Acta Pharm. **63** (2013) 209–222
DOI: 10.2478/acph-2013-0019

Original research paper

Formulation and *in vitro* evaluation of metformin hydrochloride loaded microspheres prepared with polysaccharide extracted from natural sources

HEMANTA KUMAR SHARMA*
SUNITA LAHKAR
LILA KANTA NATH

Department of Pharmaceutical Sciences
Dibrugarh University
Dibrugarh-786004, Assam, India

The present work envisages utilisation of biodegradable and biocompatible material from natural sources for the development of controlled release microspheres of metformin hydrochloride (MetH). Natural polysaccharides extracted from *Dillenia indica* L. (DI), *Abelmoschus esculentus* L. (AE) and *Bora* rice flour were used in fabricating controlled release microspheres. The microspheres were prepared by the emulsion solvent diffusion technique with different proportions of natural materials and were studied for entrapment efficiency, particle size, particle shape, surface morphology, drug excipient compatibility, mucoadhesivity and *in vitro* release properties. The prepared microspheres showed mucoadhesive properties and controlled release of metformin hydrochloride. The study has revealed that natural materials can be used for formulation of controlled release microspheres and will provide ample opportunities for further study.

Keywords: metformin hydrochloride, *Dillenia indica*, *Abelmoschus esculentus*, *Bora* rice, emulsion solvent diffusion technique, mucoadhesive, microspheres

Accepted January 17, 2013

Metformin hydrochloride is used in the treatment of noninsulin-dependent diabetes mellitus. Its biological half life is 2.5 to 3 hours. Due to its short biological half life and narrow absorption window, it is incompletely absorbed in the small intestine. It is a strong base (pK_a = 11.5), protonated depending on the physiological pH of the human body. It is also poorly absorbed in the colon. However, reduction of gastrointestinal motility enhances the drug absorption. Most of the drug is excreted unchanged in urine while accumulation in the body causes toxicity. Bioavailability is 50 to 60 % due to its site specific absorption in the body (1). Conventional oral dosage forms are the preferable route of administration because of its reasonable cost, ease of administration and patient compliance (2). Its disadvantages are that it delivers the drug to systemic circulation without

* Correspondence; e-mail: hemantasharma123@yahoo.co.in

controlling its release behaviour, short gastric residence time as well as fluctuation in the plasma drug concentration (3). Moreover, most of the drug is released in the colon and metformin hydrochloride has poor absorption in the colon. Sustained release formulation is modulated to reduce the gastric motility time, increase the gastric residence time and thus the bioavailability of the drug (4, 5). The release rates of microspheres are dependent on many factors. The polymers used and the processing conditions during microsphere preparation determine the properties of microspheres formed and also influence the distribution and release behaviour of the drug. Hence, polymers that are biocompatible, biodegradable, easily available, having good mucoadhesive properties were used in the study. Plant sources provide the largest amount of polysaccharides and therefore plant sources were selected for extraction of polysaccharides. Plants and their parts used in this study were fruits of *Dillenia indica* L. (*Dilleniaceae*) (DI), fruits of *Abelmoschus esculentus* (L.) Moench (*Malvaceae*) (AE) and *Bora* rice flour (BRF). Natural polysaccharides extracted from DI, AE and *Bora* rice flour were used to formulate microspheres. Fruits of DI, AE are used as vegetables and *Bora* rice as grains or as flour is an occasional (traditional) food of the people of Assam.

High amylopectin content and traces of amylose in *Bora* rice (6) make it a suitable polymer and binding agent in designing a sustained release drug delivery system. Compressible behaviour of rice flour has been reported (7).

Extracts of DI and AE contain mainly pectous polysaccharides (8–10), which can be extracted by precipitation with acetone. Natural mucoadhesive substances considerably swell in water and form a gelatinous mass. These gelling and mucoadhesive properties of the DI extract were first reported (11) for use in fabricating nasal gel. Pectin is used in food industry as a thickening and gelling agent (8). Physicochemical properties of AE pectin were reported by Sengkhamparn *et al.* (12) and the rheological behaviour of AE mucilage was reported by Meister *et al.* (13). Pectin is a very promising biopolymer to construct drug carriers for controlled drug delivery because of its gelling, film forming, binding properties, biocompatibility and stability towards acidic media, and non-toxicity. It would therefore be advantageous to have means of providing an intimate contact of the drug delivery system with absorbing membranes.

EXPERIMENTAL

Materials

Metformin hydrochloride was received as a gift sample from Ozone Pharmaceutical Ltd., India. *Bora* rice, DI and AE were procured from the local villages around Dibrugarh University, Assam. All other chemicals used in the study were of analytical grade and were procured commercially. They were used without testing and purification.

Extraction of polysaccharides from Dillenia indica *and* Abelmoschus esculentus

Extraction of polysaccharides was carried out by the methods described by Baveja *et al*. (14) and Wahi *et al*. (15). Extraction of natural polysaccharides was carried out using water as the extracting medium. The sample to menstrum ratio was 1:1.5 and the extraction temperature was 60–70 °C, maintained for about 5–6 hours.

