Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 7, 2015

Original Article

DEVELOPMENT OF MUCOADHESIVE CARBOHYDRATE HETEROPOLYMER MICROBEADS FOR SUSTAIN RELEASE OF THEOPHYLLINE

GAGANPREET KAUR, K. PRABHUDAS KIRAN, AMIT SINGH*

Department of Pharmaceutics, Lovely Faculty of Applied Medical Sciences, Lovely Professional University, Jalandhar-Delhi G. T. Road (NH-1), Phagwara 144402, Punjab, India
Email: amit.16784@lpu.co.in

Received: 02 Mar 2015 Revised and Accepted: 30 May 2015

ABSTRACT

Objective: The aim of present study was to develop and evaluate mucoadhesive microbeads for oral sustained release of an antiasthmatic agent "theophylline" using natural gums such as sodium alginate and sesbania gum.

Methods: The compatibility studies of drug with different polymers were investigated by using DSC (Differential Scanning Calorimeter) and FTIR (Fourier Transform Infrared Spectroscopy). Carbohydrate heteropolymer microbeads of alginate and sesbania gums were prepared by ionotropic gelation technique, where calcium chloride is used as a source of counter ions. Prepared beads were characterized for particle size, entrapment efficiency, surface morphology, swelling index, *in vitro* release studies and release kinetics.

Results: Final optimized formulation consists of a polymer blend of alginate and sesbania gum with hydroxy propyl cellulose as release modifier. Microbeads exhibited good swelling index and high percentage of drug entrapment efficiency. The developed formulation showed a maximum drug release of 92% in 11 h using 0.1 N hydrochloric acid buffer (pH 1.2). The formulation followed Korsmeyer-Peppas and Higuchi release mechanism, releasing the drug by non-fickian diffusion. Prepared beads showed significant mucoadhesion in acidic buffer.

Conclusion: The sustained release microbeads were successfully designed for oral administration of theophylline which may be used for the treatment of nocturnal asthma.

Keywords: Mucoadhesive, Natural polymers, Microbeads, Sustained release, Theophylline.

INTRODUCTION

CRSs (Controlled release systems) have been extensively developed due to their considerable therapeutic advantages over conventional forms [1]. The development of CRSs using natural hydrophilic polymers becoming popular as these offer various desired characteristics such as cost-effectiveness, safety, non-toxicity, biocompatibility and biodegradability of a drug delivery matrix. Furthermore, these polysaccharides can be conveniently tailored using chemical or biochemical tools to develop a drug delivery matrix with specific features, hence, have been extensively studied in medicine and pharmaceutical laboratories. The polymeric hydrogels are three-dimensional cross-linked networks that are able to absorb water and swell without losing their shape. One of the mostly used natural polysaccharide as drug carrier is sodium alginate, which undergoes a sol-gel transformation in response to divalent cations. Alginate is preferably used to prepare multi-unit controlled release dosage forms like microbeads and micro particles which spread over a large surface area preventing exposure of the absorbing site to high drug concentration on chronic release. These microbeads may be dispensed as filled in hard gelatin capsules or they can be compressed into tablets [2]. These microbeads are usually prepared by using polyelectrolyte complexes of alginate with other polymers as if used alone, it shows unsatisfactory drug release profiles. In the present study, sesbania gum is used with alginate as polymer matrix, which is obtained from the endosperm of *Sesbania bispinosa* seeds, family Leguminosae. It consists of a linear chain of (1, 4)-β-D-mannopyranose units with α-D-galacto-pyranose units attached by (1-6) linkages, the ratio of mannose to galactose is 2:1 [3]. The alginate-sesbania gum polymer was used only for the entrapment of theophylline, a bronchodilator used as an add-on therapy to low or high doses of inhaled glucocorticosteroids for asthma control [4]. The short biological half–life (about 3.5 h) of theophylline requires to be delivered as controlled release systems [5]. The use of sesbania gum along with alginate enhanced the entrapment efficiency and sustains release of theophylline.

