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Isolation and physicotechnical properties of microcrystalline cellulose obtained from *Phaseolus vulgaris*

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Abstract

In this study, microcrystalline cellulose, coded BH-MCC, was obtained from beans *(Phaseolus vulgaris)* husk by a two-stage sodium hydroxide delignification process followed by sodium hypochlorite bleaching and acid hydrolysis. BH-MCC was examined for its physicochemical and physicotechnical properties in comparison to those of the well-known commercial microcrystalline cellulose grade, Avicel PH 101. The extraction yield of BH-MCC was approximately 11%. The cellulose material was composed of irregularly shaped fibrous cellulose particles and had a moisture content of 7.2% and total ash of 0.14%. The true density was 1.41. The flow indices showed that BH-MCC flowed poorly. The hydration, swelling and moisture sorption capacities were 3.4, 96.4% and 24% respectively. The study revealed that the cellulose material compares favourably with Avicel PH 101 and conformed to official requirement specified in the British Pharmacopoeia 1993 for microcrystalline cellulose.

Keywords: Beans husk, microcrystalline cellulose, isolation, physicochemical and physicotechnical properties

Introduction

Microcrystalline cellulose, (MCC), is described as purified, partially depolymerised cellulose prepared by treating α-cellulose, obtained as a pulp from fibrous plant with mineral acids. It is one of the most used filler-binders in direct compression. Its popularity in direct compression is due to its excellent good binding properties when used as a dry binder. It also works as a disintegrant and a lubricant and has a high dilution potential in direct compression formulations. In addition to its use in direct compression formulations, MCC is used as a diluent in tablets prepared by wet granulation as well as a filler for capsules and spheres [1].

Commercially available MCC is derived from both gymnosperms (generally conifers) and other softwoods, and from hardwood dicotyledons. These woods differ considerably in chemical composition (proportions of cellulose, hemicelluloses and lignin) and structural organization which affect the composition of the α-cellulose extracted and the composition and crystallinity of MCC finally produced [2]. Besides the wood pulp as a source of cellulose and its derivatives, another commonest source is the purified cotton linters obtained from Gossypium species [3]. Purified cotton linters and wood pulp are obtained from plantations specially grown in temperate climates; consequently, its production is expensive and the need for exploring other sources for MCC has become imperative. Alternative sources for MCC recently investigated include agricultural wastes and other plants parts not traditionally used for MCC production [4-9].

Phaseolus vulgaris, often called Common beans or French or snap bean, is an annual, twining or bushy herbs grown for its mature, dry seeds, for its immature green or yellow pods

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('snap beans'), and occasionaly in Africa and Asia for their leaves used as vegetables. It has been domesticated in Central and South America, Europe, Africa and Asia and in these places the dried pods husk exists as a huge waste after the mature dry seeds have been harvested [10]. Literature survey reveals no report on husk of *P. vulgaris* as sources of α-cellulose and or its modified form, MCC. As part of the continuous search for locally available pharmaceutical raw materials we have in this study obtained microcrystalline cellulose, (BH-MCC), from the husk of *P. vulgaris*. BH-MCC was then assessed for its physicochemical and physicotechnical properties in comparison to those of the well-known commercial grade microcrystalline cellulose, Avicel PH 101.

Materials and methods

Materials

Sodium hydroxide (BDH, England), sodium hypochlorite as 'Jik' (Reckitt and Colman Ltd, Nigeria), hydrochloric acid (Fisons, UK), Avicel PH 101 (FMC Corporation, USA), xylene, phloroglucinol and iodine crystals (Hopkin and Williams, London) were used as obtained. All other chemicals used were of analytical or reagent grade and water was double distilled. Beans husks were collected from a farmer in Abuja, Nigeria. The microcrystalline cellulose, BH-MCC, was prepared in our laboratory as described under Methods.

Extraction of a cellulose

Beans husks, as obtained, were crushed using a mill powered by an electric motor (3.7kW/220 V) and a fraction of the powdered material passing through a sieve of 2.0 mm aperture was used for extraction of α-cellulose as described in an earlier study [7].

Production of microcrystalline cellulose (BH-MCC)

The procedure reported earlier [7], with slight modification, was used. A 50 g quantity of the α-cellulose obtained was placed in a glass container and hydrolyzed with 0.8L of 2.5 N hydrochloric acid, at a boiling temperature of 105°C, for 35 min. The hot acid mixture was poured into cold tap water which was followed by vigorous stirring with a wooden spatula and allowed to stand overnight. The resultant microcrystalline cellulose was washed with water until it is neutral to litmus test and filtered using calico cloth. It then dried in a fluidised bed dryer at an inlet air temperature of 57 60 °C for 60 min. Following further milling and sieving, the fraction passing through a sieve of 710μm aperture was obtained and stored at room temperature in a desiccator.

