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Isolation And Physicochemical Characterization Of *Bora* Rice Starch From Assam As Pharmaceutical Excipients

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ABSTRACT

Physicochemical properties of starch isolated from fruit of *Assam Bora* rice (*Oryza sativa,* family Gramineae) have been characterized. Amylose content was found to be nil. The starch granules are polygonal to spherical in shape. The granules exist as single entities. Degree of crystallinity of *Assam Bora* rice starch is significantly higher than potato starch, which reflects its resistance towards enzymatic hydrolysis during digestion in gastrointestinal tract, which also reflect its pharmaceutical use in controlled drug deliver system. *Assam Bora* rice starch can be used in colon targeted drug delivery system.

Keywords: *Assam Bora* rice starch ,True density , Hydration capacity , Degree of crystallinity,Moisture sorption.

1. Introduction:

Because of the multipurpose use and unique functionality of starch in pharmaceutical and food industry has created the search of new starch sources [1]. *Assam Bora* rice locally known as *Bora Chaval* is widely distributed throughout upper *Assam*. *Bora* rice is characterized by high amylopectin contents was introduced in *Assam* from Thailand or Myanmar by Thai-Ahoms [2] and is used as food source and preparation of dishes in religious occasion (*Bihu*) and production of special rice beer in rural *Assam*. Various organizations like *Assam* Agriculture University, Indian council of agricultural research (ICAR), International rice research institute (IRRI) has reported and maintain the various gene bank and genotype of *Assam Bora* rice [3]. Pharmaceutical application of *Assam Bora* rice in controlled drug delivery system has been reported [4]. However Information on the fine structure and physicochemical properties of *Assam Bora* rice starch are limited. The chemical composition and physicochemical characteristic of starch are essentially typical of its biological origin [5].

2. Materials and methods:

Assam Bora rice was procured from local villagers of Dibrugarh district of upper *Assam*. All others chemical used were of analytical reagent grade and used without further purification.

2.1. Isolation of starch:

Starch was isolated from *Assam Bora* rice variety *Aghuni Bora* as process described by T.E Wallis [6] with slight modification. About 1 part of broken rice (fruits of *Oryza sativa,* family Gramineae) were steeped in 2 part of 0.1 M sodium hydroxide solution. The mass was stirred every 60 min and liquor changed every 3 to 5 hr. This process was completed when grains were crushed between fingers. This treatment loosens and partially dissolves the glutinous matter that holds the starch together. The steeped rice was grounded in a mixer grinder with 2 part of glass distilled water to each part of steeped rice, which results into milky fluid. This suspension of milky fluid was diluted until it contains about 2-2.5 % of solid. This suspension was passed through series of sieves 30#, 52#, 60#, 72#, 120# respectively. The thick suspension was allowed to settle in a beaker. It was then washed with 0.0125 M NaOH several times until the supernatant solution was clear. The sediment was washed several times with freshly prepared glass distilled water to remove alkali completely to the neutral pH. The damp starch was dried in hot air oven at 40^0C for 12 hr. After 12 hr, the brownish outer layer was scraped off and dried slowly in an oven at 30^0C for 48 hr. It was then grounded and passed through sieve 120# and stored in tightly closed container. About 5 gram of dried starch was taken into round bottom flask to this excess of n-butyl alcohol (25% v/v) was added and stirred at 100^0 C for 1 hr under reflux. It was then allowed to cool down to room temperature over the period of 18 hour. After 18 hr no any amylose-butyl alcohol complex was found. This indicates the almost negligible contents of amylose in *Assam Bora* rice starch.

2.2. Physicochemical characterization:

The following properties were studied for *Assam Bora* rice starch.

- **Color,Odor, Taste:**
- **Identification test:**

1 g of starch powder was suspended in 50 ml of glass distilled water and was boiled for 2-3 min and cooled. Cloudy mucilage was formed. To the 5 ml of mucilage 0.05 ml of iodine solution was added [7-9].

