ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
Nilani P. et al, /J. Pharm. Sci. & Res. Vol.2(3), 2010, 178-184

Formulation and Evaluation of Polysaccharide Based Biopolymer – an Ecofriendly Alternative for Synthetic Polymer.

Nilani.P*, Raveesha.P, B. Rahul Nandkumar Kasthuribai.N, Duraisamy.B, Dhamodaran.P,& Elango.K
Department of Pharmacognosy, JSS College of Pharmacy, Rocklands, Ootacamund, Tamilnadu.
Email: p.nilani@jsscpooty.org

Abstract:
The Main objective of the present study was to develop polysaccharide based biopolymer films with similar properties comparable to synthetic polymer films using waste materials of plant origin ,especially by the utilization of starch and pectin from plant source namely Mangifera indica (Mango kernel starch) and Cyphomandra betacea (Tree tomato pectin) . To overcome the problems due to usage of plastics, to conserve non-renewable resources like petroleum, natural gas and coal, to maintain ecological balance and to reduce pollution it is the need of the hour to develop ecofriendly biodegradable plastics that are made from renewable resources. An effort had been taken to develop a polysaccharide based polymer film and to study the filmogenicity and biodegradability of mango kernel starch-polyvinyl alcohol cross linked film. Mango kernel starch was isolated and evaluated for the physiochemical property and biodegradability of the prepared polysaccharide based film. The isolated starch showed a good physicochemical property and film forming property with polyvinyl alcohol and pectin. The developed polysaccharide based polymer film can be used as a substitute for synthetic polymer in pharmaceutical industry.
Keywords: Polysaccharide based polymer, Mango kernel starch, Tree tomato pectin, Biodegradation.

Introduction:

Mango (*Mangifera indica*) is an important fruit crop cultivated in tropical regions, belonging to Anarcaiaceae family. India is the largest producer of mangoes in the world. The kernel obtained after decortications of mango seeds can be utilized as a supplement to wheat flour or for extraction of edible oils[1]. Besides its use in animal feed, mango kernel flour can be utilized for edible purposes. After industrial processing of mango and extraction of oil from mango kernel, considerable amounts of mango kernels are discarded as waste, usually termed as total waste, has sufficient amount starch[3, 4]. Approximately 40-60% waste is generated during processing of mango, out of which peel and kernel constitute 12-15% and 15-20%, respectively[2].

Starches from different sources vary, particularly in their qualitative and quantitative make up, as well as in some of their physiochemical functional properties. Identification of native starch sources like mango kernel starch is required for desired functionality and unique properties.

Starch is a natural polymer. Starch can be processed directly into a bioplastic, but because it is soluble in water, articles made from starch will swell and deform when exposed to moisture, limiting its use. This problem can be overcome by modifying the starch into a different polymer. Microorganisms transform starch into lactic acid, a monomer. Finally, the lactic acid is chemically treated to cause the molecules of lactic acid to link up into long chains or polymers, which bond together to form a plastic called polylactide (PLA) [5]. If these kinds of plastics are reinforced with plant fibers such as sponge gourd fiber, jute or fiber from Musa stem, which are locally available, which will improve the mechanical strength to the bioplastics

Materials and Methods:

Materials:

The following raw materials were used to develop cross linked film. Starch isolated from kernel of Indian cultivar of mango (*Mangifera indica*) i.e. Totapuri, Badam, Langra and Kuppi collected from local market, Ooty, Tamil Nadu, India. Pectin isolated from fruits of Tree tomato (Cyphomandra betacea) collected from

Herbal Garden in Coonoor, Polyvinyl Alcohol(S D fine chem. Ltd) and Gelatin(S D Fine chem. Ltd).

