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Isolation and Evaluation of Starch from *Musa balbisiana* Species as A Mucoadhesive Polymer

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Ripunjoy Bordoloi [a,b*], Abdul Baquee Ahmed [c]

[a]*Research Scholar, Assam Science & Technology University (ASTU), Tetelia Road, Jalukbari Assam, India, Pin 781013*

[b]*Assistant Professor, NETES Institute of Pharmaceutical Science, NIPS Mirza, Santipur Kamrup, Assam, India, Pin 781125*

[c]*Professor Cum Principal, Girijananda Choudhury Institute of Pharmaceutical Science, Tezpur, Assam, India, Pin 784501*

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ABSTRACT

The present study involves the isolation of starch from unripe species of Banana (*Musa balbisiana*) utilizing the technique of wet milling. The isolation was carried out in presence of distilled water along with sodium sulfite aiding the process of extraction. The starch isolated was confirmed by iodine test and this was followed by characterization for physicochemical properties like aqueous solubility, swelling capacity, flow properties, etc. Furthermore, characterization for the starch was carried out in terms of Scanning Electron Microscopy (SEM), Differential scanning calorimetry (DSC), and Infrared spectroscopy (IR). The mucoadhesive strength of the isolated banana starch was estimated by the rotating cylinder method by incorporating it with microspheres and comparison was carried out with the available mucoadhesive polymers. Mucoadhesive microspheres prepared by using this starch employing ionic gelation techniques were also characterized for preliminary parameters and it showed good results. Finally, the in vivo toxicity study carried out in rats provided the safety of the isolated polysaccharide. The outcome of the study suggested that the isolated polysaccharide is having the potential in being utilized as a natural mucoadhesive agent.

INTRODUCTION [1-7]

The concept of bioadhesion also known commonly as mucoadhesion is of greater interest for the formulation and development of various controlled release systems in improving the administration of drugs or medicaments via the buccal, nasal, and oral route. In general, the polymers having bioadhesive properties usually consist of certain functional groups like hydroxyl or carboxyl that in turn helps in the formation of hydrogen bonds with the mucin molecules present in the mucosal surface. Commercially available polymers with bioadhesive properties include Polyacrylic acid cellulose derivatives, polymethacrylic acid, lecithin, polyethylene oxide, and chitosan. The major limitation of these available polymers is their mucosal irritation when cross-linked with other polymers and hence there is always a necessity for some alternate natural polymers to overcome these limitations.

The banana fruit is either consumed in its fully grown state because of its high sugar content or in its unripe form when higher starch content is required in dishes. Starch is considered to be the second most abundant renewable polymer that is cost-effective and is fully biodegradable. Starch $(C_6H_{10}O_5)_n$ which is a combination of amylase and amylopectin is the most familiar of the naturally available hydrophilic polymer. These starch can be available from a variety of sources like grain seeds (like maize (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum vulgare* L.), plant tubers (e.g. potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz)), and plant pith (e.g. sago of the Palmae family) and the properties of starch can vary with both the source from which it is obtained and also the techniques involved in processing it.

Musa balbisiana also commonly known as “Bhim Kol” belongs to the family Musaceae. This species grows extensively in tropical and also sub-tropical regions like India, Africa, and also in the islands of the pacific.

In this present study, an effort was carried out to evaluate the potential of the hydrophilic polysaccharide isolated from *Musa balbisiana* species as a potential mucoadhesive agent. Investigations in terms of characterization of starch, physiochemical properties, flow properties, surface chemistry, thermal properties, and also the suitability of the isolated starch in utilization as a mucoadhesive polymer in the formulation of microsphere were studied and evaluated.

MATERIALS AND METHODS

ISOLATION OF STARCH [8]

Starch was isolated from the collected green and unripe banana species following the modified method of Kim et al. For isolation, the collected fruits were peeled and were cut into 5-6 cm cubes equivalent to 500g total weight and were immediately rinsed in sodium sulfite solution (1.22g/l) following gentle maceration for 2 minutes. The above-homogenized mixture was consecutively shifted through a sieve having mesh size 60# and 100# respectively. The wet mass was washed several times with distilled water until a clear filtrate is obtained. The filtrate that was collected was centrifuged at 7000rpm for 20 minutes and the upper off white sediment is scraped out. The centrifuged mass was again dispersed with distilled water and was again centrifuged for 20 minutes at 7000rpm. Finally, the white starch sediments were dried in a hot air oven at 40 ± 5 °C for 24 h. The dried mass so obtained was grounded with a mortar and pestle and was stored in a sealed container. The confirmation of the collected starch was carried out by the established iodine test in determining starch.

