RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

TITLE:

Fabrication and characterization of carboxymethylated Bael fruit gum with potential mucoadhesive applications

AUTHORS:

Atul Srivastava, Devegowda Vishakante Gowda*, Umme Hani, Chetan Govindrao Shinde and Riyaz Ali M. Osmani.

Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University,

Sri Shivarathreeshwara Nagara, Mysore-570 015, Karnataka, India

***CORRESPONDING AUTHOR:**

Dr. D.V. Gowda, Professor,

Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, Mysore-15, Karnataka, India

E-mail address: dvgowdajssuni@gmail.com

Tel.: +91 9482277850, Fax: 0821-2548359

Abstract

The study was conducted to enhance the mucoadhesive potential of bael fruit gum by carboxymethylation. Carboxymethylation of bael fruit gum was generated through its reaction with monochloroacetic acid in the presence of sodium hydroxide as a catalyst at different reaction conditions. The optimal degree of substituted carboxymethyl in the carboxymethylated bael fruit gum was found to be 0.68. The resulting product was characterized by FT-IR, DSC, XRD and SEM analysis. Results revealed that carboxymethylated derivative of bael fruit gum showed improved mucoadhesive potential as compared to unmodified gum, a slightly increased degree of crystallinity, surface roughness and decreased viscosity. Further, metformin-loaded, ionotropically gelled-beads of bael fruit gum and carboxymethylated bael fruit gum was formulated using calcium chloride as cross linking agent. An *ex vivo* bioadhesion study performed by a wash-off test using goat intestinal mucosa showed higher bioadhesion times for carboxymethylated bael fruit gum as compared to bael fruit gum. *In vitro* release study conducted using phosphate buffer (pH 6.8) showed a faster release of metformin from carboxymethylated bael fruit gum than bael fruit gum. These results have demonstrated that carboxymethylated bael fruit gum is a promising mucoadhesive excipient.

1. Introduction

The term bioadhesion in general may be characterized as the attachment or contact between two surfaces, with at least one being a biological substratum. If perhaps among the surfaces included is a mucosal layer, the term mucoadhesion is then utilized. Mucoadhesive polymers adhere to mucosal epithelial surface through non-specific interactions and have weak bioadhesion [1]. To enhance their functional properties, natural polymers are amenable to various chemical alterations, such as graft co-polymerization, oxidation, thiolation, carboxymethylation that results in imparting functional properties to these materials[2]. Among the specified methodologies, carboxymethylation is often utilized due to its ease of processing, lower chemical cost and product versatility. Carboxymethylation of the polysaccharide usually enhances the hydrophilicity and solution clarity and will result in an evident improvement in aqueous systems[3].

Bael fruit gum (BFG) is a non-ionic polysaccharide isolated from partially ripe fruits of *Aegle marmelos*, family Rutaceae. It has a backbone chain of (1→3)-linked, β-D-galactopyranosyl residues[4]. BFG is reported to contain a high content of d-galactose (71%), arabinose (12.5%), rhamnose (6.5%) and galactouronic acid (7%). The gum exhibits an optical rotation of $[\alpha]_D + 84^0$ in water[5]. BFG is widely used as adhesives, gelling agent, water-proofing substance, suspending agent, thickening agent; and also as a carrier for controlled release. Due to its easy accessibility in nature, biocompatibility, biodegradability and non-toxicity, it represents an alluring biopolymer for a number of pharmaceutical and biomedical applications[6].

Despite the fact that BFG and its derivatives have various advantages, however, like other polysaccharides they are identified with numerous drawbacks like easier susceptibility of microbial attack, pH dependent solubility and uncontrolled rates of hydration.

Carboxymethylation has emerged as a versatile modification technique not only in eliminating such drawbacks, but also improving its swelling and solubilization behavior[7].

Carboxymethylated bael fruit gum (CBFG) was characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), X-ray diffraction (XRD) and scanning electron microscopy (SEM) studies.

The degree of carboxymethyl substitution was determined in order to recognize the properties and quality of BFG, in addition to molecular weight, purity and crystallinity[8]. Diverse methods, like the coulometric, conductometric and acid wash methods[9], could be implemented in the direction of degree of substitution determination. In the present work degree of carboxymethyl substitution was determined by classical acid wash method[10].

The mucoadhesive performance of CBFG was assessed by formulating mucoadhesive beads. CBFG was comparatively evaluated with BFG for *ex vivo* bioadhesion study using freshly excised goat intestinal mucosa. Further, the beads of BFG and CBFG were comparatively evaluated for % entrapment, *in vitro* release and swelling behavior.

Metformin, an anti-diabetic drug, therapeutically indicated for the management of type-2 diabetes, has been used as a model drug because it shows a dose dependent, saturable transport with absorption limited to the upper part of the intestine. Previous studies have investigated the oral delivery of metformin using other bioadhesive polymers[11].

The objective of our study was to enhance the mucoadhesive properties of BFG by synthesizing CBFG conjugate and testing it through *ex vivo* and *in vitro* studies using metformin as a model drug.

2. Experimental

2.1 Materials

Unripe Bael fruit was obtained from the local market. Monochloroacetic acid and Calcium chloride were purchased from Merck specialties pvt. ltd., Mumbai, India. Metformin was obtained as a gift sample from Bal Pharma ltd. Bangalore. All other chemicals used were of analytical grade and obtained commercially.

2.2 Extraction of bael fruit gum (BFG)

The BFG was separated from the partially ripe bael fruits by using the method reported by Jindal *et al*., with some modifications[12]. Briefly, the gummy envelopes around each seed of partially ripe bael fruit were collected and mixed with 2% v/v glacial acetic acid solution. The suspension was kept in water bath for 45 min with continuous stirring and kept overnight. The suspension was filtered using nylon cloth to remove debris. To the clear filtrate, an excess of acetone was added where upon a brownish precipitate appeared. Finally, the precipitates were dried at 50 ^{0}C under vacuum. The BFG was obtained as a light brown fine powder.

