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Isolation and Characterisation of Some Natural Polysaccharides as Pharmaceutical Excipients

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Abstract: From the last few decades the importance of search for polysaccharides as pharmaceutical excipients for mucoadhesive drug delivery system has been increasingdue to their various advantages. The present study was aimed at determining the suitability of adhesive polysaccharides from three different plant namely Bora rice starch *(Oryza sativa)*, Tamarind seed polysaccharides *(Tamarindus indica)* and Drumstick polysaccharides *(Moringa oleifera)*, for use as Pharmaceutical excipients. The polysaccharides were isolated and Characterised for their solubility, pH, swelling index, viscosity, mucoadhesiveness, powder flow property etc.
All the polysaccharides were found to have good mucoadhesiveness with Bora rice starch showing the highest mucoadhesiveness comparatively. The polysaccharides showed suitable pH, rheological behaviour and good swelling. Determination of Carr's index and Housner ratio showed good flow properties of all lyophilised polysaccharides. Kawakita analysis for powder flow revealed that the values for kawakita constants 'a' and '1/b' were in the order of bora rice starch > drumstick polysaccharide > tamarind seed polysaccharide. Lower value of 'a' and 'b' were obtained showing better flowability and lack of cohesiveness respectively.
From the study we can conclude that the isolated polysaccharides of bora rice, tamarind and drum stick may be suitable alternative to be used for mucoadhesive and controlled drug delivery and better granule flowability.
Keywords: Natural Polysaccharides, Mucoadhesive, Kawakita Analysis, Powder Property.

INTRODUCTION:

Polysaccharides are polymers consisting of repeating carbohydrate units. They may be linear or branched and, when soluble in water, they swell and form highly viscous solutions. This capacity is used in various technological and industrial fields (particularly pharmaceutical technology and pharmacology) because polymeric high-viscosity solutions frequently cover nasal, buccal, vaginal and gastro-intestinal mucosal tissue with a thin transparent film in order to protect it and control drug absorption or prolong local drug delivery which can be advantageous in the treatment of local conditions. A bioadhesive can therefore be defined as a biocompatible substance that is capable of interacting with biological materials and being retained by them or holding them together for an extended period of time.[1, 2] In recent years, some bioactive polysaccharides isolated from natural sources have attracted much attention in the fields of biochemistry and pharmacology.[3] They exhibit various biological activities depending upon their different chemical structures.

Many reviews had been published discussing different aspects of the growing field of polymer therapeutics.

Starch is a polysaccharide composed of glucose units that occurs widely in plant tissues in the form of storage granules, consisting of amylose and amylopectin. Pharmaceutical application of Assam Bora rice in controlled drug delivery system has been reported.[4] Colon targeted delivery using bora rice starch is cited by some researchers.

Tamarind Seed Polysaccharide (TSP), derived from the seeds of *Tamarindus indica linn*. A common and most important tree of India and Southeast Asia, is composed of (1_4)-b-D-glucan backbone substituted with side

chains of a-D-xylopyranose and b-D-galactopyranosyl (1_2)-a-Dxylopyranose linked (1_6) to glucose residues. TSP can change into gel in neutral and acidic pH. The gel can be used as a thickening and stabilizing agent in food industry.[5,6] Several features make TSP an attractive candidate as a vehicle.

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. All parts of the Moringa tree are edible and have long been consumed by humans. Drug delivery using moringa bark exudates polysaccharides are yet very limited.

MATERIALS

Collection of Raw Materials

Collection of Bora rice: Assam Bora rice was procured from Mangaldoi, district of lower Assam, India.

Collection of Moringa gum exudates: The crude gum exudates were collected from the trees available indigenously from Nalbari (Batachara), district of lower Assam, India. The incisions were made on the trunk of the trees and after 2-3 days the thick gum was collected and dried in sunlight. The exudates in old trees are blackish in colour and brownish in young trees.

Collection of Tamarind seeds: Tamarind seeds were collected locally.

Chemicals and Reagents: All the chemicals used during the project are of analytical grade. Irinotecan HCL was purchased from Yarrow Chem. Products, Mumbai, India. Acetone, Hydrochloric Acid, Sodium Hydroxide, Sodium Dihydrogen Phosphate, Potassium Dihydrogen Phosphate were purchased from Merck Specialities Pvt Ltd, Mumbai. Ethanol was purchased from Thangshu Yngyuan Chemical, China.