Physicochemical Characterization of Banana starch

1. Determination of chemical composition [9-10]

The estimation of moisture content of the starch collected from banana species was calculated from the weight loss upon heating for 2 hours at 130 ± 5°C in an oven. Furthermore, the determination of protein content was done by estimating the total nitrogen content by the established Kjeldahl method.

2. Total Ash [11]

An equivalent amount of 2g of dried banana starch was taken and was weighed in a silica crucible previously tared. This was followed by incineration at a temperature of 400 ºC until free from carbon. The contents were allowed to cool and were reweighed again and the percentage of ash was calculated on the dried polymer basis.

3. Aqueous solubility [12]

For estimation of the aqueous solubility of the starch, the gravimetric method was employed. This involves accurately weighing out about 1 gm of starch in 100 ml of distilled water which was hydrated for 24 hours at room temperature. The above dispersion was then filtered

through a pre-weighed filter paper. The residue left on the filter paper was allowed to dry in hot air over at 50 o C for 24 hr and was weighed. The solubility of starch was determined by taking the difference between the weights.

4. Swelling Capacity [13]

The swelling capacity of the isolated starch was carried out by taking 5gm of starch and transferring it to a 100ml measuring cylinder. The tapped volume (Va) occupied by the powders was determined and recorded. To the above 5gm of starch, 50 ml of distilled water was added and was subjected to agitation. Finally, the volume was adjusted up to the mark with distilled water. After 24 hours of standing, the volume of the sediment (Vb) was determined and recorded. The swelling capacity was estimated using the formula:

$$\text{Swelling capacity} = V_a/V_b$$

where, V_a = tapped volume,

V_b = volume of sediment after 24 h

5. Particle size Determination [14]

The determination of particle size of the fine starch powder was carried out using the technique of optical microscopy that was previously adjusted with a calibrated stage micrometer. The sample was prepared by dissolving in glycerol and the particle size was determined.

6. The angle of repose [15]

To estimate the flow property of the starch powder, the angle of repose was determined. The fixed funnel method was used for estimating the same. An equivalent amount of sample (10g) was taken and was allowed to pass through a funnel fixed to a stand with an appropriate height in such a way that it forms a heap of powder. The measurement of the height of the pile and the radius was done and the angle of repose was measured by using the formula.

$$\text{Tan } Ø = h/r$$

h = height of the pile

r= radius of the pile

7. Bulk density determination [15]

The bulk density of the powder bed is simply the weight of the powder divided by the whole volume of the bed. In the determination of bulk density, 10 gm of powder was taken and was poured into a measuring cylinder. The bulk volume (V1) was recorded and the density was determined in triplicate. The bulk density (Do) was determined using the following equation:

$$Do = M/ V1\pi r2h$$

where, M = weight of powder (g)

V1 = volume of powder bed

r = radius of the cylinder(cm)

h = height of the powder

8. Freeze- Thaw Stability [16]

The estimation of freeze-thaw stability was carried out by taking 5ml of 5% banana starch paste subjected to a one-cycle freeze-thaw process at 20° C for 18-hour storage in a freezer following its storage at RT for 6 hours. These samples were then centrifuged at 3000rpm for 10 minutes. Finally, the percentage of water separated from the sample after the freeze-thaw cycle was measured.

9. Scanning Electron Microscopy (SEM) [17]

Scanning electron microscopy was performed for morphological characterization of the starch powder using a scanning electron microscope (SEM—LEICA, 5430, London, U.K). The isolated starch was dried and was mounted directly onto the SEM sample stub using double-sided sticking tape and coated with gold film (thickness, 200 nm) under reduced pressure (0.001 mmHg). Finally, the image obtained was collected.

10. FT-IR Spectroscopy [18]

IR spectrum of pure banana starch was recorded using Fourier Transform Infra-Red spectrophotometer (Shimadzu IR Affinity 1). The samples were previously triturated and mixed thoroughly with potassium bromide in the ratio of 1:99 (sample: potassium bromide). Then, the sample was scanned by the DRS method. The scans were obtained at a resolution

from 4000 to 400 cm-1. The response was recorded in terms of the IR spectra obtained after the completion of the scan.