2.3 Carboxymethylation of Bael fruit gum (CBFG)

The method applied is based on a procedure described by Dodi *et al*., with some modifications[13]. Briefly an aqueous dispersion of BFG (1.25%, w/v) dispersed slowly in ice cold sodium hydroxide (45%, w/w) with vigorous stirring at 0–8 ^{0} C for 30 min. To the above solution monochloroacetic acid (45%, w/v) was added with continuous stirring for 15 min. The reaction mixture was then heated in a thermostatic water bath at 75 ^{0} C and maintained for 1 h. The contents of the flask were shaken occasionally during the course of the reaction. The resulting mass, after cooling, was suspended in (80%, v/v) methanol and the suspension were then neutralized with glacial acetic acid. Finally, the mass was washed with methanol and dried initially at room temperature and then in vaccum oven at 70 ^{0} C for 60 min.

2.4 Characterization of CBFG

2.4.1 *Fourier transform infra-red spectroscopy (FT-IR)*

Both native and modified BFG samples were blended with solid KBr and subjected to FT-IR spectroscopy in a Fourier transform-infrared spectrophotometer (FT-IR, Shimadzu 8400 S, Japan) in the range of 4000-400 cm^{-1}.

2.4.2 *Differential scanning calorimetry (DSC)*

The thermograms of BFG and CBFG were recorded using differential scanning calorimeter (DSC Q2000) with a temperature range of 40-250 ^{0}C at a heating rate of 10^{0}C/min in nitrogen atmosphere. The runs were made in triplicate.

2.4.3 *X-ray diffraction analysis (XRD)*

BFG and CBFG samples were analyzed for crystallinity using a X-ray diffractometer (Bruker Focus D8). The powdered samples were scanned from 0◦ to 70◦diffraction angle (2θ) range under the following measurement conditions: source, Ni filtered Cu-Kα radiation; voltage 35 kV; current 25 mA; scan speed 0.05 ^{0}/min.

2.4.4 *Scanning electron microscopy (SEM)*

The morphological structure of BFG and CBFG were investigated using SEM analysis (JEOL, JSM-6100). The samples were coated with gold palladium alloy and mounted in a sample holder. The photomicrographs of the sample were taken at an accelerating voltage of 20 kV at different magnifications.

2.4.5 *Determination of degree of substitution*

The absolute degree of substitution was assessed employing classical acid wash method with few modifications[14]. Briefly, carboxymethylated sample of BFG (4 g) was dispersed in hydrochloric acid reagent (40 ml) for 3-4 h. The acid CBFG solution was then filtered and washed with 70 % methanol to remove the acid, followed by drying it in an oven at 70 ^{0}C until constant weight. The

dried CBFG sample was then dispersed in methanol (70 %, v/v) in Erlenmeyer flask and to this an excess of 0.5N sodium hydroxide was added. The reaction mixture was stirred for 3 h to dissolve the sample completely. The excess of sodium hydroxide was back titrated with 0.5 N hydrochloric acid using phenolphthalein as an indicator.

The degree of substitution (DS) of CBFG was calculated using the following equation:

$$DS = \frac{0.162A}{1-0.059A} \qquad (1)$$

Where, A is the miliequivalents of sodium hydroxide required per gram of the CBFG sample.

2.4.6 *Viscosity*

The viscosity profile of 2% (w/v) dispersion of BFG and CBFG was determined using Brookfield digital viscometer (Model RVDVE 230, Brookfield engineering laboratories, USA) at temperature of 25±1^0 C. The samples were prepared by dispersing BFG and CBFG in distilled water. The spindle number 63 was used at different shear rates.

2.5 Preparation of BFG and CBFG beads

Ionotropically gelled beads of BFG and CBFG were prepared using metformin as a model drug and calcium chloride as a cross linking agent. Briefly, 5 % w/v of BFG or CBFG sample was dispersed in an aqueous medium containing 0.5 % w/v of metformin. The obtained dispersion was extruded through #18G needle into an aqueous solution containing 5‑20% w/v of calcium chloride. The gelled beads were allowed to cross-link for 5 min followed by washing with distilled water and filtration. Afterwards, the beads were frozen for 4 h at -80 ^{0}C and then dried in a freeze dryer (Alpha 2-4 LD Plus, Martin Christ, Germany) at −90^{0}C, at 0.0010 mbar for 24 h.

2.6 Evaluation of BFG and CBFG beads

Metformin loaded BFG and CBFG were characterized for swelling study, entrapment efficiency, *in vitro* drug release behavior and *ex vivo* bioadhesion study.

2.6.1 *Swelling study*

To determine the swelling behavior, accurately weighed beads were soaked in 20 ml of phosphate buffer (pH 6.8) at 37± 0.5^0C and allowed to swell until a constant weight was reached. The beads were then removed and blotted with filter paper and their changes in weight were measured. The degree of swelling was calculated using the following formula,

$$\% \, Swelling = \frac{We - Wo}{Wo} X \, 100 \qquad (2)$$

where, W_e is the weight of swollen beads and W_o is the weight of dry beads.

2.6.2 *Entrapment efficiency*

Entrapment efficiency is the percentage of the actual mass of the drug loaded in the polymeric beads, related to the initial amount of loaded drug.

$$\% \, Entrapment \, efficiency = \frac{Actual \, drug \, content}{Theoretical \, drug \, content} X \, 100 \qquad (3)$$

An accurately weighed 25 mg of beads were dispersed in 100 ml of pH 6.8phosphate buffer for 30 min. Solution was filtered through 0.45 µm syringe filter and diluted appropriately. The drug contents in the beads were determined using a UV absorption spectrophotometer (Shimadzu 1801, USA) by measuring absorbance at 233nm.

