International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 5, 2023

Original Article

TAILORED *BASELLA ALBA* MUCILAGE-BASED BIPOLYMERIC HYDROGEL BEADS FOR CONTROLLED RELEASE OF DICLOFENAC SODIUM

MOUMITA CHOWDHURY[1,2], PINTU KUMAR DE[1*]

[1]Department of Pharmaceutical Technology, JIS University, 81, Nilgunj Road, Agarpara, Kolkata-700109, West Bengal, India. [2]Guru Nanak Institute of Pharmaceutical Science and Technology, 157/F Nilgunj Road, Panihati, Sodepur, Kolkata-114, West Bengal, India
[*]Corresponding author: Pintu Kumar De; [*]Email: pintu.de@jisuniversity.ac.in

Received: 09 Jul 2023, Revised and Accepted: 09 Aug 2023

ABSTRACT

Objective: The present investigation aims to convert the underutilized *Basella alba* mucilage (BAM) into a smart carrier by formulating its bipolymeric hydrogel beads for the controlled release of Diclofenac sodium (DFS).

Methods: At first, mucilage from the stem and fruits of *Basella alba* was extracted, isolated, and evaluated. *Basella alba* mucilage was chemically modified to its carboxymethyl derivative to improve its physicochemical properties. Single and bipolymeric hydrogel beads of carboxymethylated *Basella alba* mucilage (CBAM) and Sodium carboxymethyl cellulose (SCMC) were formulated by the Ionotropic gelation method using aluminium chloride ($AlCl_3$) as a cross-linking agent. A four-factor I-optimal response surface design was used to optimize the formulations. Drug and excipient compatibility was studied by Fourier transform infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) study. Scanning electron microscopy (SEM) was done to reveal the surface morphology. *In vitro* release of the drug in phosphate buffer (pH 6.8) and acidic buffer (pH 1.2) were compared for all the formulations. The effect of various formulation parameters on the release of the drug was studied, and the best-fitting model for release kinetics was determined.

Results: The degree of carboxymethylation was found to be 0.565±0.05. The bipolymeric beads were found to release 14% drug in 2 h in acidic media, minimize the release of the drug in the stomach to avoid the harsh effects of DFS and then provide controlled release in the intestine, releasing 80-90% of the drug in 10 h. The release kinetics followed the Hixon Crowell model, which suggests an erosion of the matrix to release the drug.

Conclusion: The bipolymeric hydrogel beads of tailored *Basella alba* mucilage were found to control the release of Diclofenac sodium.

Keywords: Mucilage, Carboxymethylation, Bipolymeric, Hydrogel beads, Controlled release, Formulation

DOI: https://dx.doi.org/10.22159/ijap.2023v15i5.48803. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Natura-polycystic blends natural polymer and pharmaceutics for the development of advanced drug delivery systems [1]. Therefore, plant-derived underutilized polymers like mucilages can be extensively characterized to meet the increasing industrial demand. *Basella alba* containing a higher proportion of mucilage in its leaves and stem is a potential candidate for the study. *Basella alba* L, having the synonym *Basella rubra* Roxb, belongs to the family Basellaceae [2]. Basella polysaccharide comprises mainly D-galactose, arabinose, glucose, galacturonic acid, and rhamnose. The IR spectrum of the mucilage confirms the presence of hydroxyl group, carboxyl group, aldehyde, keto, and phenol group [3]. *Basella alba* mucilage (BAM) is advocated to possess diverse pharmaceutical properties as a binding [4], disintegrating [5], suspending [6], gelling [3], encapsulating [7] and matrix-forming agent [8].

As per the literature, extensive chemical modification of polymers has been done, but very scarce data is available on the chemical modification of mucilage. Chemical modification improves the specific functional properties of polymer by introducing functional groups [9-12]. Bandopadhyay and Nayak (2023) have reported that thiolation of Fenugreek seed polysaccharide can be used as a mucoadhesive agent [13]. BAM having a long chain and lots of–OH group in its structure, is prone to chemical modification. Carboxymethylation converts the hydroxyl group into carboxymethylate [14]. In the present investigation, mucilage is extracted and isolated from the fruits and stem of *Basella alba* and chemically modified to get the carboxymethyl derivative of BAM.

Hydrogel is a polymeric network that controls the release of drug for a prolonged period [15-17]. Numerous natural and synthetic polymers are employed for designing hydrogels [18], but there is no report on the preparation of hydrogels from *Basella alba* mucilage. As per literature records, Quince seed mucilage was blended with sodium alginate to prepare sustained-release microspheres [19]. Another report states that microspheres were prepared from uriddall mucilage [20] and tamarind mucilage [21] in combination with sodium alginate. But there is no report on blending plant mucilage with sodium carboxymethyl cellulose to prepare hydrogel beads. Sodium carboxymethyl cellulose (SCMC) formed inter-penetrating network beads [22] with gelatin for controlled release of ketorolac tromethamine [23], and with xanthan gum for sustained release of aceclofenac [24]. In the present research work, carboxymethylation of BAM was done to improve its physicochemical properties. Since SCMC could bind with Al^{3+}ions to form water-insoluble gel beads, therefore, new bipolymeric hydrogel beads composed of carboxymethylated *Basella alba* mucilage (CBAM) and SCMC in different ratios loaded with Diclofenac sodium (DFS) were prepared by ionotropic gelation with Al^{3+}ions to provide controlled release of DFS. Diclofenac sodium is a phenylacetic acid derivative non-steroidal anti-inflammatory drug completely absorbed after oral administration [25].

MATERIALS AND METHODS

Materials

Diclofenac sodium was obtained as a gift sample from Micro Labs Limited, Hyderabad, India. *Basella alba* was procured from the local market, and the stem and fruit of the plant were authenticated (Specimen no-MC0105) by BSI, Central National Herbarium, Kolkata. Monochloroacetic acid (Loba Chemie Pvt. Ltd., Mumbai), Tween 80 (Loba Chemie Pvt. Ltd., Mumbai), Sodium hydroxide (Merck Specialities Pvt. Ltd, Mumbai), Acetone (SD Fine chemicals, Kolkata), Trichloroacetic acid (SD Fine chemicals, Kolkata), Methanol (SD Fine chemicals, Kolkata), Aluminium chloride hexahydrate, Hydrochloric acid, potassium sulfate, potassium chloride, Potassium dihydrogen phosphate, Glacial acetic acid (Chem Pure Pvt Ltd, Kolkata, India) were sourced commercially. All other reagents were of analytical grade and used as received. Double distilled water was prepared in the laboratory and used.

Extraction and Isolation of *Basella alba* mucilage

The stem and fruits of *Basella alba* were washed and dried under shade for three days. It was then dried at 30 °C until constant weight was obtained [26]. The dried part was ground, passed through sieve no 22, and stored in a desiccator. The resultant material was soaked in distilled water (four times the weight of powder) overnight. It was filtered through muslin cloth to remove the marc. Acetone was taken as an equal volume of the filtrate and added to it. The precipitated mucilage was separated and washed thrice with acetone to remove residual water and then dried in a hot air oven at 35 °C, powdered, and stored in a desiccator for further use [10].

Carboxymethylation of *Basella alba* mucilage

BAM was chemically modified to CBAM by carboxymethylation reaction [14]. Accurately weighed, 2 g of BAM was mixed with 8 ml of distilled water. Then 5.44 ml of ice-cold water containing 3.024 g of sodium hydroxide was added dropwise for 45 min. 1.5 g of monochloroacetic acid was dissolved in 3.32 ml distilled water, and the solution was added slowly for 1 h to the above mixture maintained at 15 °C. The mixture's temperature was raised slowly to 65 °C and stirred for at least 1 h. The wetted mass was washed with three successive amounts of 20 ml of 80% w/v ethanol for 15 min [27]. The pH of the suspension was adjusted to neutrality with glacial acetic acid. The product was finally washed with ethanol and kept for drying at 30-40 °C until three consecutive weights were the same.

