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Locust bean gum in the development of sustained release mucoadhesive macromolecules of aceclofenac

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ABSTRACT

The study shows the development and optimization of locust bean gum (LBG)–alginate mucoadhesive macromolecules containing aceclofenac through ionotropic-gelation using 3^2 factorial design. The effect of amount of LBG and sodium alginate on drug entrapment efficiency (%DEE), % mucoadhesion at 8 h (M_8) and % *in vitro* drug release at 10 h ($\%Q_{10\,h}$) were optimized. The percentage yield, average size and DEE of macromolecules were found within the range of 93.19 to 96.65%, 1.328 ± 0.11 to 1.428 ± 0.13 μm, and 56.37 to 68.54%, respectively. The macromolecules were also characterized by SEM, FTIR and DSC. The *in vitro* drug release from these macromolecules (84.95 ± 2.02 to 95.33 ± 1.56% at 10 h) exhibited sustained release (first-order) pattern with super case-II transport mechanism. The swelling and mucoadhesivity of these macromolecules were affected by pH of the medium. The design established the role of derived polynomial equations and plots in predicting the values of dependent variables for the preparation and optimization.

1. Introduction

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. In the last two decades, mucoadhesion has shown renewed interest for prolonging the residence time of mucoadhesive dosage forms through various mucosal routes in drug delivery applications. Mucoadhesive-based topical and local systems have shown enhanced bioavailability. Mucoadhesive drug delivery system (MDDS) gives rapid absorption and good bioavailability due to its considerable surface area and high blood flow. Drug delivery across the mucosa bypasses the first-pass hepatic metabolism and avoiding the degradation of gastrointestinal enzymes (Ahuja, 1997). Adhesion can be defined as the bond produced by contact between a pressure sensitive adhesive and a surface. The American Society of Testing and Materials (ASTM) has defined it as the state in which two surfaces are held together by interfacial forces, which may consist of valence forces, interlocking action or both. Mucoadhesive drug delivery systems prolong the residence time of the dosage form at the site of application or absorption. They facilitate an intimate contact of the dosage form with the underlying absorption surface and thus improve the therapeutic performance of the drug. In recent years, many such MDDS have been developed for oral, buccal, nasal, rectal and vaginal routes for both systemic and local effects (Prajapati, Mahajan, & Surana, 2011).

Dosage forms designed for MDDS should be small and flexible enough to be acceptable for patients and should not cause irritation. Other desired characteristics of a mucoadhesive dosage form include high drug loading capacity, controlled drug release (preferably unidirectional release), good mucoadhesive properties, smooth surface, tastelessness, and convenient application. Erodible formulations can be beneficial because they do not require system retrieval at the end of desired dosing interval. A number of relevant mucoadhesive dosage forms have been developed for a variety of drugs (Asane, 2008).

Natural biodegradable polysaccharides like various gums and mucilage like guar gum, karaya gum, tara gum, cassia tora gum, locust bean gum, fenugreek seed mucilage; pectin, chitosan, carrageenans and sodium alginate have been used in controlled drug delivery (Aydin & Akbuga, 1996; Hwagno, Skinner, Harcu, & Barnum, 1998; Kedziereuciz & Lemory, 1999; Kulkarni, Soppimath, Aminabhavi, & Rudzinski, 2001; Soppimth, Kulkarni, & Aminabhavi,

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2000). Multiparticulate systems obtained by ionotropic crosslinking of these polymers have been used to develop mucoadhesive and floating drug delivery system of various drugs.

Mucoadhesive drug delivery is one of the approaches to design a formulation, which can adhere to the lining of the stomach, thus retaining the drug at the absorption site for a prolonged period. This concept utilizes the phenomenon of bioadhesion—an adhesive interaction between the dosage form and the biological surface. It has been suggested that a delay in GI transit, brought about by an intimate and extended contact between bioadhesive systems and mucus/mucosal lining, will improve drug bioavailability and duration of action. A prolonged retention at the mucosal surface provides intimate contact between the dosage form and absorbing tissues which results in a prolonged period of drug exposure to the region. Therefore, an increased retention time is a desirable property of bioadhesive drug delivery systems (Lehr, 1991).

In the present study a non-steroidal anti-inflammatory drug [NSAID], aceclofenac was selected as a model drug. Aceclofenac has been indicated for various conditions like post-traumatic pain, rheumatoid arthritis, ankylosing spondylitis. It is one of the emerging NSAID molecules for arthritis treatment (Yesmin, Mesbah, Muhammad, Susmita, & Tasnuva, 2008). The molecule is practically insoluble in water, but almost totally absorbed from gastrointestinal tract, its biological half-life is 4 h and administered twice daily with single dose of 100 mg. To overcome the side effects associated with conventional administration of NSAIDs and increase the patient compliance, controlled release dosage forms have been formulated in the form of single unit and multiunit dosage forms. Compared to single unit dosage forms, multi unit drug delivery system avoid the variations in gastric emptying and different transit rates through the gastrointestinal tract (Beckett, 1980), release drugs in a more predictable manner (Follonier & Doelkar, 1992), and spread over a large area preventing exposure of the absorbing site to high drug concentration on chronic dosing (Davis et al., 1986). The successful treatment of arthritis depends on the maintenance of effective drug concentration level in the body for which a constant and uniform supply of drug is desired. Sustained release dosage forms deliver the drug at a slow rate over an extended period of time and achieve this object. Short biological half life (about 4 h) and dosing frequency more than one per day makes aceclofenac an ideal candidate for sustain release (Parfitt, 1999). Several synthetic polymers have been used to formulate multiunit dosage forms. Recently, much research efforts have been concentrated to develop drug-loaded mucoadhesive macromolecules using a natural polymer. However, its adverse effects are seen in patients with active or suspected peptic or duodenal ulcer or history of recurrent peptic or duodenal ulcer or who have gastrointestinal bleeding or other active bleedings or bleeding disorders. These concerns led to the objective of this study. The aim of the present work was to formulate aceclofenac entrapped LBG–alginate mucoadhesive macromolecules (beads) for the oral sustained release application using natural polymers (locust bean gum and sodium alginate) which could overcome the problems associated with oral sustained release systems like drug loaded calcium-alginate macromolecules, polyelectrolytes coated drug loaded alginate macromolecules.

Locust bean gum (LBG) has been utilized in the formulation of mucoadhesive macromolecules as fewer research works has been done on it (Vineet & Sokindra, 2012). Second, it exhibits an effective mucoadhesive property require for the desired formulation of mucoadhesive macromolecules. LBG is a high molecular weight branch polysaccharide and is extracted from the seeds of carob tree *Ceratonia siliqua*. It is a non-starch polysaccharides consisting of galactose and mannose in the ratio of 1:4 and hence they are known as galactomannan. It consists of a (1, 4)-linked β-D-mannopyranose backbone with branch points from their 6-positions linked to α-D-galactose. The mannose elements from a linear chain linked with branched galactopyranosyl residues at varying distance of parent chain as a function of the plant origin (Sharma, Dhuldhoya, & Merchant, 2008). The molecular weight of LBG ranges between 300,000 and 1200,000 Da. It is less soluble in water and needs heating to dissolve. Being non-ionic, its aqueous solubility is not affected by pH or ionic strength of the liquid medium. LBG is approved for food uses by the US Food and Drug Administration (FDA). Sodium alginate is chosen as an ion source (anionic) and it facilitates the mucoadhesive property of other non-ionic natural polymer. It is one of the most extensively studied natural, hydrophilic polysaccharide composed of D-mannuronic acid and L-guluronic acid residues. The interpenetrating polymeric networks (IPNs) formation between naturally occurring polysaccharides are reported in the literature for better mechanical strength and to obtained needful release behavior of entrapped drug. These include gellan gum–alginate (Rao et al., 2007), carrageenan–alginate (Mohamadnia, Zohuriaan-Mehr, Kabiri, Jamshidi, & Mobedi, 2007), guar gum–alginate (George & Abraham, 2007), carboxymethyl xanthan–alginate (Ray, Maity, Mandal, Chatterjee, & Sa, 2010) *etc*.