2.6.3 *In vitro drug release study*

The *in vitro* release study of metformin from BFG and CBFG beads was comparatively evaluated using USP XXI dissolution apparatus, type II. The beads were subjected to dissolution study in 250 ml of pH 6.8 phosphate buffer as dissolution medium, maintained at 37±0.5 ^{0}C and stirred at a speed of 50 rpm. An accurately weighed 25mg of beads were enclosed in the muslin

cloth and it was tied to the paddle. Samples of 5 ml were withdrawn at predetermined time intervals and replaced with fresh media to maintain sink condition. The drug content of the withdrawn sample was analyzed spectrophotometrically using a UV absorption spectrophotometer (Shimadzu 1801, USA) by measuring absorbance at 233 nm[15].

2.6.4 *Ex vivo bioadhesion study*

The *ex vivo* bioadhesive properties of beads were performed using wash-off test method[16]. A freshly excised goat intestine was obtained from a local abattoir house (Mysore, India) within an hour of slaughter and transported to the laboratory at 4 ^{0}C isotonic saline solution. Intestinal tissue was excised and cleaned by washing with isotonic saline to remove any luminal contents. The excised intestinal tissue was attached to a glass slide using cyanoacrylate glue. About 100 BFG and CBFG beads were attached to the intestinal mucosal tissue by applying light force with a fingertip for 30 sec. The glass slide was fixed on to the arm of USP tablet disintegrating apparatus. This assembly was immersed in a beaker containing 900 ml of Phosphate buffer (pH 6.8) maintained at 37$\pm$0.5 ^{0}C. The number of beads bioadhered to intestinal sample was determined at regular intervals up to 24 h by slow, regular up and down movement of USP tablet disintegrating apparatus.

3. Results and discussion

The carboxymethylation of the BFG was carried out by Williamson's synthesis, which is an example of SN2 reaction[17]. The main reaction proceeds in two steps.

1. In the primary reaction, sodium hydroxide deprotonates the free hydroxyl groups of the gum to provide alkoxides groups.

$$\text{BFG-OH} + \text{NaOH} \longrightarrow \text{BFG-ONa} + \text{H}_2\text{O} \qquad (4)$$

2. The carboxymethyl groups are then formed by treating gum alkoxides with monochloroacetic acid through a SN2 reaction,

$$BFG\text{-}ONa + ClCH_2COOH \longrightarrow BFG\text{-}OCH_2COONa \quad (5)$$

A side reaction also occurs simultaneously results in the formation of sodium glycolates from sodium hydroxide and sodium monochloroacetate.

$$NaOH + ClCH2COONa \longrightarrow HOCH2COONa + NaCl \quad (6)$$

The side reaction is considerably slower than the main reaction and can be neglected for the conditions applied in this study.

Figure 1 shows the FT-IR spectrum of the BFG and CBFG in the frequency region from 4000-400 cm^{-1}. The spectra of BFG show a broad absorption band at 3442 cm^{-1} that corresponds to -OH stretching band of hydroxyl group, a peak at 2927 cm^{-1} attributed to C-H stretching of alkane, a peak at 1620 cm^{-1} and 1422 cm^{-1} due to asymmetrical and symmetrical C-O stretching of carboxylic acid. A band at 1046 cm^{-1} depicts the stretching vibration of C-O group which is characteristic of polysaccharides[12].

The spectra of CBFG shows a broad absorption band at 3425 cm^{-1} attributed to -OH stretching indicating that some -OH groups were not carboxymethylated. It's a subtle twist, but the 3425 cm^{-1} band shows that –OH groups are present. Moreover, the curves are different in both width and height between the BFG and CBFG curves indicates that some of the –OH groups reacted. The absorption band located at 2975 cm^{-1} corresponds to CH group stretching. The asymmetrical and symmetrical C-O stretching of carboxylic acid was assigned to 1644 and 1439 cm^{-1}, while the C-O stretch of carboxylic acid appears at 1059 cm^{-1}. These bands confirm carboxymethylation of BFG and were in agreement with the literature[2].

Thermal properties of BFG and CBFG were investigated using Differential Scanning Calorimetry. Figure 2 represents the DSC thermograms of BFG and CBFG. The DSC curve of BFG shows a broad endotherm at 105.3^0C with a heat of fusion of 342.6 J/g. The thermal curve of CBFG shows a broad endothermic peak at 118.65^0C with a heat of fusion 312.3 J/g. The shift in the endothermic peak and variation in the heat flow provided more proof on the insertion of carboxymethyl group.

Figure 3 shows the XRD spectra of BFG and CBFG. X-ray diffractogram of BFG is typical of amorphous materials with no sharp peaks while diffractogram of CBFG shows characteristic peaks at 21.6, 29.5, 37.5, 44.6, 52.3 (2θ) scale. Similar kind of observation has been reported by Kumar et al.[14] for gum kondagogu and Ahuja et al.[18] for xanthan gum. The peak intensity of CBFG is slightly greater which indicates an increase in crystallinity over BFG.

Figure 4 reveals the shape and surface morphology of BFG and CBFG particles using scanning electron microscope. It can be observed from the photomicrographs that the BFG and CBFG particles are polyhedral in shape (Fig. 4A and Fig. 4B). The surface morphology of native BFG (Fig. 4C) was observed to have an irregular but smooth surface. As observed, the conditions of modifications brought noticeable changes to the structure of CBFG and some of the particles get attached by adhering themselves. The morphology of CBFG particles (Fig. 4D) revealed rough porous surface than the BFG. The CBFG particles showed rough porous surface due to the cross linking of BFG. There are various small alveolate holes on the surface of CBFG which looked like surface corrosion. The alkaline treatment during the carboxymethylation process is responsible for the structural changes. This result also suggests that crystallinity of BFG was altered due to the loss of crystalline structure affected by strong alkaline conditions.

