# ESTABLISHMENT OF PHARMACOPOEIAL STANDARDS FOR STARCH FROM SORGHUM BICOLOR MOENCH (*Graminae*)

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## ABSTRACT

Starch remains an important ingredient in tablet manufacturing and the availability of suitable local sources of starch is of Pharmaceutical importance. The starch prepared from the seeds of *Sorghum bicolor* Moench has been reported to be as good as corn starch BP in binding and disintegrating properties and better than acacia as binder. This study is aimed at standardizing the starch prepared from the seed of sorghum for possible inclusion in the pharmacopoeia. The starch prepared from the seeds of *Sorghum bicolor* Moench were evaluated using standard organoleptic, macroscopic, microscopic as well as physicochemical methods. Sorghum starch possesses certain unique features that can be used to distinguish it from other starches. The starch grains are off-white in colour, oval, mostly single, odourless and tasteless with a size distribution of 7-21-43 µm and 244,000 granules per milligram of starch. Mucillage formation commenced at $66^0$ C and was completed at $71^0$C. It also rotated the plain of polarized light by an average value of $+2.69^0$ (Dextrorotatory). The above results can be used as quality parameters in the correct identification as well as determination of purity and quality of the crude drug.

**Key words:** Starch, Standardization, *Sorghum bicolor*.

## INTRODUCTION

Starch constitutes the major food reserve material of higher plants. It is a carbohydrate polymer containing several hundreds and thousands of anhydro-glucose units which form two structurally distinct polymers which include amylase (Rees, 1976). The ratio of these two components of starch and the average degree of polymerization vary according to the source of the starch and method of isolation. Generally, amylase accounts for 15-30% of starch and amylopectin for 70-85%, (Saunders, 1988). Its major applications in the pharmaceutical industry are as auxiliary substances for solid dosage forms, principally binders, disintegrants, diluents/filers and glidants, (Chowdary *et al.,* 2011; Ramu *et al*, 2010; Singh *et al.*, 2010; Okunlola and Odeku, 2008; Ibezim 2005; Iwuagwu and Akerele, 2001; Subhadhirasakul *et al.*, 2001).

Most drugs generally formulated as tablets may present a number of problems during its manufacture into tablets. For example, crystalline or powdered forms of paracetamol undergo capping and lamination readily during compression (Obiorah and Shotton, 1976; Krycer *et al.,* 1982), while the particles of the powder are known to acquire negative static charge when flowing through a hopper (Singh and Nath, 2010). In addition, some reports on commercial brands of paracetamol tablets in Nigeria have suggested that the availability of the drug in this dosage form is variable and infact, some brands were shown not to disintegrate even after an hour, (Nasipuri and Obiorah, 1976). It has been shown (Esezobo and Ibiezugbe, 1982) that the proportions of disintegrant (starch) present in a capsule formulation considerably influenced the disintegration and dissolution characteristics of the capsules.

The starch prepared from the seeds of *Sorghum bicolor*, Moench (Graminae) has been evaluated as a disintegrant and binder in tablets of magnesium sulphate, calcium carbonate, sulphadimidine and chloroquine phosphate to represent soluble and insoluble substances (Desphande and Panya, 1987). The starch was as good as maize starch in binding and disintegrating properties and better than acacia as binder.

*Sorghum bicolor* is a genus of tall, annual and perennial tropical grasses easily recognized by its dense terminal particulate inflorescence and broad flat leaves. The cultivated *Sorghum* which were initially developed in Africa, are important commercial crops especially in the tropics, because they are easily grown and produce high yield of granules or fodder with little attention. This study is intended to establish diagnostic features of the starch made from *S. bicolor* which can aid in the identification, evaluation and monograph preparation of the plant.

## MATERIALS AND METHODS

Sorghum starch was prepared in our laboratory from the seeds of sorghum (*S. bicolor*), which were first washed once and dried at ambient temperature. They were milled and reduced to fine pulp by a blender after mixing with water. The pulp was filtered to a fine mesh and the starch allowed settle. It was washed several times with 0.1M Sodium hydroxide and then with purified water. The product was reduced to fine powder (African Pharmacopoeia, 1986).

