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Original Article

DEVELOPMENT OF CASHEW GUM AND ITS DERIVATIVES FOR SUSTAINED RELEASED DRUG DELIVERY SYSTEM: BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

The aim of the present study was to investigate the Cashew gum (CG) and its modifications for formulating the sustained release delivery system using theophylline (TH) as a model drug by varying the gum to drug ratio (1:1 and 3:1). Different formulations were designed, prepared and evaluated by employing response surface, optimal design of experimental technique using Design Expert® ver 8.0.1 software. The optimized formulation was identified and validated for its performance by using the numerical optimization technique. The matrix tablets were prepared by direct compression using CG, cross linked CG (CCG), carboxymethylated cashew gum (CMCG) and carboxymethylated cross linked CG (CMCCG). The modifications in CG were confirmed using FT-IR and C^{13} NMR. SEM was used to study the surface morphology of formulations. *In vitro* drug release, gum erosion and hydration studies were carried out using a dissolution apparatus (USP 1) for 12 h using specified buffer solutions (pH 1.2 and pH 6.8). The kinetic analysis of dissolution data showed a good fit in Peppas equation which confirmed anomalous non-fickian release mechanism for CCG (epichlorhydrin/ gum ratio (E/G^b = 0.15)) with TH. The physiochemical changes, solubility, metallic remains after purification, stability of crude and purified CG and the effects of crosslinking density on swelling were also accessed. The stability of the optimized formulation indicated that it was stable. Thus, it can be concluded that CG crosslinking (E/G^b = 0.15) can be effectively employed to formulate sustained release systems.

Keywords: Cashew gum, Carboxymethylation, Optimization, Validation, Sustained release systems.

INTRODUCTION

Gums are complex polysaccharides with the high molecular mass and may be classified as acidic (tragacanth, albizia, acacia), neutral (asparagus gum, plantago seed gum) or basic. Natural gums are known to be either acidic or neutral as there are no naturally-occurring basic gums

Natural gums have large variety of composition and rheological properties that cannot be easily mimicked by synthetic polymer. There are many advantages associated with the natural gum low cost, free from side effects, biocompatible, environmental friendly processing and local availability [1,2].

As a result researcher are now investigating for new natural polymer which can be used in the pharmaceutical industry. Cashew gum (CG) is one such versatile naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry [3-6].

CG have been used in the food and beverage industry as a thickening agent, a gelling agent and for many other purpose [7, 8]. Recently the role of these gums in enveloping controlled drug delivery systems has increased significantly and CG is gaining lot of attraction towards this application [9-12].

The physiochemical and binding properties of cashew tree gum in metronidazole tablet formulation has been studied and found that Purified cashew gum at concentrations of 4 – 8 % w/w could successfully be employed as binder in the preparation of tablets [13].

Apart from this a Comparative study of some mechanical and release properties of paracetamol tablets formulated with cashew tree gum, povidone and gelatin as binders has also been performed and found superior to both binders in its ability to reduce brittle fracture tendency in paracetamol tablets[14].

But no study has been yet performed to signify the role of this versatile hydrophilic polymer as a sustained release polymer. The characterization of the swelling, erosion and the drug release parameters of the cashew gum and its modification by the cross-linking is the major emphasis of this research.

MATERIALS AND METHODS

Materials

Theophylline was gifted from Darwin Formulations Pvt Limited, Vijayawada. Cashew gum (CG) was Gift from ayurvedic market. Hydrochloric acid, Sodium Hydroxide, monochloroacetic acid (MCA), Epichlorhydrin was purchased from Sigma Chemicals, Bangalore. All the reagents used were of analytical grade.

Methods

Purification of cashew gum

100 g of the crude gum powder was dissolved in 200 ml of distilled water and allowed to stand for 24 h. Using a piece of calico the gum mucilage obtained was filtered by squeezing to remove any insoluble debris or impurities. The filtered mucilage was re-filtered to ensure that all debris was removed. The filtered mucilage was purified by precipitating the gum out with about 350 mL of 96 % ethanol for 100 gm of CG and washed with di- ethyl ether and dried in the hot air oven at 50 °C for about 8 h. The dried purified gum was milled and sieved through sieve number 80. The powdered gum was used in subsequent test and analysis as purified cashew gum. The sequence of purification is shown in Figure 1.