The degree of carboxymethyl substitution in BFG was found to be 0.68 of carboxymethyl group/g as determined by classical acid wash method. The main factors affecting the DS value in

the reaction of CBFG synthesis, were investigated. It includes the molar ratio of sodium hydroxide to monochloroacetic acid (mNaOH/mMCA), the volume of 70% methanol (V/V), the reaction temperature and the time of first step and second step reaction. Taking into consideration the fact that molar ratios of sodium hydroxide to monochloroacetic acid would distinctly affect the reaction rate and DS of CM-BFG; molar ratios of sodium hydroxide to monochloroacetic acid were varied and its influence on DS was as quoted in Table 1.

With the increment of mNaOH/mMCA, DS increases to the maximal value of 0.68; however, when the ratio added is more than 0.6, the DS decreases. This could be for the reason that increasing mNaOH/mMCA ratios would lead to enhanced NaOH consumption, whereas under present reaction condition NaOH amount was unchanged[19].

During the carboxymethylation process, for the reaction along with serving as a swelling agent, NaOH provides the alkaline environment to facilitate diffusion and penetration of the etherifying agent to the granular structure of BFG. More the monochloroacetic acid is, less sodium hydroxide can react with BFG, so that higher mNaOH/mMCA ratio led to lower DS[20]. The solvent medium's effect on the extent of reaction is related to the miscibility, the ability to solubilize the etherifying agents and to swell the biopolymer and to create an environment that favors carboxymethylation rather than glycolate formation Eq. (6). In this work, for the BFG carboxymethylation process, 70% methanol is used as reaction media.

Table 1 shows that DS increase as the volume of 70% methanol increased from 18 to 20 mL; after that a sharp decline was noted. The solvent content significantly affects etherifying reagents diffusion and absorption. Adding to that, swelling of BFG is also dependent on the solvent content and this would in turn increase the surface area for the reaction. The preliminary amplification in DS is accountable to these aspects. On the other hand, higher solvent content led

to agglomeration; which reduces contact among etherifying agent and BFG molecules, consequently leading to declined DS.

Furthermore, BFG carboxymethylation reaction was carried out at diverse temperatures to assess its effect. Outcomes reflected that with a rise in reaction temperature DS was increased noticeably followed by declination, independent of reaction steps (Table 1). An increase in temperature enhanced the ionic mobility of solutes in solution and also facilitated both the swelling of the BFG molecules and the diffusion of reactants[21]. The proportion of molecules possessing higher energy than that of activation energy rises with the rise in temperature, subsequently ensuing in augmented reaction rate and DS[22]. However, it has been observed that DS has reduced at temperature higher than 35^0C; which could be attributed to reaction medium volatilization.

The reaction time's effect of the first and second step on DS was scrutinized. The DS increases with the increase in reaction time and reaches a maximum; a significant decrease is observed on prolonging the time (Table 1). The enhancement of DS by prolonging the duration of reaction is a direct consequence of the favorable effect of time on swelling of BFG as well as the diffusion and adsorption of the reactants with the ultimate effect of better contacts between the etherifying agents and BFG. Alike annotations are quoted earlier by many researchers[23]. However, longer time resulted in no further increase in DS. Several researchers contemplated that the etherifying agents have maximum accessibility independent of extended reaction time.

The viscosity profile is generally considered as one of the important parameters to evaluate the feasibility of any gum or their derivative in industries. Figure 5 shows the viscosity-shear rate profile of BFG and CBFG solutions. It can be observed that apparent viscosity decreases with increase in the rate of shear. The viscosity of CBFG was less than that of BFG. Similar results were earlier reported for carboxymethylation of cellulose[24] and xanthan gum [18].

The low viscosity of CBFG solution may be attributed to a non-specific degradation at the reducing sugar unit, by β-elimination and /or peeling reaction during carboxymethylation process. The viscosity of BFG and CBFG solutions were observed to decrease slowly with increase in the rate of shear from 1-7 rpm. Further increase in the rate of shear from 7 to 30 rpm, the viscosity decreased rapidly. No further decrease in viscosity was observed for BFG and CBFG solutions when the rate of shear was increased from 30 to100 rpm.

In the present study, CBFG was formulated as beads for exploiting its mucoadhesive application using metformin as a model drug. As cross-linker concentration was increased from 5-20%, drug entrapment was found to be higher as a result of higher degree of cross-linking between CBFG and calcium chloride resulting in more viscous gelation and high degree of drug entrapment. Considering the fact, 20% calcium chloride was used to formulate metformin loaded beads. Entrapment efficiency of metformin in BFG and CBFG was found to be 22.56% and 31.23% respectively.

The SEM microphotographs revealed that metformin loaded-BFG beads were nearly spherical in shape having smooth surface (Fig. 6A), however the shape of metformin loaded CBFG beads were distorted with non-uniform and rough porous surface (Fig. 6B) [25].

Table 2 summarizes the results of *ex vivo* bioadhesion studies performed using goat intestinal mucosa by wash off method. It is evident from the results that the beads formulated using BFG showed 74% of bioadhesion while CBFG beads showed 87% of bioadhesion, after 24 h period of the study. Thus, carboxymethylation of BFG results in improved mucoadhesive characteristics which can be further utilized for developing mucoadhesive dosage form.

The swelling study revealed that metformin loaded beads of BFG hydrated quickly; having a % swelling of 62.4 % in the first hour compared to the beads formulated using CBFG which showed % swelling of 25.8 %. The reduced swelling behavior of CBFG may be due to its

ability to interfere with free access of water to the hydroxyl group of BFG. Further the beads of BFG eroded at a faster rate than the CBFG beads. The faster swelling and erosion of the BFG beads could be one of the reasons for their short bioadhesion time.