METHODS

Isolation of Starch: About 1 part of broken rice 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 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 400ºC for 12 hr. After 12 hr, the brownish outer layer was scraped off and dried slowly in an oven at 300ºC for 48 hr. It was then grounded and passed through sieve 120# and stored in tightly closed container.[7]

Isolation of Drumstick Polysaccharide:

The gum was collected from trees (injured site). It was dried, ground, and passed through sieve no 66. Dried gum (40 g) was stirred in distilled water (1000 ml) for 6- 8 h at room temperature. The supernatant was obtained by centrifugation. The residue was washed with water and the washings were added to separate supernatant. The procedure was repeated four times. Finally the supernatant was concentrated on water bath and treated with twice the volume of acetone by continuous stirring. The precipitated material was washed with acetone and dried at 50-60° under vacuum.[8]

Isolation of Tamarind Polysaccharide:

The seeds were washed thoroughly with water to remove the adhered sand particles. The seeds were than boiled in distilled water for about 3 hours and then dried in a tray dryer. Then, the reddish testa of the seeds was removed using grinding in a glass mortar. The seeds were crushed lightly. The crushed seeds of *Tamarindus indica* were soaked in water separately for 24 hours and then boiled for 1 hour and kept aside for 2 hours for the release of mucilage into water. The soaked seeds were taken and squeezed in a muslin bag to remove marc from the filtrate. Then, equal quantity of acetone was added to precipitate the mucilage. The mucilage was separated. The separated mucilage was dried at temperature 50°C, powdered and passed through sieve number 80. The dried mucilage was powdered and stored in airtight container at room temperature.[9]

Percentage Yield:

A known quantity of crude material was weighed first. After the polysaccharide was precipitated using acetone the freeze-dried mass of the polysaccharide was determined. Yield was expressed as percentage of the mass of the dry precipitate against the mass of the whole fresh crude materials.[10]

Organoleptic Properties:

The organoleptic properties of the three polysaccharides were examined visually.

Solubility in different solvents:

The approximate solubility was checked for different common solvents depending on their polarity such as water, phosphate buffers pH 6.8, 0.1N HCL, acetone, ethanol, methanol etc. in the room temperature.[11]

pH:

The pH of the polysaccharides was determined by digital pH meter. The instrument was first calibrated with standard phosphate buffer pH 4.0, pH 7.0 and pH 9.0. 100mg powdered polysaccharide was dissolved in 10 ml of distilled water and the electrode was then dipped in to gel formulation and allowed to equilibrate for 30min. The measurements of pH of each formulation were replicated three times.[12]

Powder Properties:

The isolated polysaccharides were evaluated for the following powder characteristics- [13]

Bulk density:

A sample powder was introduced in 100 ml graduated cylinder. The volume of the material was noted on graduated cylinder. The bulk density was measured by the following formula-

Where, P_o $P_o = M/ V_o$

M is the mass of the sample powder; V_o is the volume of the powder.

Tap Density:

A sample powder was filled in 100ml graduated cylinder. The mechanical tapping was carried out and the tapped volume was V_f was noted.

$$P_t = M/ V_f$$

Where P_t is the tapped density, M is the mass of the sample powder and $V_{f\,is}$ the tapped volume of the powder.

Compressibility Index:

The bulk density and the tapped density was measured and compressibility index (CI) was calculated using the following formula-

$$\% CI = (P_t - P_o)/P_t \times 100$$

Where, C is the compressibility index, P_t is the tapped density, P_o is the bulk density.

Angle of Repose:

The angle of repose gives an indication of the flow ability of the substance. A funnel was adjusted such that the tip of the funnel lies 2 cm above the horizontal surface. The drug powder was allowed to flow from the funnel under the gravitational force till the tip of the pile just touched the tip of the funnel of orifice size 6 mm so the height of the funnel was taken as 2 cm. The diameter of the pile was determined by drawing a boundary along

the circumference of the pile and taking the average of six diameters. These values of height and diameter were then substituted in the following equation-

$$\Theta = \tan^{-1} 2h/d$$

Where, 'Θ' is the angle of repose, 'h' is the height of the pile and'd' is the diameter of the pile. The experiment was done in triplicate.

Hausner Ratio:

Hausner Ratio (HR) was calculated using the following formula-

Where P_t is $HR = P_t / P_o$ ty and P_o is the bulk density.