MATERIALS AND METHODS

Chemicals

Theophylline was kindly gifted from French Pharma, Chandigarh, India. Sodium alginate be procured from CP Kelco Pvt. Ltd, Mumbai, India. Sesbania gum was a generous gift by Govind Madhav Industries, Mehsana, India. Hydroxy Propyl Cellulose and other buffer salts were purchased from LobaChemie Pvt. Ltd, Mumbai, India. Thimerosal was supplied from Alpha Chemika, Mumbai, India.

Methodology

Analytical method development and validation

The quantitative and qualitative determination of theophylline was done using a double beam UV-visible spectrophotometer (Shimadzu, Japan). The theophylline was dissolved in 0.1 N HCl buffer (pH 1.2) and the absorption spectrum was recorded. A calibration curve was drawn by plotting concentrations of theophylline ranging from 1-8 µl/ml on X-axis and their corresponding absorbance on Y-axis. The method was validated in terms of linearity, precision, accuracy and robustness.

Drug-polymer compatibility studies

Drug–polymer interactions were confirmed using FT-IR spectroscopy. The drug was mixed separately with sodium alginate and sesbania gum in 1:1 ratio, filled in separate glass vials and then stored under three different conditions, 8 °C±2 °C,25 °C±2 °C and 40 °C±2 °C with 75% RH in a humidity chamber for one month. Vials containing pure drug and pure polymers were also kept in similar conditions. Samples were taken on 0, 7, 15 and 30th day and prepared in potassium bromide disk (2 mg sample in 200 mg KBr) with a hydraulic press by applying a pressure of 8-9 tons for 2 min. The discs were scanned over a wave number range of 4000-400 cm^{-1} and the resolution was kept at 4 cm^{-1}.

Development of formulation

Initially the microbeads of sodium alginate were prepared by ionotropic cross linking method [6]. Sodium alginate and dispersed

in distilled water at 60 °C with stirring using glass rod. Theophylline (400 mg) was dissolved and dispersed in the above prepared polymer solution after lowering the temperature upto 20 °C. Thiomersal (0.5 mg) as preservative was also added in the above mixture. This bubble free solution (5 ml) was dropped through 21 G needles into chilled calcium chloride solution (10 %) with stirring at 100 rpm on magnetic stirrer to form the gelled beads. The beads were allowed to cure for 3 h in the calcium chloride solution and then separated by filtration, washed thrice with distilled water and kept in a hot air oven at 40 °C for drying. For preparation of alginate-sesbania microbeads, varied concentrations of sesbania gum were blended a selected concentration of sodium alginate and then. Similar procedure was followed.

Differential scanning calorimetry (DSC)

Thermograms of pure theophylline and the polymer blends were recorded using a DSC (DSC 8500, Perkin Elmer, Maharashtra, India). Each sample (3–4 mg) was accurately weighed into an aluminium pan in a hermetically sealed condition. The measurements were performed in an atmosphere of nitrogen (20 ml/min) between 30–500 °C at a heating rate of 10 °C/min.

Determination of % yield

The % yield of all the formulations of microbeads was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Total weight of dried microbeads}}{\text{Total weight of polymer} + \text{drug}} \times 100$$

Estimation of drug entrapment efficiency

The amount of theophylline present in the filtrate after bead formation was measured and subtracted from the total amount taken initially to determine total entrapped drug. The drug entrapment efficiency (%) was calculated using the following formula:

$$\% \text{ Entrapment efficiency} = \frac{(\text{Total drug} - \text{Drug in filtrate})}{\text{Total drug}} \times 100$$

Micromeritic properties of microbeads

Various micromeritic properties such as particle size analysis, bulk density, tapped density, angle of repose etc. were assessed for the prepared beads using standard protocols. Particle size analysis was performed by optical microscopy using a compound microscope (Kyowa, Japan). Angle of repose was measured according to the "fixed funnel and free standing cone method". % compressibility index and Hausner's ratio were also calculated using standard formulae.

