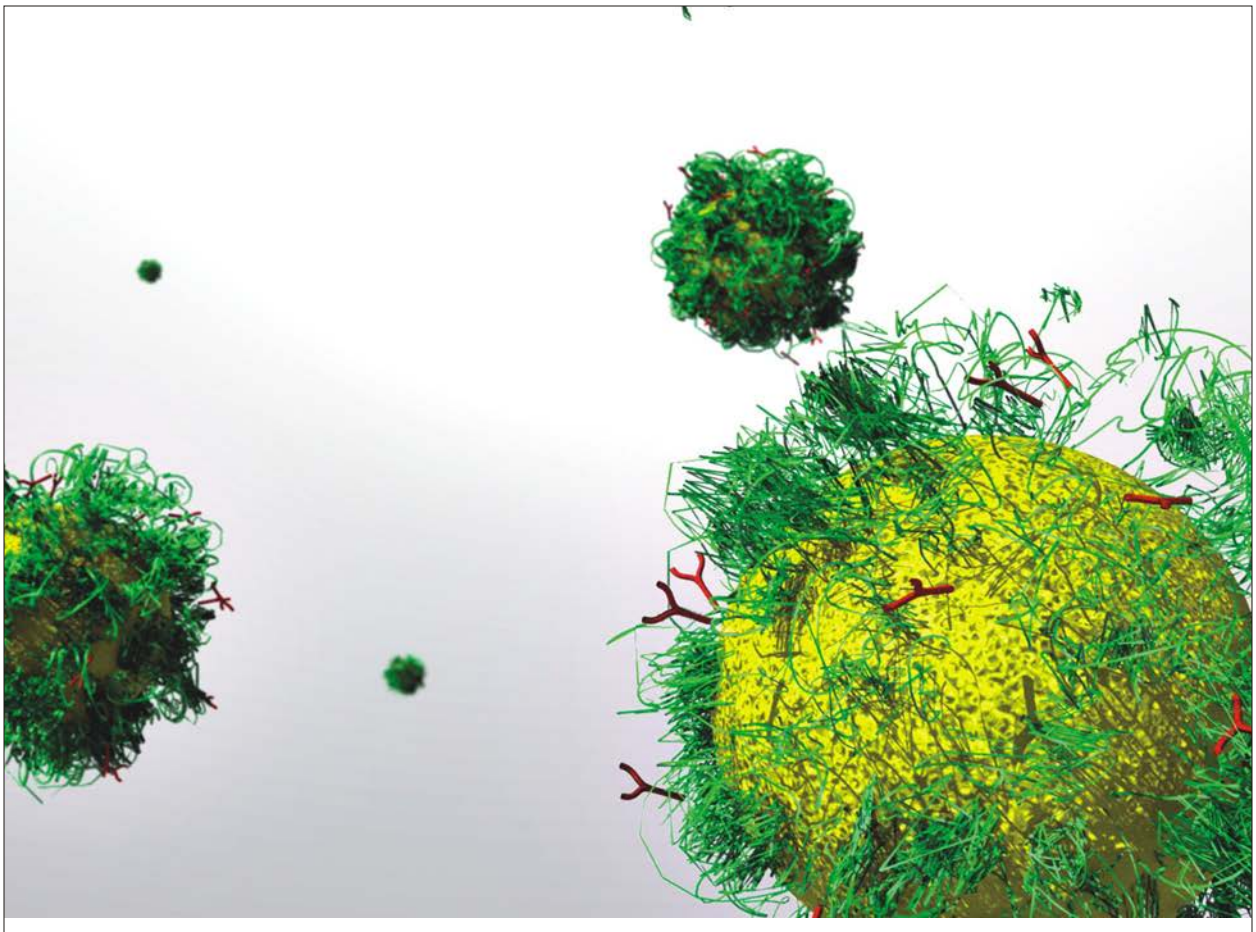

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Evaluation of Bacterial Cellulose Produced Form *Acetobacter xylinum* as Pharmaceutical Excipient

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ABSTRACT

Microcrystalline cellulose (Avicel 101, AV 101) has been widely used as the excipients for tablet formulation. The objective of this research was to produce cellulose from *Acetobacter xylinum* (*A. xylinum*) which is used as alternative excipient for tablet formulation instead of AV 101. The bacterium was isolated from kombucha tea preparation. Bacterial pellicle formation was cultivated in stationary conditions using a Herstin-Schramm nutrient (HS) medium. The produced Bacterial Cellulose (BC) was compared with AV 101 by carrying out Moisture Sorption Capacity (MSC), Loss on Drying (LOD), Scanning Electron Microscopy (SEM), FT-IR Spectroscopy, X-Ray diffraction analysis, micromeritic properties and compactibility studies. Comparisons revealed that MSC for AV 101 was higher when compared to BC due to crystalline nature of BC. LOD of BC was 4.635% which is required for good compression. The particles of BC were small sized densely packed reticulated structure consisting of fine thread like fibrils whereas AV 101 was irregularly large sized elongated structures. The IR spectra showed that there were no significant difference in the spectra of BC and AV 101. BC had higher crystallinity than AV 101. Micromeritic properties and compactibility studies showed that BC have high bulk density, better flow property, easy fragmentation of the particles and rearrangement at a lower compression load, lesser elastic recovery and higher tensile strength than AV 101. The result provides evidence that BC produced by HS medium can be suitably used as pharmaceutical excipient for tablet formulation instead of AV 101.