Hence, in present investigation LBG (non-ionic, natural mucoadhesive substance) and sodium alginate (anionic, release retardant) were used in different ratio to formulate and optimize lab-scale sustained release mucoadhesive macromolecules of aceclofenac by ionotropic gelation method using $CaCl_2$ as a crosslinker in order to extend the application of locust bean gum in mucoadhesive macromolecule formulation.

2. Experimental

2.1. Materials

Aceclofenac was received as a kind gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad–Gandhinagar, Gujarat, India. Locust bean gum powder (mean arithmetic particle size (d_{avg}) 255 μm, *M*/*G* ratio 4:1 and molecular weight 320,000 Da, specifications received from supplier) was received as a gift sample from Triveni Chemicals, Vapi, Gujarat, India. Sodium alginate (molecular weight 242 kDa, 80 cP viscosity of 0.1% (w/v) aqueous solution, particle size <375 μm) and calcium chloride were procured from Ozone Pvt. Ltd., India and Chemdyes Pvt. Ltd., India, respectively. All other chemicals and reagents were of analytical grade purchased from commercial supplier and used as received.

2.2. Characterization of powder of locust bean gum and sodium alginate

The needful physical characteristics like pH of aqueous solution, viscosity, swelling index (%w/w) and moisture content (%w/w) of both powder samples were performed individually according to following methods in order to explore their effect on desire goal of study. Both are nontoxic and safe to use for needful formulation of drugs according reports of supplier. The experiential results are shown in Table 1.

2.2.1. Determination of pH

The pH of 0.1%w/v aqueous solution of LBG and sodium alginate were noted by pH meter (EUTECH Instruments, Singapore).

2.2.2. Measurement of viscosity

0.1, 0.2 and 0.3%w/v aqueous solution of LBG (prepared by applying heat at temperature less than 50 °C and then cooled at 25 °C) and sodium alginate were prepared separately and their viscosity was determined at 25 °C, 10 rpm using TL6 spindle small sample adapter

Table 1
Observed needful characteristics like pH, swelling index (%w/w), moisture content (%w/w) and viscosity of powdered locust bean gum and sodium alginate.

Characteristics		Powdered locust bean gum	Powdered sodium alginate
pH (0.1%w/v aqueous solution at 25 °C)		5.9 ± 0.08	9.1 ± 0.22
Viscosity (cP) at 25 °C	0.1%w/v aqueous solution	70.56 ± 1.12	82.25 ± 1.34
	0.2%w/v aqueous solution	127.24 ± 2.50	139.50 ± 1.52
	0.3%w/v aqueous solution	168.30 ± 1.05	189.65 ± 2.32
Swelling index (%w/w)	pH 1.2	16.50 ± 1.45	41.45 ± 1.62
	pH 6.8	17.65 ± 0.82	52.50 ± 1.74
Moisture content (%w/w)		9.4 ± 0.17	8.8 ± 0.51

All values indicate mean ± S.D., $n = 3$.

(EXPERT L, Fungilab rotational viscometer, Barcelona, Spain). The viscosity in cPs was recorded in triplicates of each sample.

2.2.3. Measurement of swelling index (%w/w)

Equilibrium swelling measurements of both the powder samples were performed in triplicate in two different media (0.1 N HCl, pH 1.2 and phosphate buffer, pH 6.8) to exhibit the effect of pH for 2 h at ambient temperature. Accurately 0.5 g of dry samples was immersed in 10 ml of each media containing measuring cylinder, mixed thoroughly for 5 min and then left to swell for 2 h at ambient temperature. After 2 h, the supernatant media was removed from each cylinder and the swollen mass was recovered carefully. The excess media from swollen was removed using tissue paper and reweighed using a single pan electronic balance (XB 220A, Precisa Instruments Ltd., Switzerland) having accuracy up to fifth decimal. Percentage weight gained by the swollen mass was calculated using the formula (Yeole, Galgatee, Babla, & Nakhat, 2006):

$$\text{Swelling index (\%w/w)} = \frac{W_t - W_0}{W_0} \times 100 \quad (1)$$

where W_t is the weight of swollen mass at time t and W_0 is the initial dry weight of powder mass.

2.2.4. Measurement of moisture content (%w/w)

Moisture content was expressed as percentage weight loss on drying (%LOD). Two grams of powder sample (LBG and sodium alginate) was weighed and oven dried at 105 °C for 4 h to a constant weight. The experiment was done in triplicates and mean of triplicates was taken. The percentage loss on drying was then calculated using the formula:

$$\%\ \text{loss on drying} = \frac{\text{Weight of water in sample}}{\text{Total weight of wet sample}} \times 100 \quad (2)$$

2.3. Preparation of aceclofenac loaded LBG–alginate mucoadhesive macromolecules

The macromolecules were prepared by ionotropic gelation technique (Yesmin et al., 2008). According to described proportion as shown in Table 2, sodium alginate and LBG (mucoadhesive polymer) were dissolved in purified water (20 ml) to form a colloidal dispersive solution. Based on preliminary batches of drug loaded LBG–alginate macromolecules, fixed quantity of aceclofenac (0.8 g) was dispersed in above polymer dispersion and mixed thoroughly to form a smooth viscous dispersion in order to entrap added quantity of drug in polymeric macromolecules. Variables such as concentration and volume of $CaCl_2$ solution (5%w/v, 100 ml), stirring speed (50 rpm), and flow rate of addition of dispersed phase into dispersion medium (8–10 drops min^{-1}), and dropping distance from the tip of a needle to the hardening dispersion medium (3.5 cm) were fixed based on preliminary trials of batches in order to get desired strength, size and shape macromolecules. The resultant bubble free dispersion was dropped through 18 G syringe needle into calcium chloride solution (5%w/v, 100 ml), which was kept under stirring (50 rpm) to improve the mechanical strength of the macromolecules and also to prevent aggregation of the formed macromolecules. Immediate formations of LBG–alginate mucoadhesive macromolecules were developed. The developed macromolecules were retained in the calcium chloride solution for 30 min to complete the curing reaction. The formed macromolecules were collected by filtration and dried under sunlight for 1 day. The dried macromolecules were stored in tightly closed glass vials for further studies. Table 2 shows formulation composition of batches of mucoadhesive macromolecules.

2.4. Experimental design for optimization

In order to obtain "best" or an "optimized product" nine different formulations were generated using 3^2 factorial design. Amount of locust bean gum (X_1) and amount of sodium alginate (X_2) were taken as independent formulation variables while % drug entrapment efficiency (Y_1), % mucoadhesion at 8 h in phosphate buffer (pH 6.8) (M_8) (Y_2) and % drug release at 10 h ($Q_{10\,h}$) (Y_3) were considered as dependent or response variables. A statistical model incorporating interactive and polynomial terms was used to evaluate the responses. Design Expert® (Version 8.0.7.1) software was used for the generation and evaluation of the statistical experimental design.