Figure 7 represents *in vitro* release profile of metformin from BFG and CBFG beads. It was observed during our studies that CBFG showed a biphasic release pattern i.e. burst release and slow-sustained release. This indicated a combined effect of diffusion and erosion mechanism for controlled drug release. An initial burst release of drug from CBFG beads was observed with 30% of drug released within the first 30 min for immediate effect followed by the slow release of the drug over a prolonged period of time for longer duration of action. The initial burst release of drug from CBFG beads may be attributed to decreased viscosity of CBFG. Due to decrease in viscosity, a greater amount of drug adsorb on the surface of CBFG beads during the gelation of beads. The slow and sustained release phase could be attributed to degradation by erosion or hydrolysis of CBFG beads. It can be observed from the results that the release rate is almost similar with both the formulation releasing 50% of the drug in 24 h. Thus carboxymethylation of BFG providing a means of enhancing the bioadhesion time without affecting the release rate.

4. Conclusions

In the present study BFG was modified successfully to obtain its carboxymethylated derivative, which was confirmed by FT-IR spectroscopy. With a view to optimize the synthesis conditions for a high degree of substitution; the factors that influence the synthesis, such as the ratio of monochloroacetic acid to sodium hydroxide, the volume of methanol and the influence of the reaction time and temperature have been illustrated in this study. DSC and XRD analyses confirmed the crystalline nature of CBFG. Scanning electron microscopy revealed the rough porous surface of CBFG. Viscosity of carboxymethylated derivative of BFG was found to be less

viscous than the BFG. Mucoadhesive applications of CBFG were explored by formulating ionotropically gelled beads of metformin, which requires further optimization of formulation and processing variables. It can be concluded that CBFG is a promising bioadhesive and biocompatible polymer, which should be explored for further applications in pharmaceutical technology. The results obtained will help to design experiments to evaluate the improved bioadhesive nature of CBFG through appropriate *in vivo* models.

Acknowledgments

The authors express their gratitude for the financial support provided by the Dept. of Science and Technology, Government of India (New Delhi, India) under grant **SR/SO/HS-0107/2010** to Atul Srivastava**.** The authors are grateful for the excellent technical assistance of Dr. Dattatri K. Nagesha, Associate Professor, Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University.

Declaration of interest

The authors report no conflicts of interest.

References

1. G.P. Andrews, T.P. Laverty and D.S. Jones, *Eur. J. Pharm. Biopharm*., 2009, **71**, 505-518.
2. M. Ahuja, S. Singh, and A. Kumar, *Int. J. Biol. Macromol*., 2013, **53**,114-121.
3. K.S. Parvathy, N.S. Susheelamma, R.N. Tharanathan and A.K. Gaonkar, *Carbohyd. Polym.,* 2005, **62**, 137-141.
4. R.K. Basak, P.K. Mandal and A.K. Mukherjee, Carbohyd. Res., 1981, **97**, 315-321.
5. P.K. Mandal and A.K. Mukherjee, Carbohyd. Res., 1980, **84**, 147-159.
6. N. Mahammed, D.V.Gowda, R.D. Deshpande and S. Thirumaleshwar, *Arch. Pharm. Res.,* 2015, **38**, 42-51.
7. L. Yang, T. Zhao, H. Wei, M. Zhang, Y. Zou, G. Mao and X. Wu, *Int. J. Biol. Macromol.,* 2011, **49**, 1124-1130.
8. K. Kurita, *Prog. Polym. Sci*., 2001, **26**, 1921-1971.
9. R.W. Eyler, E.D. Klug and F. Diephuis, *Anal. Chem*., 1947, **19**, 24-27.
10. O.A. El Seoud, H. Nawaz, E.P. Areas, *Molecules*., 2013, **18**, 1270-1313.
11. P.P. Ige and S.G. Gattani, *Arch. Pharm. Res*., 2012, **35**, 487-498.
12. M. Jindal, V. Kumar, V. Rana and A. K. Tiwary, *Food. Hydrocolloid*., 2013, **30**,192-199.
13. G. Dodi, D. Hritcu and M.I. Popa, *Cell. Chem. Technol*., 2011, **45**,171- 176.
14. A. Kumar, and M. Ahuja, *Carbohyd. Polym*., 2012, **90**, 637-643.
15. R. Sharma and M. Ahuja, *Carbohyd. Polym*., 2011, **85**, 658-663.
16. C. M. Lehr, J.A. Bouwstra, J.J. Tukker and H.E. Junginer, *J. Control. Release*., 1990, **13**, 51-62.

17. O. S. Lawal, M.D. Lechner, B. Hartmann and W.M. Kulicke, *Starch-Starke*., 2007,**59**, 224-233.

18. M. Ahuja, A. Kumar and K. Singh, *Int. J. Biol. Macromol*., 2012, **51**, 1086-1090.

19. W. Gao, X. Lin, J. Ding, X. Huang and H. Wu, *Carbohyd. Polym*., 2011,**84**,1413-1418.

20. W. Yanli, G. Wenyuan and L. Xia, *Carbohydr. Res.,* 2009, **344**, 1764-1769.

21. O. S. Lawal, M.D. Lechner and W.M. Kulicke, *Polym. Degrad. Stabil*., 2008, **93**, 1520-1528.

22. L. Wang, S. Pan, H. Hu, W. Miao and X. Xu. *Carbohyd. Polym.,* 2010, **80**,174-179.

23. P. Goyal, V. Kumar and Sharma P, *Carbohyd. Polym*., 2007, **69**, 251-255.

24. D. R. Biswal and R.P. Singh, *Carbohyd. Polym*., 2004, **57**, 379-387.

25. B. Yaacob, M.C.I. Mohd Amin, K. Hashim, B. Abu Bakar, *Iran. Polym. J.,* 2011, **20**, 195-204.

List of Tables

Table 1

Reaction parameters investigated for the carboxymethylation of bael fruit gum.