- **Loss on drying (LOD):**

Loss on drying was carried out by using the procedure as described in USP30-NF25 “loss on drying in oven at 105^0C for constant weight” [8].

- **Ash value:**

Total ash, acid insoluble ash, and sulphated ash value of the starch was determined as described in *Indian Pharmacopoeia* 1996 [10].

- **Determination of true density:**

True density of starch sample was determined by liquid displace-

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ment method in ordinary pycnometer [11]. Using benzene as immersion fluid. True density was determined as

$$\rho = \frac{b-a}{(d+e)-c}$$

a = weight of empty pycnometer, b= weight of pycnometer + half filled with starch, c = weight of bottle + ½ filled sample + ½ filled benzene, d = weight of pycnometer filled with benzene, e = b-a.

- **pH:**

About 2 g quantity of sample was dissolved in 100 ml of glass distilled water. The pH of the solution was determined using pH meter (Mettler Toledo, MP 220, Germany)

- **Determination of acidity:**

Acidity of isolated starch was determined by process as described by *Indian Pharmacopoeia* 1996 under the monograph of starch. About 10 g of starch was dispersed in 100 ml of 70% v/v ethanol, which was previously neutralized, to phenolphthalein. It was shaken on rotary shaker for one hour, then filtered and 50 ml of filtrate was titrated with 0.1 M sodium hydroxide.

- **Determination of hydration capacity :**

Hydration capacity of starch was determined as process described by Kornblum *et al* (1973) [12] with modification. 1.0 g of accurately weighted starch sample was placed in each of four 50.0 ml capacity of centrifuge tube. 15.0 ml of glass-distilled water was added and stoppered. The contents were mixed on a cyclo vortex (Remi,India) for 2 min. It was then allowed to stand for 10 min and centrifuged at 1000 rpm on cold centrifuge (Remi C-24 cold centrifuge). The supernatant was carefully decanted, the stopper was replaced and sediment weighed. The hydration capacity was taken as ratio of weight to dry sample weight.

- **Differential Scanning calorimetry (DSC)**

The DSC gelatization properties of Assam Bora Rice starch was studied using a Mettler Toledo , Gieben, Germany). 2 g Starch of known moisture content was thoroughly mixed with distilled water to obtain a s starch: water ratio of 1:2 w/w on dry basis and sample was allowed to stand in sealed glass jar for 12 hour at room temperature before heating in DSC. Approx 50 mg of well mixed slurry was transferred into aluminum DSC Sample pans of 40 µl capacity and sealed hermetically. Empty aluminum pan was used as reference, the sealed pans were placed in DSC cell and heated from 40 ^{0}C to 240 ^{0}C at a rate of 3 ^{0}C/ min. Onset temperature (To), peak temperature (Tp), conclusion temperature (Te) of gelatization and enthalpy of gelatinization (Hgel) were evaluated by thermal analyzer software (Mettler Toledo STAR system, Micro soft corporation).

- **Viscosity characterization:**

0.5% w/v starch solution was prepared in 1 M KOH. The resulting solutions were filtered through membrane filter of pore size0.45µ. The viscosity measurement were carried out in cone plate type Brook field programmable Rheometer (model DV-III), using spindle CP41, at temperature of 25 ± 0.1 ^{0}C. All measurements were performed within torque range from 10 to 90% of its full scale. The relative viscosity (η_{rel}), specific viscosity (η_{sp}) and inherent viscosity ($ln\ \eta_{rel}/c$) were measured for the filtrate [13, 14].

- **Scanning electron microscopy:**

Scanning electron micrographs (SEM) of the starches were taken with a scanning electron microscope (Hitachi S-300N, electron Microscope). Starch sample were mounted on metal stabs with the aid of double-sided adhesive tape. The samples were gold coated (about 100 A ^{0}) in KSE24M high vacuum evaporator and scanned in scanning electron microscope. The selected region depicting distinct morphological feature were photographed

- **X-ray diffraction pattern:**

X-ray pattern of starch were obtained with copper nickel foil filtered, Kα radiation using diffractometer (Djerassis-dII, UK). The diffraction was obtained at 27mA and 50 kV. The scanning region of two theta (2θ) angle was from 4 to 50^0 with a 0.05^0 step and count time of 2 sec.

