Development and characterisation of metformin loaded spray dried *Bora* rice microspheres

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Abstract: *Bora* rice, a glutinous rice, is grown in Assam (a north eastern state of India) and is used traditionally for various purposes. The rationale of this study was to prepare and to assess Metformin loaded mucoadhesive spray dried microspheres using locally grown *Bora* rice powder.
Metformin loaded microspheres were prepared using *Bora* rice and sodium alginate by spray drying method. For the study of the consequence of parameters of spray drier on the properties of microspheres, parameters such as aspirator flow rate, temperature, feed flow rate and concentration of the spray solution were changed. The in-vitro release properties were also studied.
Almost spherical microspheres were obtained with significant swelling and mucoadhesivity. Dissolution study was carried out in phosphate buffer (pH 7.4) for 7 hrs. It was also noted to possess good mucoadhesive in such a way that about 90% of microspheres remained adherent on the surface of intestinal mucosa of pig skin. The total amount of drug released from microspheres after 7 hr. was 80%. The release of drug was not affected by the changes in parameters but was affected when sodium alginate concentration was changed. It was observed that microsphere properties changed as the parameters were changed. Smaller particles were obtained when the concentration of the spray solution, aspirator flow rate, the temperature difference between inlet and outlet and feed flow rate were lower.

Keywords: *Bora* rice, microsphere, spray drying, aspirator flow rate, mucoadhesive.

INTRODUCTION

Microsphere carrier systems made from the naturally occurring biodegradable polymers have delineated significant attention for many years in sustained drug delivery (Burges and Hickey, 1995; Woo *et al*., 2006; Capan *et al*., 2003; Gohel and Amin, 1998). However, due to their short residence time at the site of absorption the success of these microspheres is limited (Sajadi *et al*., 2003). This can be accomplished by combining mucoadhesive characteristics to the microspheres. The rate of absorption and bioavailabity is increased due to high surface to volume ratio. The intimate contact with the mucus layer and specific targeting of drugs to the site of absorption is also possible (Patel and Patel, 2005).

Sodium alginate is non-toxic, biocompatible, bypass the hepatic metabolism and can deliver the drugs to the specified site. It also helps in lowering significantly of blood glucose, insulin and C-peptide in human and non-insulin dependent Diabetes. It has been used in food, beverage and pharmaceutical industry (Gacesa, 1988). It was used in this study along with *Bora* rice powders for preparation of microsphere to have better physico-chemical and release properties.

Bora rice, a glutinous rice variety, cultivated in Assam and used traditionally for various purposes. It is not used as an ingredient of regular meal but used occasionally as a part of traditional and cultural practices. It is also used for the preparation of traditional beer.

For this study, an anti-diabetic drug Metformin hydrochloride was chosen to be microencapsulated following solvent evaporation technique by spray drier (Williams *et al*., 2002), using an intermingle of sodium alginate and *Bora* rice powder.

MATERIALS AND METHODS

Materials

Metformin Hydrochloride was a gift sample from Ozone Pharmaceutical Ltd., Assam, India. *Bora* rice was procured locally. The grains were powdered in dry state and passed through sieve no. 120. It was used as such without further treatment.

Sodium alginate (5.5±2 cps) and potassium dihydrogen phosphate (Loba Chemie Pvt. Ltd., India); Sodium hydroxide and hydrochloric acid (Rankem Pvt. Ltd, India) were commercially available. All chemicals were of analytical grade and were used as such without further testing and purification.

Methods

*Preparation of microsphere***:** Metformin loaded microspheres were prepared by aqueous solvent evaporation method. Aqueous dispersion of rice powder

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(1%) was heated at 75°C for one hr. Sodium alginate solution (0.5 %) was prepared by dissolving in hot water. The above two were mixed and drug was added to this mixture and stirred to dissolve it. The mixture was sonicated for 10 minutes. Then the mixture was spray dried using Lab spray dryer (LU-222 Advanced Labultima, India) with 0.7mm nozzle. Parameters of spray dryer were changed and products were collected separately in each run.