Equilibrium swelling studies

10 mg of microbeads were placed in 0.1 N HCl buffer (pH 1.2) and allowed to swell up to a constant weight. After this the microbeads were removed, blotted with filter paper and their change in weight was measured. The swelling index was then calculated from the following formula:

$$\text{Swelling index (\%)} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{wet weight}} \times 100$$

***In vitro* drug release study**

The drug release from alginate-sesbania microbeads was studied using a conventional USP type-II dissolution apparatus (Lab India Disso 2000, India) using 900 ml 0.1N HCl buffer (pH 1.2) at 37 °C for 12 h. A sample (10 ml) was collected from dissolution test apparatus hourly and replaced with fresh buffer. The samples were diluted to suitable concentration with HCl buffer and assayed spectrophotometrically at 272 nm.

***In vitro* release kinetics**

To investigate the drug release mechanism, *in vitro* release data was fitted with various release models such as Zero order, First order, Korsmeyer Peppas and Higuchi model. Regression coefficient values for all the release models were calculated and higher regression coefficient (>0.98) was considered as the validity of the model. The equations used for these kinetic models are listed below:

$Q_t = k_o t$(zero order)

Log C = Log C_o–kt/2.303 (first order)

$Q_t = k_H t^{1/2}$(Simplified Higuchi diffusion)

$M_t/M_\infty = Kt^n$(Korsmeyer-Peppas equation)

Where,

Q_t = amount of drug released at time (t),

k_0 = zero order release constant

C = cumulative percent of drug release

C_o = the initial concentration of drug

k_H= Higuchi-diffusion rate constant,

M_t/M_∞ = fraction of drug released at time (t),

K = constant comprising the structural and geometric characteristics of the microbeads

n = release exponent

Shape and surface morphology of microbeads

Shape and surface characteristics of microbeads were investigated using scanning electron microscopy (SEM, Hitachi S 3400 N, Japan) of the samples before and after carrying out the dissolution. The SEM samples were prepared by lightly spreading the microbeads on carbon tape, which was stucked on an aluminium stub. The stubs were coated with gold using a gold sputter coater in a high vacuum evaporator, and the samples were observed at 10 kV.

Mucoadhesion testing

The mucoadhesive properties of various formulations of theophylline loaded alginate-sesbania beads were evaluated by the *in vitro* wash-off method. Freshly excised pieces of goat gastric mucosa [1 cm×1 cm, collected from a slaughter house (permission for the same was taken from Institutional Animal Ethical Committee)] were mounted on a glass slide using thread. About 50 beads were spread out on each piece of mucosa and then hung from the arm of the tablet disintegration test apparatus. The tissue specimen was given a regular up and down movement in a 1 l vessel containing 900 ml of 0.1 N HCl (pH 1.2), maintained at 37±0.5 °C. The adherence of beads was regularly observed. The beads that remained adhered to the mucosa were counted at regular intervals for up to 10 h and % bioadhesion was calculated as:

$$\% \text{ Bioadhesion} = \frac{\text{No. of microbeads adhered at a time interval "t"}}{\text{Total no. of microbeads taken}} \times 100$$

RESULTS AND DISCUSSION

Physical description of drug

Theophylline powder was creamy white in color, odorless and bitter in taste. It showed melting point in the range of 270-274 °C which was close to the reported value, i.e. 272.19 °C (Drug bank, 2012). The purity of the drug was further confirmed by FT-IR spectroscopy. Vibrational bands present>3000 cm^{-1} in the FTIR spectra of xanthine were assigned as N–H stretching of the imidazole ring. The other two bands present in the region at 2983-3121 cm^{-1} were assigned to N–H asymmetric and symmetric stretching. Strong bands observed at 1666 cm^{-1} and at 1566 cm^{-1} in FTIR spectra were considered to be due to C=O asymmetric and symmetric stretching vibrations. Theophylline presented an intense absorbing stream around the frequency 1566 cm^{-1}, Stream that appears due to the C=N stretching vibrations. Similar observations have also been reported by Forizs *et al.* [7].