FTIR:

IR spectra of pure substance were obtained by grounding and mixing 2 mg in a mortar with 200 mg of desiccated IR grade potassium bromide (Merck) in dry box. Infra red spectra were recorded using FTIR 8300 (Shimadzu, Japan) spectrometer and spectrum was recorded in region of 4500 to 500 cm^{-1}. The procedures consist of dispersing the samples in IR grade KBr and compressing into disc by applying a pressure of 5 tons for 5 min hydraulic pressure. The pellet was placed in light path and spectrum was obtained. Results obtained were compared with data reported in literature [15].

- **Moisture sorption with desiccator method**

Moisture is known to modify the flow and mechanical properties of many powders, therefore knowledge of moisture sorption profile of starch is necessary especially in tablet compression and where controlled flow or compaction is critical [16,17]. Moisture sorption properties of dried starch were determined under condition of various relative humidities (RH) in laboratory desiccator. The various RH conditions were achieved in laboratory desiccators using saturated salt solution of magnesium nitrate, sodium chloride, potassium chloride and potassium nitrate respectively. Sample in triplicate in open glass container were allowed to equilibrate in dessicator and samples were weighted at room temperature at regular interval till equilibrium was achieved. The water sorption behavior was evaluated from moisture pickup rate (mg/g) at each storage time and was expressed as moisture uptake rate (MUR) [18, 19]

3. RESULT AND DISCUSSION:

The yield of starch from *Assam Bora* rice was 83.5% on the grain mass basis. Selected physicochemical properties of *Assam Bora* rice starch are depicted in table 1.

3.1. Physicochemical characterization:

- **Color:** White or almost white powder.
- **Odor:** Odorless or almost odorless
- **Taste:** Tasteless.
- **Identification test:** Dark blue color was produced which disappear on heating. This confirms the presence of starch.

Table 1: Properties of *Assam Bora* rice starch.

Loss on drying (%)	10.8 ± 1.55
Total ash value (%)	0.491 ± 0.05
Acid insoluble ash (%)	0.201 ± 0.003
Sulphated ash (%)	0.27 ± 0.001
Acidity	0.6 ± 0.057
Hydration capacity	4.89 ± 0.691
True density (g/ml)	1.543 ± 0.037

Each value represents the (mean ± S.D) of three determination.

- **Differential Scanning calorimetry (DSC)**

The DSC gelatinization thermogram of Assam Bora rice starch is given in Fig. 1 and table 2. The To, Tp, and Te of Assam Bora Rice starch were found to be 64 ^{0}C, 68 ^{0}C, and 78 ^{0}C respectively. The gelatinization temperature of Assam Bora rice starch is similar to other waxy rice starch [20]. The high Hgel (35.9 cal/g) of Assam Bora rice starch indicates a stronger crystalline nature of *Assam Bora* rice starch, since energy is required to melt the crystallinity of starch granules [21].