*Yield***:** The prepared microspheres were collect and weigh. The measured weight was dividing by the total solid content of feed and the percentage yield was intended as follows:

$$\% \text{ yield} = \frac{\text{Actual weight of product}}{\text{Total solid content in feed}} \times 100$$

*Determination of particle size***:** The prepared microspheres were mounted in light liquid paraffin and the diameters of 100 particles were measured by means of optical microscope fitted with a calibrated ocular micrometer. The mean diameter was calculated with the following relationship (Vehring, 2008).

$$\text{Mean particle size} = \sum nd / \sum n$$

*Swelling ratio***:** The swelling ratio of microspheres was studied in aqueous swelling media i.e. Phosphate buffer (pH 7.4), 0.1M HCl and water at 37±0.5°C. Following expression was used to calculate the swelling ratio (S_{wt})

$$S_{wt} = [(W_t - W_0)/ W_0] \times 100$$

Where, W_t and W_0 express the weight of sample swollen at time't' and weight of the original sample respectively.

*Evaluation of mucoadhesive property***:** The mucoadhesive property of microsphere was evaluated by an *in vitro* adhesion testing method known as wash off test method. Freshly excised pieces of pig intestinal mucosa (2×2cm) were mounted on to glass slides with cotton thread. About 80 microspheres were spread on to each prepared glass slide and immediately thereafter the slides were hung to USP tablet disintegration test apparatus (Tab. Machines, Mumbai), when the test apparatus was operated, the sample was subjected to slow up and down movement in the test fluid at 37°C contained in a 1 L vessel of the apparatus. At an interval of 60 min. up to 7 hrs, the machine was stopped and the numbers of microspheres still adhering to mucosal surface were counted. The test was carried out in phosphate buffer (pH 7.4). (Badri *et al*., 1999; EI-Gibaly, 2002; Lehr *et al*., 1990; Lehr *et al*., 1997).

Drug Entrapment Efficiency (DEE): Microspheres equivalent to 50 mg of the drug were taken and the amount of drug entrapped was estimated by dispersing the crushed microspheres in 100 ml of phosphate buffer (pH 7.4) by shaking on a mechanical shaker (Remi Motors, Mumbai, India) for 12 hr. Then the solution was filtered and diluted 25 times with phosphate buffer (pH 7.4) and the absorbance was measured Spectrophotometrically (UV-1700, Shimadzu, Japan) at 233nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula (Uchida *et al*., 1997).

$$\text{DEE} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

*In vitro drug release study***:** The release of drug from the microspheres was studied in phosphate buffer (pH 7.4) as medium, using USP XXVI dissolution test apparatus paddle type (Campbell Electronics, India) at 37±0.5°C with a rotating speed of 100 rpm. A sample of microspheres equivalent to 50mg of Metformin hydrochloride was used in each test. 10 ml of the sample from each was withdrawn at a time interval of 1hr for 7 hrs. The withdrawn samples were diluted to 100 times with distilled water and the absorbance of all the samples were measured at 233nm using the UV-Visible Spectrophotometer (UV-1700, Shimadzu, Japan) (Henriksen *et al*., 1996; Yuen *et al*., 1999).

Differential Scanning Calorimetry (DSC): The DSC thermograms of the pure drug, drug loaded microspheres, blank microspheres, sodium alginate and rice powder were obtained using the Perkin Elmer Jade DSC system under the condition of gas flow (N_2 purge gas 20ml.min^{-1}) and heat flow (10°C.min^{-1}) to identify any interaction between the components of formulation.

*FT-IR spectroscopy***:** FT-IR spectra of pure model drug, Polymer (Sodium alginate and *Bora* rice powder), blank microspheres and drug loaded microspheres were obtained in KBr Pellets at moderate scanning speed between 4000-200cm^{-1} in a Perkin-Elmer FT-IR Spectroscope.

Scanning Electron Microscopy (SEM): The surface morphologies of the microsphere and microspheres collected after dissolution study were investigated by using (S-3600N, HITACHI) Scanning Electron Microscope at 8KV and 20KV. Prior to examination samples were gold coated under vacuum (B10 RAD microscience division SC 502) to render them electrically conductive.

RESULTS

Particle size and yield: The composition and various processing parameters are presented in table 1 and the effects are presented in table 2.

Drug Entrapment Efficiency: The entrapment efficiency of drug in different formulations is summarized in the table 2. Metformin hydrochloride is soluble in water and had a tendency to diffuse out to the aqueous medium from the alginate coated microsphere.