Key words: Bacterial cellulose, *Acetobacter xylinum*, Avicel 101, pharmaceutical excipient, tablet

INTRODUCTION

Cellulose is the earth's major biopolymer and is of tremendous economic importance globally (Keshk and Sameshima, 2006). Microcrystalline cellulose has been widely accepted as the excipient of choice in pharmaceutical and allied industries. It has found its utility majorly in tablet formulation where in it acts as a multipurpose additive. It functions as an inert diluent, a binding agent and a disintegrating agent. Microcrystalline cellulose powders are probably the most important pharmaceutical excipients used in tableting, especially for direct compression (Yu and Atalla, 1998; Anonymous, 1996). Microcrystalline cellulose is made from fibrous cellulose (mainly wood pulp) which consists of an ordered region (microcrystal) and a less-ordered region (amorphous regions) (Yu and Atalla, 1998).

Microbial cellulose is an exopolysaccharide produced by various species of bacteria, such as those of the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*,

Azotobacter, *Rhizobium*, *Sarcina* and *Salmonella* (Rezaee *et al*., 2008; Ross *et al*., 1991; Norouzian *et al*., 2011). Production of cellulose from *A. xylinum* was first reported in 1886. This organism is nature's most productive cellulose producing bacterium. *A. xylinum* uses cellulose as a device to raise itself to the oxygen rich air at the surface of its environment. He observed that the resting cells of *Acetobacter* produced cellulose in the presence of oxygen and glucose (Brown, 1886).

The cellulose synthesized by *A. xylinum* is identical to that made by plants in respect to molecular structure; however, the secreted polysaccharide is free from lignin, pectin and hemicelluloses as well as other biogenic products which are associated with plant cellulose (Keshk and Sameshima, 2006). Additionally, extracellularly synthesized BC microfibrils differ from plant cellulose with respect to its high crystallinity and mechanical strength in the wet state (Klemm *et al*., 2001; Yoshinaga *et al*., 1997; Parthiban *et al*., 2011; Yudianti and Indrati, 2008).

A. xylinum is a non-pathogenic, Gram-negative, rod-shaped, obligate aerobe, belonging to the family Acetobacteraceae (Kersters *et al*., 2006). *A. xylinum* produces two forms of cellulose: (1) cellulose I, the ribbon-like polymer and (2) cellulose II, the thermodynamically more stable amorphous polymer (Yu and Atalla, 1996). The molecular formula of bacterial cellulose $(C_6H_{10}O_5)_n$ is the same as that of plant cellulose, but their physical and chemical features are different (Yoshinaga *et al*., 1997; Rezaee *et al*., 2008). It also has higher tensile strength and water holding capacity than that of plant cellulose, making it more suitable raw material for producing high fidelity acoustic speakers, high quality paper and dessert foods, textile industry, medical pads and artificial skin, wound healing (Klemm *et al*., 2001; Yoshinaga *et al*., 1997; Czaja *et al*., 2007).

The major industrial source of cellulose is vascular plants. Since harvesting of forests for production of cellulose has led to soil erosion, pollution etc., hence there is a need for development of cellulose from alternative source which is identical to made by plants and can be used as a pharmaceutical excipient. A substitute, to reduce the demand from plants, is the production of cellulose using a microbial system. In this study, bacterial cellulose was prepared from *A. xylinum* in static culture by using HS medium. The prepared cellulose was compared with AV 101 as a pharmaceutical excipient by carrying out MSC, LOD, SEM, FT-IR Spectroscopy, X-Ray Diffraction analysis, micromeritic properties and compactibility studies.

MATERIALS AND METHODS

Materials: AV 101, Glucose, Peptone, Yeast extract, Anhydrous disodiumphosphate, Citric acid monohydrate, Hydrogen peroxide from Loba Chemie, Mumbai. All other chemicals used were of analytical grade.

Isolation of *A. xylinum*: The bacterium was isolated from kombucha tea preparation available locally at Mysore, India. For the isolation of the bacterial strain the medium was employed with modification. Briefly, the medium consisted of (g L^{-1}): sucrose 50, yeast extract 5.0, ammonium sulphate 5.0, KH_2PO_4 3.0, $MgSO_4.7H_2O$ 0.05, citric acid 1.0 and sodium acetate 2.0. The pH of the medium was adjusted to 5.0-5.5 using 1.0 M HCl. After sterilization the liquid medium (100 mL) in 250 mL conical flask was inoculated with 0.1 mL of kombucha tea culture and was allowed to grow at 30°C for 48 h. Again from the above flask 0.1 mL of inoculum was added to another conical flask to obtain growth. This process was repeated for another five times. Pure colonies of *A. xylinum* were selected by plating 0.1 mL of the above liquid culture on 1.5% agar medium.