Chemical characterization of carboxymethylated *Basella alba* mucilage

Determination of the degree of O-carboxymethyl substitution

Five hundred milligrams of CBAM were dispersed in 5 ml 80% (v/v) methanol/water mixture. Concentrated hydrochloric acid was added in excess amounts and stirred for 2-3 h. The mixture was filtered through Whatman filter paper with a pore diameter of 11 µm, and the residue was washed with successive 5 ml of ethanol until washing showed neutrality. The residue was dried to constant weight. Accurately weighed, 200 mg of dried sample was taken in a beaker; 1.5 ml 70% (v/v) ethanol/water mixture was added and allowed to stand for a few minutes. Then 20 ml of water and 5 ml of 0.5 N sodium hydroxide were added. The mixture was shaken for 3-4 h until the sample dissolved completely. The solution was then back titrated with 0.4 N hydrochloric acid to a phenolphthalein end-point. The degree of substitution (DS) of the O-carboxymethyl group was calculated from the equation [28]:

$$DS = \frac{0.162A}{(1 - 0.058A)}$$

Where 'A' is the milliequivalent of Sodium hydroxide required per gram of sample.

The determination was done in triplicate.

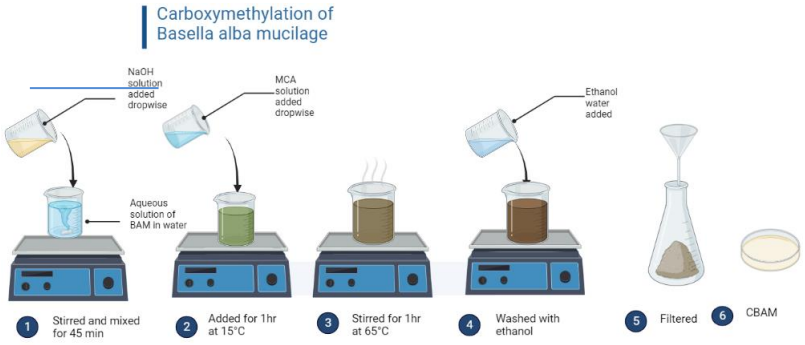


Fig. 2: Carboxymethylation of BAM

Physical characterization of carboxymethylated *Basella alba* mucilage

Viscosity measurement

The viscosity of 1% w/v aqueous solution of BAM and CBAM was determined by the Brookfield viscometer (Toki Sangyo viscometer, model no. TV-10, Japan) using spindle no. M1. The measurement of viscosity was done three times for each sample.

Preparation of hydrogel beads

SCMC and CBAM were dissolved in distilled water with constant stirring until a homogenous solution was obtained. The required amount of DFS was added to the polymer solution with constant stirring. The resulting solution was extruded through a 21 G flat-tipped needle into an aluminum chloride solution. The resultant droplets were incubated in the gelation medium for 20 min. The hydrogel beads were collected, washed, and air-dried. Finally, it was dried in a hot air oven at 30 °C until constant weight was obtained.

Experimental design and optimization

Response surface methodology is one of the best statistical approaches in experimental design to get the best response [29]. In the present work, a 4-factor I-optimal response surface design was used. The four independent variables, total polymer concentration (A), CBAM: SCMC (B), Drug loading (C), and $AlCl_3$ concentration (D), were studied at two levels. The particle size (micrometre) and drug entrapment efficiency (%) were the dependent variables/responses. Design expert software (12) was used to construct the design matrix. The quadratic model was applied with analysis of variance so that the model's significance of probability value<0.5 [30].

Physicochemical characterization of CBAM-SCMC hydrogel beads

Measurement of bead diameter

The diameter of the drug-loaded beads was measured using digital slide callipers (Asahi, India). The mean particle diameter and standard deviation were calculated using 30 beads randomly selected from each formulation. Each measurement was carried out in triplicate.

Percentage yield determination

The following formula was used to find the percentage yield of the bead [31]

$$\text{Percentage yield}(\%) = \frac{\text{Weight of beads obtained}}{\text{weight of drug taken} + \text{Weight of polymer taken}} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

The incorporation of the O-carboxymethyl functional group in BAM can be studied using FTIR. The FTIR spectra of BAM and CBAM, blank CBAM-SCMC beads, pure Diclofenac sodium, and DFS-loaded CBAM-SCMC beads were obtained using Perkin Elmer spectrometer (Spectrum RX1, UK) from 4000 to 600 cm^{-1}taking pellets of potassium bromide. The pellet of mucilage sample and potassium bromide was made in a hydraulic press (KP, Kimaya Engineers, India) by maintaining the high pressure of 125 kg/cm^2 [32].

Drug entrapment efficiency (DEE)

10 mg of dried beads were taken in a mortar and ground completely. The powder was taken in a beaker, and 100 ml of phosphate buffer pH 6.8 was added. It was kept overnight, filtered, and analyzed with a spectrophotometer at λ_{max} 276 nm [27]. The analysis was carried out in triplicate for each sample.

$$\text{Drug Entrapment Efficiency (DEE) (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Scanning electron microscopy (SEM)

Dried Blank CBAM-SCMC bead, DFS loaded CBAM: SCMC bead (2:1) (20% drug loading), and DFS loaded CBAM: SCMC bead (3:1) (20% drug loading) were assessed under a scanning electron microscope. SEM photos were taken by placing the samples in conducting stub at an acceleration voltage of 17kV. Gold sputter coater (Edwards-S 150 B model; Mfg by BOC Edwards UK) was used to form vacuum-coated gold palladium film over the sample.

Drug–polymer interaction study through FTIR spectroscopy

The FTIR spectra of blank CBAM-SCMC beads, pure DFS, and DFS-loaded CBAM-SCMC beads were obtained in Perkin Elmer spectrometer using potassium bromide pellets. The change in the characteristics peak of pure DFS was observed in the physical mixture and hydrogel beads.

Differential scanning calorimetry (DSC) study

DSC measurements of pure drug, blank polymeric beads, and drug-loaded beads were performed using a DSC4000 device (Perkin Elmer). A weighed amount (about 6 mg) of the sample was heated in a hermetically sealed aluminium pan at the rate of 10 °C min^{-1} in the range of 30–300 °C [33]. The first heating scan was the source of the curves obtained.

Assessment of *in vitro* drug release

Dissolution apparatus type II (TDP-06P Electro Lab, India) was used to conduct an *in vitro* dissolution study. The study used an acidic buffer of pH 1.2 and a phosphate buffer of pH 6.8). 50 mg drug-loaded beads weighed were taken in a dissolution flask. 500 ml acidic buffer solution was added (37±0.5 °C), and the paddle was rotated at 75 rpm. A 5 ml aliquot was withdrawn and substituted with a buffer solution of the same volume at a specific time interval. The optical density of the samples was measured at 276 nm. The amount of drug released in an acidic buffer solution was found and calculated from the standard curve. Each release study was done three times. The same procedure was carried out, taking a phosphate buffer of pH 6.8.

Drug release kinetics

The mechanism of drug release from the hydrogel beads was studied by incorporating the data obtained from the release study in different kinetic models. The cumulative percentage of drug release vs time was plotted for zero-order drug release [34], the Log percentage of drug remaining to be released vs time was plotted for first-order release [35], and the Cumulative percentage of drug release vs square root of time was plotted to follow Higuchi model [36]. The cube root of the percentage of drug remaining vs. time was plotted for the Hixon Crowell model [37]. The release kinetics was found using linear regression analysis from the value of the correlation coefficient [38].

RESULTS AND DISCUSSION

The present research work found that the mucilage extracted from *Basella alba* fruit and stem could form hydrogel beads in the presence of trivalent Al^{3+}ions, but the beads formed could not retain their shape when out of the cationic solution. Therefore, the mucilage was chemically modified by carboxymethylation to form carboxymethylated mucilage. This imparted a definite shape to the beads. Thus the gel-forming ability of *Basella alba* mucilage was utilized for designing hydrogel beads to control drug release from the formulation. Carboxymethylation increased the hydrophilicity and solution clarity of the mucilage, thereby improving mucilage's aqueous solubility. This result supports the study by Maity *et al.* (2010), where the solubility of locust bean gum improved after its carboxymethylation [27]. BAM is slightly soluble in water but becomes soluble when the water temperature is raised, whereas CBAM is soluble in water at room temperature due to the hydrophilic property of the carboxylate group. The extent of modification in the mucilage is reflected in the degree of substitution as per carbohydrate chemistry. The average value of the degree of o-carboxymethyl substitution in BAM was calculated to be 0.565±0.05, which suggests that the mean number of newly incorporated o-carboxymethyl groups per anhydrous sugar unit is 0.565, i.e., out of three hydroxyl group, 0.565 have been substituted.