Fig. 1 DSC thermogram of Assam Bora rice starch

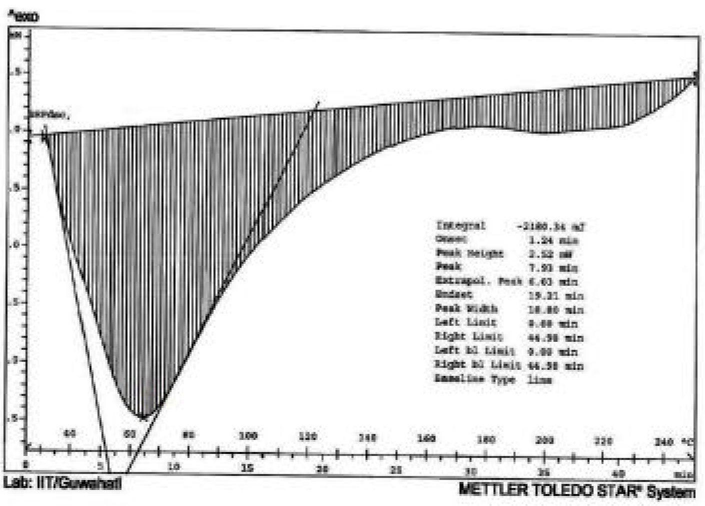

Table. 2. DSC gelatinization of Assam Bora rice starch

To	64 ^{0}C
Tp	68 ^{0}C
Te	78 ^{0}C
Hgel	35.9 cal/g

Viscosity characterization:

Table 3: Viscosity of 0.5% *Assam Bora* rice starch in 1 M KOH

RPM	Relative viscosity (η_{rel})	Specific viscosity (η_{sp})	Inherent viscosity. 2.303 log $\eta_{rel}c$
120	7.593 ± 0.1900	6.593 ± 0.1800	4.055 ± 0.0072
150	7.163 ± 0.066	6.163 ± 0.628	3.938 ±0.0026
180	7.100 ± 0.100	6.100 ± 0.946	3.9208 ± 0.0040

Each value represents the (mean ± S.D) of three determination

It is clear from the table 3 that inherent viscosity of *Assam Bora* rice starch is very high in 1 M KOH, this may be attributed to very high degree of branching and very high weight average molecular weight. This increases the internal friction among the molecules of *Assam Bora* rice starch. Since viscosity of polymer solution depends on concentration and size of dissolved polymer.

- **Scanning electron micrograph:**

Scanning electron photomicrographs of the starch are presented in figure 2 and 3. Starch from *Assam Bora* rice is spherical to polygonal in shape and some granules were of irregular shape. The surface of starch is relatively smooth with some evidence of crack and indentations. In some granules pores were observed.