White colonies adhering to the agar were lifted and again inoculated into liquid medium for growth. The purified culture was stored on Watanabe and Yamanaka agar medium for further use.

The organism was confirmed as *A. xylinum* depending on its gram-negative property and rod-hke structure in addition to its ability to utilize sucrose, glucose and sodium acetate in the presence of citric acid and lowering the pH of the growth medium down to pH 2.5-3.0 during growth with the addition of producing a thick cellulose pellicle at the surface (George *et al.*, 2005).

Production and processing of bacterial cellulose: The medium for inoculum preparation and pelhcle formation was cultivated in stationary conditions using a HS medium composed of Glucose 2% w/v, Peptone (Difco Bactopeptone) 0.5% w/v, Yeast extract (Marmite) 0.5%, Anhydrous disodium phosphate 0.27% w/v, Citric acid monohydrate 0.115% w/v, distilled water up to 1000 mL. Conical flasks used were filled with HS medium. pH of the medium was adjusted to 5.0 using 1 N acetic acid (Hestrin and Sehramm, 1954; Kouda *et al.*, 2000). The medium was inoculated with *Acetobacter xylinum*, allowed for fermentation in stationary condition for 24 h at 25, 30 and 35°C temperature. Medium was recharged with fresh decoction every 7 days and allowed it for further fermentation (Keshk and Sameshima, 2006; Pourramezan *et al.*, 2009; Norouzian *et al.*, 2011).

Polysaccharide (bacterial pellicle) was formed at the interface of air. Thickened pellicle sinks to the bottom of fermentation vessel. Bacterial pellicle isolated from culture media was thoroughly washed with distilled water and cut into small pieces. BC was weighed (500 g) and transferred to a 2.5 L round bottom flask containing 1.5 L 4% NaOH, fitted with a water condenser. It was boiled at 100±5°C for 20 min in order to remove bacterial cells. The alkali treated pellicle was washed successively with distilled water (Surma-Slusarska *et al.*, 2008).

Product obtained was bleached with 10% of hydrogen peroxide in order to achieve maximum brightness (Gupta and Johnson, 1991). Bleached product was washed thoroughly with distilled water after cooling and size was reduced using high speed mixer for 5 min at 8000 rpm. The slurry was filtered and washed with 250 mL of acetone for 8 h to remove the water content and filtered again. The weight of the BC was determined by the weight of purified BC after drying in an oven at 40°C over night. The dried product was size reduced in mixer and passed through sieve no. 120 and stored in a well closed container (Netravali, 2010).

Moisture sorption capacity: Two grams of the BC and AV 101 were accurately weighed (W_1) separately and evenly distributed over the surface of a tarred Petri dish. The samples were placed in a large desiccators containing distilled water in its reservoir (RH 100 %) at room temperature and the weight gained by the exposed sample was recorded at the end of a four day period (W_2). The amount of water absorbed was calculated from the weight difference in triplicate (Ohwoavworhua and Adelakun, 2010):

$$\text{Moisture sorption capacity (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

Loss on drying: One gram of powder samples (BC and AV 101) were transferred into a petri dish and dried in an oven at 105°C until a constant weight (W_2) was obtained. The percentage loss on drying was determined in triplicate (Ohwoavworhua and Adelakun, 2010):

$$LOD\ (\%) = \frac{W_1 - W_2}{W_2} \times 100$$

Scanning electron microscopy: Shape and surface morphology of powders (BC and AV 101) were studied using scanning electron microscopy (Joel-LV-5600, USA). Samples was put on copper support, cover with a 1 nm thick layer of gold for 60 s (Barud *et al.*, 2007).

FT- IR spectroscopy: Solid pellet technique was used to predict any significant difference in the spectra of BC and AV 101. Samples were mixed with potassium bromide separately in the ratio 1:100, triturated and compressed to prepare the pellet. Twenty scans were acquired in the 4000-600 cm^{-1} range with a resolution of 4 cm^{-1} using FT-IR spectroscopy (Shimadzu, 8400S, Japan). FT-IR spectrum of BC was compared with FT-IR spectra of AV 101.

X-Ray diffraction analysis: To determine the crystallinity of the produced BC and AV 101, the X-Ray Diffraction (XRD) pattern of the sample was collected on a Scintag PAD V theta-2-theta diffractometer (Scintag, Cupertino, CA) using a copper x-ray source. The XRD diffraction patterns were taken over 2 h in the range of 5 to 50° at a scan speed of 12° min, the step size was 0.02° and the exposure time was 10 min. MDI Jade 8 software (Materials Data, Inc., Livermore, CA) was used to process the diffraction pattern and to calculate the crystallinity of BC (Pongjanta *et al.*, 2009).

Micromeritic properties

Angle of repose: The static angle of repose (θ) was measured according to the fixed funnel and free standing cone method (Train, 1958). A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. To increase the reliability of the observations, the angle of repose were performed in triplicate. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

$$Tan\theta = \frac{2h}{D}$$

where, H is the height of the heap of powder and D is the diameter of the base of the heap of powder.