Physicochemical characterization of the polysaccharides

Determination of various physiochemical characteristics of DI and AE extracts included pH, bulk density, solubility in various aqueous and non-aqueous solvents as well as the effect of various solvents on their swelling and adhesive properties (16).

Preparation of microspheres

Metformin hydrochloride loaded microspheres were prepared by the emulsion solvent diffusion method (16). Aqueous dispersion of mixed natural extracts of *Bora* rice, AE and DI in different ratios (Table I) was heated at 75 °C for one hour. Metformin hydrochloride (1 g) was dispersed to an aqueous dispersion. The oil phase was prepared by blending methanol (7 mL) and acetone (10 mL). To 15 mL of dichloromethane, 0.5 g of ethyl cellulose was added and then added to the blend of acetone and methanol. The oil phase was kept under mechanical stirring at 500 rpm. To the oil phase, the aqueous dispersion was added dropwise using a disposable syringe with a No. 22 needle in the 1:3 ratio to form a w/o emulsion. The emulsion obtained was allowed to stabilise for one hour, followed by heating at 30 to 40 °C, which temperature was maintained for 2 to 3 hours. The products with different proportions of extracts and flour were collected in batches by filtration and were dried.

Characterisation of microspheres

Particle size determination. – Formulated microspheres were mounted in light liquid paraffin, and the diameters of 100 particles were measured using an optical microscope provided with a standardized ocular and stage micrometer. The mean diameter was determined. Results of drug concentration and varying different concentrations of polymers ratios on the average particle sizes were evaluated (18).

Table I. Composition and formulation parameters of microspheres

Composition and formulation parameters	Formulations												
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Drug (g)	1	1	1	1	1	1	1	1	1	1	1	1	1
Bora rice flour (g)	1	2	1	1	3	1	1	2	1	3	2	3	1
Polysaccharide of *A. esculentus* (g)	1	1	2	1	1	3	1	1	2	1	3	2	3
Polysaccharide of *D. indica* (g)	1	1	1	2	1	1	3	3	3	2	1	1	2
Ethyl cellulose (g)	1	1	1	1	1	1	1	1	1	1	1	1	1
Methanol (mL)	7	7	7	7	7	7	7	7	7	7	7	7	7
Dichloromethane (mL)	10	10	10	10	10	10	10	10	10	10	10	10	10
Acetone (mL)	10	10	10	10	10	10	10	10	10	10	10	10	10

Drug entrapment efficiency. – Drug entrapment efficiencies of dried microspheres were approximated by mashing dried microspheres and extracting the drug into aqueous medium in phosphate buffer of pH 7.4 by dynamic shaking with a magnetic stirrer for 24 h; the drug content was then examined. Entrapment efficiency of microspheres was calculated.

Swelling index. – Swelling indices of different batches of microspheres were analysed by determining the percentage of water retained by the microspheres after 12 hours. About 25 mg of microspheres were placed on an electronic balance and weighed. The microspheres were then dispersed in 20 mL of distilled water, and also phosphate buffer of pH 7.4 at a temperature of 37 ± 1 °C. After 12 hours, the microspheres were taken out from their respective media, air dried and weighed. The swelling index was determined by percent water uptake.

Mucoadhesive test. – Mucoadhesive properties of the formulations prepared with the natural polysaccharides used were determined following the reported procedures (11, 19).

In vitro *wash-off test. – In vitro* wash-off test was carried out by the method described by Lehr *et al.* at pH 7.4 with freshly excised goat intestinal mucosa (20).

Scanning electron microscopy (SEM). – The shape and surface morphology of microspheres were examined using a Scanning Electron Microscope JSM-6360 (JEOL, Japan). The microspheres were previously coated with a thin layer of gold under vacuum so as to make them electrically conductive. Surface morphology of *Bora* rice powder, the DI mucilaginous substance and the AE mucilaginous substance, metformin hydrochloride loaded microspheres were examined by photomicrographs at an excitation voltage of 20 kV under different magnification.

FT-IR spectroscopy. – The FT-IR spectra of the supplied drug (metformin hydrochloride), powdered *Bora* rice, powdered AE and DI extract, ethyl cellulose, physical mixture of *Bora* rice and drug, physical mixture of DI and drug, physical mixture of AE and drug, physical mixture of drug, ethyl cellulose, *Bora* rice, DI, AE, blank microsphere, drug loaded microspheres were obtained in KBr pellets using a Shimadzu FT-IR 8400 infrared spectrophotometer (Shimadzu, Japan) in the mid IR region (wavenumber from 200 to 4000 cm^{-1}), to study the drug and polymer materials interaction.

X-ray powder diffraction study (XRPD). – The X-ray diffractograms of the drug, *Bora* rice powder, AE, DI, ethyl cellulose, physical mixture of ethyl cellulose, *Bora* rice, DI, AE, blank microspheres and drug containing microspheres were obtained by (X'Pert Pro, PAN analytical, The Netherlands) to detect any variation in the crystallinity of the drug in the formulation due to polymer so as to find out the possible drug polymer interaction.