Rheological Behaviour Study:

Viscosity determination of 1% solution of polymer was carried out on a cone and plate geometry viscometer (Brookfield DV-E viscometer), using spindle no 62 and 64. Viscosity of polymer solutions were measured at different angular velocity 0.0 to 100 rpm. Evaluations were conducted in triplicate.[14]

Swelling Index Determination:

100mg of polysaccharide powder of each sample was introduced into separate 25 ml glass-stoppered measuring cylinder. The internal diameter of the cylinder was about 16 mm, the length of the graduated portion about 125 mm, marked in 0.2 ml divisions from 0 to 25 ml in an upwards direction. 25 ml of water was added and shake the mixture thoroughly every 10 minutes for 1 hour. Allowed to stand for 3 hours at room temperature. The volume was measured in ml occupied by the polysaccharide material. The mean value was calculated for the individual determinations, related to 100mg of polysaccharide material.[15]

The swelling Index was calculated using the following equation-

$$SI = (S2 - S1)/S1 \times 100$$

Mucoadhesive strength of 1% (w/v) aqueous solution of each polysaccharide was studied with Texture Analyzer (TA.XT EXPRESS, FD/I-077). Freshly excised goat intestinal membrane was attached to the upper probe of the instrument, and drop of polysaccharide solution was kept below that. The upper probe was then lowered at a speed of 10 mm/min to touch the surface of the solution. A force of 0.1 N was applied for 10 min to ensure intimate contact between the membrane and the gel. The surface area of exposed mucous membrane was 1.13 cm^2.[16]

Kawakita Analysis:

The powder flow properties were determined by kawakita analysis. The method involved pouring a 10g of powder into a 50 ml glass measuring cylinder and the heap of the particles in the cylinder was levelled off horizontally with a thin metallic spatula and the bulk volume V_0 was accurately measured. Then tapping was started mechanically and the change of volume of the powder column V_N was noted after N no of taps. The behaviour of the powders was compared using numerical constants obtained from the kawakita plots.

The flow property of powders was determined by the following kawakita equation

$$N/C = N/a + 1/ab$$

Where 'a' and 'b' are constants. 'a' describes the degree of volume reduction at the limit of tapping and is called compactability and '1/b' is considered to be a constant related to cohesion and is called cohesiveness.

$$C= (V_0-V_N)/V_0$$

C is the degree of volume reduction which is calculated from the initial volume V_0 and the tapped volume V_N as-

Numerical values of constants 'a' and '1/b' are obtained from the slope of plot of N/C vs N (N=10, 30,100,300).[17]

Fourier transforms infrared spectroscopy (FT-IR)

IR spectra of pure substance were obtained by grounding them and directly placing the sample under the pressure head of the instrument. Infra-red spectra were recorded using FTIR (Bruker; ALPHA; Model No-10059736) spectrometer and spectrums were recorded in region of 4500 to 500 cm^{-1}.[18]

RESULTS AND DISCUSSIONS:

Percentage Yield:

The % yield of the polysaccharides was found to be 8.33%, 27.5 % and 20.0% for the bora rice, drumstick and tamarind respectively. During the processing of bora rice starch isolation washing is required many times which may result in loss of dissolved polysaccharides. However extraction processes drumstick and tamarind are comparatively easy and hence gives better yield.

Organoleptic Properties:

The organoleptic properties were observed and the results are shown in Table 1.

Solubility in various solvents:

The solubility was checked in common solvents depending on their polarity such as water, phosphate buffers pH 6.8, 0.1N HCL, acetone, ethanol, methanol etc. The polysaccharides were found to be practically insoluble in organic solvents and are soluble in the aqueous solvents and swell to make a viscous solution. Hence all the polysaccharides are hydrophilic in nature.

Determination of pH:

The pH of 1% solution was found to be 7.8, 7.4 and 6.5 for bora rice, drumstick and tamarind polysaccharide. Hence the polysaccharides should be compatible with human physiological system. The results are shown in Table 1.

Powder Properties:

The polysaccharides were subjected to the analysis of powder flow properties such as bulk density, tap density, Carr's index (CI) and Hausner ratio (HR). Direct compression of powders requires materials exhibiting good flowability, comapactibility and compressibility. These parameters become more critical when the formulation contains large amount of active substances with poor compressional properties. Wet granulation method is selected for production of porous and free-flowing granules, which enables to form tablets with high mechanical strength at low compression pressure. The %CI range in between 26-31 stands for poor flow. Again HR range in between 1.35-1.45 also possess poor flow. From the investigation the values were found to be in this range for all three polysaccharides. Hence they are of poor flow properties. The reason may be due to the high intermolecular force of attraction among the particles. It may be due to insufficient freeze drying of the product resulting in slightly fluffy powder which are difficult to be sieved. The results are shown in Table1.

Rheological Behaviour:

The viscosities of the polysaccharide solutions were determined using Brookfield viscometer at different shear. All the polysaccharides showed sufficient viscosity at different shear rate and shows pseudoplastic flow. The viscosity decreases correspondingly with increasing shear, however in case of drumstick polysaccharide, it shows plastic behaviour. An initial high threshold shear is required for drumstick polysaccharide to break the strength of the gel and once it reaches the maximum it causes abrupt lowering of viscosity. On the other hand tamarind and starch possess consistent change behaviour upon application of pressure. The results are shown in Fig.3.