Bulk density: The bulk density of each cellulose material at zero pressure (loose density) was determined by pouring a known quantity of the powder (W) into a 250 mL measuring cylinder and the volume (V_0). The bulk density was calculated as $B_d = W/V_0$. The results presented are the mean of three replicates determinations (Ohwoavworhua and Adelakun, 2010).

Tapped density: Tapped density was determined by placing a graduated cylinder containing a known mass of powder on a mechanical tapper apparatus (Electro lab tap density tester). Samples were tapped until no further reduction in volume of the sample was observed (Lachman *et al.*, 2008). To increase the reliability of the observations, the tapped density were performed in triplicate.

True density: The true densities (D_t) of cellulose powders were determined by the liquid displacement method using xylene as the immersion fluid and computed according to the following equation:

$$D_t = \frac{w}{[(a+w)-b]} \times SG$$

where, w is the weight of powder, 'SG' is specific gravity of solvent, 'a' is weight of bottle+solvent and 'b' is weight of bottle+solvent+powder (Ohwoavworhua and Adelakun, 2010). To increase the reliability of the observations, the true density were performed in triplicate.

Hausner ratio: The Hausner ratio was determined as the ratio of tap and bulk density of the samples (Ohwoavworhua and Adelakun, 2010).

Porosity: Based on the apparent bulk density and true density, the percentage porosity of the BC and AV 101 were calculated in triplicate (Ohwoavworhua and Adelakun, 2010; Franklin-Ude *et al.*, 2008):

$$\text{Porosity (\%)} = \frac{\text{True density-Bulk density}}{\text{True density}} \times 100$$

Compressibility or carr's index: Based on the apparent bulk density and the tapped density, the percentage compressibility of the polymers was determined in triplicate (Franklin-Ude *et al.*, 2008):

$$\text{Carr's index} = \frac{\text{Tapped density-Bulk density}}{\text{Tapped density}} \times 100$$

Particle size analysis: Particle-size analysis was carried out by microscopic method. Size of 100 particles of each BC and AV 101 were analyzed and average size was determined.

Compactibility studies: Tablets of BC and AV 101 were prepared using hydraulic press (KBr Press) fitted with pressure gauge (1-6 Tonnes). Three hundred mg of powder was compressed at different predetermined pressure for 30 sec. Tablets were stored over silica gel in desiccators. Weights (w) and dimensions of tablets were determined and their relative densities (D) were calculated from the equation:

$$D = w/V_t . \rho_s$$

where, w is the weight of the tablet, 'V_t' is the volume (cm^3) of the tablet and $\tilde{n}_s$ is the true density ($g\ cm^{-3}$) of the solid material (Apeji *et al.*, 2010).

Heckel analysis: Heckel equation has been most widely used to describe the compaction of pharmaceutical powders. Compressibility of powder is characterized from the density- compression pressure relationship according to the heckle plot. Dimensions of the tablets of BC and AV 101 were

measured using micrometer screw gauge (Mitotoyo, Japan). Average of three determinations was considered for the studies. Relative densities at different compression load were calculated and Heckel plots of ln [1/(1-D)] Vs applied load (P) were constructed for BC and AV 101 tablets (Apeji *et al.*, 2010).

The relative density 'D_A' was calculated from the intercept using the equation, $D_A = 1\text{-}e^{-A}$. While 'D_B' is the relative density describing the phase of densification occurring during rearrangement of the powder bed at low pressure and is given as; $D_B = D_A\text{-}D_0$ (Emeje *et al.*, 2007).

Determination of elastic recovery of the tablets: Thicknesses of five tablets of each BC and AV 101 were analyzed by screw micrometer at 0, 1, 10 and 24 h after 10 days of tableting. The percentage (%) elastic recovery of tablets made at different compression loads after 10 days was calculated by using the following formula:

$$ER = [He\text{-}Hc/Hc]\times 100$$

where, Hc: Minimum thickness of tablet i.e., at 0, 1, 10 and 24 h; 'He': Maximum thickness of tablets i.e., on 10th day (Parida, 2010). The plots of % ER versus applied load (P) were constructed for BC and AV 101 tablets.

Tablet tensile strength test: The prepared tablets of BC and AV 101 were kept in a desiccator for about 24 h and then a hardness tester (Erweka tablet hardness tester, Ahmedabad) was used to measure a load across the diameter of each tablet at a specific compression speed to find the hardness F when crushing. The following equation was then used to calculate the tensile strength T:

$$T = 2F/\pi dL$$

where, L is breaking hardness (kg cm^{-2}), d is diameter (cm) and F is the tablet thickness (cm). Plots of tensile strength versus compression load for BC and AV 101 (Parida, 2010).

RESULTS AND DISCUSSION

Production of bacterial cellulose: During the breeding of *A. xylinum* bacteria in stationary conditions, BC was synthesized in the form of film on the surface of the HS medium. The yield of the biosynthesis process depends on many factors mainly temperature and time. Hence keeping the concentration of HS medium constant, the temperature and time for breeding was varied to check the highest percentage yield.