11. Differential Scanning Calorimetry (DSC) [18]

The thermal behavior of isolated banana starch was studied using a differential scanning calorimeter (JADE, Perkin Elmer, USA,). For the study, 0.85 mg of starch was weighed into an aluminium pan & 70 % moisture content was adjusted by adding de-ionized water. The pan was hermetically sealed & was left for equilibration for 1hr at room temperature. An empty sealed pan, in the same way, was used which served as the reference. The sample was scanned from temperature 30°C to 300°C at a rate of 10°C/min under a nitrogen flow of 10mL /min. Gelatinization temperature was determined by automatically computing initial temperature (Ti), maximum peak temperature (Tp), final temperature (Tf) & gelatinization enthalpy (ΔH) from the resulting Thermogram.

12. Acute oral Toxicity study of banana starch [19]

To access the safety of the isolated banana starch, an acute oral toxicity study was carried out on mice. The study was by the protocol described in Institutional animal ethics as per the animal ethical committee of NETES Institute of pharmaceutical science, Mirza, India NIPS with the approval number NIPS/AH/20/19 under CPCSEA, India. The study protocol was carried out as per the OECD guidelines. Mice weighing between 20-25 gm of either sex were taken in groups of five (n=5). Before the study, the animals have fasted for 4 hours with free access to water only. The isolated starch in form of mucilage was administered orally to the animals in doses of 2000mg/kg in 0.5 ml of distilled water to all the groups. The animals were further observed for 14 days for mortality and any changes in physical behavior.

13. Preparation of microsphere [20]

The microsphere containing was formulated by utilizing the ionic gelation technique. Initially, Sodium Alginate and the previously isolated banana starch polymer were dissolved in 50ml of deionized water to produce a homogenous polymer solution by using a homogenizer (Remi Motors, India). Finally, the resultant dispersion containing d the polymer was manually added dropwise via a 26-gauge needle to a solution of 10% $CaCl_2$ solution. Similarly, an equivalent amount of chitosan was combined along with sodium alginate, and the microsphere was prepared by using the same technique.

14. Determination of the mucoadhesive properties of banana starch [21]

The assessment of the mucoadhesive property of isolated banana starch was determined by the falling liquid film technique. This methodology utilizes a freshly cut piece, 5 cm long, of goat nasal mucosa obtained from a local slaughterhouse within 2 h of killing the animal was cleaned by washing with isotonic saline solution. Accurate weight of microspheres was placed on the mucosal surface, which was attached over a polyethylene plate that was previously fixed at an angle of 45° relative to the horizontal plane, and pH 6.8 phosphate buffer warmed at 37°C was peristaltically pumped at a rate of 5mg/min over the tissue. One hour after administration of microparticles, the concentration of the drug in the collected perfusate was spectrophotometrically determined. The microparticles amount corresponding to the drug amount in the perfusate was calculated. The adhered microparticles amount was estimated from the difference between the applied microparticles and the flowed microparticles amount. The ratio of the adhered microparticles was computed as percentage mucoadhesion.

RESULTS AND DISCUSSION

1. Isolation and Physiochemical properties

The starch was isolated as per the techniques described above. The fine white starch powder obtained was subjected to initial identification with Iodine solution which gave a brown to black colour confirming the presence of starch (Figure 1). Several other physiochemical parameters of the starch were evaluated and the results for the same are described in Table 1.

Table No. 1: Physiochemical Properties of isolated starch

Sl no	Parameters Evaluated	Results
1	Moisture Content [%w/w]	0.428 ± 0.0045
2	Total Ash [%]	2.25 ± 0.25
3	Aqueous Solubility[mg/ml]	0.813 ± 0.0065
4	Swelling Capacity	0.42
5	Particle Size [µm]	25.95 - 30.44
6	Angle of Repose [θ]	36.49° ± 0.021
7	Bulk Density [g/cm^3]	0.218± 0.048
8	Freeze Thaw Stability [ml]	1.21 ± 0.057