The matrix of the design including investigated responses *i.e.* %DEE, M_8 (%) and $Q_{10\,h}$ (%) are shown in Table 2. The effects of independent variables were modeled using a quadratic mathematical equation generated by a 3^2 factorial design such as

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (3)$$

where Y is the response; b_0 is the intercept, and b_1, b_2, b_{12}, b_{11}, b_{22} are regression coefficients. X_1 and X_2 are individual effects; ${X_1}^2$ and ${X_2}^2$ are quadratic effects; X_1X_2 is the interaction effect. One-way ANOVA was applied to estimate the significance of models ($p < 0.05$). Individual response parameters were evaluated using the F-test. The surface response plots, and contour plots were analyzed to reveal the effect of independent factors (amount of LBG and sodium alginate) on the measured responses (%DEE, M_8 and $Q_{10\,h}$).

2.5. Percentage yield (%w/w)

The percentage yield of mucoadhesive macromolecules of each batch was calculated using the following formula:

$$\%\ \text{yield} = \frac{\text{Weight of dried beads}}{\text{Weight of drug} + \text{Weight of polymer}} \times 100 \quad (4)$$

2.6. Morphology and particle size analysis

Particle size of the prepared macromolecules was determined using a digital calibrated vernier caliper (For-Bro Engineers,

Table 2
Experimental plan of 3^2 factorial design (coded values in bracket) with observed response values for different formulations.

Experimental formulations batch code	Normalized levels of factors		%DEE (Y_1)	Responses (Y_1, Y_2, Y_3)	
	Amount of LBG (g) (X_1)	Amount of sodium alginate (g) (X_2)		M_8 (%) (mean ± S.D., n = 3) (Y_2)	Q_{10h} (%) (mean ± S.D., n = 3) (Y_3)
F1	0.1 (−1)	0.7 (−1)	56.37	60 ± 1.62	84.95 ± 2.02
F2	0.1 (−1)	0.8 (0)	63.33	65 ± 1.32	93.12 ± 1.99
F3	0.1 (−1)	0.9 (+1)	67.95	70 ± 1.66	89.47 ± 1.54
F4	0.2 (0)	0.7 (−1)	58.48	70 ± 1.57	85.02 ± 1.89
F5	0.2 (0)	0.8 (0)	62.52	75 ± 1.32	95.33 ± 1.56
F6	0.2 (0)	0.9 (+1)	68.54	80 ± 1.59	89.95 ± 2.11
F7	0.3 (+1)	0.7 (−1)	58.30	75 ± 1.62	86.25 ± 1.99
F8	0.3 (+1)	0.8 (0)	62.18	75 ± 1.58	87.23 ± 2.21
F9	0.3 (+1)	0.9 (+1)	66.45	85 ± 1.83	90 ± 1.54

DEE is the drug encapsulation efficiency; M_8 is the percentage mucoadhesion at 8 h in phosphate buffer (pH 6.8); Q_{10h} is the percentage cumulative drug release from LBG–alginate beads at 10 h.

Mumbai, India). Size of 20 dried macromolecules from each batches were measured for calculating the mean diameter of macromolecules (n = 3). Shape and surface morphological examination of the surface structure of dried macromolecules was carried out by scanning electron microscopy (JEOL, JSM 5160, New Delhi, India).

2.7. Drug entrapment efficiency (%DEE or PDEE)

Accurately weighed 100 mg drug loaded macromolecules were crushed in glass mortar and pestle and the powdered mass was transferred in a volumetric flask (50 ml) containing few ml of methanol. It was diluted to the mark using methanol and kept it for 10 min. It was allowed to stir for 2 h at 100 rpm on magnetic stirrer (5 MLH Plus, Remi Laboratory Instruments, India), then it was filtered using a 0.45 μm filter paper (MF-Millipore membrane filter). Drug content in the filtrate was determined by spectrophotometrically. %DEE was calculated using the formula:

$$\text{Entrapment efficiency} = \frac{\text{Actual drug content (\%)}}{\text{Theoretical drug content (\%)}} \times 100 \quad (5)$$

2.8. Drug excipients compatibility study

Drug excipient compatibility study was carried out by FTIR and differential scanning calorimetry studies.

2.8.1. Fourier transform infrared (FTIR) spectroscopy

Drug excipients interaction was checked out by comparing the IR spectra of pure drug aceclofenac, polymers (LBG and sodium alginate) and IR spectra of the formulation. All samples in powders were triturated in a small size mortar and pestle until the powder became fine and uniform. The spectral scanning was carried out by ATR (Bruker Opus, Germany) in the wavelength region between 4000 and 600 cm^{-1} and at a resolution 4 cm^{-1} by keeping dried sample on Zinc selenide crystal. Stack all the IR spectra using Opus software (Vineet & Sokindra, 2012).

2.8.2. Differential scanning calorimetry (DSC) study

Differential scanning calorimetry (DSC) was performed on pure drug, locust bean gum, sodium alginate, placebo mucoadhesive macromolecules (beads) and drug-loaded LBG–alginate mucoadhesive macromolecules (beads). DSC measurements were done on modulated DSC (Shimadzu, Japan). About 3.0 mg of sample was placed in an aluminum pan and then hermetically sealed with an aluminum lid. The thermograms were obtained at a scanning rate of 5 °C min^{-1} over a temperature range of 0 to 300 °C under an inert atmosphere flushed with nitrogen at a rate of 20 ml min^{-1} (Yassin, Alsarra, & Al-Mohizea, 2006).

2.9. Swelling study

Swelling extent was measured in terms of percent (%) weight gain by the macromolecules in triplicate as a function of pH. An amount of 20 mg macromolecules of each batch were immersed in petri plates containing 4 ml of 0.1 N HCl (pH 1.2) triplicately. At the end of 1 h, macromolecules were withdrawn from respective petri plates and reweighed after removing the excess water using blotting papers. The weight measurements of swollen macromolecules was determined using a single pan electronic balance (XB 220A, Precisa Instruments Ltd., Switzerland) having accuracy up to fifth decimal. For every 1 h weight of macromolecules was noted, and process was continued till the end of 10 h. The macromolecules were handled carefully in order to avoid any mass loss due to breaking or erosion. Percentage weight gained by the macromolecules was calculated using Eq. (1) (Yeole et al., 2006). Same procedure was carried out in triplicate using phosphate buffer (pH 6.8) and percentage weight gained by macromolecules was calculated.

2.10. In-vitro wash off test

Mucoadhesive property of various formulations of aceclofenac loaded macromolecules was evaluated by the *in vitro* wash-off method. A freshly excised piece of goat intestinal mucosa (3×3 cm^2) was mounted on a glass slide using thread. About 50 macromolecules were spread out on each piece of mucosa and then it was hanged from the arm of the tablet disintegration test apparatus. A regular up and down movement was give to the tissue specimen in a vessel containing 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5 °C. The adherence of macromolecules was regularly observed. The macromolecules that remained adhered to the mucosa was counted at regular intervals for up to 10 h. Same procedure was followed by replacing 0.1 N HCl with phosphate buffer of pH 6.8. Disintegration test apparatus was stopped at different time intervals up to 10 h and the number of macromolecules still adhering to the tissue was counted and % mucoadhesion was calculated (Ritesh, Anuratha, & Anil, 2012).

2.11. In-vitro drug release study

In-vitro release of aceclofenac from the macromolecules of each batch was carried out in the simulated gastrointestinal condition by the pH change method at 37 ± 2 °C (Yesmin et al., 2008). A solution of 0.1 N HCl (0.1% SLS, pH 1.2) was used to represent the gastric condition; pH 6.8 (phosphate buffer) is a compromise condition between the pH of the gastric and small intestine. Dissolution process was carried out in USP apparatus-I (basket method) at 50 rpm by taking macromolecules equivalent to 200 mg aceclofenac in

900 ml of 0.1 N HCl containing 0.1% SLS media for first 2 h, followed by 900 ml of pH 6.8 phosphate buffer for 10 h. Total process was continued for 12 h. Aliquots of 5 ml were withdrawn every half an hour and replaced an equivalent amount of fresh dissolution media equilibrated at the same temperature. The aliquots solution was diluted suitably, filtered and analyzed at 274 nm using a UV visible spectrophotometer. All the release studies were conducted in triplicate ($n = 3$).