Differential scanning calorimetry (DSC). – Possible drug and polymer interactions were detected in the DSC thermograms of pure drug, *Bora* rice, AE, DI, physical mixture of pure drug, *Bora* rice, AE, powdered DI, blank and drug loaded microspheres using a JADE DSC System (Perkin Elmer, UK) with the N_2 purge gas flow rate of 20 mL min^{-1} and heat flow rate at 10 °C min^{-1}.

In vitro *drug release study*

Release of metformin hydrochloride from the microspheres was studied in phosphate buffer pH 7.4 using an IP/BP/USPXXVI dissolution rate test apparatus of basket type (Campbell Electronics, India) at 37 ± 0.5 °C with the rotating speed of 100 rpm. The

study was carried out in intestinal pH because of the presence of pectous matter in the extract used in the formulation. Moreover, the polysaccharides showed a good swelling property in alkaline pH. Dissolution test was carried out in triplicate to get reproducible results and was carried out for 8 hours. A sample of microspheres equivalent to 80 mg of metformin hydrochloride was used. At preset time intervals, 10 mL of the sample was withdrawn and replaced with an equal volume of fresh phosphate buffer of pH 7.4 maintained at the same temperature. The withdrawn samples were filtered through a membrane filter (0.45 μm) and were analysed for drug content spectrophotometrically at 233 nm using a UV-Visible spectrophotometer (UV-1800, Shimadzu).

Modelling and comparison of release profiles

Data obtained from *in vitro* release studies were inserted in various kinetic equations to find out the drug release mechanism of the microspheres. The kinetic models used were the zero order equation, first order equation and the Higuchi and Korsmeyer--Peppas models.

RESULTS AND DISCUSSION

Preparation and characterization of microspheres

The microspheres of metformin hydrochloride were prepared by the emulsion solvent diffusion technique according the procedure described in Experimental, with different proportions of natural polysaccharides of DI, AE and *Bora* rice. The used polysaccharides were found to be insoluble in organic solvents due to the presence of the acetone insoluble precipitate. They formed colloidal mucilaginous dispersion in water, saturated saline and showed a good swelling property in phosphate buffer of pH 7.4. The pH of 1 % aqueous dispersion of DI and AE was found to be 5.0 and 6.1, respectively, which indicated that it was compatible to the alkaline pH of the intestine. These properties were significant for its use in controlled drug delivery.

Mean particle sizes of different batches of prepared microspheres were found to be within the range from 77 ± 1 to 190 ± 10 μm, as shown in Table II. The microspheres were found to be uniformly sized and spherical. Particle sizes were slightly bigger for polymer blends containing a larger amount of *Bora* rice. The results also indicated a more uniform distribution of particle size and shape at 500 rpm while increased rpm caused non-uniform distribution. Variation in the concentration of polymers influenced particle size and shape. Ethyl cellulose caused smoothening of the surface of prepared microspheres. Increase in curing time decreased the particle size of microspheres. The results also revealed that the size of microspheres was increased at lower rpm of the mechanical stirrer.

Drug entrapment efficiency (DEE) of the drug loaded microspheres is shown in Table III. It was found that the curing time as well as the mechanical stirrer rpm affected the drug entrapment efficiency of microspheres. Maximum drug entrapment efficiency of the microspheres was 84.7 ± 2.3 %. It was found that an increase in curing time caused lower entrapment efficiency because the drug was highly water soluble and there was a

Table II. Particle size and size distribution

Formulation	Particle size (μm)[a]
F1	77 ± 1
F2	78 ± 1
F3	78 ± 2
F4	85 ± 3
F5	90 ± 5
F6	101 ± 2
F7	112 ± 1
F8	124 ± 4
F9	130 ± 2
F10	145 ± 1
F11	156 ± 2
F12	162 ± 2
F13	170 ± 1
F14 (blank)	152 ± 1
F15 (blank)	190 ± 0_2

[a] Mean ± SD (n = 100).

Table III. Drug entrapment efficiency (DEE) of microspheres

Formulation	DEE (%)[a]
F1	60.7 ± 1.2
F2	80.7 ± 1.2
F3	66.7 ± 1.5
F4	73.7 ± 1.5
F5	82.0 ± 1.0
F6	62.7 ± 1.5
F7	74.0 ± 2.0
F8	77.3 ± 1.2
F9	77.3 ± 0.6
F10	84.7 ± 1.5
F11	63.7 ± 0.6
F12	84.7 ± 2.3
F13	64.7 ± 0.6
F14	Blank
F15	Blank

[a] Mean ± SD (n = 3).