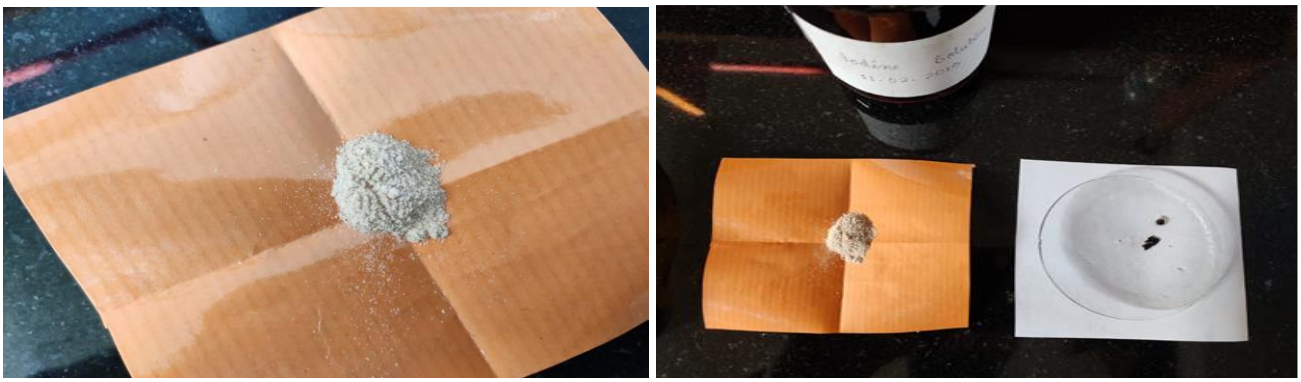

Figure No. 1: Isolated starch and Confirmatory test

2. Scanning Electron Microscopy

The morphological characteristics of the isolated banana starch were estimated by Scanning electron microscopy. As seen in the SEM image (Figure no 2) the surface characteristics show a smooth texture with oval to polygonal shape. The size of the banana starch granules ranged from 25.95 µm- 30.44 µm. In general, the size and shapes of starch granules have an impact on the overall physiochemical, functional, and nutritional characteristics as larger granules seem to develop high viscosity paste and the smaller granules owe high digestibility.

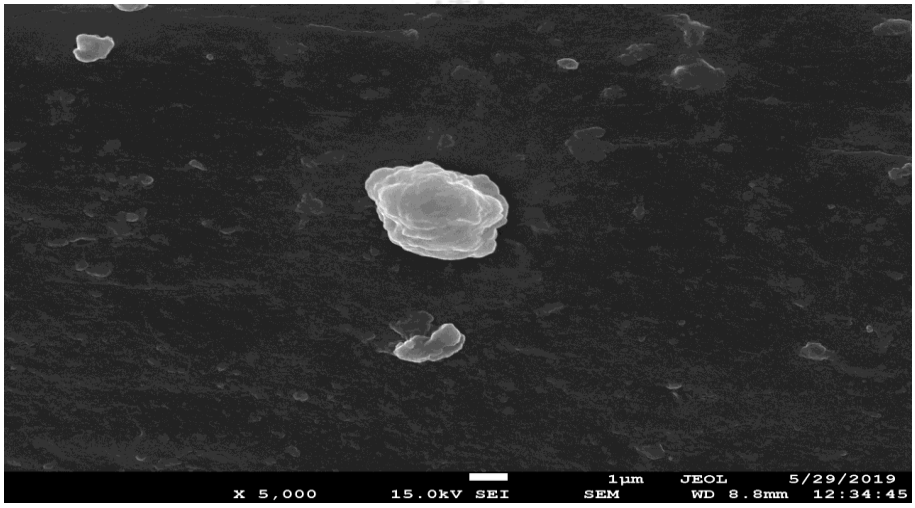

Figure No. 2: SEM image of isolated starch

3. FT-IR Spectroscopy

The FT-IR Spectroscopic spectrum of banana starch (Figure 3) shows a band occurring at 3251 cm-1 and this was because of the presence of hydroxyl (-OH) groups. Also, peak was seen at 2921.49 cm-1 because of the stretching mode of the C-H bonds of methylene groups

(-CH2). Moreover, the absorption bands seen at 997.35 cm-1 indicates the presence of C-O stretching of ether groups present in starch.