Isolation of Starch:

Mangoes were washed and peeled, the seeds were separated from pulp using pulper and seeds were washed to remove any traces of adhering pulp. The seeds were dried at sunlight for 2 days; kernels of each cultivar were removed from seeds after breaking them open. Kernels were cut into small pieces and dried in oven at 40°C for 10 hours .The dried kernels are made into powder by using pulverizer. The mango kernel starch powder is mixed with distilled water (1:5). Stirred well and filtered through muslin cloth. The residue was washed thoroughly with distilled water. The slurry was allowed to stand for 1hour. Supernatant liquid was removed by suction and settled layer was resuspended in distilled water and centrifuged at 2800 rpm for 5 min. Upper non white layer was scrapped off. The white layer is resuspended in distilled water and recentirfuged 3-4 times. The starch was collected and dried at in a vacuum oven at 50°C/100 mmHg for 6-7 hours.

Identification of isolated starch:

The quantitative microscopical evaluation was performed using Motic image system microscope. Fig -1 (A).

I.Physical properties of Isolated Mango kernel starch

Moisture content[9]:

Moisture content of the isolated starch was determined by using Sartorius Electronic Moisture Balance. Table-1

Total ash[9]:

2g of air dried starch was accurately weighed, in previously ignited and tarred silica crucible. The material was spread in an even layer and ignited by gradually increasing the temperature to 500-600°C. Cooled in desiccator and weighed. Percentage total ash was calculated. Table-1

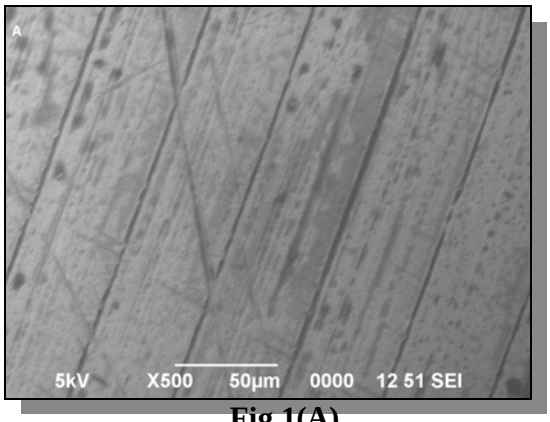

Fig 1(A)

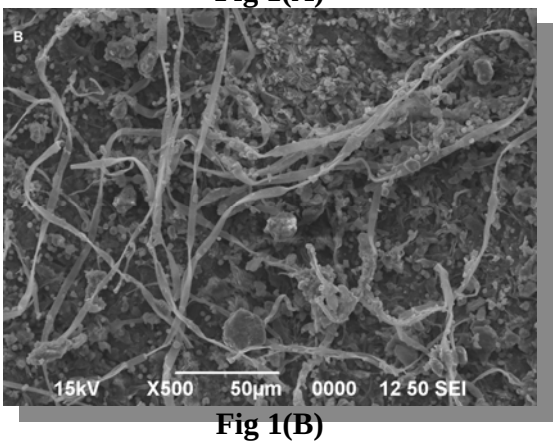

Fig 1(B)

Fig 1 (A) & 8(B). Scanning electron microscope (SEM) image of Mango kernel starch based polymer film.
(A) Before biodegradation, (B) After 100 days of biodegradation.

Particle size and shape[8]:

The starch grains were suspended in iodine water for 4-5 min and then mounted on the microslide, observed under system microscope-Motic images plus, the size and shape of the grains were determined. Table-1

Swelling Index[9]:

1g starch was introduced in to 25-ml glass-stoppered measuring cylinder (Internal diameter-16mm, Length of graduated portion 125 mm, marked in 0.2 ml divisions). 25 ml water was added and the mixture was shaken thoroughly every 10

min for 1 hour and allowed to stand for 3 hours at room temperature.