2.12. Analysis of in vitro drug release kinetics and mechanism

In order to investigate the mechanism of drug release, the data were fitted to various release kinetic model equations such as zero order (cumulative % aceclofenac release *vs.* time), first order (log cumulative of % drug retaining *vs.* time), Higuchi's square root of time model (cumulative % aceclofenac release *vs.* square root of time), Hixson Crowell cube root plot (cube root of drug % remaining in matrix *vs.* time) and Korsmeyer–Peppas kinetic plot (fraction release of drug *vs.* time).

The zero order rate Eq. (6) describes the system where the drug release rate is independent of its concentration. The first order Eq. (7) describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (8). The Hixson–Crowell cube root law Eq. (9) describes the release from systems where there is a change in surface area and diameter of particles. The Korsmeyer–Peppas exponential model Eq. (10) describes the drug transport mechanism.

$$C = K_0 t \tag{6}$$

where K_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$\text{Log}\, C = \frac{\text{Log}\, C_0 - Kt}{2.303} \tag{7}$$

where C_0 is the initial concentration of drug and K is the first order rate constant.

$$Q = Kt^{1/2} \tag{8}$$

where Q is the amount of drug released at time t and K is the diffusion rate constant.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} t \tag{9}$$

where Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in the bead, and K_{HC} is the rate constant for Hixson–Crowell rate equation.

$$\frac{M_t}{M_8} = Kt^n \tag{10}$$

where M_t/M_8 is the fractional release of the drug, t is the release time, K is a constant incorporating structural and geometric characteristic of the release device and the diffusional exponent 'n' is dependent on the geometry of the device as well as the physical mechanism for release. The values of n for a cylindrical shaped device are 0.43 for Fickian release. For non-Fickian (anomalous) release, 'n' value is 0.5 to 1.0; for quasi-Fickian diffusion, $n < 0.5$; for Fickian diffusion (square root of time kinetics), $n = 0.5$; for zero order release case II transport (relaxation controlled), $n = 1.0$; for super case transport II, $n > 1.0$.

3. Results and discussion

3.1. Characterization of powder of locust bean gum and sodium alginate

The pH of 0.1%w/v aqueous solution of LBG powder and sodium alginate were found to be 5.9 ± 0.08 and 9.1 ± 0.22, respectively, as shown in Table 1 indicating LBG as slightly acidic and sodium alginate as alkaline in nature.

According to Table 1, the viscosity of aqueous solution of LBG as well as sodium alginate increased gradually with their proportion at low rpm indicating their nature as highly branched random coils in composition and also due to water uptake capacity of their polysaccharide units. LBG is composed of galactose and mannose units (mannose to galactose content 4:1) and the longer the galactose side chain greater will be the viscosity. The viscosity of sodium alginate solution was dependent on its concentration and length of alginate molecules or the number of monomer units in the chain. The characteristic of viscosity was helped in the preparation of aqueous dispersion containing LBG and sodium alginate, selection of their proportion with respect to needful quantity of addition of drug as well as its effect on selection of needle size for extruding them in continuous phase without blockage. Based on viscosity characteristics, 18 G needle and proportion of LBG to sodium alginate were fixed keeping fixed amount of aceclofenac (0.8 g) in optimization batch preparation.

From the swelling study (Table 1), it was observed that LBG powder showed least swelling in both pH 1.2 and pH 6.8 media (16.50 ± 1.45%w/w and 17.65 ± 0.82%w/w, respectively). In both the media, LBG powder did not any significant difference in swelling behavior. This was due to its non ionic nature. Natural gums generally on hydration swell rapidly forming the viscous gel layer on the surface, which increases the diffusion path length and slows down the solvent imbibition. Moreover, as it is a galactomannan, it has lesser tendency to interact with water molecule due to its steric bulkiness and random coil nature (Lundin & Hermansson, 1995). On the other hand, sodium alginate in powder form exhibited higher swelling in both pH 1.2 and pH 6.8 media (41.45 ± 1.62 and 52.50 ± 1.74%w/w, respectively) in comparison to LBG powder. Being polyelectrolyte, sodium alginate was exhibited higher swelling in acidic media as compared to LBG powder. The higher swelling of sodium alginate powder in pH 6.8 with compare to pH 1.2 might be due to its acid resistant nature toward acidic pH. The swelling extent of sodium alginate will be affected during blending with LBG and crosslinking with $CaCl_2$ following their concentration. This study was helped in selection of ratio of LBG to sodium alginate with respect to $CaCl_2$ as cross-linker to get desired oral sustained release mucoadhesive macromolecules of aceclofenac.

The moisture content was calculated as percentage loss on drying to determine purity of both polymers. It was found to be 9.4 ± 0.17% for LBG and 8.8 ± 0.51% for sodium alginate (Table 2). Pharmacopoeial lime for moisture content of natural gums has been set at $\leq$15.0%. The moisture content of the LBG and sodium alginate was within the limit set for natural gums. If natural materials like gums contain excess moisture it is therefore, important to assess the moisture content of natural gums which indicate to certain extent its stability.

3.2. Optimization

A 3^2 factorial experimental design technique was employed to investigate the effect of independent variable on dependent variables like % drug entrapment efficiency, % mucoadhesion at 8 h and

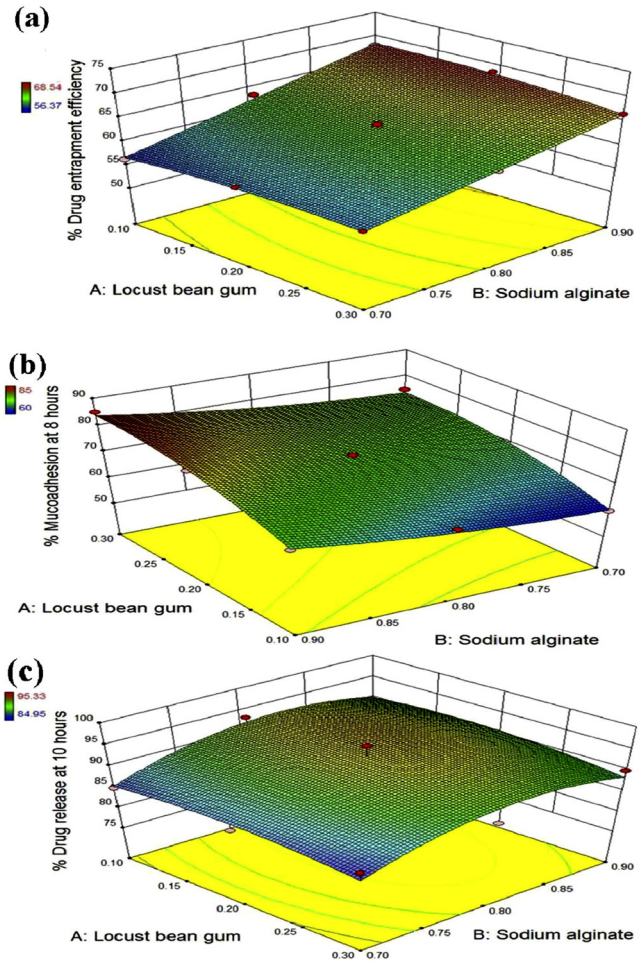

Fig. 1. Three-dimensional response surface plots showing the effect of amount of LBG (g) 'X_1' and sodium alginate (g) 'X_2' on responses (a) DEE (%), (b) mucoadhesion at 8 h 'M_8' (%), and (c) $Q_{10\,h}$ (%).