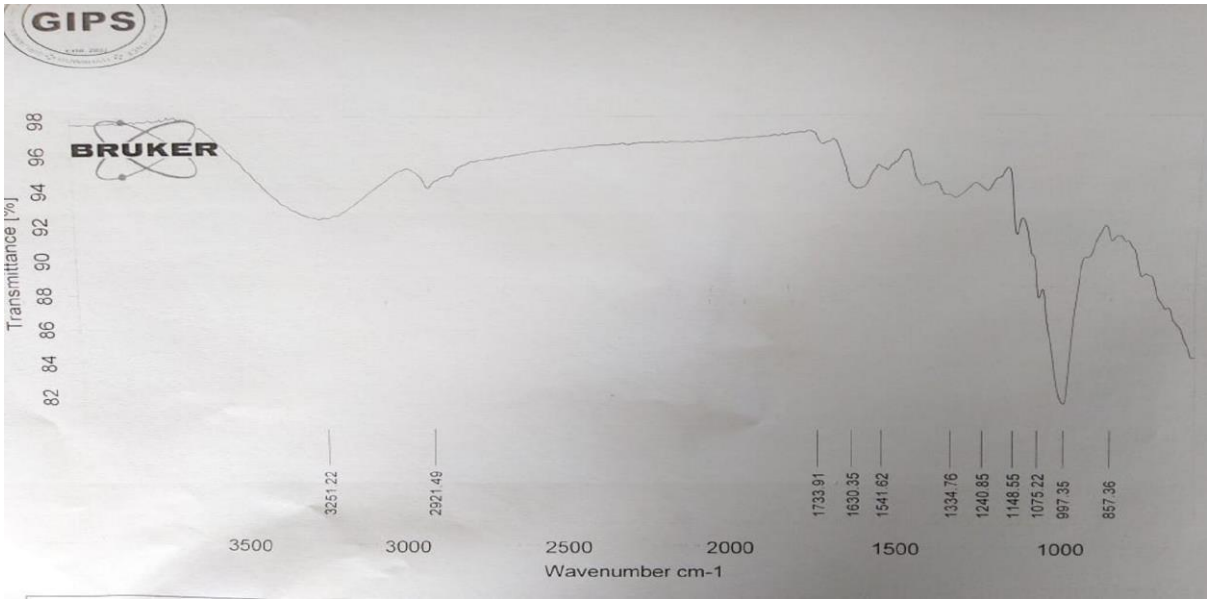

Figure No. 3: IR Spectra of isolated starch

4. Differential Scanning Calorimetry

The DSC thermogram of isolated banana starch (Figure 4) gave an idea regarding the peak temperature (Tp). The peak temperature corresponds to the temperature required for gelatinization and was found to be 117.84°C. Similarly, gelatinization enthalpy (DH) corresponds to the amount of energy required for gelatinization to take place. For the isolated starch, the gelatinization enthalpy was found to be 1039.1603 J/g.

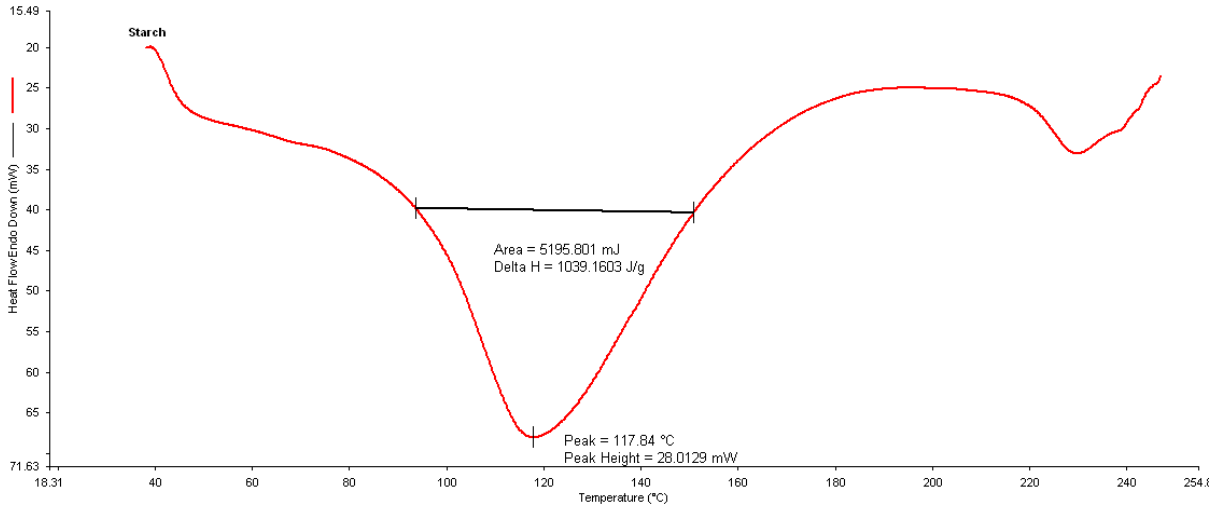

Figure No. 4: DSC Thermogram of isolated starch

5. Toxicity Study

The safety of the isolated starch was estimated out by conducting an oral toxicity study in Mice. The animals were subjected to a dose of 2000 mg/kg body weight and were checked for any abnormal changes for one month. No such death or abnormal behavior was recorded which supports the safety of the polymer. Furthermore, as the species of banana is traditionally consumed by the local people, this also supports the safety of the species. Thus, it can be claimed that the starch is safe for use and in particular, the amount used here is safe for further study.