On the basis of preliminary research, temperature of 30°C and time of 7 days were chosen as optimal conditions for breeding (Fig. 1). The pellicle grown in HS medium was whitish in color (Fig. 2). The thickness of the pellicle was in the range of 0.5-2 cm. The percentage yield of BC production was found to be 25.65%.

Moisture sorption capacity: MSC is a measure of moisture sensitivity of the material. MSC for AV 101 was higher (13.9%) when compared to BC (6.012%). Ohwoavworhua and Adelakun (2010) reported moisture content (AV 101) of 16.60%. It has been reported that crystalline portion of

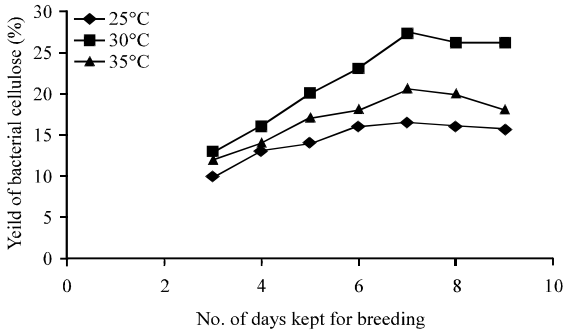

Fig. 1: Effect of the temperature and time (in days) of *A. xylinum* bacteria breeding

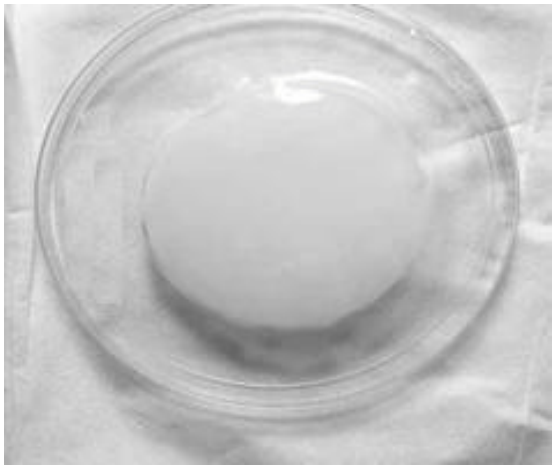

Fig. 2: Bacterial pellicle grown in herstin-schramm nutrient medium

cellulose do not absorb water when compared to amorphous portion of cellulose (Stamm, 1964). Since BC is more crystalline its MSC is less compared to AV 101.

Loss on drying (LOD): LOD for BC and AV 101 were found to be 4.635 and 2.923%, respectively. The LOD limit for MCC in Indian pharmacopoeia is 6% while that in USP is 7%. It has been technically proved that the plasticizing property which is required for good compression is maximum at LOD range of 4 to 5% in the excipient. A lower LOD tends to lead to problem of cracking of tablet and friability while a high LOD leads to soft tablet, in a standard formulation. However, some hygroscopic formulations do require excipients with LOD limit of 2% to less than 1% (Ranadive, 2011). BC having LOD of 4.635% within the range; hence formed a good compressed tablet.

Scanning electron microscopy: The structure of BC (Fig. 3a) and AV 101 (Fig. 3b) are shown in Fig. 3. The BC showed a significant difference in appearance when compared to AV 101. The particles of BC were small sized densely packed reticulated structure consisting of fine thread like fibrils whereas AV 101 was irregularly large sized elongated structures (Chawla *et al.*, 2009). The difference in the morphology of the particles was evident in the packing and flow characteristics of the material.

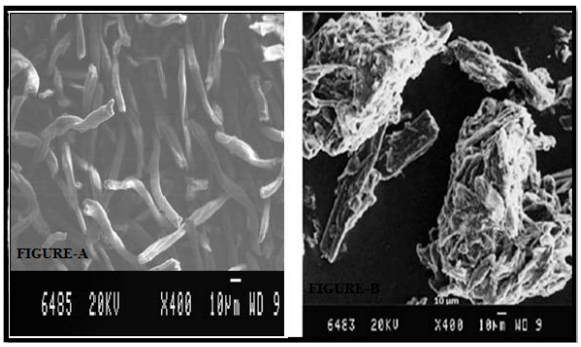

Fig. 3(a-b): Scanning electron micrographs illustrating micro structures of (a) bacterial cellulose and (b) Avicel 101