Table 1: Particle Size, Shape& Proximate Analysis

Studies	Particle Size (µm)	Particle Shape	Total ash	Foaming index	% Moisture content
Isolated Mango Kernel Starch	52.4	Elliptical	98.19	**Less than 100 (0.63)**	**11.67**

Table 2: Gelatinization Temperature

Temperature(ºC)	Observations
40	No change
50	Some starch grains were swelled.
60	Some starch grains were swelled.
70	Some starch grains were swelled and some bursting occurred.
80	**Gel Formation**

Table 3: pH, Viscosity, Bloom Strength and Total Bacterial Count

Physicochemical Parameters	Starch Based Polymer Film Solution	Gelatin Soft Capsule Grade	Gelatin Hard Capsule Grade
pH	3.9	3.5 – 7	3.5 – 7
Viscosity (mps)	41.55	34 – 45	40 – 48
Bloom strength (gm)	65	150 - 170	210 – 255
Total bacterial count (per gm)	60	NMT 1000	NMT 1000
E. coli, Salmonella, Liquefiers, Clostridium (per gm)	ABSENT	ABSENT	ABSENT

The volume in ml occupied by the starch was measured. The mean value was determined.

Foaming Index[9]**:**

1g of coarsely powder starch was transferred to a 500ml conical flask containing 100 ml of boiling water and continued to boil for 30 min. The solution was cooled and filtered into 100ml volumetric flask and the volume was made up to 100ml with distilled water. Flask was shaken for 15 seconds and allowed to stand for 15 min and the foaming index was calculated. Table-1

Gelatinization temperature:

1g of starch was heated with 5ml of water at different temperature ranges from 40-50ºC, 50-60ºC, 60-70ºC, 70-80ºC, 80-90ºC and the above samples were observed under Motic Image System Microscope to observe swelling ,bursting and gel formation property starch. Table-2.

II.Physical evaluation of starch based biopolymer film

Film formation property[11]**:**

Starch based biopolymer solution was prepared by dissolving 0.5 g PVOH in 100 ml (15.625 %) of 50˚C degassed distilled water and stirred for 30 min at 50±5ºc. About 0.2 g of pectin (6.25%) was added and stirred for 30 min at 50± 5ºc. Pectin was reported to increase film elasticity and reduced the viscosity of film forming solution. To this solution 3.5 g of starch (78.125%) was added and stirred at 75±5ºc until it completely gelatinized and a clear, viscous solution is obtained. Water was added continuously to compensate any evaporation during the heating process. The

Physical and Microbial evaluation of Starch based biopolymer solution[11, 12, 13]

pH:

The pH of the starch based film solution was determined by Systronics – pH meter pH system 361. Table-3

Viscosity:

A programmed heating and cooling cycle was used where the samples were heated from 50 to 95ºC at a heating rate of 6.5ºC/min, held at 95ºC for 4 min. Cooled to 50ºC at 7.5ºC/min, and then held at 50ºC for 1 min and the viscosity was determined by pipette viscometer. Table-3

Bloom strength:

The starch based biopolymer solution was kept at the temperature 10±1ºC for 17 hours and the bloom strength was determined by Stevens LFRA Texture Analyzer. Table-3.

Total bacterial count:

The total bacterial count of the starch based biopolymer solution was determined by using standard plate count method. The plates were prepared by using nutrient agar medium. The solution were applied to the plate and incubated at 37 ± 2ºC for 24 h. The developed colonies were counted by using Quebec colony counter. Table-3.

prepared films were evaluated by following parameters.

Uniformity of weight:

The biopolymer film was dried at 40 ^{0} C to 50 ^{0} C for 3 hours. And three different films of the individual batch with uniform size were selected randomly and the average weight was calculated. The individual weight should not deviate significantly from the average weight of the three. Table -4 (a).

Thickness measurement:

Film thickness was measured with Mitutoyo Digimatic Caliper (ID-C112, Mitutoyo Corp., Kawasaki, Kanagawa, Japan) at five

random positions on each film specimen to an accuracy of ± 1µm. Table -4 (a).