Swelling Index:

The polysaccharides showed good swelling showing swelling index (% SI) of 100%, 125% and 136% for bora rice, drumstick, and tamarind polysaccharides respectively. The substantial swelling shown by these polysaccharides may be useful for achieving controlled drug release behaviour from polysaccharides matrix. The results are shown in Table.1.

Mucoadhesive Test:

From Texture Analyzer studies of mucoadhesive tests, it was found that the adhesive property of the polysaccharides are greater than that of HPMC (Fig.1). The observations for the three polysaccharides holds considerable significance for use in mucoadhesive drug delivery. The relative mucoadhesiveness was observed as bora rice starch > tamarind > drumstick > HPMC. Hence they are suitable as mucoadhesive drug carriers and can be used in place of synthetic excipients.

Kawakita Analysis:

Kawakita constants indicate the behaviour of the powder from the bulk density state to the tapped density state. The kawakita analysis revealed that the values for kawakita constants 'a' and '1/b' were in the order bora rice > drumstick > tamarind as shown in Table.4. Lower value of 'a' indicate less compactability property of the particles and lower value of 1/b indicate less cohesiveness among the particles. So bora rice starch has more compactability as well as cohesiveness and hence suitable for direct compression. Whereas lower value of '1/b' for tamarind shows that it is less cohesive than granule. It will produce better flowability in granular form than its powder form. This property is essential during the formulation of tablets. Results are shown in Fig.2.

FTIR Spectrum: The IR spectrums of individual polysaccharides are shown in the figures 4, 5, 6 and their respective structural assignments are described in tables 5, 6, 7. Different stretch and bend vibrations showed their respective peaks. From the groups present on their chemical structures it may be concluded that the mucoadhesive substances are of polysaccharides family.

Table.1: Comparative Physicochemical properties of the polysaccharides

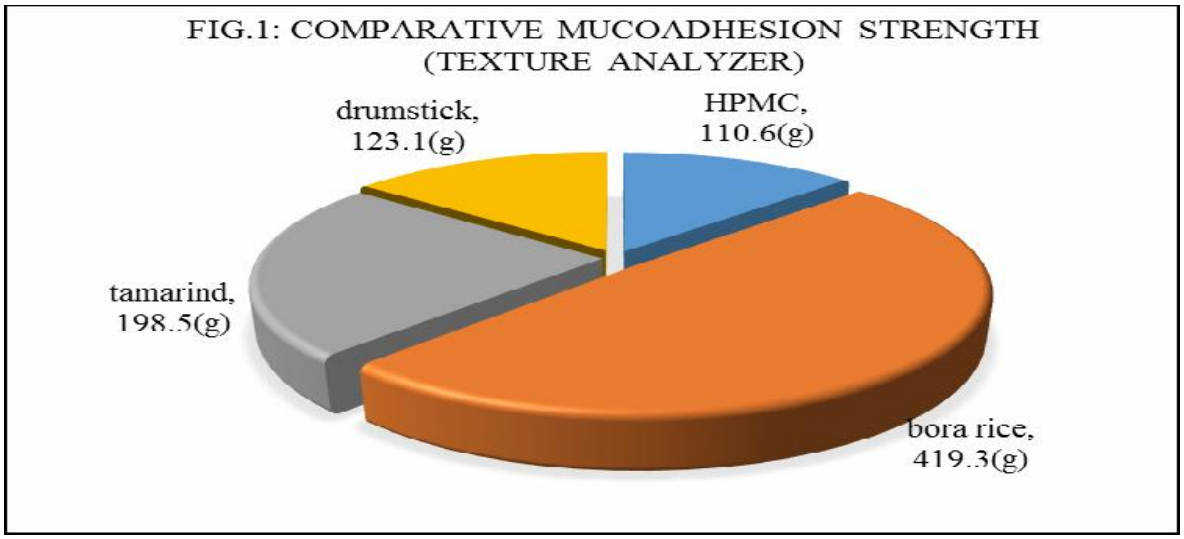
Samples	Colour	Odour	Taste	% Swelling Index	pH
Bora Rice Starch	White	Odourless	Tasteless	100	7.8
Tamarind Polysaccharide	Greyish White	Characteristic	Characteristic	136	6.5
Drumsticks Polysaccharide	Pinkish White	Odourless	Tasteless	125	7.4