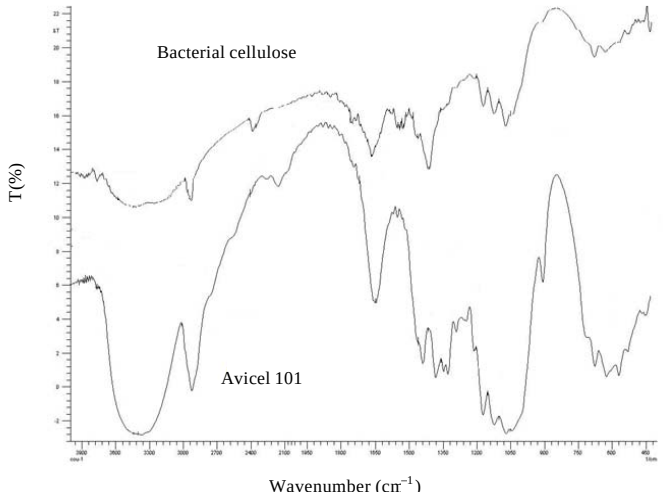

Fig. 4: FT-IR Spectra of avicel 101 and bacterial cellulose

FT-IR spectroscopy: All the peaks of AV 101 and BC (Fig. 4) were comparable and there were no significant difference in the spectra of BC and AV 101. However there is a slight difference in spectrum of BC and AV 101 which may be due to the strong hydrogen bonding in BC than AV 101. The obtained peaks of BC were similar to characteristic absorption bands of BC such as 2942 cm^{-1} (shoulder) for C-H stretching, 3348 cm^{-1} for hydroxyl groups, 1161 cm^{-1} which corresponds to C_1OC_4 stretching at **β**-glycosidic linkage as in case of plant cellulose (Surma-Slusarska *et al.*, 2008; Oh *et al.*, 2005).

X-Ray diffraction of bacterial cellulose powder: Random orientation of a crystal lattice in a powder sample causes the x-rays to scatter in a reproducible pattern of peak intensities at distinct angles (θ) relative to the incident beam. Each diffraction pattern is characteristic of a specific

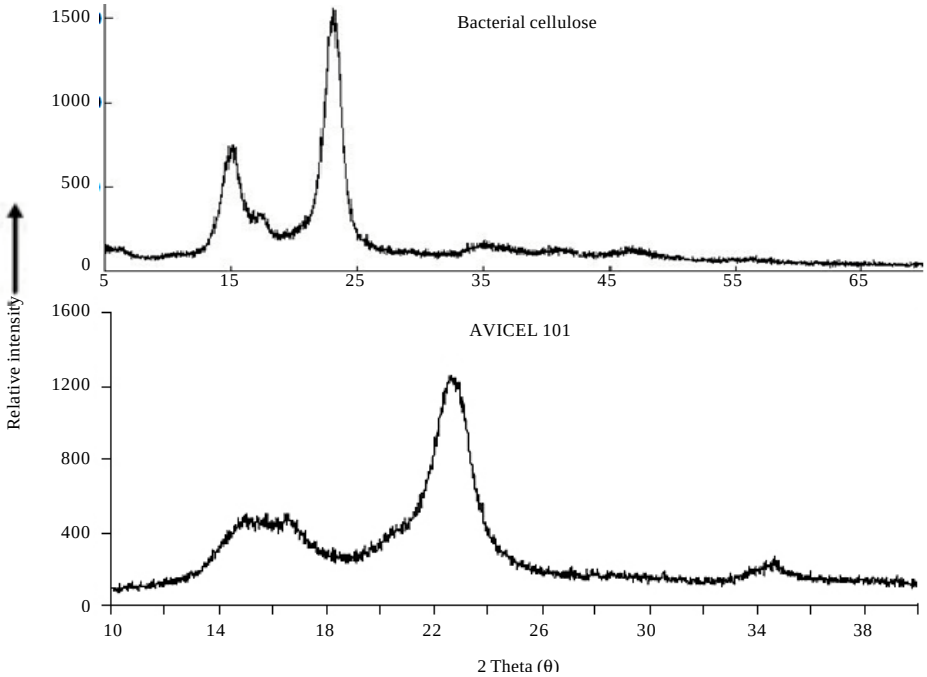

Fig. 5: X-Ray diffraction pattern of bacterial cellulose and avicel 101 powder

crystalline lattice for a particular compound. An amorphous form does not produce a pattern. XRD patterns of BC obtained demonstrated three main characteristic peaks standing for crystal plane $<\overline{110}>$, $<\overline{110}>$ and $<\overline{002}>$ (Fig. 5). The peaks were found at 15, 16 and 23° 2θ due to $<\overline{110}>$, $<\overline{110}>$ and $<\overline{002}>$ reflections which were also seen in cellulose I which is represented by the native cellulose (Isogai, 1994). The crystalhnity index of BC produced by static culture in the HS medium was found 93% which may arise as a result of longer polymerization of BC whereas the crystalhnity index of AV 101 was 71.4%. The peaks obtained for AV 101 was different when compared with the peaks of BC. The crystal size of $<\overline{002}>$ crystal plane for BC sample was found around 5.2 nm. Soh *et al.* (2004) and Kumar and Kothari (1999) in their research reported the obtained crystallinity of AV 101 as 69.56 and 72.23%, respectively. Yudianti and Indrati (2008) reported high crystanallity index of BC produced form fermentation of coconut water by *A xylinum* under static condition.