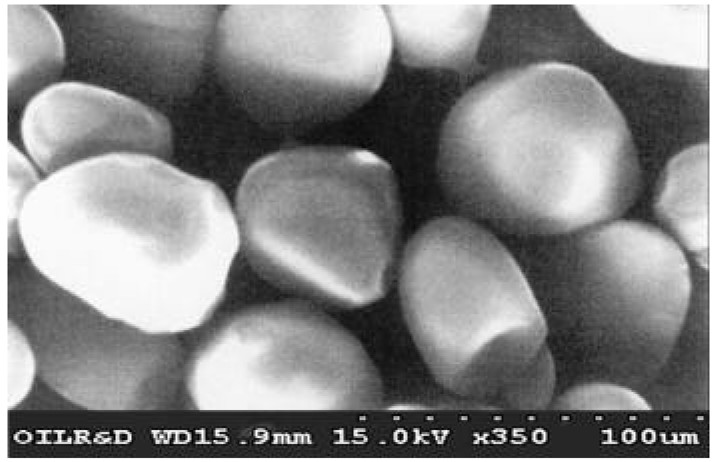

Figure 2: Scanning electron micrographs of *Assam Bora* rice

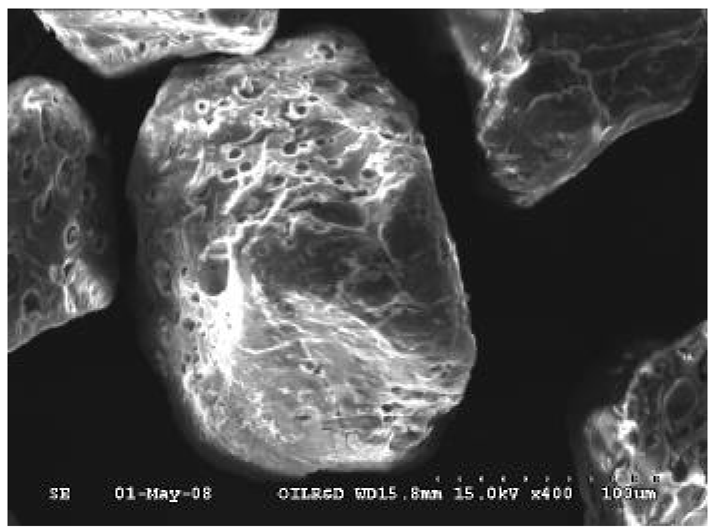

Figure 3: Scanning electron micrographs of *Assam Bora* rice showing pores

- **X-ray diffraction pattern and degree of crystallinity:**

The X-ray diffrectogram of starch from *Assam Bora* rice and their crystallinity value are presented in figure 4: while the scattering angle at which diffraction intensities observed was 2θ, the d spacing was used to discriminate the planes of different sites. The percent of starch granules crystallinity was estimated from ratio of crystalline area to the total area drawn under major diffraction peak [22, 23]**.** *Bora* rice starch exhibit typical A type of diffraction pattern with maximum X-ray diffraction peak at 17.320^0 (2θ) and 24.040^0(2θ). The crystallinity level of starch from *Assam Bora rice* was significantly high i.e. 37.09%. This is significantly higher than potato starch. The Starch granules was found to be semicrsytalline in nature and crystallinity has been assigned to the well ordered structure of amylopectin molecules inside the granules, since the crystallinity level of starch granules is reported to be influenced by amylopectin [24]. The degree of starch granules crystallinity influences various properties of starch such as gelatinization, resistance to hydrolysis (both acid and enzyme) and reactivity during chemical modification [25].