Formulation Code	Molar ratio (NaOH/MCA)	70%Methanol (V/V)	Temperature (T_1,^{0}C)	Time (t_1,min)	Temperature (T_2,^{0}C)	Time (t_2,min)	Degree of Substitution
CBFG-A	0.4	20	35	45	60	15	0.271
CBFG-B	0.5	20	35	45	60	15	0.486
CBFG-C	0.6	20	35	45	60	15	0.68
CBFG-D	0.7	20	35	45	60	15	0.623
CBFG-E	0.6	18	35	45	60	15	0.516
CBFG-F	0.6	19	35	45	60	15	0.561
CBFG-G	0.6	21	35	45	60	15	0.479
CBFG-H	0.6	22	35	45	60	15	0.443
CBFG-I	0.6	20	30	45	60	15	0.485
CBFG-J	0.6	20	40	45	60	15	0.461
CBFG-K	0.6	20	45	45	60	15	0.425
CBFG-L	0.6	20	35	40	60	15	0.543
CBFG-M	0.6	20	35	50	60	15	0.518
CBFG-N	0.6	20	35	60	60	15	0.478
CBFG-O	0.6	20	35	45	40	15	0.386
CBFG-P	0.6	20	35	45	50	15	0.528
CBFG-Q	0.6	20	35	45	70	15	0.512
CBFG-R	0.6	20	35	45	60	5	0.387
CBFG-S	0.6	20	35	45	60	10	0.476
CBFG-T	0.6	20	35	45	60	20	0.412

Table 2

Ex vivo bioadhesion study of metformin loaded BFG and Carboxymethylated BFG (n=3)

Time (h)	% Mucoadhesion of BFG	% Mucoadhesion of CBFG
0	100±0.24	100±0.35
2	96±0.41	98±0.33
4	93±0.19	97±0.27
8	88±0.32	95±0.18
16	81±0.21	91±0.31
24	74±0.26	87±0.28

Legends for the Figures

Fig. 1. FT-IR spectrum of BFG and CBFG.

Fig. 2. DSC thermograms of BFG and CBFG.

Fig. 3. XRD pattern of BFG and CBFG.

Fig. 4.SEM microphotographs showing the shape of (A) BFG, (B) CBFG and surface of (C) BFG, (D) CBFG

Fig. 5. Rheological behavior of aqueous solution of BFG and CBFG**.**

Fig. 6. SEM microphotographs of (A) BFG and (B) CBFG beads.

Fig. 7. *In vitro* release profile of metformin from BFG and CBFG beads

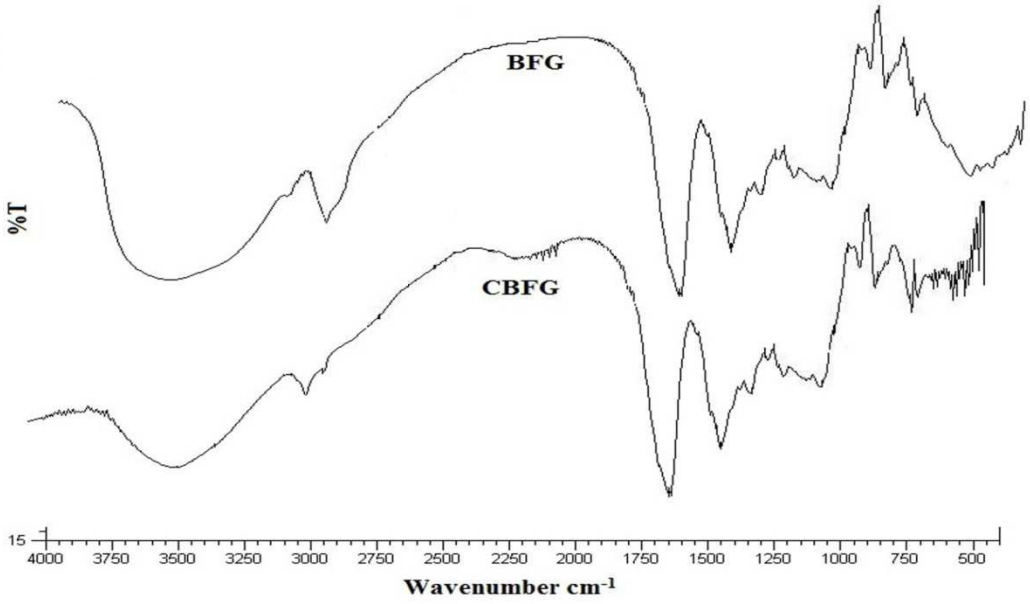

Fig. 1. FT-IR spectrum of BFG and CBFG.