Table IV. Water sorption of microspheres after 48 hours

Bora rice/ *A. esculentus*/ *D. indica*	Sorption medium	m_t (mg)	m_0 (mg)	Water sorption (%)
1:1:1	HCl[a]	55	50	10
	Water	60	50	20
1:2:1	Phosphate buffer pH 7.4	75	50	50
	HCl[a]	54	50	8
	Water	65	50	30
1:1:2	Phosphate buffer pH 7.4	80	50	60
	HCl[a]	56	50	12
	Water	68	50	36
2:1:1	Phosphate buffer pH 7.4	78	50	56
	HCl[a]	58	50	16
	Water	69	50	38
	Phosphate buffer pH 7.4	81	50	62

[a] c = 0.1 mol L^{-1}

chance of drug diffusion in water. Decreased curing time led to an increase in the drug entrapment efficiency. Hence, an optimum curing time was selected. Increased mechanical stirrer rpm also caused drug diffusion to the external fluid. Thus, optimum mechanical stirrer rpm had to be selected.

Microspheres containing a high proportion of *Bora* rice in the physical mixture showed the highest swelling property in water sorption study, as shown in Table IV.

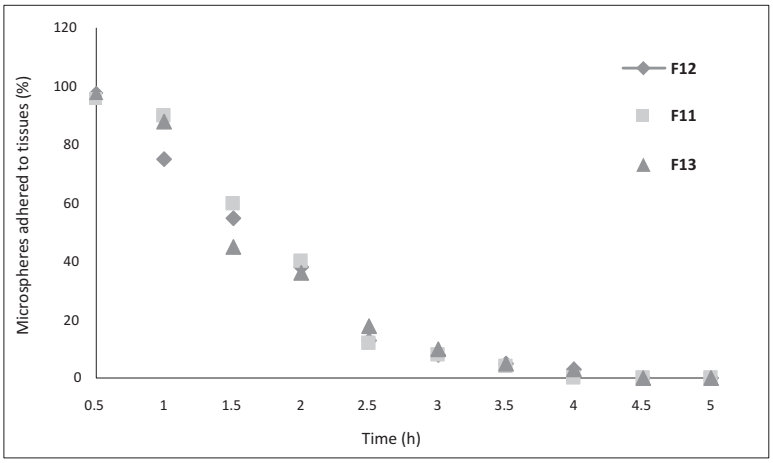

Fig. 1. *In vitro* wash off test of the microsphere formulations showing mucoadhesion at phosphate buffer of pH 7.4.

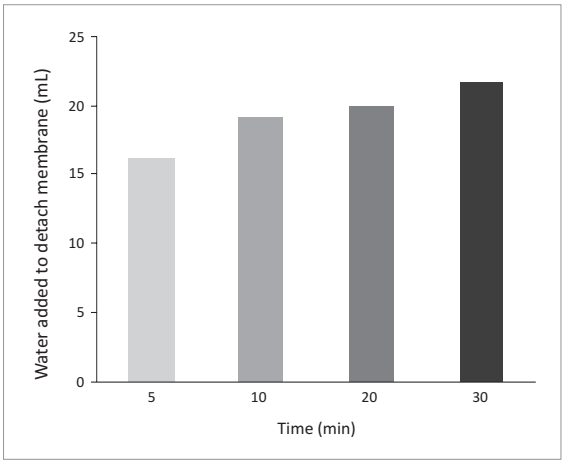

Fig. 2. *Ex vivo* mucoadhesive study by the Park Robinson method of formulation F12.

Mucoadhesive properties of DI and AE have been reported (11, 19). In this study, mucoadhesive properties of the formulations prepared with the natural polysaccharides used were determined. The study indicated that microspheres possessed good mucoadhesive properties (Fig. 1). The polymer ratio and the pH of the medium also affected mucoadhesivity, since an increase in polymer concentration increased the adherence time of the microspheres to the intestinal mucosal surface. Also, higher concentration of *Bora* rice in polymer blends displayed more mucoadhesion than other polymers. Its wash-off in phosphate buffer pH 7.4 was not easy; it rather formed a gel and adhered to the intestinal mucosal surface for a long time. Mucoadhesivity of microspheres to the goat intestinal mucosa increased with increasing time (Fig. 2).

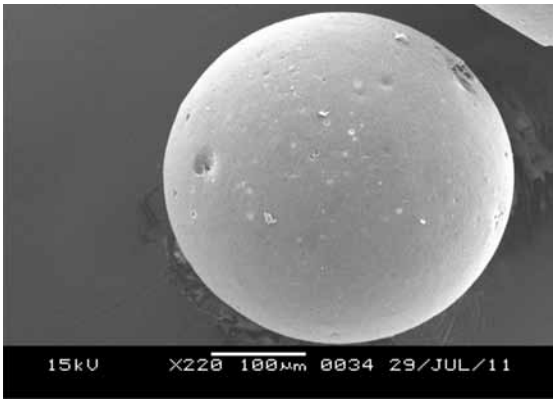

Fig. 3. SEM of a metformin hydrochloride loaded microsphere.