Micromeritic properties: The powder properties of BC and AV 101 investigated by several investigators (Ohwoavworhua and Adelakun, 2010; Soh *et al.*, 2004; Kumar and Kothari, 1999) were presented in Table 1. Bulk densities of powders are dependent on the size, shape and packing geometry of particles. Bulk density of BC was 0.57±0.01 gm mL^{-1} while for AV 101 its 0.25±0.01 gm mL^{-1}. Density differences are due to different crystal habits, shapes leading to different contact points and packaging arrangements. Particle size analysis revealed that particles of BC were small sized (39-43 μm) whereas AV 101 were irregularly large sized (50-65 μm). BC powder because of small particle size, showed more bulk density and showed higher packaging ability.

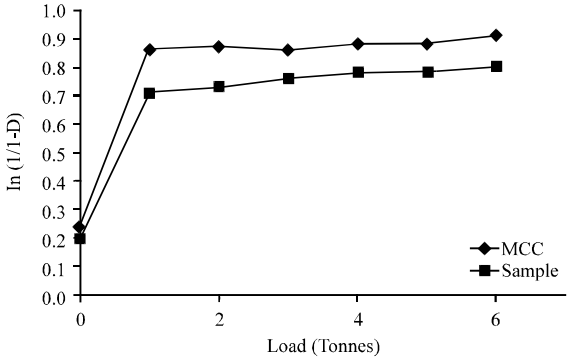

Fig. 6: Heckel plots for avicel 101 and bacterial cellulose

Angles of repose were able to provide gross measurements of the flowability of powders. The flow properties of a powder are essential in determining its suitability as a direct compression excipient. Most free flowing materials have angle of repose ≤30°. Powders with angle of repose = 40° have flow problems and are suggested as poorly flowing material (Lachman and Liberman, 1981). BC exhibited better flow property than AV 101. AV 101 showed 47±1.2° angle of repose hence exhibited little adhesiveness and were sticking to the funnel. Angle of repose for BC was found to be less (15±1.5°) because of their particle size and smooth surface. Porosity of AV 101 and BC has very less difference between them of 83.8±1.3 and 82.2±1.0, respectively.

Carr's showed the relationship between the compressibility index and flowability. The compressibility index is a simple and fast method for estimating flow of powder. As the values of carr's indices increase, the flow of the powder decreases. BC has lower Carr's indices of 20.83±0.1% than AV 101 which has 31.69±0.3% this indicates that BC has fair flow character whereas AV 101 has poor flow character (Gregory *et al.*, 2009). Hausner ratio is 1.26±0.02 for BC which indicate passable flow character whereas AV 101 has 1.46±0.01 which indicate very poor flow character. Hence AV 101 needs a glidant to improve the flow character.

Heckel analysis: The Compactibility may be defined as the ability of a powder to form a coherent tablet as a result of compression (Larry and Stephen, 2008). The Heckel plot for both materials used for the study is displayed in Fig. 6. The values obtained were tabulated in Table 1. The BC and AV 101 plot shows an almost linear relationship at all applied pressures suggesting that they deform principally by plastic deformation. The slope of Heckel plot (K) is indicative of the plastic behavior of the material. A larger value for the slope is related to a greater amount of plasticity in the material.

The mean yield pressure P_Y is inversely related to the ability of the substances to deform plastically under pressure. BC deformed at a much lower yield pressure compared to AV 101. This low yield value reflects low resistance to pressure, good densification and easy compression (Jivraj *et al.*, 2000). This agrees that a large value of slope is an indication that the onset of plastic deformation occurs at relatively low pressure (Shangraw *et al.*, 1981).

The value of Do which represents the degree of initial packing in the die as a result of die filling, increased with increased binding property. BC showed higher value than AV 101. This indicates that BC exhibited a higher degree of packing in the die as a result of die filling than AV 101.

Table 1: Micromeritic properties and heckel values of Avicel 101 and Bacterial cellulose

Parameter	Avicel 101[a]	Bacterial cellulose	Avicel 101[b]	Avicel 101[c]	Avicel 101[d]
Particle size range (μm)	54.34- 58.46	22.6-29.9	76.53	-	-
Angle of repose (θ°)	47.00±1.2°	15±1.5°	-	-	41.20
Bulk density (g mL^{-1})	0.25±0.01	0.57±0.01	0.31	0.315	0.31
Tapped density (g mL^{-1})	0.36±0.01	0.72±0.02	0.43	0.401	0.42
True density (g mL^{-1})	1.55±0.02	3.20±0.02	-	1.577	1.40
Hausner ratio	1.46±0.01	1.26±0.02	-	-	1.35
Porosity (%)	83.8±1.3	82.2±1.0	-	75	78
Carr's Index (%)	31.69±0.3	20.83±0.1	-	-	26
A	0.466±0.01	0.565±0.02	-	-	-
D_O	0.36±0.01	0.420±0.02	-	-	-
K	0.071±0.02	0.073±0.01	-	-	-
P_Y	14.08±0.01	13.6±0.02	-	-	-
D_A	0.372±0.02	0.432±0.02	-	-	-
D_B	0.0125±0.02	0.0012±0.01	-	-	-

Mean±SD, n = 3, A: Intercept, D_0: Relative density of powder at zero applied load, D_A: Relative density at different load (= 1- e^{-A}), K: Slope of the straight line portion, Py: Mean yield pressure (= 1/K), Avicel 101[a]: Results obtained in our study, Avicel 101[b]: Results obtained by Soh *et al.* (2004), Avicel 101[c]: Results obtained by Kumar and Kothari (1999), Avicel 101[d]: results obtained by Ohwoavworhua and Adelakun (2010)

It has been shown that both particle size and shape have great effects on relative density values (York, 1978). A decrease in particle size and an optimal presence of water in the powder mass will result in stronger tablets especially with plastic deforming material (Korhonen *et al.*, 2002).