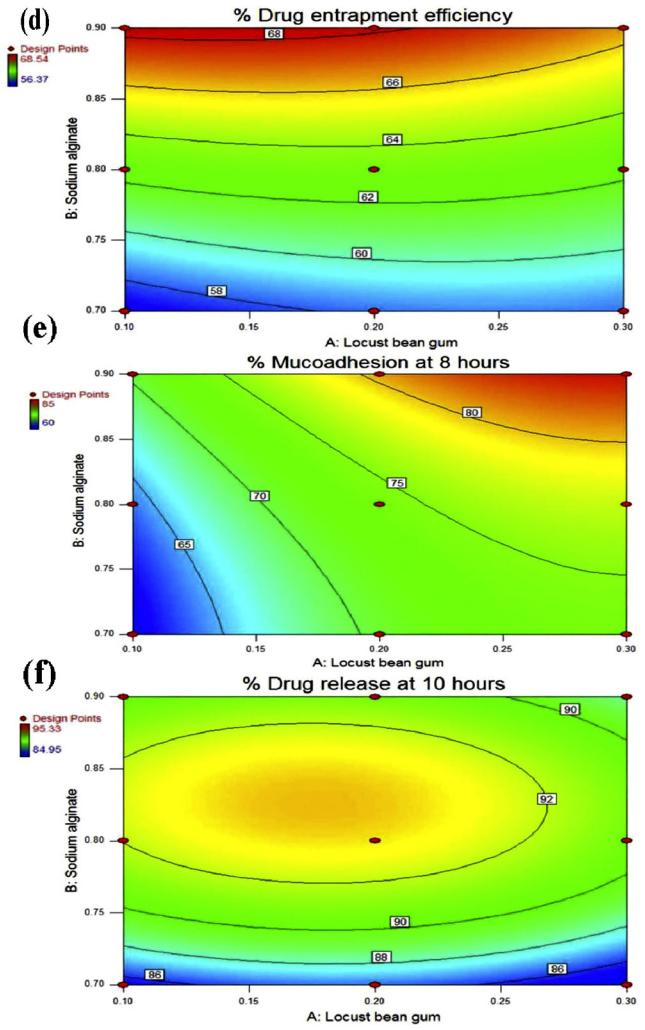

Fig. 2. Contour plots showing the effect of amount of LBG (g) 'X_1' and sodium alginate (g) 'X_2' on responses (d) DEE (%), (e) mucoadhesion at 8 h 'M_8' (%), and (f) $Q_{10\,h}$ (%).

% drug release at 10 h using the Design Expert® Software (Version 8.0.7.1).

3.2.1. *Effect of formulation variable on % Drug entrapment efficiency (Y_1)*

The R^2 (0.9896) was high indicating the adequate fitting of the quadratic model. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; *i.e.* positive or negative. The % drug entrapment efficiency of all batches was within 56.37 to 68.54%. Among the nine batches F6 showed highest % drug entrapment efficiency, this may be due to a high level of sodium alginate which allows the drug to get entrap more as compared to formulation containing a low level of sodium alginate. Locust bean gum does not affect the % drug entrapment efficiency as it is neutral polymer and only shows mucoadhesive property. F3, F6 and F9 contain the highest concentration of sodium alginate so they showed highest % drug entrapment efficiency than other batches. The % drug entrapment efficiency increases with increase in the concentration of sodium alginate. The effect of formulation variable on % DEE is presented by response surface plot and contour plots in Figs. 1(a) and 2(d), respectively. The polynomial equation for % drug entrapment efficiency was calculated using the equation:

$$Y_1 = 8.51 + 95.15X_1 + 63.30X_2 - 83.50X_1X_2 - 73.50X_1^2 + 2.0X_2^2 \quad (11)$$

3.2.2. *Effect of formulation variables on % mucoadhesion at 8 h in phosphate buffer pH 6.8 (M_8) (Y_2)*

The R^2 (0.9756) was high indicating the adequate fitting of the quadratic model. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; *i.e.* positive or negative. The % mucoadhesion at 8 h of all batches was within 60 ± 1.62 to 85 ± 1.83%. Among the nine batches F9 showed highest % mucoadhesion at 8 h in phosphate buffer pH 6.8, this may be due to a high level of locust bean gum. The batches containing higher level of locust bean gum showed significantly greater mucoadhesive property in phosphate buffer (pH 6.8) may be due to the presence of a certain amount of unionized hydroxyl groups within locust bean gum which forms a strong gel network with the mucus glycoprotein network of the intestinal mucosa, which results in formation of stable mucoadhesive joint and explains the large force required to detach the mucoadhesive dosage form from the mucosal surface. Macromolecules of batch F6 showed 80 ± 1.59% mucoadhesion at 8 h (M_8) although containing medium level of locust bean gum because of it contains the highest level of sodium alginate as it shows little mucoadhesive property. The effect of formulation variable on M_8 (%) is presented by response surface plot and contour plots in Figs. 1(b) and 2(e), respectively. The polynomial equation for % mucoadhesion at 8 h in phosphate buffer (pH 6.8) was calculated

using the equation:

$$Y_2 = 113.88 + 200.0X_1 - 216.66X_2 - 3.77X_1X_2 - 333.33X_1^2 + 166.66X_2^2 \quad (12)$$

3.2.3. Effect of formulation variables on % drug release at 10 h (Q_{10h}) (Y_3)

The R^2 (0.7227) was high indicating the adequate fitting of the quadratic model. The polynomial equations can also be used to draw conclusions considering the magnitude of co-efficient and the mathematical sign it carries; *i.e.* positive or negative. The % drug release at 10 h of all batches was within 84.95 ± 2.02 to 95.33 ± 1.56%. Among the nine batches F5 showed highest % drug release at 10 h, this may be due to a entrapment of drug occurs in an ideal way and medium concentration of sodium alginate cannot get entrapped whole amount of drug and so some particles of the drug remained on the surface which releases first while dissolution. In case of batches containing highest concentration of sodium alginate entrapped whole amount of drug and releases drug slowly than other batches and whole amount of drug available lately. The effects of formulation variables on $Q_{10\,h}$ (%) are presented by response surface plot and contour plots in Figs. 1(c) and 3(f), respectively. The polynomial equation for % drug release at 10 h was calculated using the equation:

$$Y_3 = -207.10 + 72.50X_1 + 711.71X_2 - 19.25X_1X_2 - 159.66X_1^2 - 428.66X_2^2 \quad (13)$$

Validation of factorial design was carried out using Design Expert® software (Version 8.0.7.1) and check point was generated. Amount of two variables were found and new batch of LBG–alginate mucoadhesive macromolecules was prepared by using that amount of two variables. Batch F6 showed actual value nearby to new generated batch and so batch F6 was confirmed as optimized batch (Table 3).

3.3. FTIR study

The characteristics absorption peaks of aceclofenac were obtained at 1566.92, 3314.98, 1247.11, 1442.41, 1766.70, 1710.17, 1340.95, 742.91 and 664.28. The principle peaks obtained for the combination were almost similar to that of the drug. The possibility of interaction was ruled out as there was no major shift in the absorption bands of drug and the formulation. The frequencies of functional groups of drug aceclofenac remained intact in IR spectra of formulation so it was concluded that there was no interaction occurred between the drug and excipients used in the study (Fig. 3).