Melting point:

The melting point of the starch based polymer film was determined in open capillaries by using Veego VMP-1 apparatus. Table -4 (a)**.**

Table 5(a): Uniformity of Weight & Thickness Measurement

Studies	Thickness (mm)	Uniformity of weight (mg)	Melting Point (ºC)	Moisture Content (%)	Folding Endurance
Starch based Biopolymer film	0.15	24.23	117.3	11.68	335.6

Table 5 (b): Disintegration Test and Dissolving Test

Samples	Disintegration Time (Min)	Status	Dissolving Time (Min)		Status
			pH-(1.2)	pH-(7)	
Gelatin film	07.15	Completely Disintegration	09.48	07.50	Dissolved Completely
Polyvinyl alcohol film	0.4.00	Completely Disintegration	06.25	05.15	Dissolved Completely
Starch based polymer film	0.5.00	Complete Disintegration	08.00	06.00	Complete Disintegration

Moisture content:

Moisture content of the starch based film was determined by using Sartorius Electronic Moisture Balance. Table- 4(a)**.**

Folding endurance:

The folding endurance was measured manually for the prepared films. A strip of film (3×3 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance. Table -4 (a)**.**

Disintegration test[14]**:**

Disintegration test for gelatin and starch based biopolymer was done according to Indian Pharmacopoeia. The same size (1×1cm) was cut and the disintegration time was determined for both gelatin and mango kernel starch film at 37±2ºC. Table-5 (b)**,** Graph-1.

Dissolving test[14]**:**

The objective of the dissolving test is to find out whether samples are soluble in neutral or in slightly acidic solution, within 10 min in 50 ml of water or in 50 ml HCl solution at 37 ± 2ºC and pH 1.2. Table- 4(b)**.**

III.Biodegradation[15]

The mango kernel starch biopolymer film and a synthetic polymer film of Polyvinyl Alcohol were cut into similar sizes (1X1cm). The films were weighed separately and observed under system microscope-Motic image plus and by scanning electron micrography. The films were kept in the small cloth bags made up of muslin cloth, and again weighed. All the weights were recorded.

The bags were buried in the layers of soil; soil used was loamy, containing some extent of moisture. After every 10 days the weights and the microscopic images of the film were observed for 100 days. The weight differences and structural differences in the microscopic images were observed. Fig 1(A)&1(B).

IV.In vivo acute oral toxicity study[16]

The study was carried out according to the OECD guidelines 423.

Female albino mice of weight (25-30 g) were taken for the study and kept for overnight fasting. Next day, body weights were taken and the starch based polymer film administered orally at a dose of 2000mg/kg in distilled water. Then the animals were observed for mortality and morbidity at an interval of 0, ½, 1, 2, 4, 6, 8, 12 and 24 h. Feed was given to the animals after 4 h of the dosing and the body weight was checked at 6 h after dosing. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. The animals were observed twice daily for 14 days and body weights were taken. The same experiment will be repeated once again on 3 mice (preferably females). IAEC project proposal No.: JSSCP/IAEC/M. Pharm/Ph.cog/06/2008-2009.

Results and Disscussion:

The starch isolated from the kernels of *Mangifera indica* L. were subjected for the physicochemical evaluation. The mango kernel starch granules around from 20.4 to 28.6 µm in width and from 58.3 to 65.4 µm in length.

Starch based polymer solution was prepared to obtain a clear, viscous solution, and casted on a glass plate (9x15 cm), and it showed better film forming property and flexibility.

The polymer solution was tested for pH, bloom, viscosity and total bacterial count and compared with the standards for gelatin used for the production of both soft capsule and hard capsule. The results were compatible with gelatin but the bloom strength of the gel needs improvement.

The formulated film was found to be 24.233 mg at thickness of 0.15 mm and moisture content on an average of 11.69%.

Disintegration and Dissolution test for the starch based polymer film was performed and the results were comparable with gelatin film. The results compiled with the monographic standard.

Biodegradation studies for prepared starch based biopolymer and Polyvinyl Alcohol (PVOH) film were carried out in loamy, moist soil for 100 days and the physical changes and reduction in weights in both the films were observed in every 5 days. The starch based biopolymer showed rapid weight reduction when compared with the PVOH film.