Mucoadhesion testing: The adhesion of microspheres to the intestinal mucosa of pig was evaluated as the mean percent of microspheres remain adhered after a defined period of washing. The results are presented as table 3.

Table 1: Composition of the formulation with different processing parameters

Formulations / Parameters	F1	F2	F3	F4	F5	F6	F7
DRUG(mg)	800	600	------	500	600	-------	600
Bora rice powder(mg)	550	600	800	400	300	900	600
Sodium alginate(mg)	400	400	500	300	350	800	600
Inlet temp.(°C)	180	175	185	175	190	195	190
Outlet temp.(°C)	120	110	110	120	140	115	120
Feed flow rate (ml/min)	1.5	1.6	2	1.4	1.2	2.5	1.8
Aspirator flow rate (Nm^3/ml)	24	25	27	23	22	28	26
Nozzle size(mm)	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Pump speed(rpm)	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Water(ml)	200	200	200	200	200	200	200

Table 2: Particle size, yield and Entrapment efficiency of differenent formulations

Formulation Code	Mean particles size (µm) Mean ± SD	Mean Drug Entrapment Efficiency (%) ± SD	Yield of products (%)
F1	90±12.70	96.667±1.10	59
F2	101±10.95	96.815±1.05	57
F3	112±11.44	96.915±1.08	52
F4	80±13.86	96.425±1.48	61
F5	67±15.38	96.325±0.41	62
F6	130±10.57	97.11±1.02	49
F7	110±12.50	96.865±1.30	54

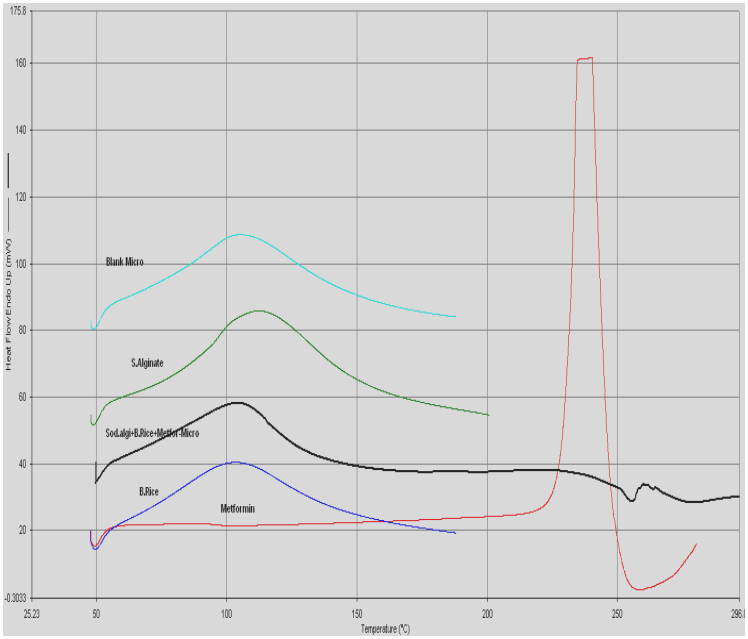

Fig. 1: Comparative DSC thermograms of *Bora* rice powder, sodium alginate, drug loaded microsphere, microsphere without drug and model drug (metformin hydrochloride)

Table 3: Results of mucoadhesive studies (wash off test) of microsphere

S. No.	Time (hr)	% of microspheres remain adhered
01	01	79.41
02	02	79.59
03	03	87.17
04	04	78.46
05	05	40.74
06	06	96.07
07	07	81.25

Swelling behaviour: The swelling behaviour of microsphere was determined gravimetrically and presented in table 4.

*DSC analysis***:** DSC thermograms of metformin, sodium alginate, and *Bora* rice powder, a mixture of alginate and *Bora* rice powder and a combination of metformin, alginate, *Bora* rice powder and are presented in fig. 1. Under the experimental condition, the DSC peak was observed for alginate, *Bora* rice powder, metformin and blank microsphere.