The SEM of the drug loaded microsphere in Fig. 3 showes spherical and smooth surfaced microsphere. However, deepening was found due to loss of solvent during drying. It was found that polymer concentration, rpm of the mechanical stirrer, temperature and curing time affected the shape and size of microspheres. An increase in polymer concentration formed smooth surfaced microspheres and ethyl cellulose also smoothened the surface of microspheres. Optimum mechanical stirrer rpm and curing time were required because an increase in rpm formed smaller sized microspheres with non-uniform distribution while reduced mechanical stirrer rpm formed bigger sized microspheres. The presence of small pores on the microsphere surface indicated solvent diffusion to the external fluid.

Drug excipients compatibility

Drug excipients compatibility was studied by FT-IR spectroscopy. Characteristic peaks of metformin hydrochloride appeared at 3367.34, 3298.05, 3169.04, 1627, 1222, 1064.63, 636.47 cm^{-1} in metformin hydrochloride loaded microspheres, showing C-N vibrations, C=N vibrations, C=N stretching, N=N stretching, C-H stretching, as shown in Fig. 4a. Disappearance of some of the metformin hydrochloride peaks in the formulated microspheres, shown in Fig. 4b, was due to encapsulation of the drug in the physical mixture.

Thermal analysis was applied to study the physical state of the drug in the formulation and to check the drug-excipient compatibility. DSC was done of the drug, DI, AE, *Bora* rice, physical mixture of polymers, blank microspheres and the drug loaded microspheres, as shown in Fig. 5. The sharp endothermic peak of metformin hydrochloride at 238.73 °C indicated relative purity. *Bora* rice also showed an endothermic peak at 108.03 °C, DI showed an endothermic peak at 120.23 °C, whereas AE showed an endothermic peak at 126.12 °C. As AE and DI had close endothermic peaks, they consumed almost the same quantity of heat flowing through, while *Bora* rice absorbed less heat than DI and AE. Natural mucilaginous substances (NMS) of *Bora* rice, AE, DI were found to be of relative purity. The physical mixture (DI, AE, *Bora* rice, ethyl cellulose and metformin hydrochloride) showed an endothermic peak at 110.91 °C. The less intense endothermic peak of the physical mixture (DI, AE, *Bora* rice, ethyl cellulose and metformin hydrochloride) was due to solid transitions. Decrease in the intensity of drug loaded microspheres was due to conversion of the crystalline form of the drug to its amorphous form. Disappearance of peaks in drug loaded microspheres showed that the drug was uniformly dispersed in the polymer matrix at the molecular level (4).

XRPD study was performed to study the phase transition of the drug in formulated microspheres due to the effect of the polymers. The XRPD of metformin hydrochloride and drug loaded microspheres are shown in Fig. 6. The result showed peaks of metformin hydrochloride at 2θ of 13, 18, 23, 24, 28, 29, 30, 32; the same diffraction was also observed in the case of drug loaded microspheres, exhibiting similar peaks but with notably decreased intensity of the signal, showing no signs of chemical interaction between the drug and other formulation components. Reduced intensity of the diffraction signal was probably caused by the dilution effect and decreased intensity of the drug was due to encapsulation into the polymer matrix.

Metformin hydrochloride displayed sharp peaks showing the crystalline phase while the drug containing microspheres showed less intense peaks indicating conversion of the drug crystalline phase to its amorphous phase.