6. Preparation of Microsphere and estimation of Mucoadhesive property of Starch

Following the technique of ionic gelation, the microspheres were prepared using isolated banana starch and combine with another polymer like sodium alginate and calcium chloride as the cross-linking agent. The microsphere prepared were having a good spherical shape with adequate size (Figure no 5). Similar technique along with the same concentration was also utilized in preparing microsphere containing chitosan to compare the mucoadhesive strength with the isolated banana starch polymer. The falling liquid film technique provides an idea about the mucoadhesive strength with mucoadhesion ranging for around 6 hours for banana starch microsphere and 12 hours for chitosan microsphere (Figure 6) when studied in phosphate buffer pH 6.8. These polymers being hydrophilic tend to absorb water, thereby swelling up leading to mucoadhesion with the mucosal membrane layer. Moreover, certain functional groups like hydroxyl and carboxyl present in the starch can also form hydrogen bonds with the mucin molecules present in the intestinal tissues. The concentration of starch used here can be utilized in further study as a natural mucoadhesive agent.

Figure No. 5: Microspheres prepared by ionic gelation technique

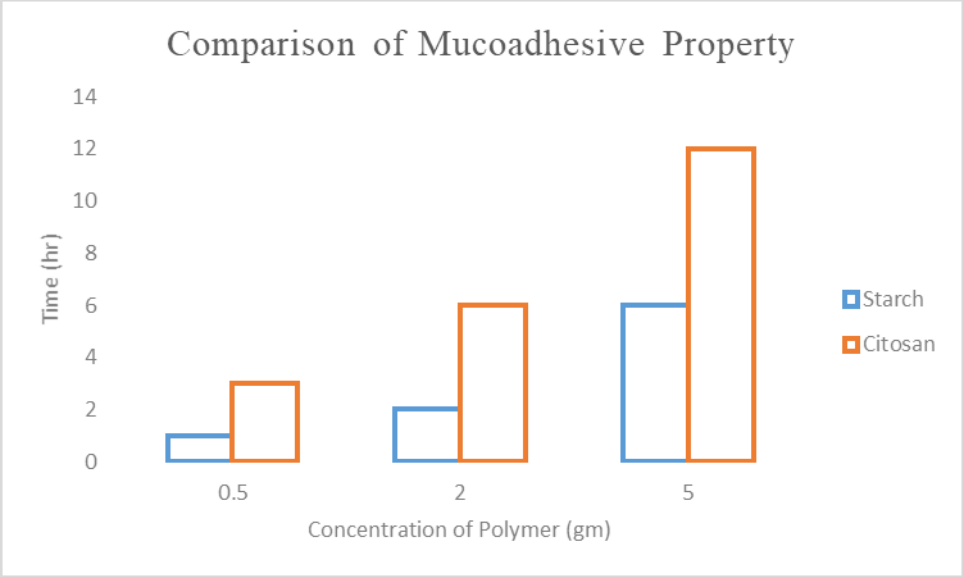

Figure No. 6: Comparison of mucoadhesive property of isolated starch with chitosan

SUMMARY AND CONCLUSION

Banana starch has been isolated from the unripe fruit of the plant *Musa balbisiana* (Family-*Musaceae*). The characterization in terms of Swelling capacity, ash value, flow property study was found in acceptable limits. Furthermore, the IR spectra, DSC thermogram, and SEM analysis provided fruitful information regarding this isolated polymer. The safety of this polymer was conducted using an Acute oral toxicity study following the OECD guidelines. Microspheres formulated using this polymer by ionic gelation technique were of discrete size and shape and were found to possess a good mucoadhesion on examining by already available model. Thus, it can be concluded that the isolated starch from this species of banana has a promising mucoadhesive property and can be utilized in the formulation of the various dosage form in enhancing the drug bioavailability and also for site-specific delivery.

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