Physicochemical properties of BH-MCC

The organoleptic characteristic, identification tests, solubility, and presence of organic impurities, starch and dextrin, and water-soluble substances were carried out in accordance with BP specifications [11]. An optical microscope, Nikon model Larphot 2 (Nikon Inc. Japan) was used for preliminary assessment of the nature of particles in BH-MCC. The combination of low and high power objective lenses of x100 and x400 magnification, respectively, were used [4].

pH determination: This was carried out by shaking 2 g of the powder material with 100 ml of distilled water for 5 min and the pH of the supernatant liquid determined using a pH meter (Corning, model 10 England) [7].

Total ash determination: Ash content was estimated by measurement of the residue left after combustion in a furnace at 550 ^{0}C [7].

Physicotechnical properties

Particle size analysis

A sieves shaker, (Endicott's Ltd UK) was used for this assessment. Test sieves ranging from 1.18 mm to 75 μm aperture size were arranged in a descending order. A 20 g quantity of BH-MCC powder was placed on the top sieve and the set-up was shaken for 5 min. The weight of material retained on each sieve determined. The average diameter was calculated as reported by Ansel et al [12] using the equation:

Average diameter = ε (% retained) x (mean aperture)]/100 1

True density

The true densities, D_t, of cellulose powders were determined by the liquid displacement method using xylene as the immersion fluid [6].

Flow properties

Angle of repose

The static angle of repose, α, was measured according to the fixed funnel and free standing cone method [13]. 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. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation: $\text{Tan } \alpha = 2h/D$....2 Where h is the height of the heap of powder and D is the diameter of the base of the heap of powder.

Bulk and tap densities

A 10 g quantity each of the powder samples was placed into a 50 ml clean, dry measuring cylinder and the volume, V_0, occupied by each of the samples without tapping was determined. After 500 taps using Stampfvolumeter (Model STAV 2003 JEF, Germany), occupied volumes, V_{500}, were determined. The bulk and tap densities were calculated as the ratio of weight to volume (V_0 and V_{500}, respectively) [7].

Other flow properties such as Hausner and Carr's indices were calculated as reported earlier [7].

Powder porosity

This was derived from the values of true and bulk densities when fitted into the equation:

$E = 1-B_b/D_t \times 100$....3

Where B_b is the bulk density, D_t is the true density and e is the porosity [7].

Hydration capacity

The method of Kornblum and Stoopak [14] was used. A 1.0 g each of the samples was placed in each of four 15 ml plastic centrifuge tubes and 10 ml of distilled water added and stoppered. The contents were mixed on a vortex mixer (Vortex-Gennie Scientific Industry, USA) for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged

at 1000 rpm for 10 min. on a Gallenkamp bench centrifuge (Gallenkamp, England). The supernatant was carefully decanted and the sediment weighed. The hydration capacity was taken as the ratio of the weight of the sediment to the dry sample weight.

Swelling capacity:
This was measured at the same time as the hydration capacity and calculated as follows:
$S = (V_2 \ V_1) / V_1 \times 100$ ....4 where S is the % swelling capacity, V_2 is the volume of the hydrated or swollen material and V_1 is the tapped volume of the material prior to hydration [7].

Moisture sorption capacity
Two grams of the cellulose material was accurately weighed and evenly distributed over the surface of a 70 mm tarred Petri dish. The samples were then placed in a large desiccator containing distilled water in its reservoir (RH = 100%) at room temperature and the weight gained by the exposed samples at the end of a five-day period was noted The amount of water sorbed was calculated from the weight difference [6].

Moisture content
Five grams of powder samples was transferred, each, into a Petri dish and then dried in an oven at 60 °C until a constant weight was obtained. The % moisture content was then determined as the ratio of moisture loss (g) to weight of sample expressed as percentage [6].

Statistical analysis:
The data were analysed statistically using Excel 2000 Window™ (Microsoft Corporation).

Results and discussion

The yield of α-cellulose was about 15% w/w of the original material. The yield of the microcrystalline BH-MCC, obtained from α-cellulose was approximately 70% w/w. Thus the yield of BH-MCC was approximately 11% w/w of the starting dry plant material. The % yield of BH-MCC (relative to the dry material) was low when compared to 29% yield of microcrystalline cellulose obtained from the dried fruits of *Luffa cylindrica* [7].
The results of the physicochemical properties investigated are shown in Table 1. The results indicate a high level of purity of the cellulose material.
The organoleptic properties of the BH-MCC produced were good as the material was odourless, tasteless, white and granular in texture. The value obtained for the total ash was very low possibly because cellulosic materials are almost free of inorganic compounds. When vegetable plants are incinerated, they leave an inorganic ash which in the case of many drugs varies within wide limits. The total ash value is of importance and indicates to some extent the amount of care taken in the preparation of the substance [3].