Bipolymeric hydrogel beads were prepared (F2 to F16) by varying the total concentration of polymer, ratio of CBAM: SCMC, $AlCl_3$ concentration, and drug loading (20%w/w and 30% w/w). Single polymeric beads (F1, F17) were prepared to compare the drug release with the bipolymeric beads. Formulation F1 was formulated using 3%w/w SCMC and F17 was prepared using 10%w/w CBAM with 20% drug loading and 20%w/v $AlCl_3$ solution.

Table 1: Properties of pure and modified *Basella alba* mucilage

Sample	Solubility in water	Viscosity (cP) of 0.1% (w/v)	Degree of carboxymethylation
BAM	Slightly soluble	68.5±0.98	Nil
CBAM	Soluble	20.2±1.05	0.565±0.05

*Data were expressed as mean±SD, where n=3

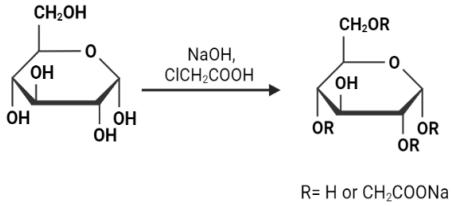


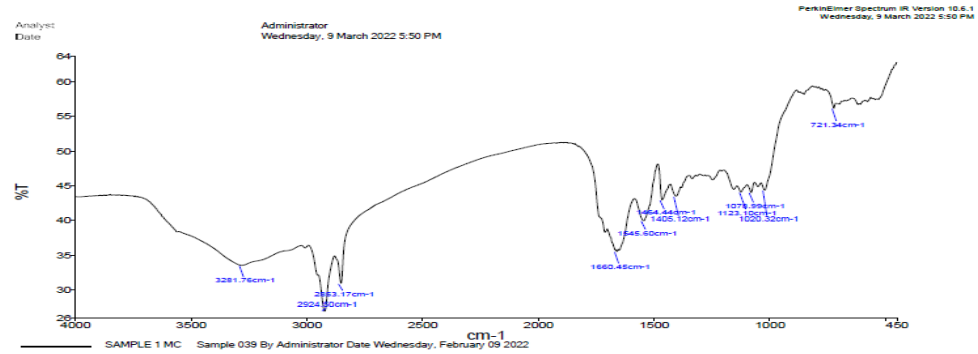
Fig. 2: Probable carboxymethylation results in *Basella alba* mucilage

FTIR analysis

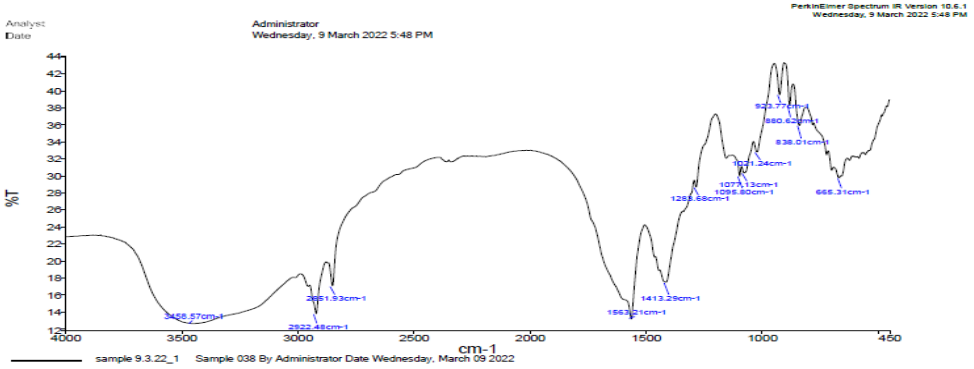
FTIR analysis also confirms the incorporation of O-carboxymethyl group in the original BAM. In the IR spectrum of BAM,-OH and–CH stretching was obtained at 3281.76 cm^{-1} and 2924.50 cm^{-1}, respectively (fig. 3A). The two bands at 1563.21 (asymmetric) and 1413.29 (symmetric) cm^{-1} in CBAM were due to C=O stretching in COO-ions, which was also observed in the carboxymethyl derivative of starch [39]. Due to the formation of sodium ion of carboxymethylate (-COO-Na) rather than the acid form (-COOH), the carboxyl group band was not observed at about 1724-1750 cm^{-1}. This is in accordance to the study where the carboxyl group band was not obtained when locust bean gum was derivatized to its carboxymethyl form [14]. The occurrence of carboxymethylate groups in the CBAM was reflected by the peak at 923.77 cm^{-1} due to symmetric C-O-C stretching (fig. 3B). Carboxymethylation reduced the viscosity of 0.1 %w/v BAM from 68.5 poise to 20.2 poise.

FTIR spectrum showed specific peaks at 3388.11 cm^{-1} as a result of N-H stretching of secondary amine in DFS,–C-O stretching of carboxyl ion was responsible for the peak at 1574.57 cm^{-1} and 1305.60 cm^{-1}due to C-N stretching. The peaks at 3037.21 cm^{-1} and 3080.24 cm^{-1}(above 3000 cm^{-1}) correspond to the presence of

aromatic rings. Aromatic ring presence was also confirmed by 3 to 4 peaks observed in the 1400 cm^{-1}-1550 cm^{-1} range. Therefore, peaks at 1454.84 cm^{-1}, 1510.91 cm^{-1}, and 1557.15 cm^{-1} also supported aromatic ring presence in DFS. This is in accordance with the FTIR report on the investigation of Diclofenac sodium done in the literature [40].



a



b

Fig. 3: FTIR spectrum of (a) BAM (B) CBAM

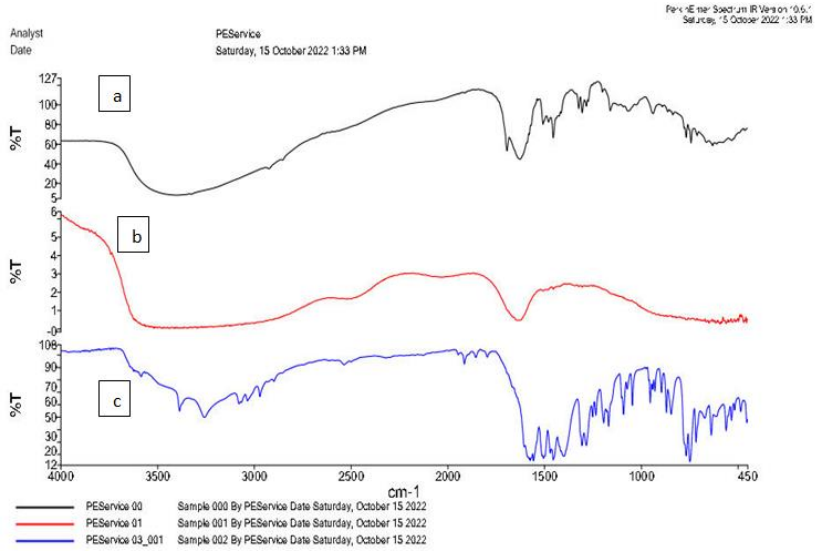


Fig. 4: FTIR spectrum of (a) DFS loaded CBAM-SCMC bead (b) Blank CBAM-SCMC bead (c) pure DFS

Diameter of beads

The prepared hydrogel beads' size appeared to depend on the formulation factors. Keeping the total polymer concentration, drug load, $AlCl_3$ concentration, and gelation time constant, substituting SCMC with an increasing amount of CBAM increased the average diameter of beads. Similarly, maintaining constant value for all variables, an increase in the CBAM concentration increased the beads' size. The high viscosity of the polymer solution resulted in big droplets of the polymer solution from the needle, increasing the size of the beads. An increase in drug load also caused beads with larger diameters. An increase in $AlCl_3$ concentration tended to reduce the size

of the beads. The reason may be due to the release of water from the beads when placed in a concentrated solution due to hyperosmosis. The decrease in size of bead with increasing concentration of cross-linking agent has been reported by many researchers [23].

