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Original Article

Extraction and Characterization of Microcrystalline Cellulose Derived from *Luffa cylindrica* Plant

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In this study microcrystalline cellulose coded LC-MCC, was obtained from dried fruits of an annual vegetable plant, *Luffa cylindrica*. LC-MCC has been examined for its physicochemical and powder properties. The powder properties of LC-MCC were compared to those of best commercial grade Avicel PH 101. The extraction yield of LC-MCC was 29%. The cellulose material was composed of irregularly shaped fibrous cellulose particle with a moisture content of 4.3% and total ash of 0.19%. The true density was 1.67. The flow indices showed that LC-MCC flowed poorly. The hydration and swelling capacities were 4.7 and 51.3 respectively. The study also revealed that the cellulose material compares favourably with Avicel PH 101 as well as official requirements as specified in the British Pharmacopoeia and Handbook of Pharmaceutical excipients for microcrystalline cellulose.

Keywords: *Luffa cylindrica*, Microcrystalline cellulose, Extraction, Characterization

INTRODUCTION

At present, more than 80% of the raw materials and finished drug products used in developing countries are imported, thus making them very expensive. As a result of this, pharmaceutical industries in these countries have recognized the importance of utilizing local and naturally occurring materials. One group of naturally occurring product that has enjoyed extensive use in the pharmaceutical industry is cellulose. This is because cellulose and its numerous derivatives are multifunctional excipient in drug formulations. It is against this background that a number of investigators (1 –7) have examined agricultural wastes and other sources besides cotton and wood-trees for cellulose. Although considerable work has been done in sourcing alpha cellulose from sources other than cotton and wood, only few workers have studied the possibility of obtaining microcrystalline cellulose (MCC) from these sources. MCC has been widely used as an additive for direct compression because of its good flowability, compactibility and compressibility(8). Alfa (6) investigated the physico-technical and tableting properties of microcrystalline cellulose derived from Andropogon and Sorghum plants while, Ofoefule and Chukwu (7) obtained microcrystalline cellulose from Indian bamboo. They examined its blend with cissus gum in the formulation of zinc oxide and sulphadimidine suspensions.

Luffa cylindrica (Cucurbitaceae) is an annual plant which is naturalized in most parts of Nigeria (rarely cultivated). It is commonly known as vegetable sponge and the fibre of the fruit is a possible paper material (9). Literature survey reveals no report on the use of fresh or dry fruits of *Luffa cylindrica* as a possible source of MCC. And as part of the continuous search for locally available pharmaceutical raw materials, a grade of MCC coded LC-MCC was prepared from alpha cellulose content of the dry fruit of *Luffa cylindrica*. The prepared LC-MCC was characterised both for its physicochemical and powder properties. The powder properties of LC-MCC were compared to those of best commercial grade Avicel PH 101.

MATERIALS

Sodium hydroxide (BDH, England), sodium hypochlorite as 'Jik' (Reckitt and Colman Ltd, Nigeria), Hydrochloric acid (Fisons, UK), Avicel PH 101 (FMC corporation, USA), Kerosene (obtained from a petrol station in Abuja, Nigeria), Phloroglucinol and iodine crystals (Hopkin and Williams, London) were used as obtained. All other chemicals used were of analytical reagent grade and water was double distilled.

Mature dried fruits of *Luffa cylindrica* were collected from the surrounding of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