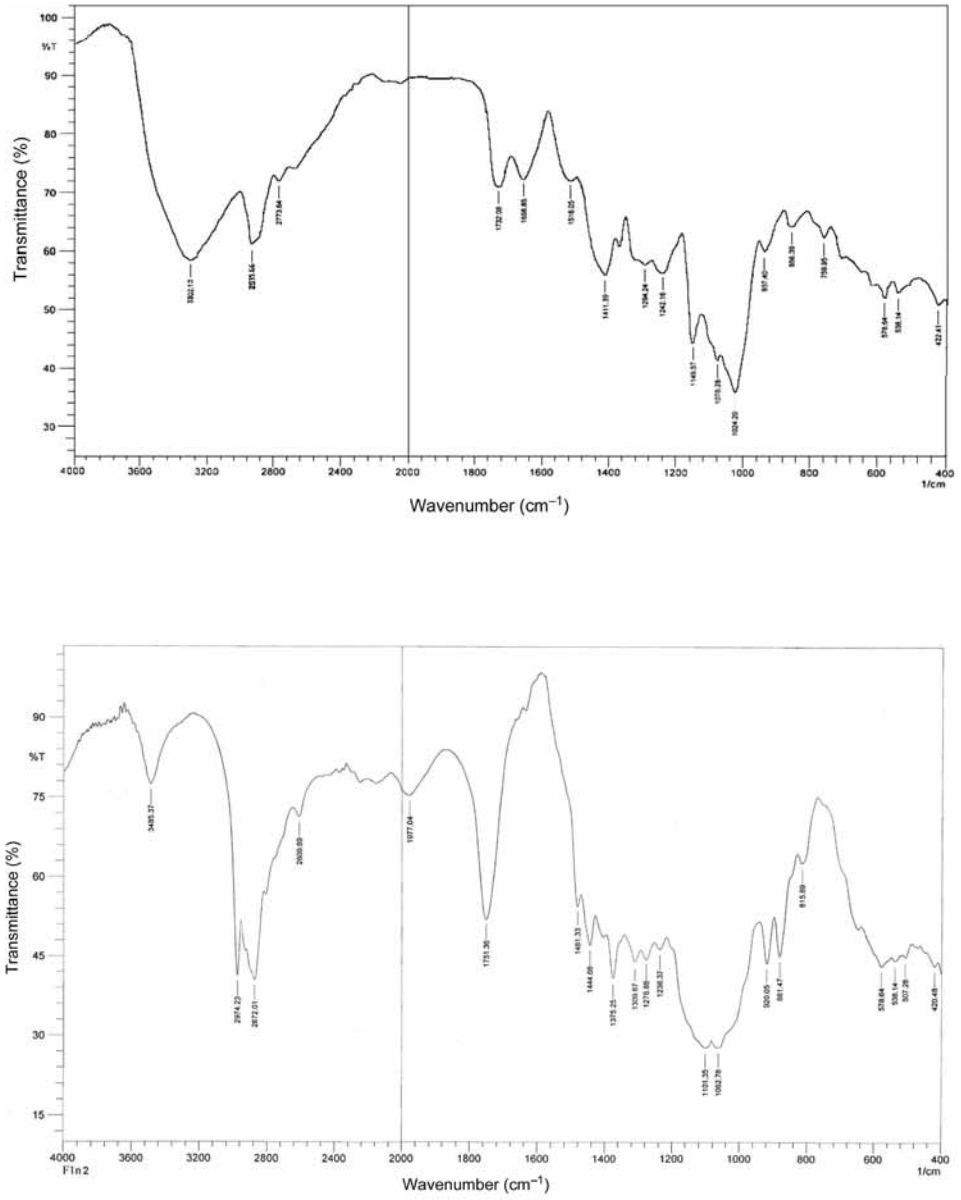

Fig. 4. FT-IR of: a) model drug (metformin hydrochloride), b) metformin hydrochloride loaded microspheres.

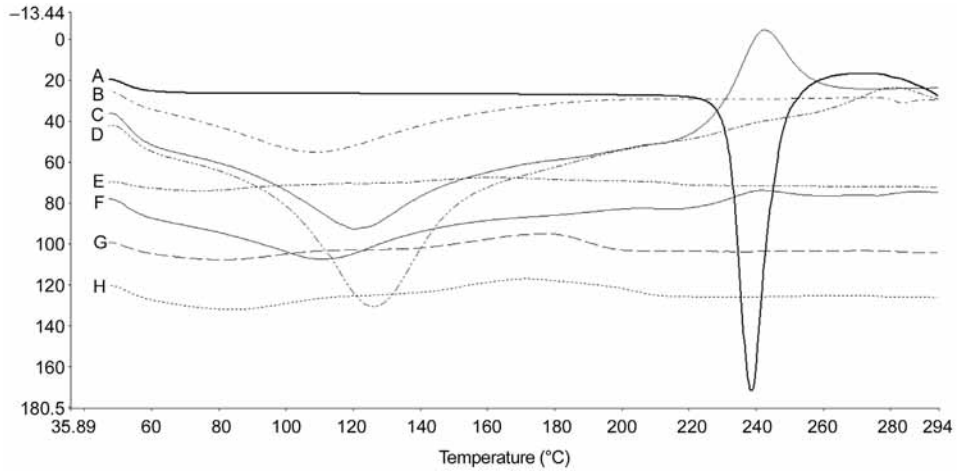

Fig. 5. The DSC thermogram of metformin hydrochloride (A), *Bora* rice (B), *Dillenia indica* (C), *Abelmoschus esculentus* (D), ethyl cellulose (E), physical mixture of metformin hydrochloride, *Bora* rice flour, polysaccharides of *Abelmoschus esculentus* and *Dillenia indica*, ethyl cellulose (F), blank microspheres (G), metformin hydrochloride microspheres (H).

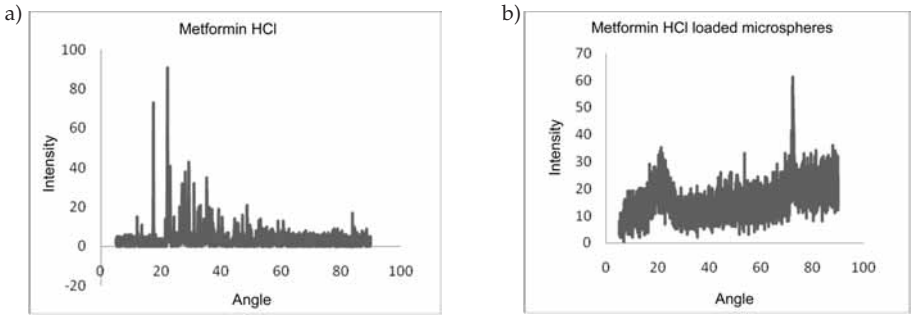

Fig. 6. XRPD of: a) metformin hydrochloride, b) drug loaded microspheres.