Drug entrapment efficiency (DEE)

DEE of CBAM and SCMC beads were found to be low. Substitution of SCMC with CBAM increased the DEE of bipolymeric beads. An increase in CBAM concentration increased the DEE, which may be due to the increased viscosity of the solution. Further increase in CBAM concentration reduced DEE, possibly due to high viscosity, which led to non-uniform drug dispersion. DEE decreased significantly when cross-linking agent ($AlCl_3$) concentration was increased from 5 to 20 % w/v. This may be due to an increase in gel porosity with an increase in the concentration of $AlCl_3$. Leaching of the drug might be the cause of overall low DEE [24].

Formulation optimization

In the present study, the particle diameter and Drug entrapment efficiency showed p value<0.0001which indicates that A, B, C, and D are significant model terms. The model F value of 18124.63 for particle diameter and 601.27 for DEE suggests the model is significant. There is a 99.99% chance that the value of F is not large due to noise [41]. The study indicated that the predicted value is in good agreement with actual values. The 2FI model was appropriate for particle diameter and DEE. The response surface methodology also correlated independent factors with a response by 2D contour and 3D surface graphs.

Table 2: Independent variables and respective levels

Factor	Independent variables	Units	Minimum (-1)	Maximum (+1)
A	Total Polymer concentration	% (w/v)	3	8
B	CBAM: CMC		0:1	3:0
C	Drug Loading	% (w/w)	20	30
D	$AlCl_3$ concentration	% (w/v)	5	20

Table 3: Formulation code of hydrogel beads with independent variables and observed responses

Code	Factor 1	Factor 2	Factor 3	Factor 4	Response 1	Response 2
	Total polymer concentration (A) % (w/v)	CBAM: SCMC (B)	Drug loading (C) % (w/w)	$AlCl_3$ concentration (D) % (w/v)	Particle diameter[a] (µm)	DEE[b] (%)
F1	3	0:1	20	20	984.56±0.12	50.66±0.32
F2	6	1:1	20	20	702.34±0.14	51.29±0.85
F3	6	1:1	30	20	731.35±0.11	51.87±0.26
F4	6	2:1	20	5	1607.26±0.16	63.27±0.52
F5	6	2:1	20	10	1575.92±0.14	61.86±0.43
F6	6	2:1	20	15	1535.54±0.08	59.97±0.74
F7	6	2:1	20	20	1504.76±0.07	58.17±0.25
F8	6	2:1	30	5	1624.66±0.12	63.75±0.42
F9	6	2:1	30	10	1597.53±0.07	62.26±0.63
F10	6	2:1	30	15	1550.27±0.12	60.77±0.47
F11	6	2:1	30	20	1524.36±0.14	59.08±0.52
F12	8	3:1	20	5	1623.26±0.16	49.42±0.62
F13	8	3:1	20	20	1517.86±0.09	44.19±0.85
F14	8	3:1	30	5	1631.46±0.15	50.27±0.32
F15	8	3:1	30	15	1564.27±0.17	46.73±0.32
F16	8	3:1	30	20	1541.36±0.12	45.87±0.26
F8'	6	2:1	30	5	1604.66±0.22	60.75±0.42
F3'	6	1:1	30	20	730.35±0.11	49.87±0.26
F6'	6	2:1	20	15	1530.54±0.08	55.97±0.74
F12'	8	3:1	20	5	1603.26±0.16	46.42±0.62

('F8', F3'. F6', F12' are the replicate values of F8, F3, F6, F12), [a]Data were expressed as mean±SD, where n=30, [b]Data were expressed as mean±SD, where n=3

Table 4: Statistical model parameters by ANOVA to optimize hydrogel beads

Response	Model	r^2	Adjusted r^2	Adequate precision	f-value	p-value	
Particle diameter	2FI	1.00	0.999	352.5768	18124.63	<0.0001	significant
Particle diameter = 1351.98+-948.964 *A+1212.55 *B+9.73898 *C+-50.0843 *D+-37.9849 *AB+2.00391 *AC+11.6757 *AD+-5.05959 *BC+-10.9933 *BD+2.28085 *CD							
DEE	2FI	0.9985	0.9968	75.1137	601.27	<0.0001	significant
DEE(%) = 60.5592+-16.2787 *A+13.8621 *B+0.173686 *C+-2.06524 *D+-10.7752 *AB+0.647886 *AC+0.505157 *AD+-0.29755 *BC+-0.896387 *BD+0.0432673 *CD							

Scanning electron microscopy

Scanning electron micrographs shows both the blank and drug-loaded beads as spherical. The surface of the blank beads is not continuous (fig. 6a), whereas the drug-loaded bead (fig. 6b and 6c) shows a smooth and uniform surface with no distortion as in the case of blank beads. However, the number of depressions on the surface lowered with increased concentration of CBAM in the beads.

The low viscosity of CBAM-SCMC produced less mechanical strength in the beads, resulting in a slight depression on the surface of the beads when dried. Adding SCMC with a higher amount of CBAM increased the solutions' viscosity, providing a more uniform surface. A similar result was observed when hydrogel beads were prepared from SCMC and sodium carboxymethyl xanthan [24]. Boppana and his coworkers (2010) reported that hydrogel beads of carboxymethyl cellulose cross-linked with aluminum showed a spherical shape with a rough, cracked, fissured surface [21].