3.4. DSC study

In an effort to investigate the possible physical and chemical interactions between drug and polymer, DSC study was carried out of pure aceclofenac, locust bean gum, sodium alginate, placebo LBG–alginate mucoadhesive macromolecules (beads) and drug loaded LBG–alginate mucoadhesive macromolecules (beads). The results are displayed in Fig. 4. The DSC thermogram showed a sharp endothermic peak at 149.32 °C for pure aceclofenac as the melting point of the drug. The DSC thermogram showed a endothermic peak at 197.12 and 119.45 °C for locust bean gum and sodium alginate, respectively.

Table 3
Results of experiments for conforming optimization capability.

Experimental formulation batch code	Factors		Responses (Y_1, Y_2, Y_3)									
	Amount of LBG (g) (X_1)	Amount of sodium alginate (g) (X_2)	%DEE (Y_1)				M_8 (%) (Y_2)				Q_{10} (%) (Y_3)	
			PV	AV	Error (%)	PV	AV	Error (%)	PV		AV	Error (%)
F10	0.25	0.9	67.33	66.85	−0.712	83.01	82.67 ± 3.21	−0.409	90.06		89.39 ± 1.25	−0.743

DEE is the drug encapsulation efficiency; M_8 is the percentage mucoadhesion at 8 h in phosphate buffer (pH 6.8); $Q_{10\,h}$ is the percentage cumulative drug release from LBG–alginate beads at 10 h; LBG is the locust bean gum; SA is the sodium alginate; PV is the predicted value; AV is the actual value.

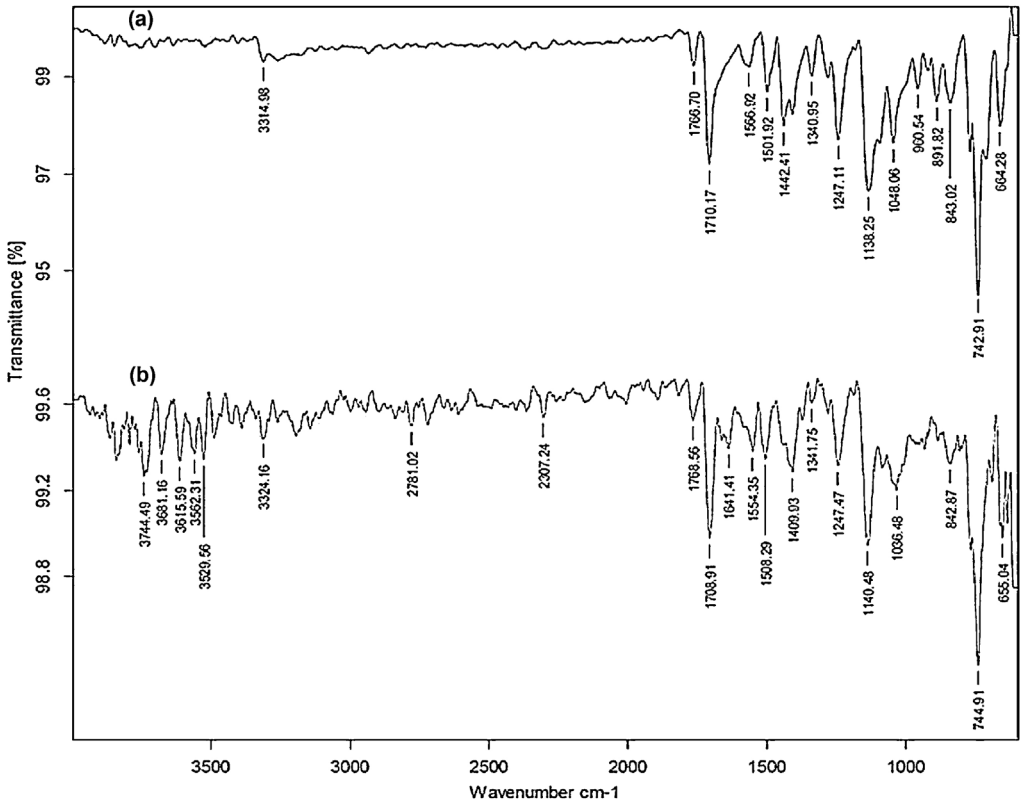

Fig. 3. FTIR Spectrum of (a) aceclofenac and (b) aceclofenac loaded LBG–alginate mucoadhesive macromolecules.

3.5. Percentage yield

The production yields of prepared formulations were in the range of 93.19 to 96.65%. This high yield of production is because all amount of the polymer is available for gelation into cross linking agent.

3.6. Morphology and Particle size analysis

Particle size of the 20 macromolecules was measured and average was calculated. The particle size of prepared formulations was in the range of 1.328 ± 0.11 to 1.428 ± 0.13 mm. The macromolecules were found to be discrete, uniform in size, spherical and free flowing. The SEM photographs (Fig. 5) indicated that the macromolecules were spherical and completely covered with the coat polymer, with a size range of 1000–1100 μm. The increase in polymer (sodium alginate) concentration showed increase in particle size and spherical nature of macromolecules might be due to more viscous nature of polymer solution. Also, the difference in the shape of macromolecules was observed, as the macromolecules containing higher amount of sodium alginate (Batch F6–F9) were more spherical and regular as compared to that of macromolecules having lower percent of sodium alginate (Batch F1–F3). Thus, sodium alginate has a great influence on the characteristics of macromolecules. The drug-loaded macromolecules were spherical and brownish in appearance.

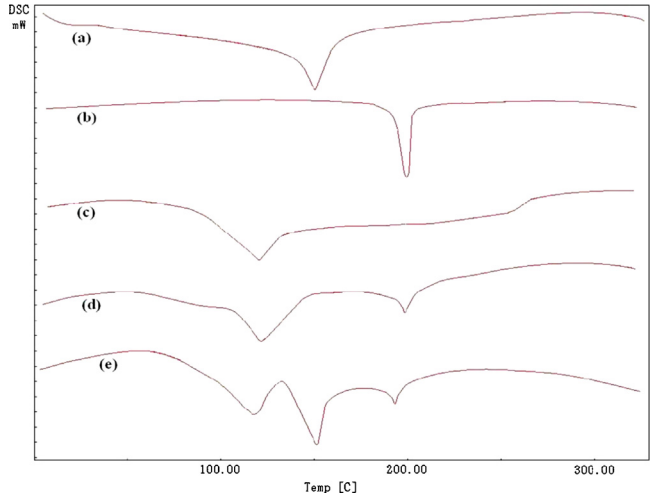

Fig. 4. DSC thermograms of (a) aceclofenac, (b) LBG, (c) sodium alginate, (d) placebo LBG–alginate mucoadhesive macromolecules and (e) aceclofenac loaded LBG–alginate mucoadhesive macromolecules.

3.7. Drug entrapment efficiency

Encapsulation efficiency of the macromolecules was dependent mainly on the concentration of sodium alginate; it was found that by increasing the concentration of sodium alginate, the encapsulation efficiency of the microcapsules also increases. It was found from literature that 5% $CaCl_2$ concentration was suitable for preparing macromolecules to provide proper hardness. Thus, increasing alginate concentration and taking 5% $CaCl_2$ concentration, increased microencapsulation efficiency of negatively charged drug. It was also assumed that gelation proceeds radially from the surface of the microcapsule to its centre. As gelation proceeds, water is expelled due to cross-links formed by the cations and the contraction of the gel volume. The entrapment efficiency was in the range of 56.37 to 68.54%.