Analytical method development and validation

The absorption maximum (λ_{max}) of theophylline in HCl buffer (pH 1.2) was found to be 272 nm which was good agreement with the

previously reported literature (271 nm, Patel *et al.*, 2011). The standard curve of theophylline showed regression coefficient (R^2) as 0.9978 with the line equation of y= 0.118x+0.0004. The method developed was found to be accurate, precise and robust as the calculated R. S. D. values were less than 2. Intra-day and inter-day precision studies revealed that the method gives repeatedly the same results irrespective to the day and time of study.

Drug-polymer compatibility studies

Drug-polymer interactions were studied as described in methodology. During this study, no physical change in any of the mixture was observed. The samples were further checked by FT-IR spectroscopy. FT-IR spectra of the drug and polymer combination showed the bands around 3448, 1666, 1425 and 1049 cm^{-1}, indicating the stretching of O–H, COO– (asymmetric), COO– (symmetric) and C –O–C, respectively. All these bands were corresponding to sodium alginate [8]. The characteristic peaks of theophylline were observed at 1666, 1568 and 3120 cm^{-1}corresponding to the carbonyl group stretching, vibration of the pyrimidine ring and asymmetric aliphatic CH_2, respectively. Identical peaks were also observed in FT-IR spectra of drug+polymer blend which indicated that no major interaction occurred between the functional groups of the studied drug and polymer blend upon mixing [7].

Development and evaluation of formulation

Alginate-sesbania microbeads were developed following the procedure mentioned in the methodology section. The basic aim of formulation development was to enhance the overall yield of beads formation, drug entrapment in the beads, complete release of the entrapped drug with extended release time. The yield of beads formation and entrapment efficiency was calculated as described in the methodology section. The release profile of the drug was observed through in-vitro release studies using standard dissolution apparatus USP type-II. Table 1 represents compositions of polymers used in formulations along with their % yield, drug entrapment efficiency (%) and maximum release (%) of the drug along with the time required for maximum release.

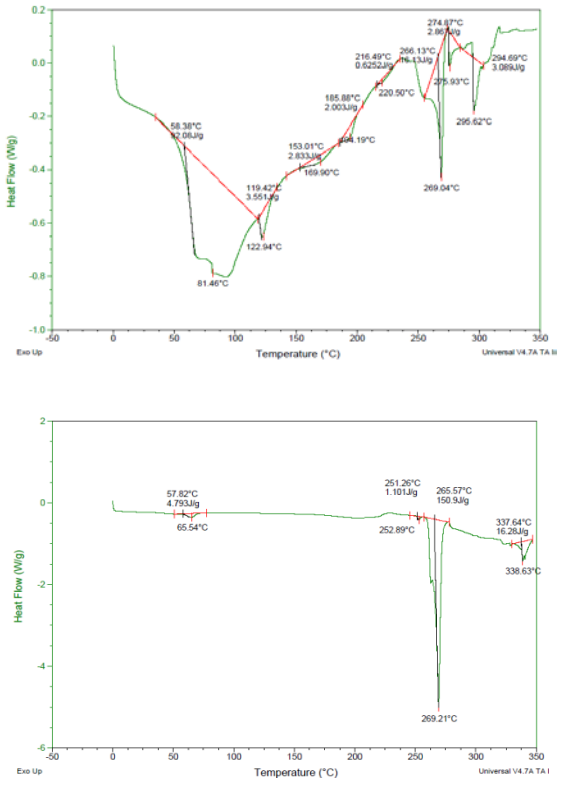
Table 1: Use of alginate and sesbania gum as support matrices