METHODS

EXTRACTION OF ALPHA-CELLULOSE

The dried outer coat of the fruit was removed and the fibrous fruit manually sawed into four parts longitudinally and the seeds removed. It was further chopped into small bits, which were oven dried at 60 °C until brittle to touch. This was milled into chips using a blender (Model BL 350, Kenwood Ltd, UK). A 300 g quantity of this material was placed in a stainless steel container to which was added 4 L of 2% w/v sodium hydroxide and digestion was effected for 3 h at 80 °C in a water bath (FGL 1083 Karl Kolb scientific). This step, also removes lignin in the form of soluble complexes. Following thorough washing and filtration, it was bleached (thrice) with 2.6 L of a 1:1 aqueous dilution of sodium hypochlorite for 30 minutes at 100 °C. The washed and filtered material was then treated with 2.4 L of 17.5 % w/v sodium hydroxide at 80 °C for 1 h. The resulting alpha-cellulose was washed thoroughly. The extraction process was completed by whitening with a 1:1 aqueous dilution of sodium hypochlorite for 15 min. at 80 °C and was washed until it is neutral. The cellulose material was filtered, pressed and manually reduced to small lumps, which were dried in a fluid bed dryer (laboratory model, Copley) at an inlet air temperature of 57 – 60 °C for 60 min.

PRODUCTION OF MICRO-CRYSTALLINE CELLULOSE (MCC)

A 50 g quantity of the alpha cellulose obtained was place in a glass container and hydrolyzed with 1.2 L of 2.5N hydrochloric acid at 100 °C for 30 min. The hot acid mixture was poured into cold tap water which was followed by vigorous stirring with a glass rod. The microcrystalline cellulose obtained by this process was washed with water until neutral, pressed and dried in a fluid bed dryer at an inlet air temperature of 57 – 60 °C for 60 min. Following further milling and sieving, the fraction passing through 1.18 mm sieve was obtained and stored at room temperature in silica gel desiccators.

PHYSICOCHEMICAL PROPERTIES OF LC-MCC

The organoleptic characteristic, identification, organic impurities, starch and dextrin, solubility, total ash and water-soluble substances were carried out in accordance with BP (10) specifications. An optical microscope, Nikon model Larphot 2 (Nikon Inc. Japan) was used for preliminary assessment of the nature of particles in LC-MCC. The combination of low and high power objective lenses of 100 and 400 times magnification was used.

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

Total ash determination: Ash content was estimated by measurement of the residue left after combustion in a furnace at 550 °C

Elemental analysis: One gram of the LC-MCC was taken and reduced to ash in a furnace. The residue was mixed with concentrated nitric acid and further ashed. The residue was dissolved in water and made up to 100 ml. The elements present and their concentrations were determined using atomic absorptions spectrophotometer (AA-680 Atomic Absorption/Flame emission spectrophotometer, Shimadzu).

POWDER PROPERTIES

Particle size analysis

An Endicott's sieves shaker, (Endicott's Ltd UK) was used for this. Test sieves ranging from 1.18 mm to 75 μm were arranged in a descending order. A 40 g quantity of LC-MCC powder was placed on the top sieve and was shaken for 5 min. and the weight of material retained on each sieve determined.

True Density

The densities of cellulose powders were determined by the liquid displacement method using kerosene as the immerse fluid (1).

Flow Properties

Angle of Repose

The static angle of repose, *a*, was measured according to the fixed funnel and free standing cone method (11). 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:

$$\text{Tan } a = \frac{2h}{D} \quad \ldots\ldots\ldots\ldots \quad 1$$

Where h is height of heap of powder and D is the diameter of the base of heap of powder.

Bulk and Tap Densities

A 30 g quantity of powder samples were each, placed into 250 ml clean, dry measuring cylinder and the volume, V_o, 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 determined from these volumes (V_0 and V_{500}) using the equation:

$$\text{Density} = \frac{weight\ \ of\ \ cellulose}{Volume\ \ of\ \ cellulose} \quad \ldots \quad 2$$

Hausner Index

This was calculated as the ratio of tap density to bulk density of the samples.

Compressibility Index (C%)

This was calculated using bulk and tap densities data when fitted into the equation:

$$(C\%) = \frac{\text{Tapped density - bulk density}}{\text{Tapped density}} \times 100\% \cdots 3$$

Powder Porosity

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

$$e = \frac{1 - B_b}{B_p} \times 100 \quad \cdots \cdots \quad 4$$

Where B_b is the bulk density , B_p is the true density and e is the porosity

Hydration Capacity

The method of Kornblum and Stoopak (12) was used. A 1.0 g each of the samples was placed in each of four 15 ml plastic centrifuge tubes and 10 ml distilled water was added from a 10 ml measuring cylinder and then 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

Swellability

This was measured at the same time as the hydration capacity determination using the method of Okhamafe *et al* (1).