3.8. Swelling study

The results of swelling studies, shown in Fig. 6(a) and (b), indicate that swelling varied with the pH of the medium. The

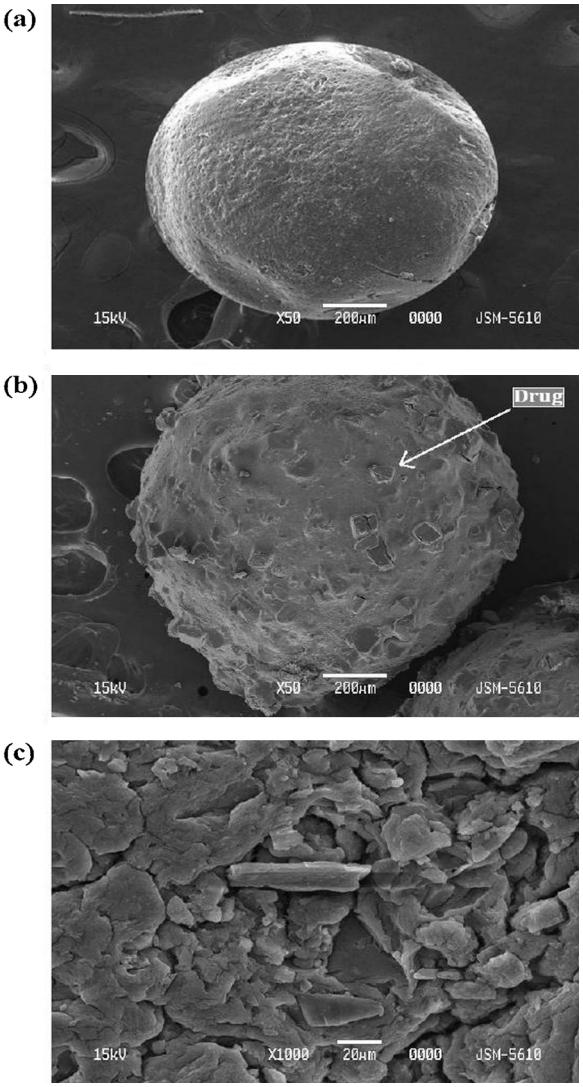

Fig. 5. SEM microphotographs of (a) placebo LBG–alginate muacoadhesive macromolecules, (b) drug loaded LBG–alginate mucoadhesive macromolecules and (c) surface of drug loaded LBG–alginate macromolecules.

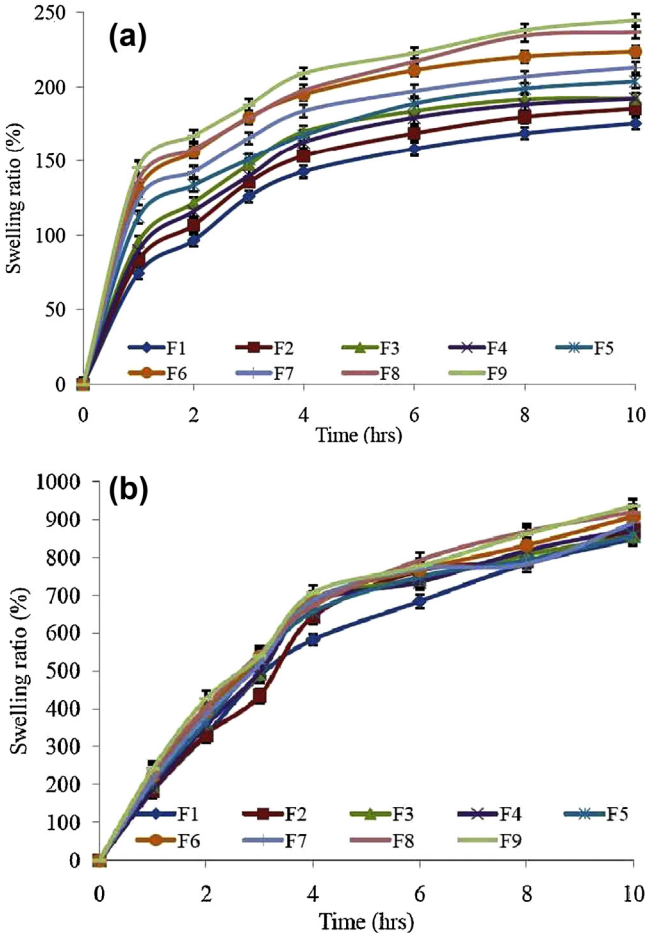

Fig. 6. Swelling behavior of LBG–alginate mucoadhesive macromolecules of all batches in 0.1 N HCl, pH 1.2 (a) and in phosphate buffer, pH 6.8 (b).

macromolecules did not show any sign of disintegration in both the media at pH 1.2 and 6.8 over a period of 10 h. However the swelling ratio of the macromolecules at pH 6.8 was considerably greater than that at pH 1.2. The swelling ratio of the formulations ranged from 175.65 to 244.85% in pH 1.2 and 848.70 to 935.55% in pH 6.8. The formulation F9 exhibited the highest swelling index in both the media. From this result it was observed that the swelling ratio was enhanced due to increased in polymer concentration in the formulations. Locust bean gum readily hydrates; absorb water with good degree of swelling. Diffusion of the drug significantly depends on the water content of macromolecules. As polymer chain becomes more hydrated and gel becomes more diluted, the disentanglement concentration may be reached (*i.e.* the critical polymer concentration below which the polymer chain disentangles and detaches from the gelled matrix) which result in swelling. Consequently, faster and greater swelling of macromolecules might lead to increased dimension of macromolecules resulting to an increasing diffusion pathway and thus, a reduction in diffusion rate. So, the drug release was found to be high initially and then gradually decreased.

3.9. *In-vitro wash off test*

The *in-vitro* wash off test using goat intestinal mucosa for assessing mucoadhesivity of LBG–alginate macromolecules containing aceclofenac was performed at both gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 6.8) for 10 h. In 0.1 N HCl, the percentage of macromolecules adhering to the goat intestinal mucosal tissue varied from 30 ± 1.29 to 45 ± 1.76% (Fig. 7(a)) over 8 h. Whereas, this was varied from 60 ± 1.62 to 85 ± 1.83% in phosphate buffer (Fig. 7(b)). The less mucoadhesion of locust bean gum–alginate macromolecules containing aceclofenac in 0.1 N HCl (pH 1.2) may be due to the erosion of calcium ion. The significantly greater mucoadhesive property of locust bean gum–alginate macromolecules in phosphate buffer (pH 6.8) may be due to the presence of a certain amount of unionized hydroxyl groups within locust bean gum which forms a strong gel network with the mucus glycoprotein network of the intestinal mucosa, which results in formation of stable mucoadhesive joint and explains the large force required to detach the mucoadhesive dosage form from the mucosal surface. Hence mucoadhesive property depends on concentration of polymers and polymer type. Therefore, the results of the wash off test indicated that the locust bean gum–alginate macromolecules containing aceclofenac possessed good mucoadhesivity in intestinal pH.