FC*	Na-Alg (%w/v)	SG (%w/v)	CMC (% w/v)	HPC (% w/v)	% Yield	Drug entrapment efficiency (%)	Maximum drug release (%)
F-1	1	-	-	-	72.50	68.63	-
F-2	2	-	-	-	75.00	74.00	-
F-3	3	-	-	-	77.25	77.25	81.2 (8 h)
F-4	4	-	-	-	80.80	81.75	83.8 (9 h)
F-5	5	-	-	-	81.20	80.63	83.6 (10 h)
F-6	4	1	-	-	81.57	81.86	79.1 (10 h)
F-7	4	2	-	-	82.05	83.65	74.7 (10 h)
F-8	4	3	-	-	85.61	87.33	72.07 (11 h)
F-9	4	4	-	-	85.50	87.00	68.3 (11 h)
F-10	4	3	1	-	84.25	87.15	66.46 (12 h)
F-11	4	3	-	1	86.33	87.55	86.3 (11 h)
F-12	4	3	-	2	88.30	92.75	92 (11 h)
F-13	4	3	-	3	88.25	87.34	87.2 (11 h)

F1 to F5 consisted of only alginate gum in varying concentrations as microbeads forming matrix, whereas the F6 to F9 were made up of alginate-sesbania gun blend. As evident from the table 1, with increasing concentration of the support matrices, the yield and drug entrapment efficiency increased, along with the time of maximum drug release. For alginate microbeads, the loading efficiency and drug entrapment efficiency increased with increasing concentration of alginate up to 4% (w/v, F4), after that, no significant change was observed. In this formulation (F4), 83.8 % of the entrapped drug was released in 9 h. After blending with increasing concentrations of sesbania gun (F6-F9), the yield, entrapment efficiency and release time increased but the maximum drug release (%) decreased. This was expected, since on increasing sesbania gum concentration, interaction between two polymers had increased, forming a closer network, which decreased the diffusion of the drug outwards from the interior of the microbeads [8, 9]. The F8, consisting of 3% sesbania gum with 4% alginate showed good drug entrapment efficiency (87.33%) with extended release time (11 h) was selected as the best formulation. To enhance the drug release from F8, HPC and CMC were tried (1%, w/w) as release modifiers (F10 and F11). Among these, the CMC showed no enhancement, rather decreased the drug release (table 1), hence, acted as a support matrix only, however, the HPC considerably enhanced the drug release (86.3%). The concentration of HPC was further enhanced (F12), the entrapment efficiency and release of drug increased from 2% HPC in formulation to 92.75 % and 88.30 %, respectively. The formulation consisting of 3% HPC (F13), showed decreased entrapment and release similar observations have been reported by Iglesias *et al.* [10].

Differential scanning calorimetry (DSC)

The compatibility of theophylline and the polymer blend (alginate+sesbania gum+HPC) was checked using DSC. The thermograms have been shown in fig. 1. Theophylline exhibited a sharp endothermic peak at 269.21 °C, corresponding to its melting transition point (fig. 1a). The physical mixture of drug and polymer showed three endothermic transitions peaks at 122.94 °C, 269.04 °C and 295.62 °C (corresponding to sodium alginate) as shown in (fig. 1b).



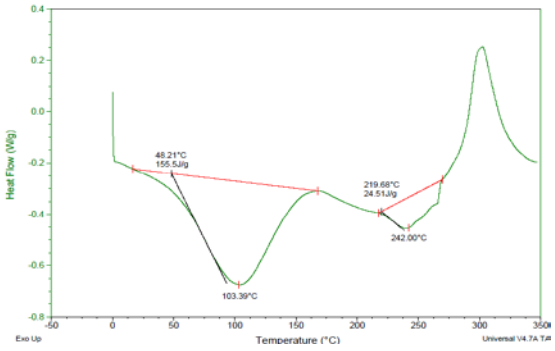


Fig. 1: DSC thermogram of (a) pure theophylline (b) Physical mixture of drug, alginate and sesbania gum (c) drug loaded microbeads

Weaker melting endotherm of theophylline appeared because the polymers might have inhibited melting of some drug crystals [11]. However, the prepared formulation of microbeads did not show any endothermic peak close to the drug melting point (fig. 1c). It may be indicated that drug was uniformly dispersed at the molecular level of polymers. But the prepared formulation showed early broad endothermic peak at 103.39 °C, probably due to evaporation of water. Hence, it may be concluded that there was no interaction between drug and excipients.