FT-IR spectroscopy: The FT-IR was used to categorize the possibility of any interaction between the formulation components. There was no significant variation in the FT-IR spectra of the drug and the drug loaded microsphere when compared to the spectra of individual components. The uniqueness N-H stretching band of the drug remained unchanged in case of the drug loaded microsphere. Also the peak corresponding to C-N vibration, C=N stretching, N=N stretching and C-H (alkane) stretching were retained in the FT-IR spectrum of the drug loaded microsphere reflecting the identity features of the metformin hydrochloride (figs. 3-6).

Scanning Electron Microscopic studies: SEM analysis of the sample revealed that all microspheres prepared were spherical in shape with a few elongated particles as exception.

In vitro drug release study: The drug-polymer ratio was found to affect the drug release characteristics of the prepared spray dried microspheres. At higher drug-

Table 4: Swelling behaviour of different formulations

Formula-tion Code	Sorption media	Weight of microspheres before sorption (mg)	Weight of microspheres after sorption (mg)	% of water sorption
F1	0.1N HCl, Water, Phosphate buffer (pH7.4)	50,50,50	61,73,85	22,46,70
F2	0.1N HCl, Water, Phosphate buffer (pH 7.4)	50,50,50	63,76,87	26,52,74
F3	0.1N HCl, Water, Phosphate buffer (pH 7.4)	50,50 ,50	70,84,92	40,68,84
F4	0.1N HCl, Water, Phosphate buffer (pH 7.4)	50,50,50	60,71,84	20,42,68
F5	0.1N HCl, Water, Phosphate buffer (pH 7.4)	50,50,50	58,69,81	16, 38,62
F6	0.1N HCl, Water, Phosphate buffer (pH 7.4)	50,50, 50	74,85,94	48,70,88
F7	0.1N HCl, Water, Phosphate buffer (pH 7.4)	50, 50,50	68,81,91	36,62,82

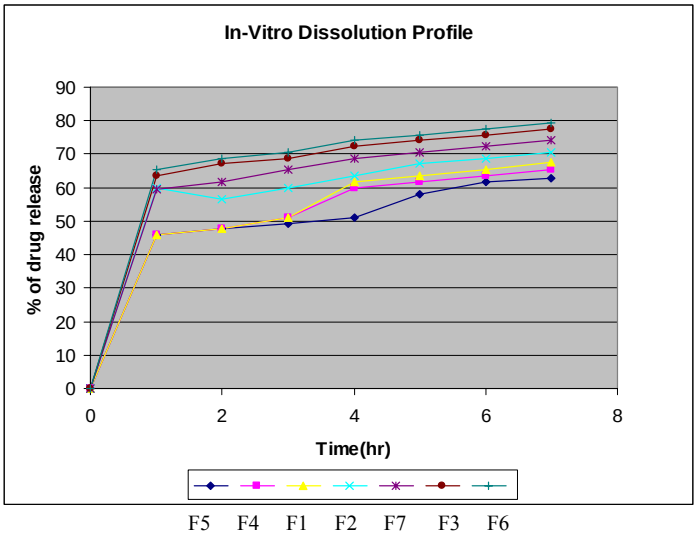

Fig. 2: Effect of drug polymer concentration on drug release

polymer ratio the drug released from the microsphere was faster as compared to lower drug-polymer ratio (fig. 2). This may be due to increase in the ratio of drug-polymer.

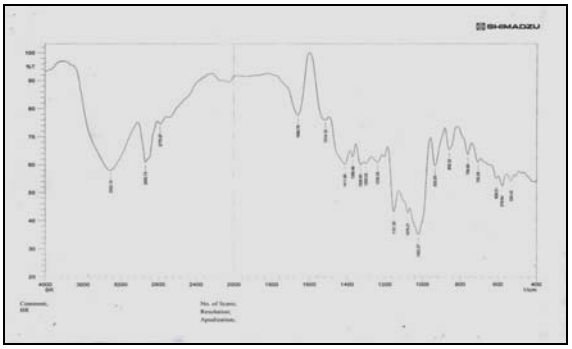

Fig. 3: FT-IR spectra of *Bora* rice powder.