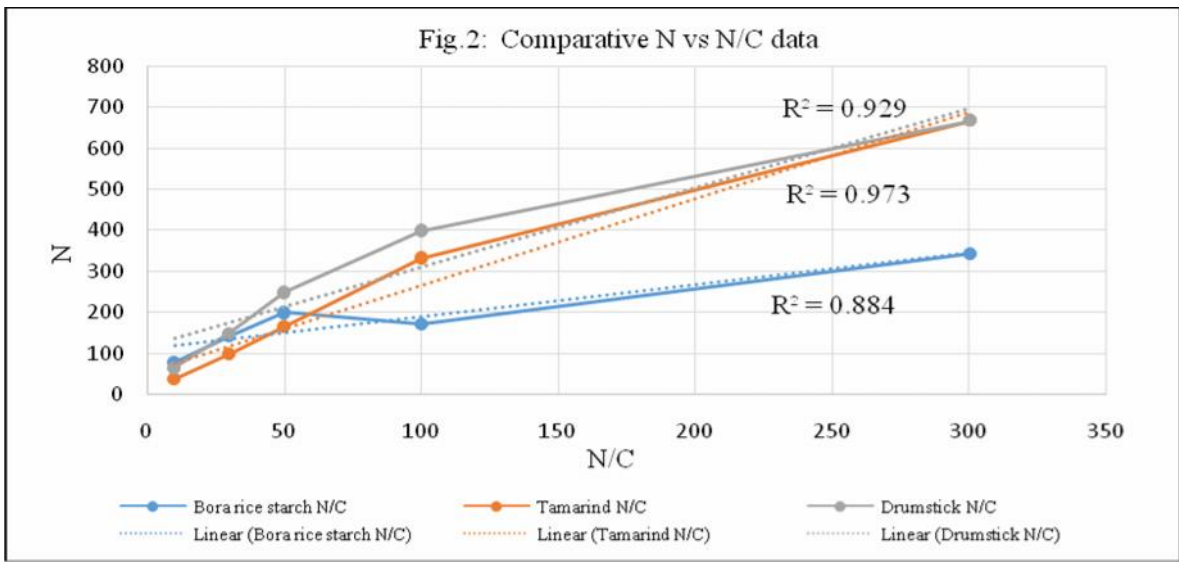
Table.2: Comparative Powder flow properties of the polysacharides

Samples	Bulk Density	Tap Density	% Compressibility Index	HR	Angle of Repose
Bora Rice Starch	0.42	0.60	28.58	1.4	30^0
Tamarind Polysaccharide	0.24	0.32	23.53	1.3	40^0
Drumsticks Polysaccharide	0.71	1.00	28.59	1.4	28^0

Table.3: Comparative Viscosity Data of % 1 dispersion of the polysaccharides

RPM	Viscosity (cps)		
	Bora rice starch	Tamarind polysaccharide	Drumsticks polysaccharide
10	2283	2220	3500
20	1073	1428	282
30	919	855	248
50	510	468	199
60	374	345	180
100	250	265	145