Evaluation of microbeads

Micromeritic properties

Particle size of formed beads was measure using optical microscopy fitted with stage micro meter, and their flow properties were determined. The data is presented in table 2 shows comparative micromeritic properties of F4, F8 and F12.

Table 2: Micromeritic properties of various selected formulations

Formulation Code	Particle size (µm)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's Ratio	Compressibility Index (%)
F-4	865±0.75	0.228±0.01	0.251±0.15	1.10±0.45	9.16±0.08
F-8	894±0.01	0.264±0.10	0.311±0.47	1.17±0.01	15.11±0.50
F-12	908±0.03	0.292±0.07	0.367±0.64	1.23±0.01	20.43±1.01

Table 3: Release kinetic analysis data of theophylline from prepared microbeads

Formulation code	Regression coefficient (r^2)				n	Release mechanism
	Zero Order	*First Order*	*Higuchi*	*Peppas*		
F-4	0.805	0.911	0.956	0.946	0.877	Non-fickian diffusion
F-8	0.824	0.924	0.947	0.935	0.819	Non-fickian diffusion
F-12	0.831	0.900	0.968	0.987	0.830	Non-fickian diffusion

The particle size analysis of the formulations revealed that the particle size of the microbeads increased with increasing polymer concentration. This may be due to the increased viscosity at a higher polymer concentration resulting in enhanced interfacial tension with diminished shearing efficiency. Similar observations have previously been reported in literature [12]. The Hausner's ratio also increased with increasing polymer concentration, but was less than 1.25 indicating good flow properties of microbeads [13]. Good flow properties of the formulations were also revealed by compressibility index which was 15.11 and 20.43 for F-8 and F-12 indicating excellent compressibility and packaging properties [14]. Hence the microbeads have an excellent flow characteristic which means they can be easily handled during capsule filling operations.

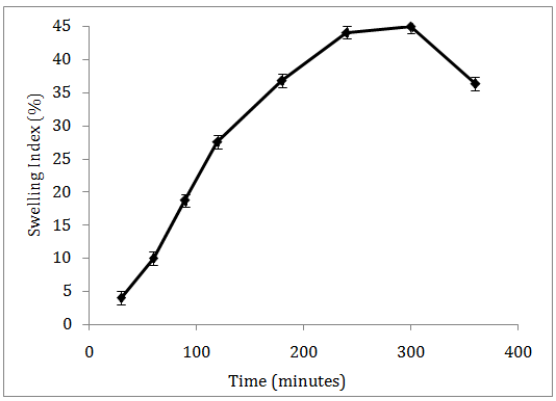


Fig. 2: Swelling behavior of microbeads in 0.1 N HCl buffer

Equilibrium swelling studies of microbeads

Swelling behavior of polymeric beads is one of the major factors controlling the drug release from the beads. For this reason, the swelling behavior of beads (fig. 2) was evaluated in 0.1 N HCl buffer (pH 1.2).

Maximum swelling of beads was noted at 6-7 h in the buffer after which erosion and breakdown took place resulting in decline in the index. Similar observations have been reported by Larson *et al.* [15]. Such behavior results from slow erosion of cross-linked microbeads due to a slight degradation of alginate backbone into smaller fragments [16].