Physicotechnical properties
The physicotechnical properties of BH-MCC and Avicel PH 101 are presented in Table 2 while the particle size analysis of BH-MCC powder is as shown in Figure 1. The figure represents a bimodal frequency distribution and the particle size is in the range of 70-1000

μm, as such BH-MCC powder belongs to the classification conventional powder [15]. Over 70 percent of the particles population is within 0-375 μm, and the calculated average diameter was 259 μm.

The true density of BH-MCC was comparable ($p<0.05$) to that of Avicel PH 101 (Table 2). Stamm [16] had pointed out that a direct correlation exists between the degree of crystallinity of cellulose and its true density when determined in a non-polar liquid. Consequently, the true density values for both cellulose materials suggest that they might have the same degree of crystallinity.

The moisture content of BH-MCC was about 7.2% which is slightly below the official limit of 8 % stated in British Pharmacopoeia, 1993 [11]. This low value is indicative of the suitability of BH-MCC as a diluent in the formulation of hydrolysable drugs such as aspirin.

The flow properties of a powder are essential in determining the suitability of a material as a direct compression excipent. The angle of repose, Hausner index and Carr's percent compressibility are considered as indirect measurements of powder flowability [17]. The high angles of repose for both BH-MCC and Avicel PH 101 (Table 2) are indicative of poor flow [18], while the Hausner index is indicative of interparticle friction, and the Carr's index shows the aptitude of a material to diminish in volume [17]. As the values of these indices increase, the flow of the powder decreases. In general however, Hausner ratio greater than 1.25 indicates poor flow and Carr's compressibility index below 16 % indicates good flowability while values above 35 % indicate cohesiveness [17]. The flow indices showed that BH-MCC and Avicel PH 101 powders have poor flow. Consequently, a glidant will be needed when these materials are to be used in solid dosage production processes.

Swelling which is generally accepted as an indication of tablet disintegration ability [19] can be assessed by the determination of hydration capacity, swelling capacity and moisture sorption profile. The hydration capacity value obtained for BH-MCC, (Table 2), indicates that it is capable of absorbing more than three times its own weight of water. The swelling capacity, which reflects the increase in volume of cellulose following water sorption, was 96 % (Table 2). This is an indication that only a small portion of absorbed water actually penetrated the individual cellulose particles causing them to swell. The bulk of the absorbed water probably exists in a 'free' state between the particles. Thus, if the cellulose was incorporated in tablet formulation as a disintegrant it would probably produce tablet disintegration by two mechanisms: capillary or wicking due to interparticulate water and swelling. In addition, the higher hydration and swelling capacities values observed for BH-MCC irrespective of comparable powder porosity values of BH-MCC and Avicel PH101 (Table 2) could possibly be due to difference in the proportion of amorphous cellulose present in the cellulose powders. Stamm [16] has reported that the amorphous portion is responsible for uptake and swelling of cellulose materials.

The moisture sorption capacity is a measure of moisture sensitivity of material. The moisture capacity value for BH-MCC was significantly higher ($p<0.05$) than that of Avicel PH 101 (Table 2). It has been reported that the crystallite portion of cellulose does not adsorb water and that the extent of water adsorption by cellulose should thus be proportional to the amount of amorphous cellulose present [16]. Thus, the results showed that BH-MCC might be lower in degree of crystallinity when compared to Avicel PH 101. Also, study of water sorption is of importance since it reflects the relative physical stability of tablets made from BH-MCC when stored under humid conditions. In general, this property showed that the cellulose powders are sensitive to atmospheric moisture and should therefore be stored in air tight container.

Table 1: Some physicochemical properties of BH-MCC

TESTS	BH-MCC
Organoleptic	Odourless, white, tasteless, coarse powder
Identification	Turns violet-blue with iodinated $ZnCl_2$
Organic impurities	Nil
Starch and dextrins	Nil
pH	7.2
Solubility (in ammoniacal solution of copper tetra-amine)	Complete and no residue
Water soluble substance	< 0.2%
Total ash (%)	0.14 (0.01)
Microscopy	Irregularly shaped fibrous particles which are mixture of primary particles and spherical aggregates.