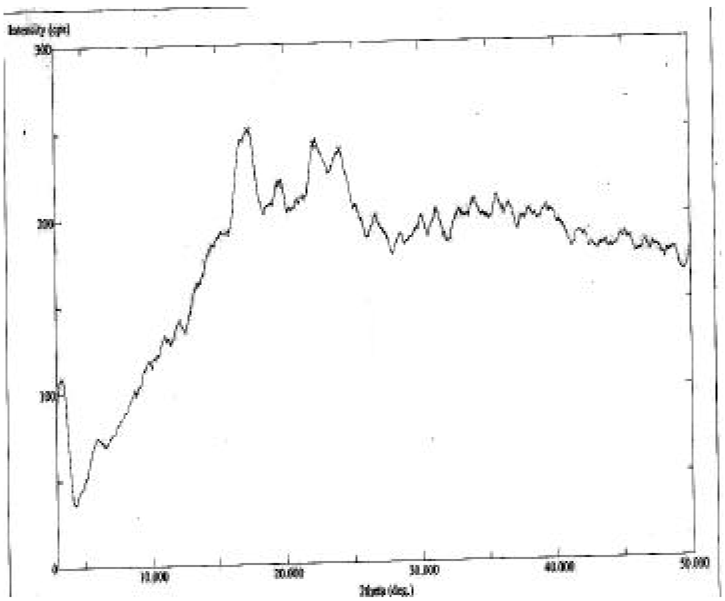

Figure 4: X-ray diffraction pattern of *Assam Bora* rice starch

FT-IR

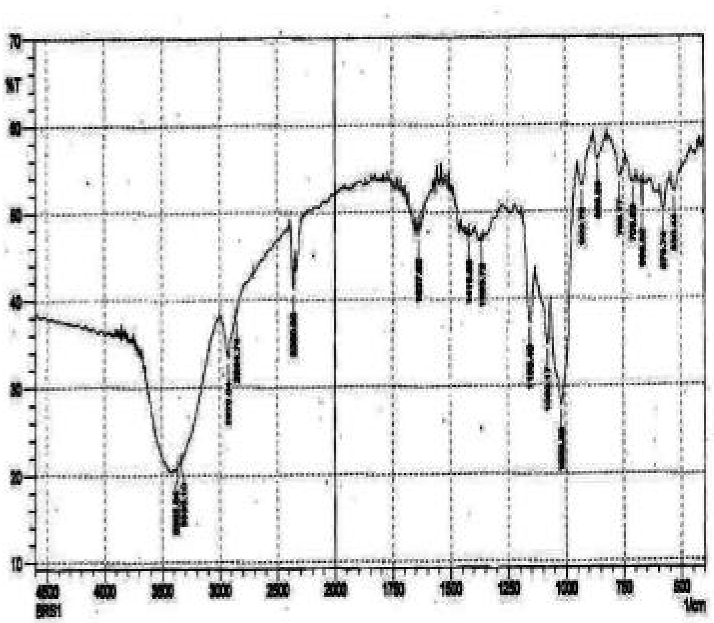

Figure 5: IR Spectra of *Assam Bora* rice starch

Table 3: Wave number (cm^{-1}) and Structural assignment of *Assam Bora* rice starch

Wave number (cm^{-1})	Structural assignment
3362.04, 3333.10	OH stretch
2928.04, 2854.74	CH stretch
1419.66	CH_2 bend
1362.72, 1240.06	OH plane bend
1155.40	Glycosidic COC asymmetric
1080.17, 1020.38	Coupled CO stretch, CC stretch & OH bend
927.72	Ring vibration
860.28	C_1 group vibration
765.77	Ring breathing vibration
709.83, 669.32, 576.74, 530.44	Low frequency ring vibration

- **Moisture Sorption:**

Moisture plays an important role in the stability of many pharmaceutical solid. The physical and chemical properties of pharmaceutical solids are critically dependent on the presence of water: e.g. flow, compaction, dissolution, stability, storage, processing into formulations and final product packing[26]. Figure 6: shows the moisture sorption of *Assam Bora* rice starch equilibrated at various humidity levels. Moisture sorption property of starch increases with increase in relative humidity.

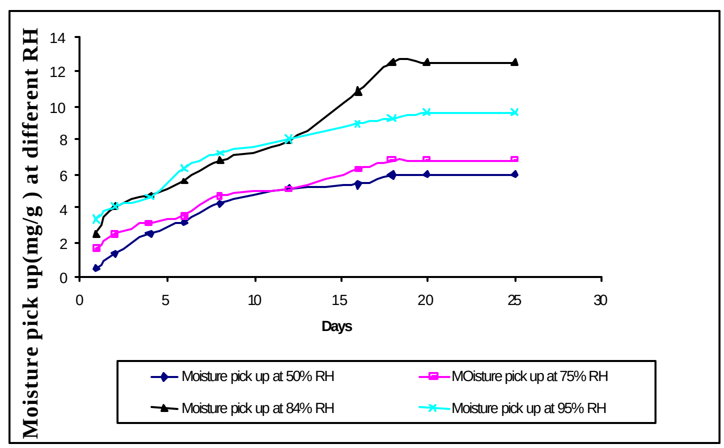

Figure 6: Moisture pickup data

4. Conclusion:

Starch isolated from *Assam Bora* rice has revealed that physicochemical properties are quite similar to rice starches. Amylose content was almost negligible. Shape of *Assam Bora* rice starch is polygonal to spherical with relatively smooth surface. IR spectrum is almost similar to standard IR spectra for starch. Inherent viscosity of Assam *Bora* rice starch is significantly high in 1 M KOH. Moisture pickup rate of *Assam Bora* rice starch increases with increase in relative humidity. The crystallinity level of *Assam Bora* rice starch is significantly high. The rigid structure of *Assam Bora* rice starch granules possibly explain the undigested nature of *Bora* rice diet, which remain as bulk and having therapeutics advantage. Because of the resistance of *Assam Bora* rice starch towards enzymatic hydrolysis finds good use in colon targeted dug delivery system.

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