Moisture Sorption Profile

Two grams of the cellulose materials were 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. At various time intervals, over a five-day period, the weight gained by the exposed samples were recorded and the amount of water sorbed was calculated from the weight difference.

Moisture Content

Five grams of powder samples were 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 weight of moisture to weight of sample expressed as percentage.

RESULTS AND DISCUSSION

The yield of the microcrystalline LC-MCC, obtained from alpha-cellulose was approximately 65 percent w/w. Thus the yield of LC-MCC was approximately 29 percent w/w of the starting dry plant material.

The results of some of the physicochemical properties investigated are shown in Table 1. The results indicate high level of purity of the cellulose material.

The organoleptic qualities of the LC-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 figure is of importance and indicates to some extent the amount of care taken in the preparation of the substance (13).

The British Pharmacopoeia gives limits for a number of possible contaminants in pharmaceutical raw materials, which may be, introduced into the finished products during processing (10). Such tests include those for lead, arsenic, calcium, iron, potassium, aluminum, halogens and a host of others. Although Pharmacopoeia requirements are not categorical on the exact tolerable level of any possible contaminant, it should not be presumed that unusual impurities are tolerated. As shown in Table 1, the heavy metals that are known to be highly undesirable in pharmaceutical raw material such as

lead and manganese were not present in the cellulose powder.

Table 1: Some physicochemical properties of LC-MCC

TESTS	LC-MCC
Organic impurities	Nil
Starch and dextrins	Nil
pH	5
Solubility (in ammonical solution of copper tetrammine)	Complete and no residue
Water –soluble substance	< 0.2%
Total ash (%)	0.19 (0.5)
Elemental analysis:	**% Elemental constituent in 1.0 g sample**
Iron	0
Selenium	0
Manganese	0
Magnesium	0.0006
Lead	0
Calcium	0.0007
Sodium	0.0058
Potassium	0.0146
Zinc	0
Copper	0

Standard deviation is listed in parenthesis.

Table 2: Powder properties of LC-MCC and Avicel PH 101

Parameters	LC-MCC	Avicel PH 101
True density (g/ml)	1.67 (0.06)	1.59 (0.12)
Bulk density (g/ml)	0.25 (0.0)	0.31 (0.04)
Porosity (%)	85	80
Flow properties:		
(a) Angle of repose	43.61 (0.56)	41.20 (0.46)
(b) Hausner index	1.64	1.45
(c) Compressibility index (%)	39	32
Hydration capacity	4.7. (0.19)	4.82 (0.21)
Swelling capacity (%)	51.3 (0.21)	54.5 (0.16)
Moisture content (%)	4.3 (0.58)	5.2 (0.4)

Standard deviation is listed in parenthesis.

POWDER PROPERTIES

The powder properties of LC-MCC and Avicel PH 101 are presented in Table 2 while the result of particle size distribution for LC-MCC is shown in Figure 1.