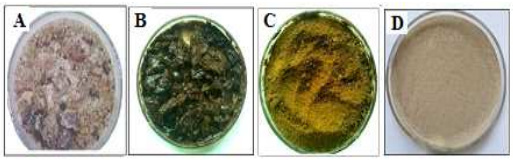


Fig. 1: Stages of Cashew gum purification (A) Crude Cashew gum, (B) Precipitated CG, (C) Dried and milled CG, (D) Cashew gum powder

Evaluation of colour change

The samples of Purified cashew gum (PCG) gum (2 % w/v) were mixed with 1 ml of 0.1% thioglycolyic acid and 1 ml of 0.1 % sodium

metabisulphite as an antioxidant. Both the samples were poured on watch glass and exposed to sunlight as well as kept in dark along with controlled samples without any antioxidant for a period of 24 hr. After 24 hr they were observed for the change in colour.

Evaluation of pH of Cashew gum

The pH of 2 % w/v PCG is more when compared to2 % w/v Crude cashew gum (CCG) indicating that crude cashew gum is more acidic than the purified gum. The study shows the effect of temperature and storage time on the pH of the CCG and PCG. The temperature was taken as cold (0 °C to 8 °C), cool (8 °C to 25 °C), room temperature (25 °C) and warm (25 °C to 50 °C) whereas the storage was done for six weeks. Two sets of CCG were prepared. One unpreserved and another preserved. Similarly two sets of PCG were prepared. One unpreserved and another preserved. Preserved sets have sodium benzoate (0.2 % w/v) and pH was the pH determined at weekly intervals for six weeks, using a standardised pH meter.

Moisture content of the Cashew gum

Powdered cashew gum (2 gm) samples were weighed accurately into a porcelaincrucible which had previously been dried and weighed. The gum was placed in a hotair oven and maintained at a temperature of 105 °C drying overnight to constant weight. Gum was removed and cooled, after which it was placed in a desiccators for 30 min. The weight of the crucible and the gum was recorded. The determination was done in triplicate. The moisture content was expressed as a percentage of the cashew gum sample. The entire process was repeated for purified cashew gum and its modification.

Evaluation of Metallic ion content in the Cashew gum

Powdered CCG and PCG (each 1 gm) was weighed into 250 ml beaker and 25 ml of concentrated nitric acid was added. The sample was digested on a hot plate, cooled and 1 ml perchloric acid (70% $HClO_4$) added. About 30 ml distilled water was added to the digest and the mixture boiled for about 10 min and filtered hot into a100 ml volumetric flask. Solution was then made to the mark with distil water. One ml of this digest was used to determine the content of calcium, magnesium, zinc and iron in the sample using an Atomic Absorption Spectrophotometer (AAS) fitted with an acetylene flame which was fitted with zinc and iron LED lamps and magnesium and calcium using CHCL lamps set at wavelength lamps of 213.86 λ, 248.33 λ, 285.21 λ and 422.67 λ, respectively. Two ml of the digest was used in the determination of sodium and potassium using the flame photometer method.

Cross-linking of Cashew gums

The gum (1.00 g) was mixed with 1.2 ml of 5 M NaOH and distilled water (2.4 ml –0.70 ml) until a homogeneous paste was formed. The epichlorohydrin (volume in the range of 0.1 ml –1.0 ml) was then added to the mixture and, was heated at 42 °C for 12 h, followed by a second heating time of 24 h at 70 °C. The cross linked gel was dialysed with distilled water for three to four washings initially for 1 hr for and then freeze dried[15].