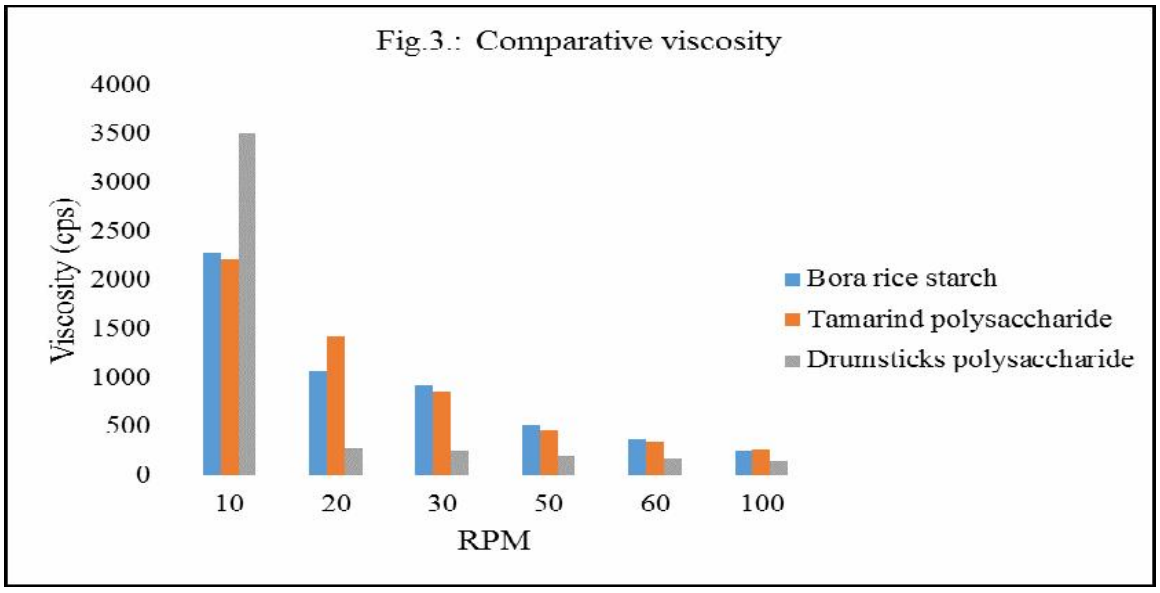


Table.4: Summary of Kawakita analysis of different polysaccharides

Sample	Value of 'a'	Value of 'b'	Value of '1/b'
Bora rice starch	0.8343	0.0127	78.6519
Tamarind polysaccharide	0.3017	1.1571	0.8642
Drumsticks polysaccharide	0.3232	0.0473	21.1259