Value is mean and standard deviation is in parenthesis, Number of replicates, N =3

Table 2: Physicotechnical properties of BH-MCC and Avicel PH 101

Parameters	BH-MCC	Avicel PH 101
True density (g/ml)	1.41 (0.05)	1.40 (0.06)
Bulk density (g/ml)	0.36 (0.01)	0.31 (0.04)
Tapped density (g/ml)	0.63 (0.0)	0.42 (0.12)
Porosity (%)	74.4	78.0
Flow properties:		
(a) Angle of repose	47.86(2.04)	41.20 (0.46)
(b) Hausner index	1.73	1.35
(c) Compressibility index (%)	42.24	26.00
Hydration capacity	3.40 (0)	2.17 (0.01)
Swelling capacity (%)	96.0 (5.05)	21.4 (0.03)
Moisture sorption capacity (%)	24.0 (0.16)	16.6 (0.24)
Moisture content (%)	7.2 (0.6)	7.4 (0.4)

Values are mean and standard deviations are in parenthesis; Number of replicates, N=3

Conclusion

The microcrystalline cellulose product, BH-MCC, obtained from beans husk (Phaseolus vulgaris) conformed to the official specifications in the British Pharmacopoeia (1993). The physicotechanical properties indicate that BH-MCC and Avicel PH101 are comparable, hence BH-MCC, is a potential tablet binder and direct compression diluent.

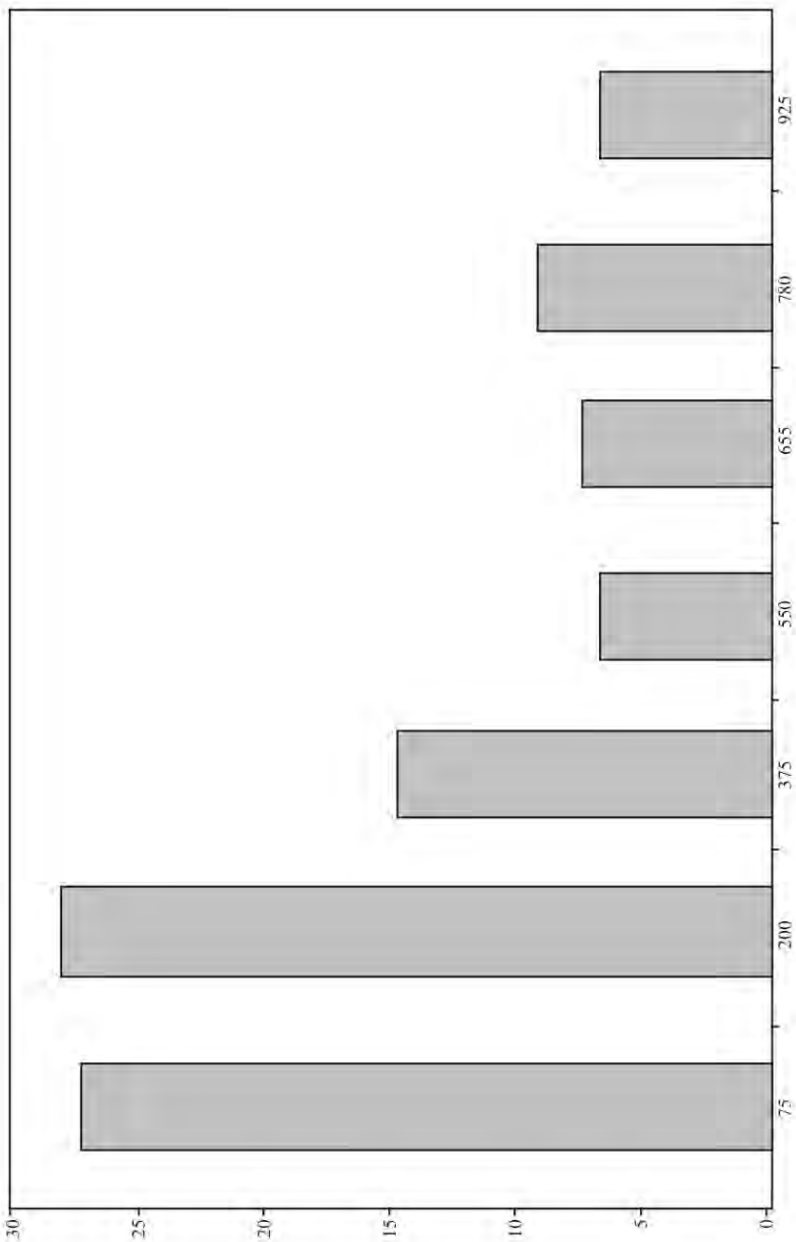

Figure 1: Particle size distribution of microcrystalline cellulose powder obtained from Phaseolus vulgaris (BH-MCC) husk

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