Organoleptic and macroscopical standardization of Sorghum starch were evaluated using its form: - irregular, angular masses or as a white powder, reaction with iodine, gelatinization temperature, effects of formalin on paste formation and solubility in organic solvents (Evans, 2006; Harborne, 1992). The odour, taste, and hydrolysis of the starch were also determined.

Microscopic evaluation involved the determination of the microscopic characters by mounting the starch in water and Smiths starch reagent (equal parts of water, glycerin and 50 % acetic acid). The size, shape and structure of the starch granules were determined. The numbers of starch granules per milligram of starch using the lycopodium spore method (Wallis, 1986) as well as the measurement of the size distribution of starch grains were also determined.

Physicochemical properties of sorghum starch were determined by comparing the polarization effects with those of potato and maize starches. Starch possess the power of rotating the plane of polarized light through a certain angle, hence are said to be optically active. The angle and sign of rotation are characteristic of the type of starch.

## RESULTS

Sorghum starch is an off-white powder, which is oval and mostly single. Mucilage formation started at $66^0$C and was completed at $75^0$C. However this temperature was reduced by the presence of formalin (Table 1). It is partially soluble in water and 70% ethanol, but soluble in chloroform. A suspension and mucilage of the starch gave a blue-black colouration with iodine. On hydrolysis, the starch gave a bluish colouration, which later turns violet, then reddish and finally colourless. Sorghum starch is odourless and tasteless.

The size distribution of sorghum starch is 7-21-43 μm (Table 2) and the shape of the granules is oval. The granules are mainly simple with few aggregates of 2-4 compound granules, that are colourless and without striations. The hilum is centrally placed and appeared as 2-, 3-, 4-, 5- and 6- stellate cleft. The number of sorghum starch granules contained in one milligramme is 244,000 compared to 94,000 spores per milligramme for Lycopodium powder (Table 4).

Starch possess the power of rotating the plane of polarized light through a certain angle and sorghum starch rotates this plane of polarized light by an average value of $+2.69^0$.

**Table 1. Effect of Formalin on paste formation of Sorghum Starch**

| Solution | Temperature at which paste formation started. | Temperature at which paste formation ended. |
|---|---|---|
| 5%v/v | $60.0 \pm 2.0^0$C | $67.0 \pm 2.0^0$C |
| 10%v/v | $58.0 \pm 2.0^0$C | $66.0 \pm 1.0^0$C |
| 20%v/v | $54.0 \pm 1.0^0$C | $64.0 \pm 2.0^0$C |
| 30%v/v | $52.0 \pm 2.0^0$C | $61.0 \pm 2.0^0$C |
| 40%v/v | $50.0 \pm 2.0^0$C | $58.0 \pm 1.0^0$C |
| 50%v/v | $50.0 \pm 1.0^0$C | $55.0 \pm 1.0^0$C |

**Table2: Determination of Size Distribution of Sorghum Starch Granules**

| Divisions | Frequency | Actual size |
|---|---|---|
| 0.5 | 4 | 7.20 μm |
| 1.0 | 9 | 14.29 μm |
| 1.5 | 6 | 21.44 μm |
| 2.5 | 14 | 28.58 μm |
| 3.0 | 7 | 42.87 μm |
| | 40 | 7-21-43 μm |

Number of small divisions on eye-piece micrometer corresponding to forty (40) starch granules and their actual sizes using the high power objective lens (x 40).

**Table 3: Microscopical Characters of Sorghum starch**

| Properties | Inferences |
|---|---|
| Shape of granules | The granules are oval in shape |
| Aggregation of granules | Mainly as simple granules with a few aggregates of 2-4 compound granules |
| Colour of Granules | Colourless |
| Striations | Absent |
| Hilum | Centrally placed and appeared as 2-,3-,4-,5-, and 6- stellate cleft |
| Size | 7-21-43 µm |