Carboxymethylation of Cashew gums

The purified cashew gum (5.00 g) was mixed with 5 ml Water until a homogeneous paste was formed. Ten molar of NaOH solution (volume of 2.7 ml) was added and the mixture was kneaded for 10 min. After that, monochloroacetic acid (2.62 g) was mixed thoroughly with the paste. The mixture was heated at 55 °C, during 3 h. The system was neutralised with 1 M HCl and dialysed against distilled water until all remained reagents and added salt were eliminated (4–5 days). The solid carboxymethylated products were recovered by freeze-drying and denoted as CMCG.

Carboxymethylation of cashew gum crosslinked gel

Crosslinked gum (CGgel 7 sample, 5.00 g) was immersed in 15 ml ethanol and allowed to swollen. Ten molar of NaOH (2.5 ml) was then added and mixed thoroughly with the swelled gel. After homogenisation, 2.43 g monochloroacetic acid (MCA) was added and the mixture was heated for 3 h at 50 °C with magnetic stirring. After this time the product was washed with distilled water, dialysed for 72 h and freeze-dried. This sample was denoted as CMCGgel 7[16].

The yield was calculated using the formula:

Percentage yield = (M_{gel} / M_{gum})x 100

Where M_{gel} is the mass of the freeze dried gel while,

M_{gum} is the mass of the gum.

Scanning Electron Microscopy (SEM)

The surface morphology of the optimized formulations before and after dissolution was analysed by scanning electron microscopy (JEOL-JSM-804A, Japan) after 2 h, 4 h, 6 h and 8 h of the dissolution study. The samples were put on a graphite surface and coated with gold using ion sputter and observed under SEM.

Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8033 (USA). Samples were transformed to the acid form in order to improve the accuracy in the determination of degree of substitution. All other analyses were performed with salt form polysaccharides as they were prepared. Samples were dispersed in KBr powder and the pellets were made by applying 5 ton pressure. FT-IR spectra were obtained by powder diffuse reflectance on FT-IR spectrophotometer.

^{13}C solid-state nuclear magnetic resonance spectroscopy (^{13}C-CP/MAS NMR)

[13]C solid-state nuclear magnetic resonance spectroscopy of samples was carried out using a 300 BRUKER instrument, operating at 75 MHz. The cross-polarization pulse sequence was used for all samples, which were spun at the magic angle at 4.5 kHz. A contact time of 1 ms and a pulse repetition time of 3 s was used. Averages of 1100 scans were accumulated for each sample.

Preparation of Slugs

Tablets were prepared by direct compression utilizing drug model, Theophylline (ratios of gum: drug of 3:1 and 1:1). Each tablet weighing 300 mg. tablets were compressed (Rimek mini press I) using 9 mm biconvex shape punches. The prepared slugs is shown in the figure 2.

Fig. 2: Image of CG tablets

Water uptake and erosion determination.[17]

Measurement of hydration and erosion rates of the formulations were carried out. After the immersion of the tablets in the test medium (pH 1.2 and pH 6.8), to relate the observed phenomenon of drug release with the rates of polymer hydration. Weighed tablets were placed in the baskets of the dissolution apparatus rotating at 50 rpm, with the dissolution medium of phosphate buffer pH 6.8 at 37±5 °C. After 0.5, 1, 2, 3, 4, 5, 6, 7 h, each dissolution basket containing sample was withdrawn, blotted to remove excess water and weighed on an analytical balance (Digital balance, Shinko Sansui, Japan). The wet samples (basket + sample) were then dried in an oven at 60 °C for 18h, cooled in a dessicator (silica gel) and dried residue was weighed. The heating-cooling weighing process was repeated until constant weight was achieved. The experiment

was performed in triplicate for each time fresh samples were used for individual time point.

The increase in weight due to absorbed liquid (Q) was estimated at each time point from the following equation:

$$Q = (W_w - W_f) / W_f \times 100$$

Where W_w is the mass of the hydrated samples befor drying and W_f the final weight of the same dried and partially eroded sample. The percentage of erosion (E) was estimated from the following equation:

$$E = (W_i - W_f) / W_f \times 100$$

Where W_i is the initial dry sample weight.