The in vivo acute oral toxicity study for the prepared starch based biopolymer film showed no toxicity or observable changes in any mice when observations twice daily for 14 days.

Conclusion:

Various natural polymers have been investigated for their application in Pharmaceutical Industries. The present communication is an investigation of the physicochemical characteristics, film forming property and biodegradation of the polysaccharide based biopolymer obtained

from natural origin and to compare the characteristics with the synthetic polymer.

The present study helps and encourages the environmentalist, Industrialists and botanists in developing biodegradable polymers from polysaccharides of plant origin. Biopolymers have several economic and environmental advantages. The major advantage is that it can be composted. The environmental benefits of biodegradable polymers must be reflected in cost advantages, if large scale applications are to become feasible. The future work based on this research will reveal the functional advantages of biodegradable polymer rather than its composition and ability to compost.

The physiochemical and filmogenecity property of the mango kernel starch encourages the selection of mango kernel starch as a suitable candidate for the formulation of natural polymer.

It can be concluded that polysaccharide based biopolymer was found to be biodegradable, nontoxic and also showed good film forming property. Hence it may prove as a suitable alternative or a substitute for conventional plastics and traditionally used gelatin capsule materials and can also be suitable as a drug carrier for sustained release dosage forms with suitable modification.

Reference:

[1] Arogba, S. S. (1997). Physical, chemical and functional properties of Nigerian mango (*Mangifera indica*) kernel and its processed flour. *Journal of the Science of Food and Agriculture*, 73, 321-328.

[2] Augustin, M. A., & Ling, E. T. (1987), composition of mango seed kernel. *Pertanika*, 10, 53-59.

[3] Bahaa El-Din, H. M. (1979). Studies on possible industrial uses of mango seed. M.Sc. thesis, Faculty of Agriculture, Al-Azhar University, Egypt.

[4] El-Soukkary, F. A. H., El-Sahn, M. A., & Mohamad, H. M. A., (2000). Physico-chemical and nutritional evaluation of mango seed kernel and its utilization for pan bread supplementation. *Zagazig Journal of Agriculture and Research*, 27, 1319-1342.

[5] Heller J. Controlled release of biologically active compounds from bioerodible polymers. Biomaterials 1980; 1: 51 -57.

[6] Averous L. Biodegradable multiphase systems based on plasticized starch: a review. J. Macromol. Sci-Part C. Polymers Reviews 2004, 3,231-274.

[7] S. Nazaneen parveen, Biodegradable plastics-ecofriendly plastics, Agrobios newsletter, VII(4), sep. 2008, 23-24.

[8] T.E. Wallis, Pharmacognosy Text Book, 5th edition, 13-16.

[9] WHO, Quality control methods for medicinal plants materials, Geneva, 2002, 45-46.

[10] Leon Lachman, Heartbeat. A, Liberman, Joseph.L.Kanig. The theory and practice of industrial pharmacy, II ed, 2000:599.

[11] Liang Chen, Syed H. Imam, Sherald H. Gordon and Richard V, Greene, Starch- Polyvinyl Alcohol Crosslinked Film-Performance and Biodegradation, Journal of Environmental Polymer Degradation, vol. 5, No.2, 1997, 111-117.

[12] American Federal Standard No. 285A. Capsules (For medicinal purposes),Washington DC 20407 General Services Administration, 1976.

[13] B.E.Jones, K. Ridgway, Hard Capsules; Development and Technology, 2nd ed. 1997, 61-67.

[14] Indian Pharmacopoeia, 1996, Volume- II, A 80-A 84.

[15] J Aruthchelvi, M suhakar, Ambika Arkatkar, Mukesh Doble, Sumit Bhaduri.Biodegradation of polyethylene and polypropylene.

[16] OECD guidelines for testing of chemicals, OECD/OCDE No. 423; 2001.