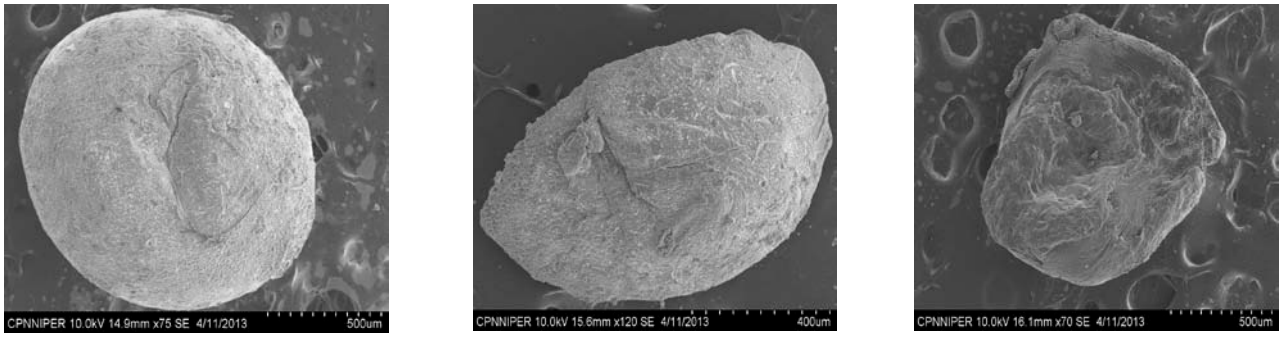
***In vitro* release kinetics**

To investigate the kinetics and mechanism of theophylline release from the polymer beads, the release data were fitted to zero, first order, Higuchi and Korsmeyer-Peppas models, as described in the methodology section. The regression coefficient of all the models was calculated for formulations selected for further modification during the study (F-4 and F-8) and finally selected formulation (F-12). The data have been represented in table 3.

According to the regression coefficients (R^2), the *in vitro* release data was in favor of Higuchi-diffusion kinetics (F-4 and F-8) and Korsmeyer Peppas model (F-12). If "n" is 0.45 or less, the release mechanism follows Fickian diffusion, but here the values obtained are higher (0.45<n<0.89). Therefore mass transfer followed a non-Fickian model (anomalous transport), where release is controlled by a combination of diffusion and polymer relaxation. Thus, selected formulations delivered their active ingredient by coupled diffusion and erosion [17]. This erosion of the micro beads was confirmed using Scanning Electron Microscope (SEM). It was evident from the SEM pictures (fig. 3) that the micro beads of hetero-polymer eroded during delivery of theophylline.

Mucoadhesivity testing

The muco-adhesiveness of the prepared formulations was checked, as described in the methodology section and the data is presented in fig. 4. All the polymers used in the formulation development were having mucoadhesive property, hence, % bioadhesion increased as other polymers were mixed in sodium alginate.



(A) **(B)** **(C)**

Fig. 3: Outer surface morphology of: (A) intact micro bead before dissolution; (B) Micro bead after 6h of dissolution; (C) Micro bead after 11h of dissolution

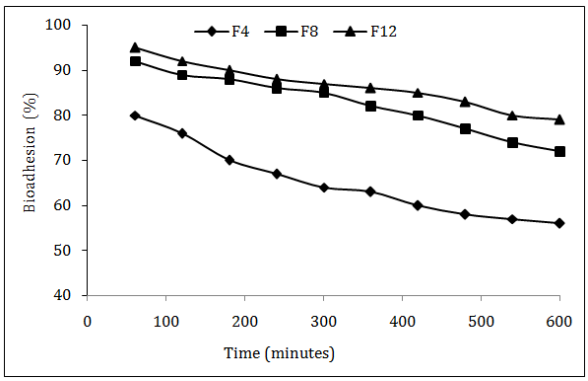


Fig. 4: Mucoadhesiveness of prepared formulations (F4, F8 and F12)

Maximum bioadhesion was shown by F12, where 3 polymers were used. This result suggested that the developed formulation has good bioadhesiveness which is desired for extended release formulations so that they do not expel from GIT before complete drug release.