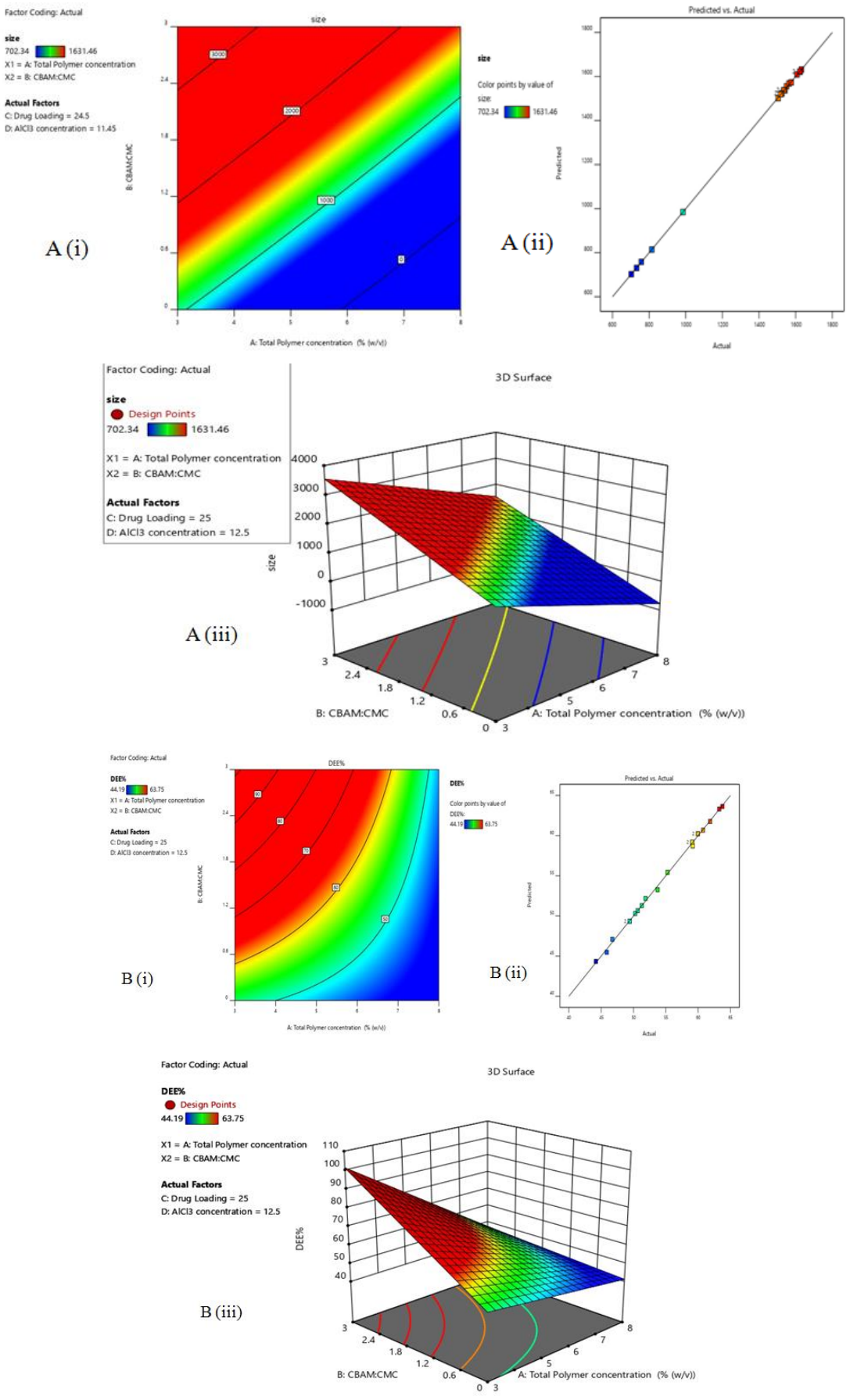


Fig. 5: (i) Contour graph 2D, (ii) predicted vs actual plot and (iii) response surface 3D graph for (A) Effect of independent variables on size of beads (B) Effect of independent variables on DEE

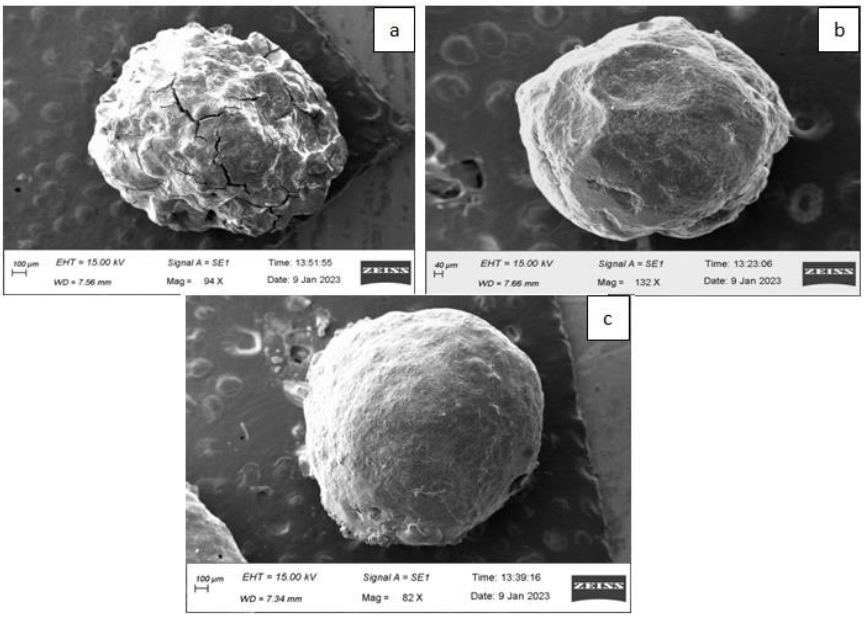


Fig. 6: (a) Blank CBAM: SCMC bead (magnification-94X) (b) DFS loaded CBAM: SCMC bead (2:1) (20% drug loading) (magnification-132X) (c) DFS loaded CBAM: SCMC bead (3:1) (20% drug loading) (magnification-82X)

Differential scanning calorimetry

Melting endotherm of drug was observed at 103.77 °C (fig. 7c) from DSC thermogram. But, the same endothermic transition was absent in drug-loaded beads (fig. 7b) and in blank beads (fig. 7a). DFS-loaded beads' thermogram showed a strong endotherm at 200 °C. At 189.46 °C, a similar endothermic transition was observed in the thermogram of blank particles, which may result from the melting of the blank CBAM-SCMC complex. Therefore, it can be concluded that the drug was present in the beads in amorphous form due to the absence of a principal melting endotherm of DFS crystals in drug-loaded beads. Due to its amorphous nature, this physical form may have high solubility and dissolution rate [27].

***In vitro* drug release**

In an acidic buffer, drug release from SCMC beads and CBAM beads was rapid, releasing 24.49% and 26.40% of the loaded drug in 3 h (fig. 9). The incorporation of SCMC with a higher amount of CBAM in the beads lowered the release of the drug to 14.18% in 3h (fig. 11). Al-CBAM and Al-SCMC may be converted into their corresponding acid forms in a solution of low pH. The unionized carboxyl groups in CBAM exert insignificant repulsive forces electrostatic in nature; hence, relaxation of the macromolecular chain does not occur [42]. As a result, the beads do not swell in acidic solution to a great extent, and drug release occurs slowly. The gel strength of CBAM beads may be expected to be less due to a smaller number of COOH (degree of substitution 0.565).

Substitution of SCMC with an increasing amount of CBAM increased the gel strength, lowering the drug's release (fig. 10, 11). Higher concentrations of $AlCl_3$ retarded the release of the drug at pH 1.2 and pH 6.8 buffer solution by forming a dense and rigid matrix due to a greater extent of cross-linking (fig. 12, 13). An increase in drug load promoted the release of the drug. Since the drug was loaded on a weight basis, an increase in the amount of drug per unit weight lowers the polymer amount, and this causes the network structure to become weak. This is in accordance with the reports researchers gave for different drug-loaded polymeric beads [23, 24, 36].

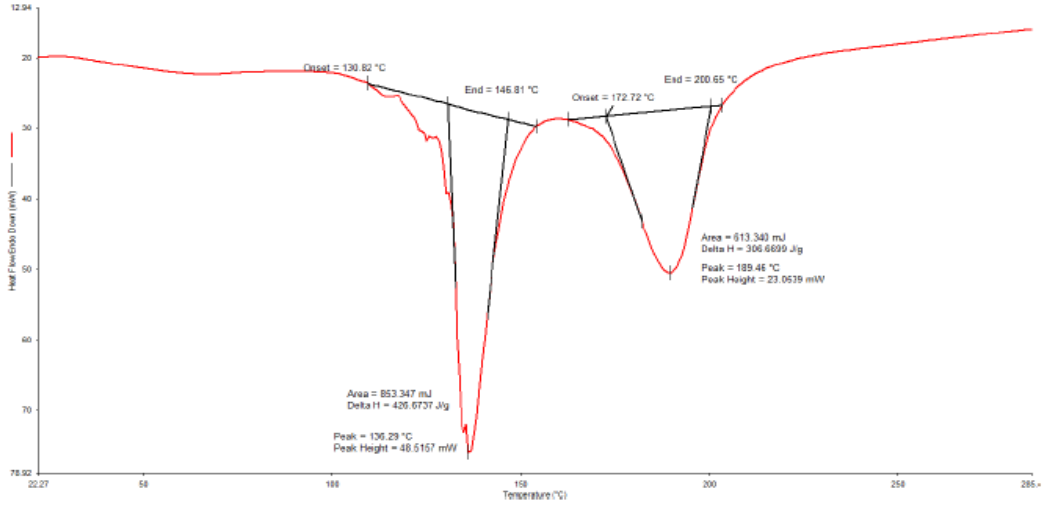
In addition, greater drug loading may increase the free space due to low polymer concentration within the network and create an easy pathway for water to penetrate into beads. An increase in total polymer concentration up to a certain value decreases the drug release, and a further increase in polymer concentration increases drug release. The reason may be because of the high viscosity of the blended polymer, which did not increase the cross-link density of the matrix due to the hindrance in an influx of Al^{3+} ions into the beads. The structure of non-homogeneously gelled beads breaks down quickly and thus produces faster drug release [24, 42].