In vitro *drug release*

The *in vitro* release study (Fig. 7) of the drug loaded microspheres showed that erosion of the microsphere surface caused diffusion of the drug to the dissolution fluid. However, a number of fluctuations were observed in drug release. Fluctuation, or release in phases, was prominent in case of formulations F11 and F13 with the composition of 2:3:1 and 1:3:2 of *Bora* rice and extracted polysaccharides of DI and AE, respectively (Table I). This indicated that erosion of the microspheres took place in phases and was related to the amount and type of the polysaccharide incorporated. Incorporation of higher amount of

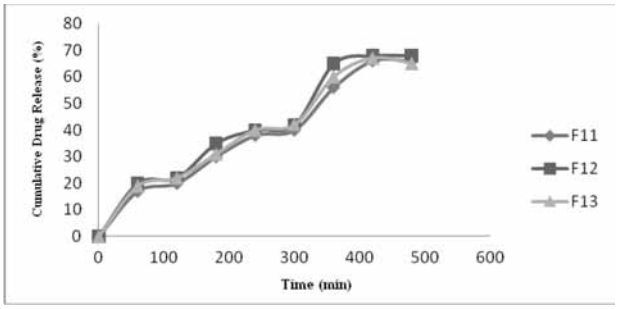

Fig. 7. *In vitro* release study of formulations F11–F13.

Bora rice flour caused comparatively sharper fluctuation than the other two polyssacharides as indicated by F12 with the composition of 3:2:1.

In order to understand the mechanism and kinetics of drug release, the *in vitro* dissolution study data were analyzed using various kinetic equations (Table V). The release profile followed all the kinetic models: zero order, first order and Higuchi model. The best model with the highest correlation coefficient was shown in the Higuchi model followed by a zero order equation. The obtained correlation coefficient values indicated that drug release followed the diffusion control mechanism from the microspheres. The data supported the findings that the drug incorporated in the swelling matrix device was mainly released by a diffusion mechanism. The data obtained were inserted in the Korsmeyer-Peppas model in order to find out the *n* value. The release showed a high correlation with the Korsmeyer-Peppas model. The *n* value ranged from 0.639 to 0.682, which suggested that drug release from the mixture of polymers was non-Fickian diffusion controlled and was influenced by both diffusion and swelling of the polysaccharides used.

Table V. Results of the parameters of model equations for in vitro release kinetics

Formulation code	Zero order		First order		Higuchi model		Korsmeyer-Peppas model	
	R^2	k_0 (mg mL^{-1} min^{-1})	R^2	k_1 (mg mL^{-1} min^{-1})	R^2	k_n (mg mL^{-1} min^{-1})	R^2	n
F2	0.906	0.131	0.895	–0.002	0.911	2.503	0.838	0.654
F7	0.882	0.127	0.862	–0.005	0.940	2.379	0.822	0.643
F9	0.936	0.146	0.808	–0.004	0.913	2.667	0.864	0.666
F5	0.910	0.123	0.881	–0.004	0.887	2.344	0.923	0.639
F11	0.916	0.148	0.882	–0.004	0.831	3.143	0.663	0.643
F12	0.928	0.159	0.868	–0.004	0.925	3.077	0.919	0.682
F13	0.927	0.145	0.890	–0.002	0.829	3.080	0.657	0.631

R^2 – regression coefficient, k_0 – zero order release constant, k_1 – first order release constant, k_n – Higuchi dissolution constant, n – release exponent, which characterises the release mechanism of drug.

CONCLUSIONS

Natural mucilaginous polysaccharides from edible sources possessed good swelling properties in the intestinal pH and also exhibited good mucoadhesivity. They can be used in encapsulating drugs which are sensitive to gastric pH, so as to deliver the drug at the intestinal pH. Extracted polysaccharides may be also used as taste masking agents for bitter drugs and hence may help improve paediatric formulations. The controlled release behaviour may be attributed to amylopectin in *Bora* rice and pectous polysaccharides in DI and AE extracts. Besides controlled release formulation, due to the presence of pectous polysaccharides the extract may be considered, for colonic delivery of drugs.

Acknowledgement. – The authors thank the All India Council for Technical Education (AICTE), F. No. 8023/BOR/RID/RPS-238/2008-09, and the University Grants Commission (UGC), F. No. 34-52/2008 (SR), for the financial support and M/S Ozone Pharmaceutical Ltd., Guwahati, Assam, India, for providing the model drug as a gift sample.