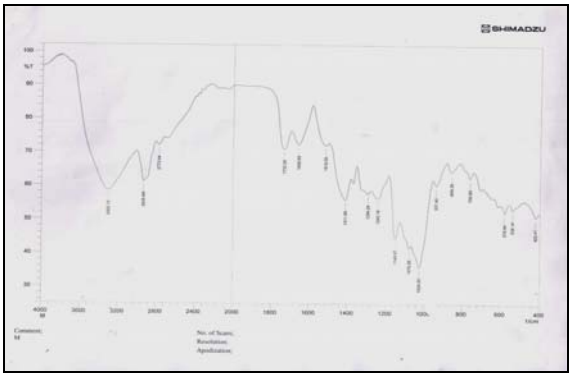

Fig. 4: FT-IR spectra of Metformin hydrochloride.

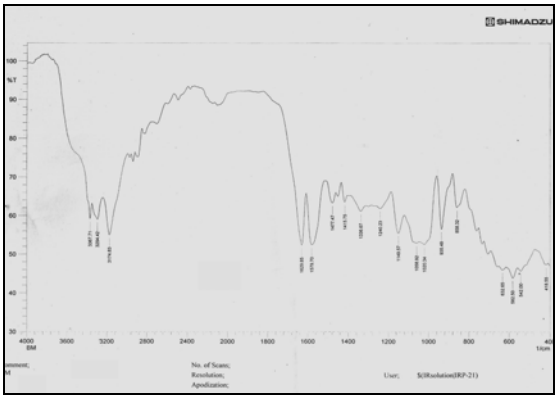

Fig. 5: FT-IR spectra of the physical mixture of *Bora* rice and Metformin hydrochloride.

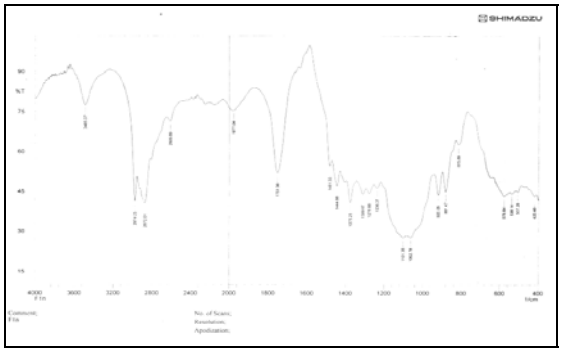

Fig. 6: FT-IR spectra of Metformin hydrochloride loaded microspheres.

DISCUSSION

The results obtained from the study on various parameters revealed that the increase in the aspirator flow rate caused an increase in the particle size due to the shorter residence time of liquids in the drying chamber. The entrapment efficiency was found to be increased at increased concentration of alginate and *Bora* rice powder as the viscosity of resulting gel was increased. The results of Mucoadhesion testing indicated that the polymer to drug ratio had significant effect on the mucoadhesive property. Greater the concentration of the polymer associated with rice powder–alginate matrix, greater was the adhesion. However, increased drug load had no such effect on mucoadhesive property. The swelling characteristics of microsphere were low in 0.1M HCl, medium in water and higher in phosphate buffer (pH 7.4). The amount of polymer also affected the swelling characteristics of microspheres. When the polymer concentration was increased the swelling ratio was also proportionately increased. The higher percentage of alginate and rice powder in microspheres rendered high swelling and gel formation. So the inclusion of rice powder in alginate gel opens an option for the manufacture of cross linked matrix device for gastro-intestinal delivery. Disappearance of the peak of the drug in DSC study indicated that the drug was molecularly dispersed inside the matrix of alginate. The FT-IR spectroscopy study indicated that there was no chemical interaction between the drug and the polymer backbone and the drug entrapment and modulation of release. The drug release study revealed that amount of drug released per unit area of exposed surface of the polymer matrix was higher. The continuation of the release rate of drug from the microsphere with increase of sodium alginate and rice powder concentration reflected the simultaneous increase in gel strength, which was a determining factor. The release of drugs by diffusion through the pores of polymer matrices can be altered by reducing the pore size by increasing the polymer concentration.

CONCLUSION

The result of this work demonstrated that the parameters selected for the preparation of microsphere were adequate and did not elicit any change in the physico-chemical stabilities of the microparticles. Drug distribution into the microparticles was found to be homogenous with a steady molecular dispersion in the polymer matrices of microparticles. This result was supported by DSC analysis. It was observed that microsphere properties changed as the parameters were changed. Smaller particles were obtained when the concentration of the spray suspension was low. The release of drug was not affected by the changes in parameters but was affected when sodium alginate and rice powder concentration were changed.

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