Dynamic drug release

The formulation F7 was found to release the drug slowest ($t_{80\%}$ in phosphate buffer 594±2.82 min) compared to other formulations. Therefore, F7 was transferred in phosphate buffer solution (pH 6.8) following the dissolution study in an acidic solution (pH 1.2) for 2 h. Only 14% drug was released in 2 h in an acidic solution, which increased to 28% in phosphate buffer in 10 min, and a further 80% drug was released within 10 h in a phosphate buffer solution (fig. 14). The greater the drug load, the faster the drug release from the beads, which may decrease the drug release in the stomach and provide controlled release in the intestine [27]. Such release behaviour is desirable to reduce the gastric irritation of NSAIDs. The correlation coefficient values of the different mathematical models by curve fitting were found. The value of r^2 ranges from 0.9745-0.9993 in the case of the Hixon Crowell model. Therefore, the release kinetics followed the Hixon Crowell model, which suggests matrix erosion to release drugs.

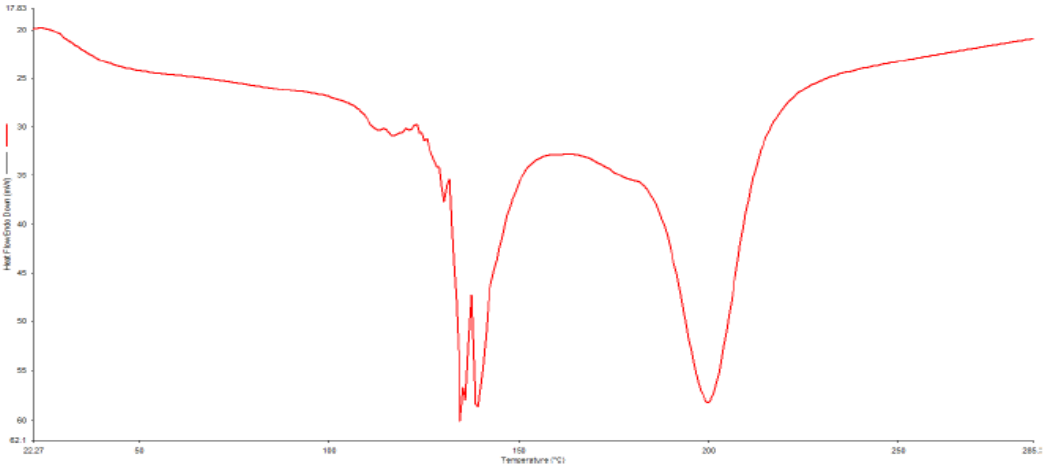
Table 5: Release kinetics of DFS from CBAM-SCMC hydrogel beads

Formulation code	$t_{80\%}$ in phosphate buffer (pH 6.8) min mean±SD (n=3)	Correlation coefficient (r^2)				
		Zero-order	First order	Higuchi model	Korsmeyer peppas	Hixon crowell
F1	370±3.87	0.9741	0.6891	0.9045	0.9123	0.9756
F2	410±2.87	0.9687	0.7234	0.9378	0.9423	0.9862
F3	391±4.15	0.9745	0.7159	0.9267	0.9387	0.9921
F7	594±2.82	0.9860	0.7481	0.9726	0.9826	0.9993
F11	530±4.21	0.9812	0.7582	0.9668	0.9746	0.9956
F13	470±3.78	0.9756	0.8342	0.9731	0.9634	0.9823
F17	150±3.46	0.9723	0.8276	0.9231	0.9134	0.9745



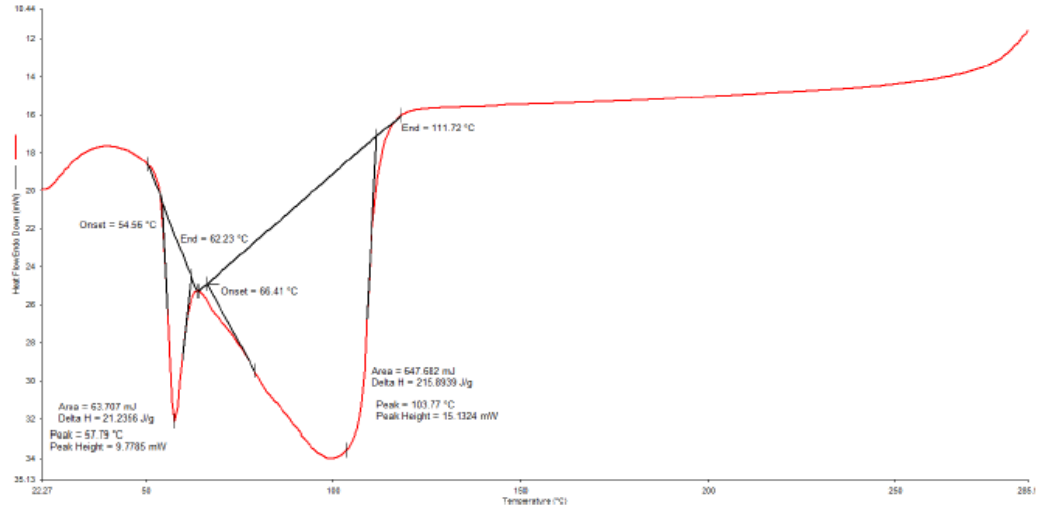
Blank Bead

a



Drug Loaded Bead

b



Drug

c

Fig. 7: DSC curves (a) Blank bead (b) DFS loaded beads (c) Pure drug

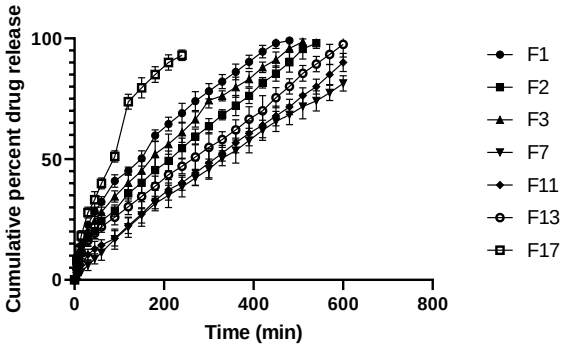


Fig. 8: Release of DFS from formulations in phosphate buffer (pH 6.8), *Graph plotted from data as mean±SD (n =3)

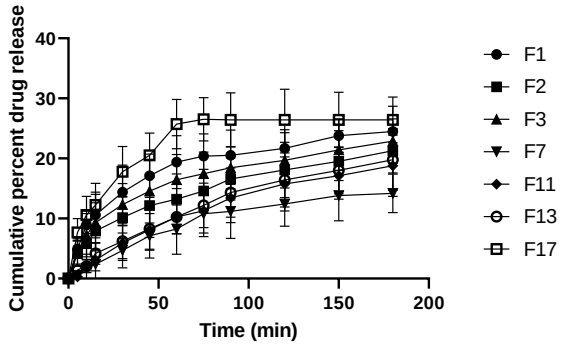


Fig. 9: Release of DFS from formulations in acidic buffer (pH 1.2), *Graph plotted from data as mean±SD (n =3)

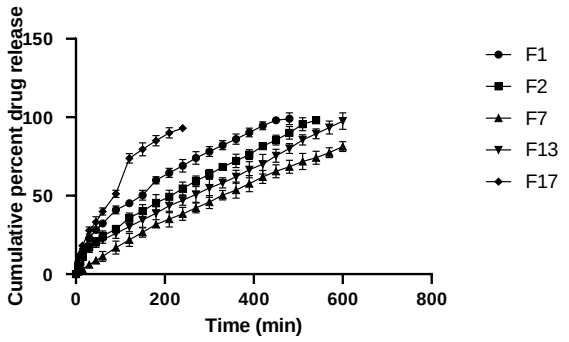


Fig. 10: Effect of polymer concentration on release of DFS in phosphate buffer (pH 6.8), *Graph plotted from data as mean±SD (n =3)