***In vitro* dissolution studies [17]**

The dissolution test was carried using apparatus 1 USP (model Electrolab, TDT 08L, Mumbai) at 50 rpm. In order to reproduce digestive physiological phases, 900 ml of dissolution medium with different pH environments at temperature 37±0.5 °C was performed. The dissolution medium consists of a mixture of 50 mM hydrochloric acids, 50 mM glacial acetic acid and 50 mM phosphoric acid (pH 1.2). After 1 h, the pH was increased to pH 6.8, both by the addition of drops of 20 M sodium hydroxide. At suitable intervals, samples were withdrawn, filtered, neutralized at pH 6.8, diluted when necessary with buffer pH 6.8, and analysed spectrophotometrically (UV-1800, Shimadzu Co, Japan) at 268 nm. Studies were performed in triplicate and the mean cumulative percentage of drug calculated (±S. D) and plotted against time. During the drug release studies, all the formulations were observed for physical integrity at different time.

The solubility of theophylline in the potassium dihydrogen phosphate buffer pH 6.8 at 37 °C± 5 was found to be 3.65 mg ml^{-1}.The dissolution apparatus was connected to a flow through UV spectrophotometer (UV-1800, Shimadzu Co, Japan) and the absorbance measured at 268 nm in a 10 mm cell at 30 min time intervals over a 12 h time period. Studies were performed in triplicate and the mean cumulative percentage of drug calculated (±S. D) and plotted against time.

Optimization of the prepared formulation using DoE Software

Based on the results of studies carried out to select suitable polymer and its concentration, different formulations were prepared to optimize polymer concentration. The quantity of drug was constant in all these formulations.

The experiments were designed by using an experimental design software (Design Expert®, v 8.0.1, Stat-Ease, USA). An I-Optimal Response Surface historical data Design was employed to obtain 8 different factor combinations (Stat-Ease, 2010). Different factor combinations that were obtained and experimentally run to measure the responses R1 (Hydration and erosion), R2 (Release 6hr) are given in Table 5[18].

RESULT AND DISCUSSION

Purification of cashew gum

The purification of the crude CG was till now attempted using only ethanol (95%). But when an attempt was made, wherein purification was done using acetone, it resulted in a much lighter product with good percentage yield which is shown in the table 1 and figure 4.

Table 1: Calculation of percentage yield of CG

Initial weight of gum (before purification) I	Final weight of gum (after purification) F		Percentage yield of purification process $Y = \frac{F \times 100}{I}$
100 gm	Ethanol (95 %) 66.433 gm	Acetone 82.541 gm	66.43 % 82.541 %