Fig.4 FTIR Spectrum of Bora Rice Starch

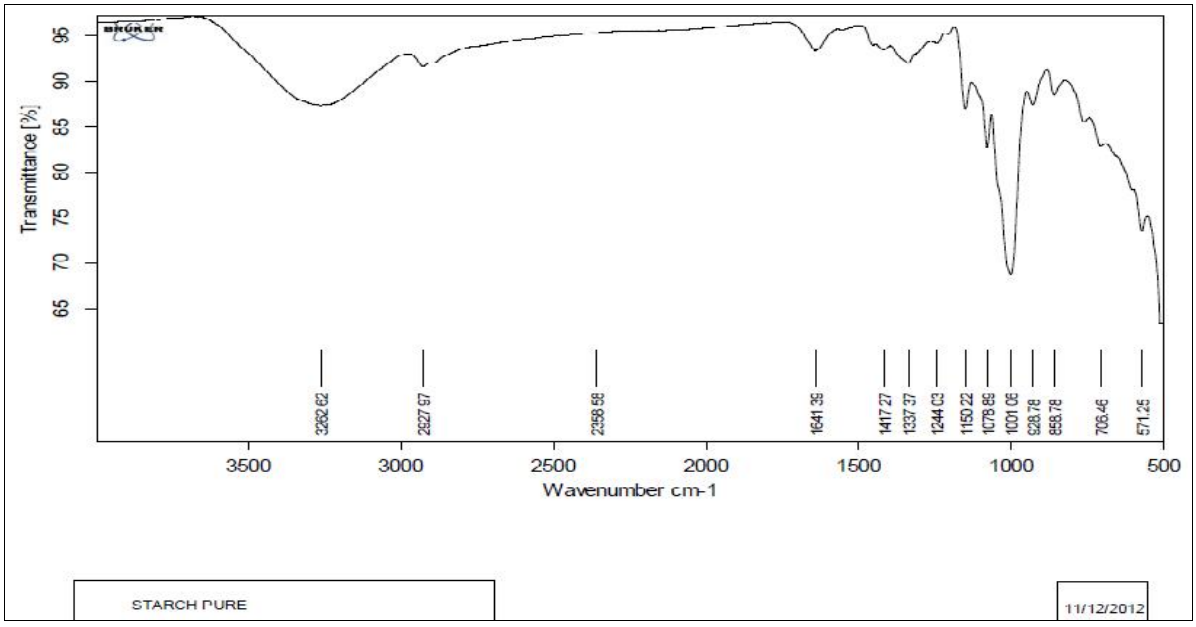


Fig.5 FTIR Spectrum of Tamarind Seed Polysaccharide

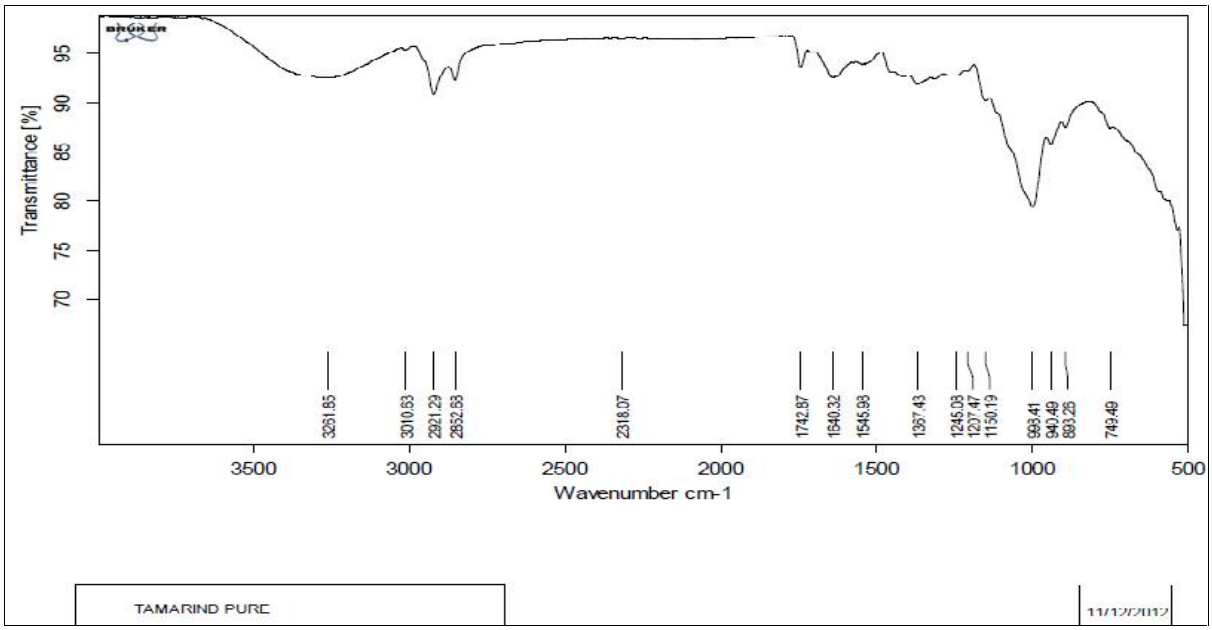


Fig.6 FTIR Spectrum of Moringa Polsaccharide

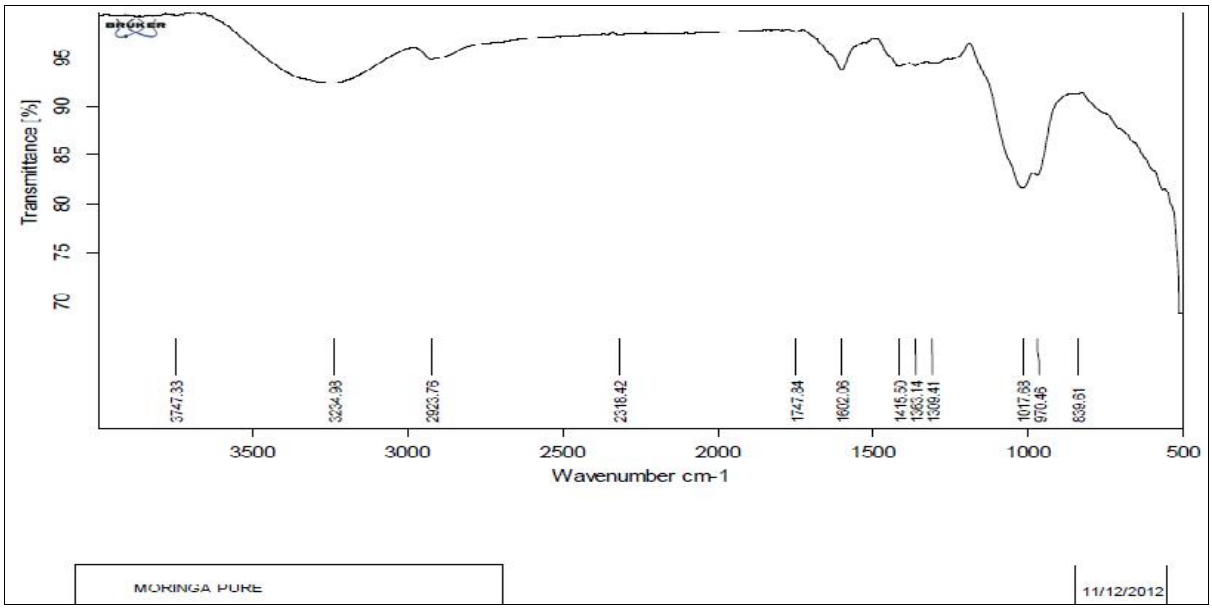


Table.5 Structural Assignment of Bora Rice Starch from FTIR data.

Wave number (cm^{-1})	Structural assignment (Bora rice starch)
3262.62	OH stretch
2927.97	CH stretch
1417.27	CH_2 bend
1337.37, 1244.03	OH plane bend
1150.22	Glycosidic C-O-C stretch
1001.06	Coupled CO stretch, , CC stretch & OH bend

Table.6 Structural Assignment of Tamarind Seed Polysaccharide from FTIR data.

Wave number (cm^{-1})	Structural assignment (Tamarind)
3261.85	OH stretch
2921.29, 2852.68	CH stretch
1367.43	CH_2 bend
1245.08, 1207.47	OH plane bend
1150.19	Glycosidic C-O-C stretch
998.41, 940.49, 893.26	CH bend

Table.7 Structural Assignment of Moringa Polysaccharide from FTIR data.