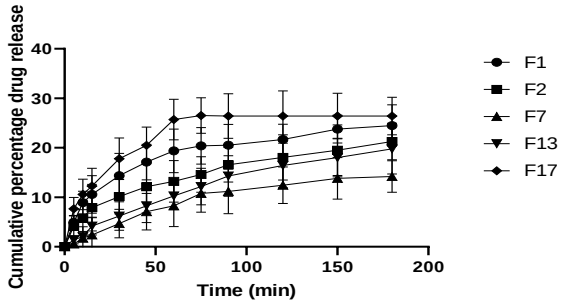


Fig. 11: Effect of polymer concentration on release of DFS in acidic buffer (pH 1.2), *Graph plotted from data as mean±SD (n =3)

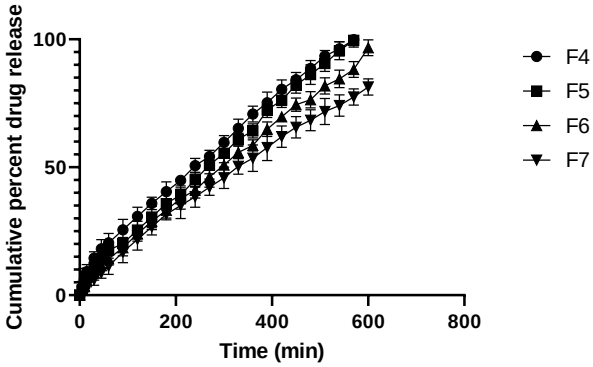


Fig. 12: Effect of $AlCl_3$ concentration on release of DFS in phosphate buffer (pH 6.8), *Graph plotted from data as mean±SD (n =3)

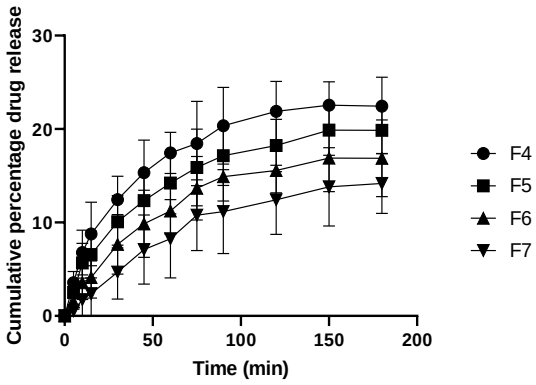


Fig. 13: Effect of $AlCl_3$ concentration on release of DFS in acidic buffer (pH 1.2), *Graph plotted from data as mean±SD (n =3)

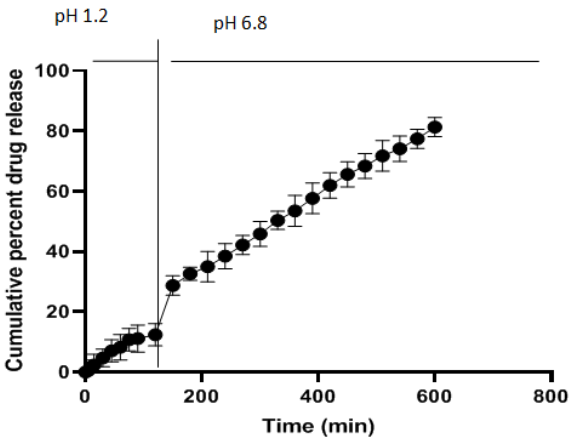


Fig. 14: Dynamic release of DFS from F7

CONCLUSION

The carboxymethylation process chemically modified the mucilage extracted from *Basella alba* fruit and stem to form carboxymethylated *Basella alba* mucilage, and it was found that it was capable of forming hydrogel beads in an $AlCl_3$ solution. Carboxymethylation increased the hydrophilicity and solution clarity of the mucilage, thereby improving the physicochemical properties of the mucilage. FTIR and DSC studies confirmed no interaction between the drug and the mucilage. Further, FTIR also proved the presence of the carboxymethyl group in the mucilage.

The *in vitro* release study revealed that increased concentration of CBAM and greater concentration of $AlCl_3$ slowed drug release from beads. But very high concentrations of polymer further increased the drug release. The carboxymethyl derivative of the mucilage combined with SCMC generated bipolymeric hydrogel beads with higher controlled release properties than the single polymeric beads. The CBAM-SCMC beads were found to provide controlled release in the intestine, thus eliminating the harsh effect of the drug in the stomach.

ACKNOWLEDGMENT

The authors sincerely thank the Guru Nanak Institute of Pharmaceutical Science and Technology and JIS University for providing facilities to complete the research.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