REFERENCES

1. D. Stepensky, M. Friedman, W. Sour and A. Hoffmann, Preclinical evaluation of pharmacokinetic-pharmacodynamic rationale for oral CR metformin hydrochloride formulation, *J. Control. Release* **71** (2001) 107–115; DOI: 10.1016/S0168-3659(00)00374-6.
2. S. Yasunori, K. Yashiaki, T. Hirofumi and H. Yamamoto, *In vitro* evaluation of floating and drug release behaviour of hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method, *Eur. J. Pharmac. Biopharmac.* **57** (2004) 235–243; DOI: 10.1016/S0939-6411(03)00185-1.
3. M. Kouchak and F. Atyabib, Ion-exchange. An approach to prepare an oral floating drug delivery system for diclofenac, *Iran. J. Pharm. Res.* **2** (2004) 93–97.
4. P. Salunke, B. Rane, S. Bakliwal and S. Pawar, Floating microcarriers of an antidiabetic drug: Preparation and its *in-vitro* evaluation, *J. Pharm. Sci. Technol.* **2** (2010) 230–240.
5. C. E. Beneke A. M. Viljoen and J. H. Hammam, Polymeric plant derived excipients in drug delivery, *Molecules* **14** (2009) 2602–2620; DOI: 10.3390/molecules14072602.
6. B. Shaptadvipa and R. N. Sarma, Study on apparent amylose content in context of polymorphism information content along with indices of genetic relationship derived through SSR markers in *Birain*, *Bora* and *Chokuwa* groups of traditional glutinous rice (*Oryza sativa* L.) of Assam, *Asian J. Biochem.* **4** (2009) 45–54; DOI: 10.3923/ajb.2009.45.54.
7. H. K. Sharma, A. Mukherjee and L. K. Nath, Evaluation and comparison of treated-untreated Assam Bora rice flour for use as directly compressible agent, *Int. J. Cur. Biomed. Phar. Res.* **1** (2011) 173–177.
8. C. Rolin and Pectin, *Industrial Gums: Polysaccharides and Their Derivatives* (Eds. Whistler and J. N. Bemillar), Academic Press, New York 1993, pp. 257–293.
9. S. S. Hunda and V. K. Kapoor, *Textbook of Pharmacognosy*, 2nd ed., Vallabh Prakashan, Delhi 2003, pp. 217–218.
10. H. K. Sharma, Sunita Lahkar and L. K. Nath, Extraction, characterisation and compatibility study of polysaccharides from *Dillenia indica* and *Abelmoschus esculentus* with metformin hydrochloride for development of drug delivery system, *Int. J. Pharm. Tech. Res.* (in press).
11. H. K. Sharma, Preparation and in vitro permeation study of nasal gel of oxytocin with natural mucoadhesive substance, Masters Thesis, Jadavpur University, Kolkata, 2003.

12. N. Sengkhamparn, L. M. C. Sagis, Renko de Vries, H. A. Schols, T. Sajjaanantakul and A. G. J. Voragen, Physicochemical properties of pectins from okra (*Abelmoschus esculentus* (L.) Moench), *Food Hydrocolloid.* **24** (2010) 35–41; DOI: 10.1016/j.foodhyd.2009.07.007.
13. J. J. Meister, K. Anderle and G. Merriman, Rheology of aqueous solutions of okra mucilage F, *J. Rheol.* **27** (1983) 37–46; DOI: 10.1122/1.549721.
14. S. K. Baveja, K. V. Ranga Rao and J. Arora, Examination of natural gums and mucilages as sustaining materials in tablet dosage forms, *Indian J. Pharm. Sci.* **50** (1988) 89–92.
15. S. P. Wahi, V. D. Sharma, V. K. Jain and P. Sinha, Studies on suspending property of mucilage of Hygrophila Spinosa T. Anders and Hibiscus esculentus Linn, *Indian Drug* **22** (1985) 500–502.
16. K. Gould, H. Desai and T. M. P. Kumar, Preparation and evaluation of a novel buccal adhesive system, *AAPS Pharm. Sci. Tech.* **5** (2004) 1–9; DOI: 10.1208/pt050335.
17. B. V. Deore, H. S. Mahajan and U. V. Deore, Development and characterisation of sustained release microspheres by quasi emulsion solvent diffusion method, *Int. J. Chem. Tech. Res.* **1** (2009) 634–642.
18. P. T. Tayade and R. D. Kale, Encapsulation of water-insoluble drug by a cross-linking technique: Effect of process and formulation variables on encapsulation efficiency, particle size, and in vitro dissolution rate, *AAPS PharmSci.* **6** (2004) 112–119; DOI: 10.1208/ps060112.
19. A. A. Attama, M. U. Adikwu and C. J. Amorha, Release of indomethacin from bioadhesive tablets containing carbopol 941 modified with *Abelmuschus esculentus* (okra) gum, *Boll. Chim. Farm*. **142** (2003) 298–302.
20. C. M. Lehr, J. A. Bouwstra, J. J. Tukker and H. E. Junginger, Intestinal transit of bioadhesive microspheres in an in situ loop in the rat – A comparative study with copolymers and blends based on poly (acrylic acid), *J. Control. Release* **13** (1990) 51–62; DOI: 10.1016/0168-3659(90)90074-4.