**Table 4: Number of Lycopodium spores and equivalent Sorghum starch granules in 10 fields of view**

| Field of view | Number of lycopodium spores | Number of Sorghum starch granules |
|---|---|---|
| 0-0 | 5 | 4 |
| 2-2 | 6 | 8 |
| 4-4 | 4 | 14 |
| 6-6 | 6 | 17 |
| 8-8 | 3 | 12 |
| 10-10 | 2 | 13 |
| $2^1$-$2^1$ | 9 | 11 |
| $4^1$-$4^1$ | 1 | 8 |
| $6^1$-$6^1$ | 2 | 10 |
| $8^1$-$8^1$ | 4 | 12 |
| **10 fields** | **42** | **109** |

*Average number of lycopodium spores per field = 4.2 spores.*
*Average number of Sorghum starch granules per field = 10.9 granules.*
*On calculating, the number of Sorghum starch granules contained in one miligramme is 244,000 compared to 94,000 spores per miligramme for lycopodium powder.*

**Table 5: Polarizing effect of Sorghum, Maize and Potato Starch**

| Readings | Sorghum Starch | Maize Starch | Potato Starch |
|---|---|---|---|
| Blank reading | $0.00^0$ | $0.00^0$ | $0.00^0$ |
| First reading | $+2.96^0$ | $+5.40^0$ | $+6.99^0$ |
| Second reading | $+2.70^0$ | $+5.41^0$ | $+7.00^0$ |
| Third reading | $+2.69^0$ | $+5.40^0$ | $+6.99^0$ |
| Fourth reading | $+2.69^0$ | $+5.40^0$ | $+6.99^0$ |
| Fifth reading | $+2.69^0$ | $+5.39^0$ | $+7.00^0$ |

## DISCUSSION

Starch remains an important ingredient in tablet manufacturing and the availability of starch is of Pharmaceutical importance (Obiorah, 1986). The behaviour of starch in water is temperature and concentration dependent. When starches are heated with water, the granules first swell and then undergo gelatinization. The temperatures at which these changes commence and are completed vary with different starches and are characteristic of the starch. Sorghum starch gelatinization commences at $66^0$C and is completed at $75^0$C. This correlates with FOA Corporate Document Repository values of $68.5^0$C and $75.0^0$C respectively. Starch granules also undergo gelatinization when treated with caustic potash or concentrated solution of chloral hydrate. As with other starches, the gelatinization temperature of Sorghum starch was reduced in the presence of formalin.

The size, shape and structure of the starch granules from any particular plant only vary within definite limits, so that it is possible to distinguish between the starches derived from different species. The size range of Sorghum starch granules which were oval in shape was 7-21-43 µm. They were mainly single granules with few aggregates of 2-4 compound granules. The description of a starch granule as 2-, 3-, or 4- compound refers to the number of component granules present in one compound granule. In some cases the compound granule is formed by the aggregation of a large number of simple granules (e.g. rice and cardamon).

The starting point of formation of the granule in the amyloplast is marked by a hilum, which may be central or eccentric. Granules with an eccentric hilum are usually longer than broad. On microscopical examination, the hilum of Sorghum starch was centrally placed and appeared as multiple stellate cleft. The starch granule is built up by the deposition of successive layers around the hilum, and concentric rings or striation are often clearly visible in larger granules e.g. Potato. The position and form of the hilum and the presence or absence of well-defined striations are of importance in the characterization and standardization of starches. Sorghum starch has no striations.

Lycopodium spores are exceptionally uniform in size and 1 mg of lycopodium contains an average of 94,000 spores. The number of spores per miligramme was determined by direct counting and by calculation based on specific gravity and dimensions of the spores. The method is of importance as it can be extended to cover not only simple estimation of the frequency of a single type of particle in a drug e.g. starch grains in powdered drugs. It can also be used for the determination of the proportions of different powders in a mixture.

Starch granules showed double refraction when examined between crossed Nicols, the granules appearing in the dark field as illuminated objects marked by a dark cross, the bars of which intersect the hilum. When examined along with potato and maize starches, +6.990 and +5.400 respectively, Sorghum starch rotates the plain of polarized light by an average value of +2.690 to the right (Dextrorotatory).

In conclusion, the above parameters can be used as diagnostic factors in the identification, standardization and quality control of *Sorghum bicolor*, a veritable source of binder as well as dinsintegrant for tables containing both soluble and insoluble drugs.

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