The D_B of AV 101 was slightly higher than that of BC. This translates to a greater degree of fragmentation with AV 101. BC showed easy fragmentation of the particles and rearrangement at a lower compression load. High D_B values are caused by fragmentation while low D_B values are typically connected to plastically deforming materials (Doelker, 1988). The D_A represents the total degree of packing at zero and low pressures. BC had a higher relative density of plastic deformation than that of Av101.

Determination of elastic recovery of the AV 101 and BC: The ER (change in tablet height after ejection from the die) increases on increase in load and AV 101 showed higher ER than the BC (Fig. 5 and Table 2) i.e., the growth of ER during storage was much higher for tablets of the AV 101 than BC. The BC tablet relaxes faster. Higher the particle size higher will be the elastic recovery which was seen in AV 101.

Tablet tensile strength test: The BC showed higher tensile strength than AV 101 (Fig. 6 and 2). This might be due to the decrease of the surface roughness and cohesiveness of particles of samples which was observed from its flowability. The tensile strength increases with increase in compression pressure (load) to an extent, later it decreases which may be due to structural fragmentation of particles of AV 101 and BC. At the same compression load, BC produced the hard compacts, whereas AV 101 produced the soft compacts because the tensile strength of BC is more than AV 101. The slight difference of tensile strength among BC and AV 101 may be attributed to their different moisture content, particle size and particle shape. The tablet relaxation after compaction results in a decrease of the tensile strength; since AV 101 showed increased elastic recovery its tensile strength is decreased. The relationship between mechanical strength and pore

Table 2: Percentage elastic recovery and tensile strength of avicel 101 and bacterial cellulose

	% Elastic recovery		Tensile strength* (kg cm^{-2})	
Load (Tonnes)	Avicel 101	Bacterial cellulose	Avicel 101	Bacterial cellulose
0	0	0	0	0
1	4.07±0.43	3.41±0.20	55.30±0.63	74.03±0.25
2	4.58±0.89	3.95±0.69	115.47±0.6	126.32±0.75
3	5.03±0.77	4.31±0.27	127.41±0.65	144.11±0.44
4	6.50±0.68	5.67±0.70	137.80±0.53	154.66±0.65
5	6.89±0.65	5.85±0.36	150.26±0.23	163.44±0.34
6	7.56±0.90	6.27±0.84	128.87±0.5	130.96±0.7

Mean±SD, n = 3

structure appears to be ambiguous. For example, a linear relationship between porosity and the logarithm of the strength of tablets has been reported (Ryskewitch, 1953). This suggests that tablets of low porosity will have high mechanical strength. George *et al.* (2005) reported tensile strength of dried cellulose pellicle, NaOH treated, KOH treated, Na_2CO_3 treated and K_2CO_3 treated was found to be 67, 43.68, 50, 57 and 56 MPa, respectively.

CONCLUSION

The percentage yield of BC produced by *A. xylinum* using HS medium was found to be 25.65% at optimized temperature of 30°C and time of 7 days. MSC for BC (6.012%) was lower when compared to AV 101 (13.9%) due to crystalline nature of BC. LOD for BC and AV 101 were found to be 4.635 and 2.923%, respectively. The particles of BC were small sized (39-43 μm) densely packed reticulated structure consisting of fine thread like fibrils whereas AV 101 were irregularly large sized (50-65 μm) elongated structures. The IR spectra of BC showed peak at 1161 cm^{-1} which corresponds **β**-glycosidic linkage as in case of plant cellulose. The crystallinity index of BC was found 93% which may arise as a result of longer polymerization of BC whereas the crystallinity index of AV 101 was 71.4%. BC powder because of small particle size, showed more bulk density and showed higher packaging ability. Angle of repose for BC was found to be less because of their decreased particle size. Hausner ratio indicated that BC had passable flow character whereas AV 101 had poor flow character. Hence AV 101 needs a glidant to improve the flow character. Heckel plot showed that BC deformed at a much lower yield pressure compared to AV 101. BC showed higher Do value than AV 101 indicating that BC exhibited a higher degree of packing in the die as a result of die filling. The D_B of AV 101 was slightly higher than that of BC which indicates a greater degree of fragmentation with AV 101 than BC. BC had a higher relative density of plastic deformation than that of AV 101. The slight difference of tensile strength among BC and AV 101 may be attributed to their different moisture content, particle size and particle shape. Form the study it revealed that BC produced from *A. xylinum* can be used as excellent pharmaceutical excipient in case of tablet formulation instead of Avicel 101.

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