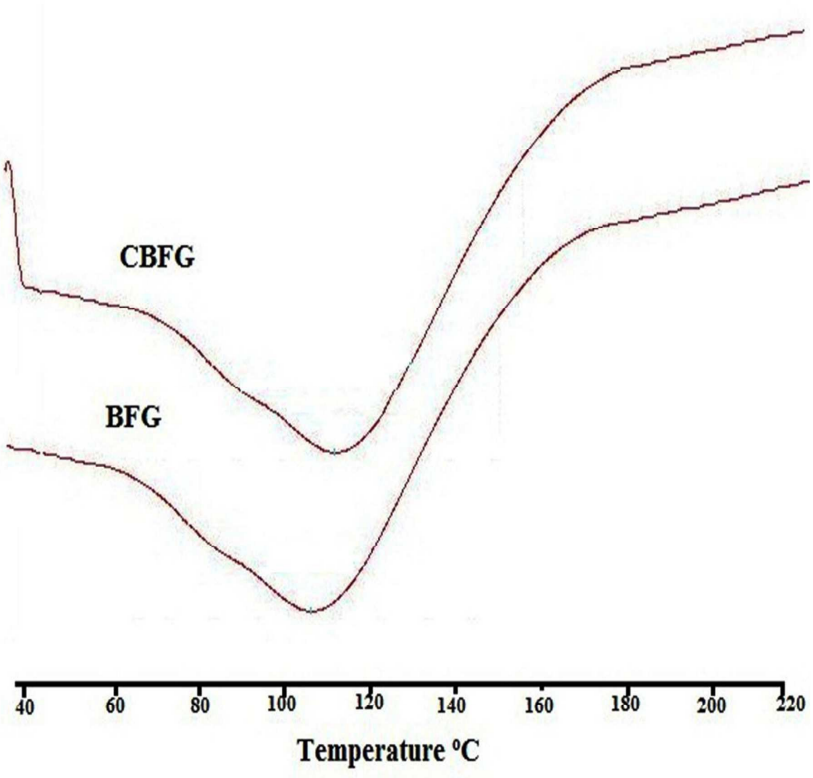

Fig. 2. DSC thermograms of BFG and CBFG.

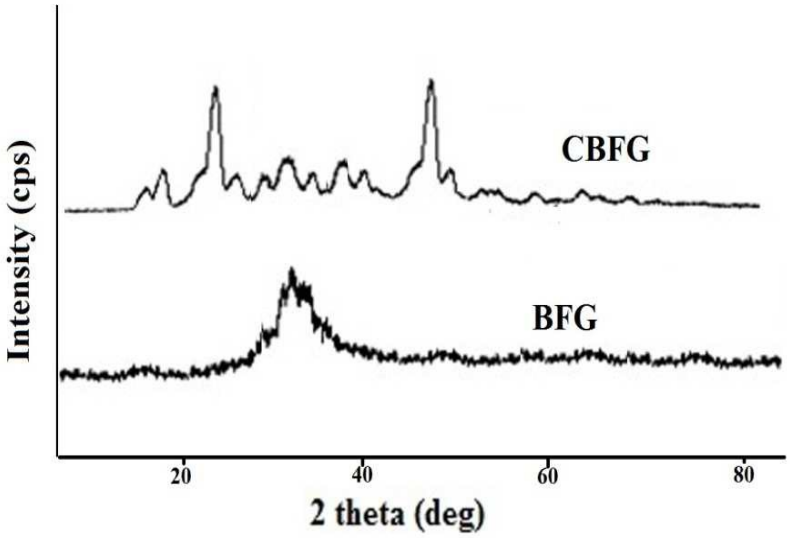

Fig. 3. XRD pattern of BFG and CBFG.