CONCLUSION

The use of naturally occurring polysaccharides, especially sodium alginate, functioning as biopolymers has been successfully used for sustained released hydrogel formulations. The entrapment and sustained release of the drug from alginates beads can further be improved by using other copolymers. In the present study, the sesbania gum and HPC were used as copolymers which enhanced entrapment efficiency, maximum drug release from the beads at extended time. Also it showed better adhesion to gastric mucosa, which is desired for extended release formulations. The developed formulation may be successfully used for sustained delivery of theophylline.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Deepak N. Kapoor (Department of pharmaceutics, Lovely Professional University) for helpful suggestions.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Rao A, Shivalingam MR, Reddy YV, Sunitha N, Jyothibasu T, Shyam T. Design and evaluation of sustained release microcapsules containing diclofenac sodium. Int J Pharm Biomed Res 2010;1:90-3.
2. Mangond BS, Sreedhar V, Baraskar W, Kulkarni RV. Development and evaluation of gellan gum based hydrogel microbeads for controlled release of ketoprofen. Indian J Novel Drug Delivery 2009;1:32-5.
3. Mathur V, Mathur NK. Fenugreek and other lesser known galactomannan polysaccharides-Scope for development. J Sci Ind Res 2005;64:475-81.
4. Weinberger M, Hendeles L. Slow-release theophylline: rationale and basis for product selection. N Engl J Med 1983;8:760-4.
5. Tandon MK, Kailis SG. Bronchodilator treatment for partially reversible chronic obstructive airways disease. Thorax 1991;46:248-51.
6. Garg R, Gupta GD. Progress in controlled gastro retentive delivery systems. Trop J Pharm Res 2008;7:1055-66.
7. Forizs E, Patrut A, Cozar IB, David L. Synthesis, structure elucidation of palladium complexes with theophylline. Academia Romana Roum Chim 2010;55:697-704.
8. Bhagwat DA, Bhutkar MA, Todkar SS, Mohite SK, Gattani YS. Formulation and evaluation of controlled release microspheres of isosorbide dinitrite. Int J Pharm Technol Res 2009;1:125-8.
9. Wan Ngah WS, Fatinathan S. Adsorption of Cu^{2+}ions in aqueous solution using chitosan beads, chitosan-GLA beads and chitosan alginate beads. Chem Eng J 2008;143:62-72.
10. Iglesias B, Coronilla R, Concheiro A, Lorenzo C. Preparation of chitosan beads by simultaneous cross linking/insolubilisation in basic pH/Rheological optimization and drug loading/release behavior. Eur J Pharm Sci 2005;24:77-84.
11. Maiti SB, Somdipta R, Ray SM. Al^{+3} ion cross-linked and acetylated gellan hydrogel network beads for prolonged release of glipizide. Carbohydr Polym 2011;85:164-72.
12. Jagtap YM, Bhujpal RK, Ranade AN, Ranpise RS. Effect of various polymer concentrations on physicochemical properties of floating microspheres. Indian J Pharm Sci 2012;74:512-20.
13. Lachman L, Lieberman HA, Kanig JL. The theory and practice of industrial pharmacy. 3rd ed. Mumbai: Varghese Publishing House; 1987.
14. Sinko PJ. editor. Martin's physical pharmacy and pharmaceutical science. 5th ed. Baltimore: Lippincott Williams and Wilkins; 2006.
15. Larson I, Dass CR, Choong PF, Dunstan DE. A chitosan-dipotassium orthophosphate hydrogel for the delivery of doxorubicin in the treatment of osteosarcoma. Biomaterials 2009;2:213-20.
16. Nayak AK, Hasnain MS, Beg S, Alam M. Mucoadhesive beads of gliclazide: Design, development and evaluation. Sci Asia 2010;36:319-25.
17. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm 1983;15:25-35.