Wave number (cm^{-1})	Structural assignment (Moringa)
3234.98	OH stretch
2923.76	CH stretch
1415.50	CH_2 bend
1363.14	OH plane bend
1309.41	Glycosidic C-O-C stretch

CONCLUSION:

The polysaccharides were isolated and characterized in terms of various physicochemical parameters and powder properties. From the preformulation characterization it was observed that they possess considerable viscosity and good mucoadhesion property. The mucoadhesion was found to be higher for bora rice starch compared to other two polysaccharides whereas the viscosity was found to be higher in drumstick polysaccharide. The other properties like pH, solubility, flow property, bulk density etc. also found to be satisfactory. The kawakita analysis showed that polysaccharides exhibited good compactability, cohesiveness and should produce better flow properties in their granular form. It may be concluded that these polysaccharides can be chosen for fabrication of mucoadhesive drug delivery as carrier materials. They may be utilized for the colon targeting, mucoadhesive nanoparticulate drug delivery, nasal drug delivery etc for better efficacy of drug. The properties also make them suitable for use in oral solid dosage formulation for sustained drug delivery action.

REFERENCES:

1. Park K., Park H., (1990) Test methods of bioadhesion. In V. Lenaerts & R. Gurny (Eds), Bioadehsive drugs delivery systems. CRC Press Inc, Boca Raton, FL. 43-64.
2. Park K., Cooper S.L., Robinson J.R., (1987) Bioadhesive hydrogels. In N.A. Peppas (Ed), Hydrogels in Medicine and Pharmacy, CRC Press Inc, Boca Raton, FL. 3(8)
3. Yang L., Zhang L., (2009) Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. Carbohydr. Polym. 76(3) 349-361.
4. Bhattacharya A., Sachan N., (2006) Evaluation of Assam Bora rice starch as possible natural mucoadhesive polymer in formulation of microparticulate control drug delivery system. J Assam Sci Soc. 47 34-41.
5. Burgalassi S., Panichi L., Saettone M.F., Jacobsen J., Rassing M.R., (1996) Development and in vitro/ in vivo testing of mucoadhesive buccal patches releasing benzydamine and lidocaine. Int J Pharm, 133 1-7
6. Saettone M.F., Burgalassi S., Boldrini E., Bianchini P., Luciani G., (1997) International patent application PCT/IT97/00026
7. Ahmad M.Z., Bhattacharya A., (2009) Isolation and Physicochemical Characterization of Bora Rice Starch From Assam as Pharmaceutical Excipients, Journal of Pharmacy Research 2(8) 1299-1303.
8. Panda D., Si S., Swain S., Kanungo S.K., Gupta R., (2012) Preparation and Evaluation of Gels from Gum of Moringa oleifera, http://www.ijpsonline.com IP: 117.211.109.140.

9. Chandramouli Y., Firoz S., Vikram A., Padmaja C., Chakravarthi R.N., (2012) Design And Evaluation Of Controlled Relelese Matrix Tablets Of Acyclovir Sodium Using Tamarind Seed Polysaccharides, Journal Of Pharmaceutical Biology 2(2) 55-62.
10. James C.R., (1995) Analytical Chemistry of Foods. London: Blackie Academic and Professional 37 53-63.
11. BP 2009 pg-36
12. Chi S.C., Jun H.W., (1991) Release rate of Ketoprofen from Polaxomer Gels in a membrane less diffusion cell. J. Pharm Sci 80(3) 281-3.
13. Shaikh A.A., Pawar Y.D., Kumbhar S.T., (2011)Preformulation and rheological characterization of various polymers for mucoadhesive drug delivery. IJPRD 4(1) 1-9.
14. Narayana R.C., Harish N.M., Gulzar M.A., Prabhakara P., Singh A.K., Subrahmonium E.V.S., (2009) Formulation And In Vitro Evaluation Of In Situ Gels Containing Secnidazole For Vaginitis, Yakugaku Zasshi 129(5) 569-574.
15. Quality control methods for herbal materials, Updated edition of Quality control methods for medicinal plant materials, World Health Organization (2011).
16. Basu S., Bandyopadhyay A.K., Development and Characterization of Mucoadhesive in Situ Nasal Gel of Midazolam Prepared with Ficus carica Mucilage, AAPS Pharm SciTech 11(3) (2010).
17. Yamashiro M., Yuasa Y., Kawakita K., An experimental study on the relationship between compressibility, fluidity and cohesion of powder solids at small tapping numbers, Powder Technology, 34 (1983) 225-231.
18. Hary G. Brittain (Ed). Analytical profile of drug substances and excipient starch, 24, New York; Academic press; (2006) 523- 577.

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