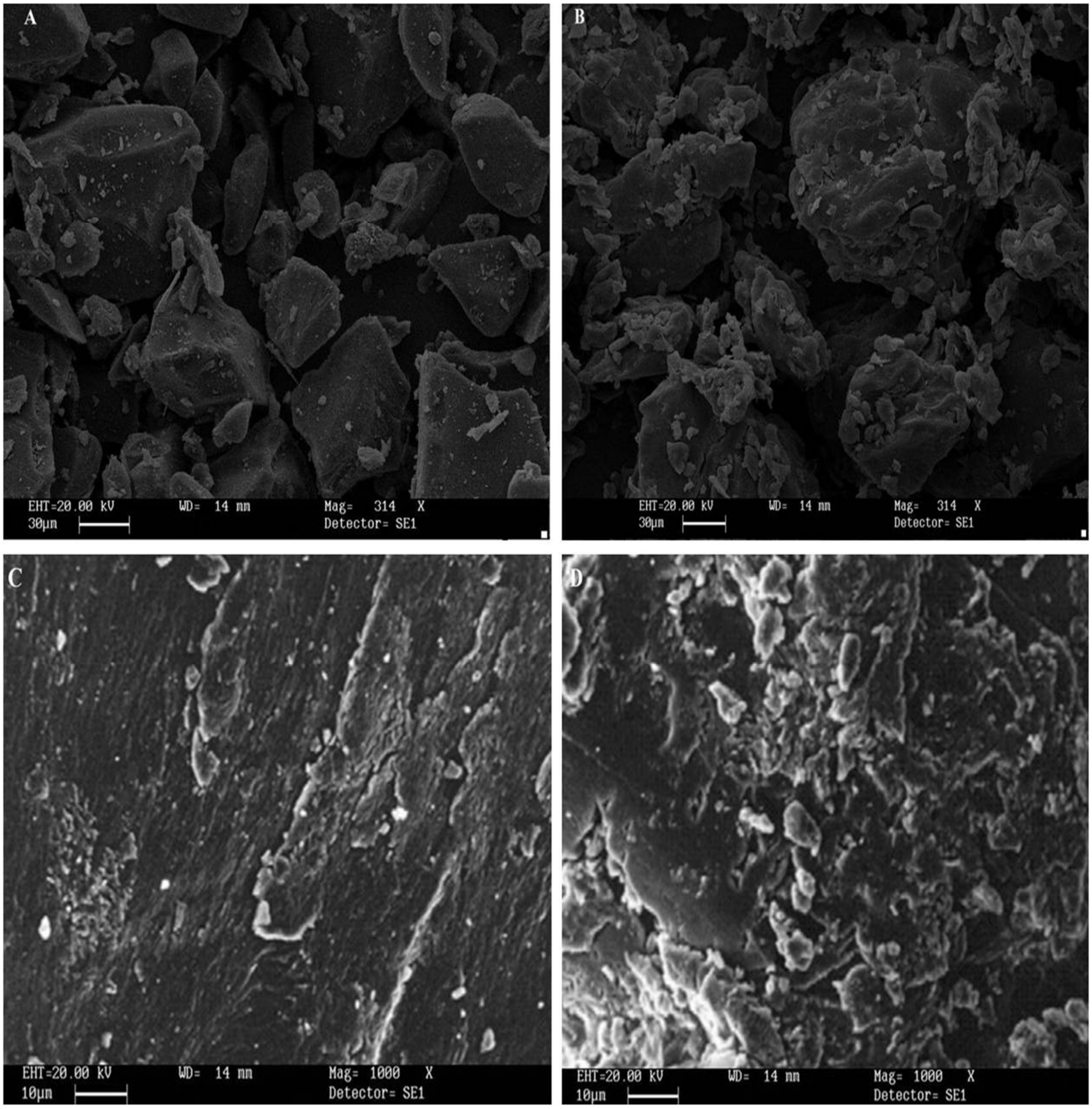

Fig. 4. SEM microphotographs showing the shape of (A) BFG, (B) CBFG and surface of (C) BFG, (D) CBFG

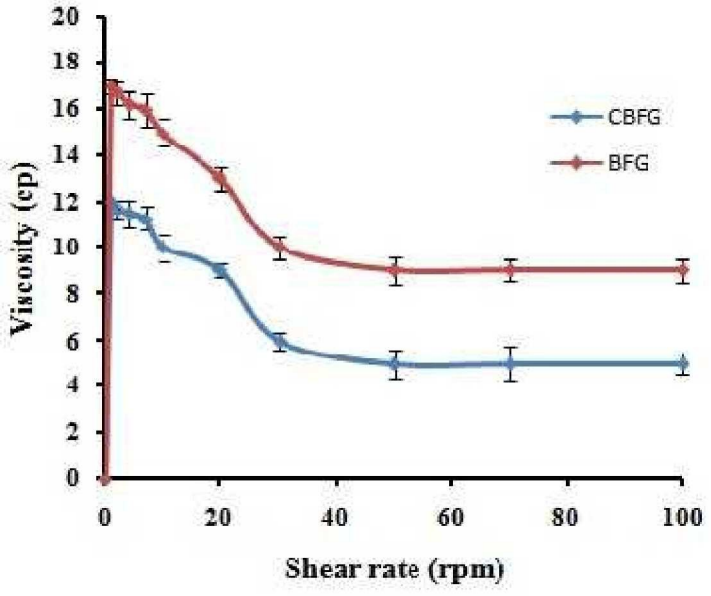

Fig. 5. Rheological behavior of aqueous solution of BFG and CBFG (each point represents mean ± SD of three replicates).

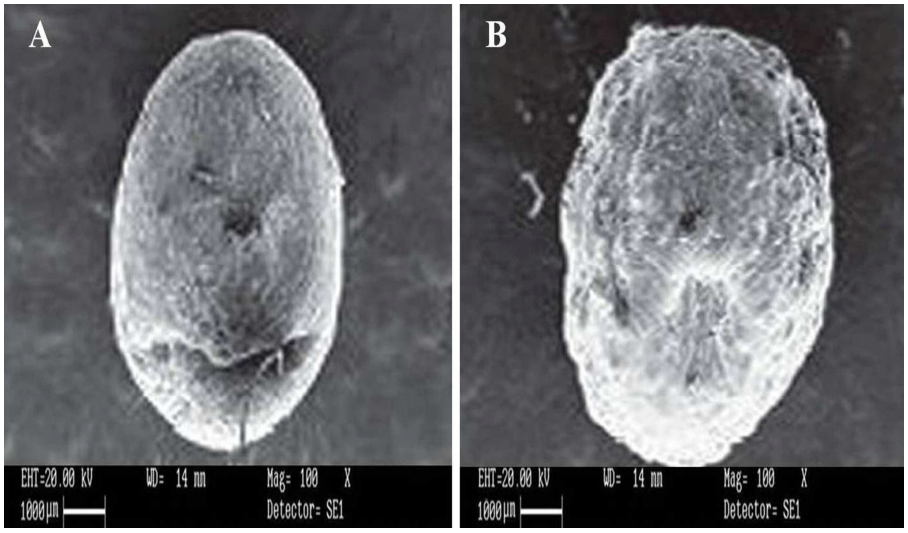

Fig. 6. SEM microphotographs of (A) BFG and (B) CBFG beads.

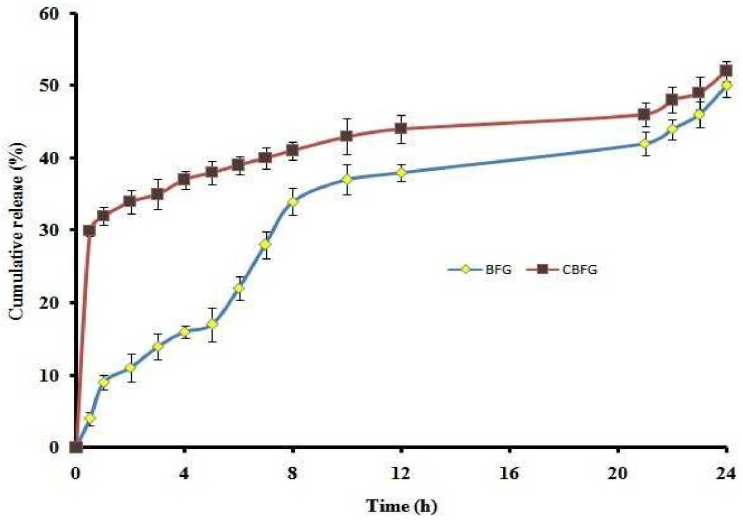

Fig. 7. *In vitro* release profile of metformin from BFG and CBFG beads