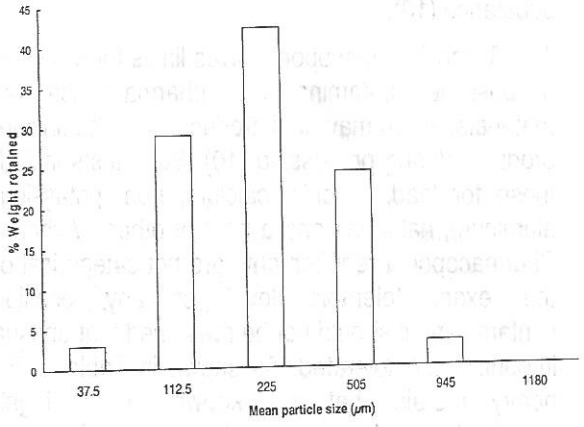

Figure 1: Particle size distribution for LC-MCC

The true density for LC- MCC (Table 2), 1.67, is high when compared to 1.59 for Avicel PH 101. This high value for LC-MCC is therefore suggestive that LC-MCC has higher degree of crystallinity than Avicel PH 101. Stamm (14) has reported that the greater the degree of crystallinity of a cellulose, the greater will be the true density of its substance determined in a non-polar liquid.

The moisture content of LC-MCC produced was about 4.3% which is well below the official limit of 8 % (10, 15). This low value is indicative of the suitability of LC-MCC as a diluent in the formulation of hydrolysable drugs such as aspirin.

The flow properties of the powder is 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 (16). The angle of repose of LC-MCC is high (Table 2), which is indicative of very poor flow (17). While the Hausner index is indicative of interparticle friction, the Carr's index (%) shows the aptitude of a material to diminish in volume (16). As the values of these indices increase the flow of the powder decreases. In general however, Hausner ratio greater than 1.25 indicate poor flow and Carr's compressibility index below 16 % indicate good flowability while values above 35 % indicate cohesiveness (16). Thus, the flow indices (Table 2) showed that both LC-MCC and Avicel flowed poorly. As such a glidant will be needed when they are to be used in solid dosage formulation.

The hydration capacity value (Table 2) indicates that LC-MCC is capable of absorbing more than four and a half time its own weight of water. The swellability, which reflects the increase in volume of cellulose following water absorption was 51.3 % (Table 2). It seems therefore, that only a small portion of absorbed water actually penetrated the individual cellulose particles causing them to swell. The bulk of the absorbed water would exist 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.

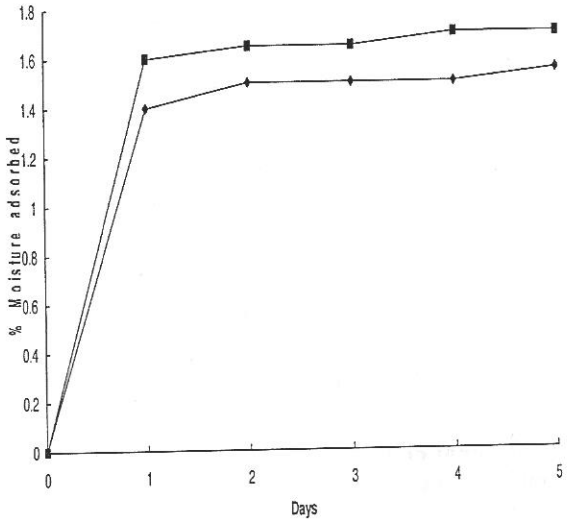

Figure 2: Moisture sorption profiles of microcrystalline cellulose powders; (■) – LC-MCC, (♦) – Avicel PH 101

Figure 2 shows the moisture sorption profile for LC-MCC and Avicel PH 101. This property is a measure of moisture sensitivity of material. The result shows that LC-MCC has low rate of water sorption. Stamm (14) 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. Thus, the result is indicative of the high crystallinity expected of this material. This inference was supported by the report of Wei *et al* (18), who stated that hydrocellulose, a highly crystalline cellulose, prepared using 2.5 N HCl solution at boiling temperature for 30 min. (the same conditions adopted in the acid hydrolysis of alpha cellulose) was used as 100 % crystalline standard in their investigation. Additionally, larger surface area due to small particle size of Avicel powder could have, in part, accounted for the higher amount of moisture taken up by it. Also, study of water sorption is of importance since it reflects the relative physical stability of tablets made from LC-MCC when stored under humid condition. Moreover, the lower the rate of moisture sorption the lower will be the deteriorating effect on incorporated drugs that undergo hydrolytic decomposition.

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