3.10. *In-vitro drug release study*

The *in-vitro* drug release from the macromolecules in 900 ml 0.1 N HCl (0.1% SLS) for 2 h, followed by 900 ml phosphate buffer pH

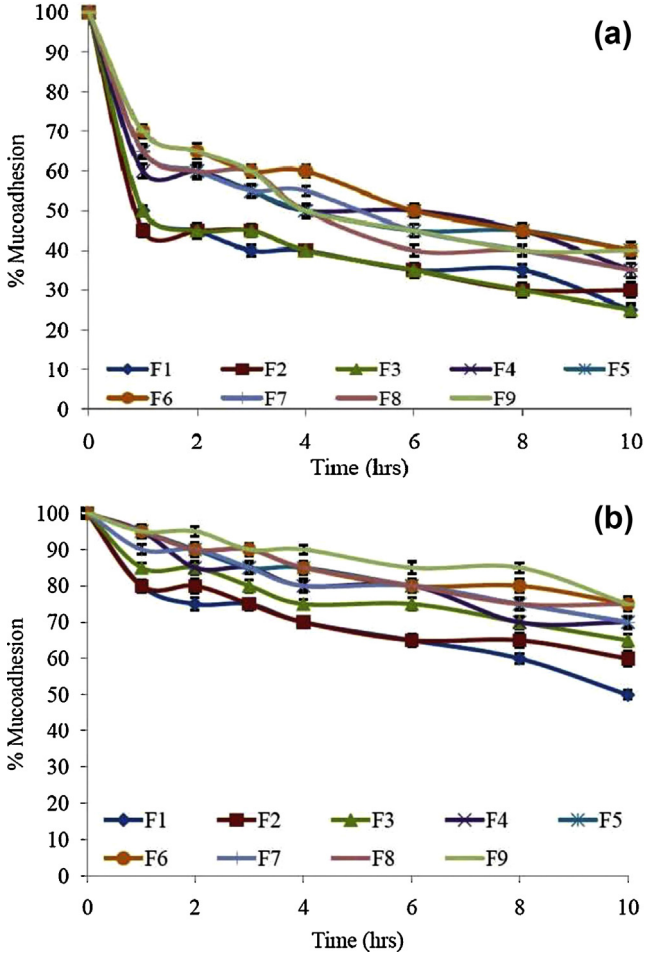

Fig. 7. Results of *in-vitro* wash off test of LBG–alginate mucoadhesive macromolecules of all batches in 0.1 N HCl, pH 1.2 (a) in phosphate buffer, pH 6.8 (b).

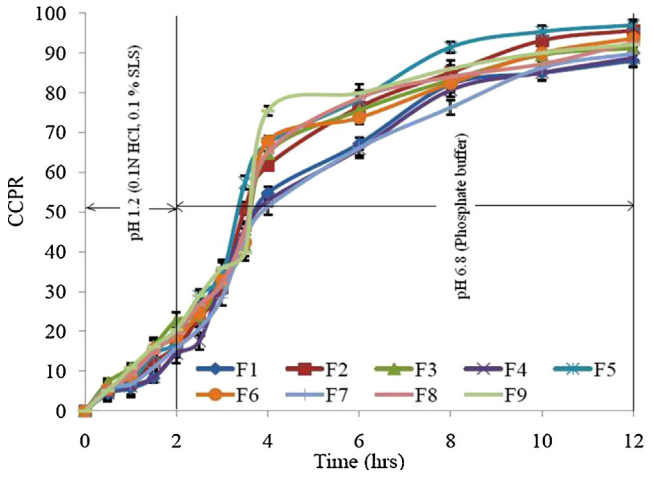

Fig. 8. Comparative *in vitro* dissolution profile of aceclofenac from LBG–alginate mucoadhesive macromolecules of all batches.

6.8 for 10 h was performed using the USP I Dissolution test apparatus. Aceclofenac release from the LBG–alginate mucoadhesive macromolecules (beads) was slow and depended on the composition of the coat. The differences in the drug release characteristics of various macromolecules are due to the differences in the porosity of the coat formed and its solubility in the dissolution fluid. It was observed that with the increase in the concentration of sodium alginate, the release of drug from macromolecules also increases as sodium alginate act as a release rate retardant. At pH 1.2, there was no discernible release of aceclofenac from macromolecules and but maximum drug releases in pH 6.8.

The *in-vitro* dissolution results showed that macromolecules were sustaining the drug release. The macromolecules were able to achieve the maximum solubility of the encapsulated drug. This may be due to the crosslinking. The reason for the sustained release might be due to the entanglement of polymer chains in the macromolecules because of strong ionic interactions. Thus, the penetration of the dissolution medium into the hydrogel macromolecules was made difficult. The drug release from these macromolecules was characterized by an initial phase of low release (burst effect) due to poor solubility of aceclofenac in pH 1.2. However, as gelation proceeded, the remaining drug was released at a slower rate followed by a phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics. Comparison of CCPR of all batches is illustrated in Fig. 8. The cumulative percentage drug release from the macromolecules of the all formulations ranged from 88.05 to 96.99% at 12 h time period. The formulation F5 exhibited the highest drug release at 12 h.

3.10.1. Kinetics and mechanism of drug release

In order to investigate the mechanism of drug release, the data were fitted to models representing zero-order, first order, Higuchi's square root of time, Korsmeyer–Peppas kinetic plot and Hixson–Crowell cube root plot. The examination of the coefficient of determination (R^2) indicated that drug release from the prepared macromolecules followed a diffusion controlled mechanism, since the R^2 values for first order (from 0.942 to 0.987) was always higher compared to zero-order (from 0.852 to 0.935), Higuchi model (from 0.893 to 0.927) and to the Hixson–Crowell ones (from 0.916 to 0.983). Most conventional dosage forms exhibits first order dissolution mechanism. Some modified release preparation, particularly prolonged release formulations, adheres to this type of dissolution pattern. Since the release from the prepared macromolecules followed a biphasic profile, it was decided to use a more stringent test

Table 4
Results of curve fitting of the *in vitro* aceclofenac release data from LBG–alginate mucoadhesive beads.

Experimental formulations batch code	Regression coefficient (R^2)					Release exponent (n)
	Zero order	First order	Higuchi	Hixson Crowell	Korsmeyer–Peppas	
F1	0.910	0.978	0.921	0.965	0.813	1.303
F2	0.902	0.983	0.912	0.976	0.795	1.275
F3	0.895	0.972	0.919	0.955	0.721	1.134
F4	0.915	0.982	0.914	0.970	0.814	1.293
F5	0.879	0.982	0.909	0.968	0.783	1.280
F6	0.895	0.977	0.914	0.963	0.762	1.206
F7	0.935	0.987	0.927	0.983	0.788	1.224
F8	0.884	0.971	0.913	0.951	0.759	1.204
F9	0.852	0.942	0.893	0.916	0.748	1.200

in order to distinguish between the mechanisms of drug release. The release data were fitted to the Peppas exponential model which characterizes the drug transport mechanism. The *n* values were in the range of 1.206 to 1.303, indicating that all the prepared formulations followed the first-order with Super case II transport release mechanism of aceclofenac ($n > 1.0$) (Table 4).

4. Conclusion

LBG–alginate mucoadhesive macromolecules (beads) containing aceclofenac were successfully prepared by ionotropic gelation method. This type of system shows a sustained drug release behavior due to the diffusion, swelling and mucoadhesive properties. By the result of optimization study the desired amount of natural polymer was obtained to make the mucoadhesive macromolecules which have good mucoadhesive property and shows better sustain release of drug. The particle size analysis revealed that the size of the macromolecules was found to be increase with increase in the concentration of polymers. SEM showed that the macromolecules were almost spherical and had rough surface. FTIR studies showed the presence of functional groups. No significant chemical interaction was observed. The *in-vitro* drug release study showed a slow release profile for LBG–alginate mucoadhesive macromolecules. From the *in-vitro* drug release study it was observed that the drug release decreased with increase in the sodium alginate concentration at some level. Drug release was diffusion controlled and followed first order kinetics. There was a significant difference in the release could be seen in 0.1 N HCL (0.1% SLS) and phosphate buffer (pH 6.8). The release rate was slower in acidic media than buffer media was observed. The macromolecules showed considerable slow swelling behavior in phosphate buffer (pH 6.8), which helped to release drug slowly and gave sustained drug release behavior.

Finally this study demonstrated the observations of spherical to oval shaped particles with high % DEE, strong mucoadhesive property and a possible sustained drug release profile from the LBG–alginate mucoadhesive macromolecules (beads). Thus, the prepared drug delivery system could be utilized for a possible sustained release product of aceclofenac.

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