REFERENCES

1. Ngwuluka NC, Ochekpe NA, Aruoma OI. Naturapolyceutics: the science of utilizing natural polymers for drug delivery. Polymers. 2014;6(5):1312-32. doi: 10.3390/polym6051312.
2. Deshmukh SA, Gaikwad DK. A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (Basellaceae). J Appl Pharm Sci. 2014;4(1):153-65. doi: 10.7324/JAPS.2014.40125.
3. Chatchawal C, Nualkaew N, Preeprame S, Porasuphatana S, Priprame A. Physical and biological properties of mucilage from *Basella alba* L. stem and its gel formulations. Int J Plant Sci. 2010;6:104-12.
4. Ramu G, Krishna Mohan G, Jayaveera KN. Preliminary investigation of patchaippasali mucilage (*Basella alba*) as tablet binder. Int J Green Pharm. 2011;5(1):24-7.
5. Bhat V, Nayak R, Praveena MB. Isolation and evaluation of disintegrating properties of *Basella alba* linn. Leaf mucilage in tablet formulations. J Biomed Pharm Res. 2015;4(2):29-42.
6. Pal D, Nayak A, Kalia S. Studies on *Basella alba* L. leaves mucilage: evaluation of suspending properties. Int J Drug Discov Technol. 2010;1:15-20.
7. Das S, Alam MN, Batuta S, Ahamed G, Fouzder C, Kundu R, Mandal D, Begum NA. Exploring the efficacy of *Basella alba* mucilage towards the encapsulation of the hydrophobic antioxidants for their better performance. Process Biochemistry 2017; 61:178-88.
8. Harika B, Shanmuganathan S, Gowthamarajan K. Formulation and evaluation of controlled release cefixime nanoparticles prepared using *Basella alba* leaf mucilage and chitosan as matrix formers. J Pharm Sci Res. 2016;8(2):92-9.
9. Parvathy KS, Susheelamma NS, Tharanathan RN, Gaonkar AK. A simple non-aqueous method for carboxymethylation of galactomannans. Carbohydr Polym. 2005;62(2):137-41. doi: 10.1016/j.carbpol.2005.07.014.
10. Das S, Ghosh A, Changder A, Nandi G, Ghosh LK. Quality-by-design approach for the development of sustained-release multiple-unit beads of lamotrigine based on an ion-cross-linked composite of pectin and okra mucilage: an *in vitro* appraisal. Int J Biol Macromol. 2020;163:842-53. doi: 10.1016/j.ijbiomac.2020.07.033, PMID 32653379.
11. Raj V, Shim JJ, Lee J. Grafting modification of okra mucilage: recent findings, applications, and future directions. Carbohydr Polym. 2020;246:116653. doi: 10.1016/j.carbpol.2020.116653. PMID 32747285.
12. Sen G, Pal S. A novel polymeric biomaterial based on carboxymethyl starch and its application in controlled drug release. J Appl Polym Sci. 2009;114:2798-805.
13. Bandyopadhyay PK, Nayak AK. Thiolation of fenugreek seed polysaccharide; utilization as a novel bio mucoadhesive agent in drug delivery. Int J App Pharm. 2023;15(1):290-7. doi: 10.22159/ijap.2023v15i1.46459.
14. Maiti S, Chowdhury M, Datta R, Ray S, Sa B. Novel gastro ulcer protective micro(hydro)gels of sulfated locust bean gum-aluminum complex for the immediate release of diclofenac sodium. J Drug Target. 2013;21(3):265-76. doi: 10.3109/1061186X.2012.745548, PMID 30952178.
15. Mohammadi H, Kamkar A, Misaghi A. Nanocomposite films based on CMC, okra mucilage and ZnO nanoparticles: physico mechanical and antibacterial properties. Carbohydr Polym. 2018;181:351-7. doi: 10.1016/j.carbpol.2017.10.045. PMID 29253983.
16. Ghumman SA, Bashir S, Noreen S, Khan AM, Riffat S, Abbas M. Polymeric microspheres of okra mucilage and alginate for the controlled release of oxcarbazepine: *in vitro* & *in vivo* evaluation. Int J Biol Macromol. 2018;111:1156-65. doi: 10.1016/j.ijbiomac.2018.01.058.
17. Liu H, Xie X, Chen C, Firempong CK, Feng Y, Zhao L. Preparation and *in vitro*/*in vivo* evaluation of a clonidine hydrochloride drug–resin suspension as a sustained-release formulation. Drug Dev Ind Pharm. 2021;47(3):394-402. doi: 10.1080/03639045.2021.1890110, PMID 33615926.
18. Maity S, Mahapatra SK. Preparation and evaluation of gellan gum-linseed gum blend microbeads for sustained release of aceclofenac. Int J App Pharm. 2022;14(3):49-55. doi: 10.22159/ijap.2022v14i3.43652.
19. Ghumman SA, Mahmood A, Noreen S, Rana M, Hameed H, Ijaz B. Formulation and evaluation of quince seeds mucilage–sodium alginate microspheres for sustained delivery of cefixime and its toxicological studies. Arab J Chem. 2022;15(6):103811. doi: 10.1016/j.arabjc.2022.103811.
20. Gada SG, Anandkumar Y, Setty CM. Design and optimization of zidovudine loaded uriddall mucilage microspheres using box behnken method. Int J Pharm Sci Res. 2019;10(4):1856-64.
21. Gada S, YA, Setty CM. Preparation, evaluation and stability of lamivudine-loaded alginate-tamarind mucilage microspheres. Int J App Pharm. 2019;11(4):365-70. doi: 10.22159/ijap.2019v11i4.33808.
22. El-Sherbiny IM, Abdel-Bary EM, Harding DRK. Preparation and *in vitro* evaluation of new pH-sensitive hydrogel beads for oral delivery of protein drugs. J Appl Polym Sci. 2010;115(5):2828-37.
23. Rokhade AP, Agnihotri SA, Patil SA, Mallikarjuna NN, Kulkarni PV, Aminabhavi TM. Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine. Carbohydr Polym. 2006;65(3):243-52. doi: 10.1016/j.carbpol.2006.01.013.
24. Kundu T, Mukherjee K, Sa B. Hydrogel beads composed of sodium carboxymethyl xanthan and sodium carboxymethyl cellulose for controlled release of aceclofenac: effect of formulation variables. Res J Pharm Technol. 2012;5(1):103-13.
25. Adedokun M, Nkanta C. Optimized delivery of diclofenac sodium formulated in a sustained release *Raphia africana* hydrocolloid matrix. Int J App Pharm. 2018;10(1):109-14. doi: 10.22159/ijap.2018v10i1.22925.
26. Das S, Ghosh A, Das R, Nandi G, Ghosh LK. Development of quality control parameters for standardization of a novel mucilage obtained from okra (abelmoschus esculentus (l.) moench) fruit. Int J Curr Pharm Sci. 2021;13(3):28-41. doi: 10.22159/ijcpr.2021v13i3.42091.
27. Maiti S, Dey P, Banik A, Sa B, Ray S, Kaity S. Tailoring of locust bean gum and development of hydrogel beads for controlled oral delivery of glipizide. Drug Deliv. 2010;17(5):288-300. doi: 10.3109/10717541003706265, PMID 20350054.
28. Shivashankar M, Mandal BK. Design and evaluation of chitosan-based novel pH-sensitive drug carrier for sustained release of cefixime. Trop J Pharm Res. 2013;12(2):155-61. doi: 10.4314/tjpr.v12i2.4.
29. Kumar A, Raizaday A, Gopal UM, Moin A. Development of cashew gum and its derivatives for sustained released drug delivery system: by response surface methodology. Int J Pharm Pharm Sci. 2014;6(10):476-84.
30. Maiti S, Ray S, Mandal B, Sarkar S, Sa B. Carboxymethyl xanthan microparticles as a carrier for protein delivery. J

Microencapsul. 2007;24(8):743-56. doi: 10.1080/02652040701647300, PMID 17926166.

31. Patel AK, Mishra MK, Gupta J, Ghoshal S, Gupta R, Kushwaha K. Guar gum-based floating microspheres of repaglinide using 3^2 factorial design: fabrication, optimization, characterization, and *in vivo* buoyancy behavior in albino rats. Assay Drug Dev Technol. 2021;19(2):63-74. doi: 10.1089/adt.2020.1006. PMID 33090876.
32. Boppana R, Kulkarni RV, Setty CM, Kalyane NV. Carboxymethylcellulose aluminium hydrogel micorbeads for prolonged release of simvastatin. Acta Pharm Sci. 2010;52(2):137-43.
33. Sakhare SS, Sayyad FJ. Design, development, and characterization of *Ocimum basilicum* mucilage-based, modified release mucoadhesive gastrospheres of carvedilol: *Ocimum basilicum* mucilage-based gastrospheres of carvedilol. Asian J Pharm Clin Res. 2019;12(10):218-25. doi: 10.22159/ajpcr.2019.v12i10.34291.
34. Babu RJ, Sathigari S, Kumar MT, Pandit JK. Formulation of controlled release gellan gum macro beads of amoxicillin. Curr Drug Deliv. 2010;7(1):36-43. doi: 10.2174/156720110790396445, PMID 19863487.
35. Ata S, Rasool A, Islam A, Bibi I, Rizwan M, Azeem MK. Loading of cefixime to pH-sensitive chitosan-based hydrogel and investigation of controlled release kinetics. Int J Biol Macromol. 2020;155:1236-44. doi: 10.1016/j.ijbiomac.2019.11.091. PMID 31730964.
36. Kulkarni AR, Soppimath KS, Aminabhavi TM, Rudzinski WE. *In vitro* release kinetics of cefadroxil-loaded sodium alginate interpenetrating network beads. Eur J Pharm Biopharm. 2001;51(2):127-33. doi: 10.1016/s0939-6411(00)00150-8, PMID 11226819.
37. Lin N, Huang J, Chang PR, Feng L, Yu J. Effect of polysaccharide nanocrystals on structure, properties, and drug release kinetics of alginate-based microspheres. Colloids Surf B Biointerfaces. 2011;85(2):270-9. doi: 10.1016/j.colsurfb.2011.02.039, PMID 21440426.
38. Shende MA, Marathe RP, Khetmalas SB, Dhabale PN. Studies on the development of sustained release diltiazem hydrochloride matrices through jackfruit mucilage. Int J Pharm Pharm Sci. 2014;6(7):72-8.
39. Mendyk A, Jachowicz R, Fijorek K, Dorożyński P, Kulinowski P, Polak S. Kinet DS: an open source software for dissolution test data analysis. Dissolution Technol. 2012;19(1):6-11. doi: 10.14227/DT190112P6.
40. Manikandan A, Nemani SC, Sadheeshkumar V, Arumugam S. Spectroscopic investigations for photostability of diclofenac sodium complexed with hydroxypropyl-B-cyclodextrin. J App Pharm Sci. 2016;6(04):98-103. doi: 10.7324/JAPS.2016.60414.
41. Neelam S, Meenakshi B. Formulation and evaluation of polymeric microspheres using box–behnken design. Asian J Pharm Clin Res. 2022;15(10):47-55. doi: 10.22159/ajpcr.2022.v15i10.45250.
42. Bajpai SK, Sharma S. Investigation of pH-sensitive swelling and drug release behavior of barium alginate/carboxymethyl guar gum hydrogel beads. J Macromol Sci Part. A: Pure and Applied Chemistry. 2006;43(10):1513-21. https://doi.org/10.22159/ajpcr.2022.v15i10.45250.