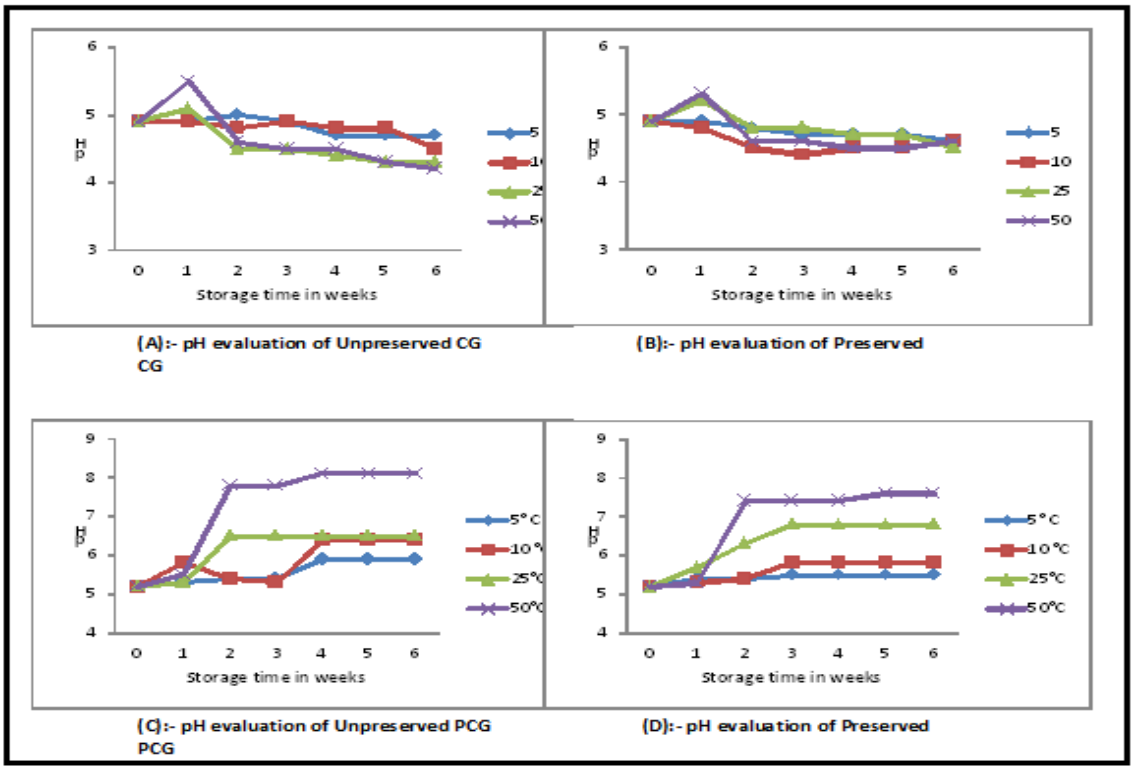


Fig. 3: The pH evaluation of the cashew gum.

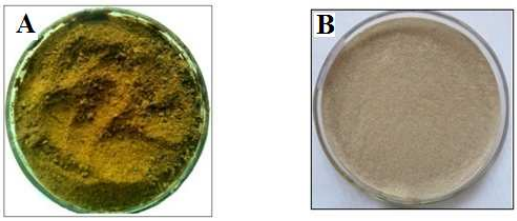


Fig. 4: (A) CG purified using Ethanol (95 %), (B) **CG purified using Acetone.**

Evaluation of colour change and pH

The investigation for colour change revealed that the presence of antioxidant does not prevent colour change. The samples exposed to sunlight were found to show more colour change both in test and control sets. The crude gum was found to be dark amber in colour with much plant debris in it and the colour may range from orange to dark brown depending upon its source of procurement. In commercial valuation of gums, strong preference is always shown for those that are light coloured. Colour is mainly due to the presence of impurities and the age factor of the tree from which the cashew gum is obtained. The purified gum is initially off white in colour, but changes to reddish brown or yellowish brown on exposure.

Evaluation of pH of Cashew gum

The pH of the unpreserved CG (fig 3 (a)) was found to increase but its variation was less evident at 5 ^{0}C and 10 ^{0}C while for preserved CG (fig 3 (b)), both increase and variation were found to be stable at 5^{0}C and 10^{0}C but showed prominent fluctuations at 25^{0}C and 50^{0}C. The figure 3 also showed the influence of gum purification on pH of cashew gum mucilage. For both the unpreserved (fig 3 (c)) and preserved (fig 3 (d)) mucilage, the pH of the purified gum mucilage was less stable compared to the crude gum on storage. The purification process appears to have removed some stability-inducing chemical constituents from PCG making the mucilage less stable compared to the crude gum. Addition of a preservative to the gum mucilage appeared to have little influence on the pH upon storage, except CG. It is therefore advisable to store cashew gum mucilage at low temperature Thus, the changes in pH observed upon storage of the gum mucilage, may reflects, chemical instability rather than microbial degradation of the crude or purified gum.

Moisture content evaluation

The evaluation of moisture content suggested that the modification of CG enhances the stability by decreasing moisture uptake compared to natural CG which is shown in figure 5.

Evaluation of Metallic ion content in the Cashew gum

Crude Cashew gum contains a variety of cationic impurities as found from the earlier works done on the constituents of this versatile gum. Hence, attempts were made to find the reduction in the cationic constituents in the purified gum so as to facilitate the croslinking.

The moisture content evaluation (Table 2) suggested that purification of crude CG, reduces the cationic content from the purified CG and promotes and easier crosslinking reaction.

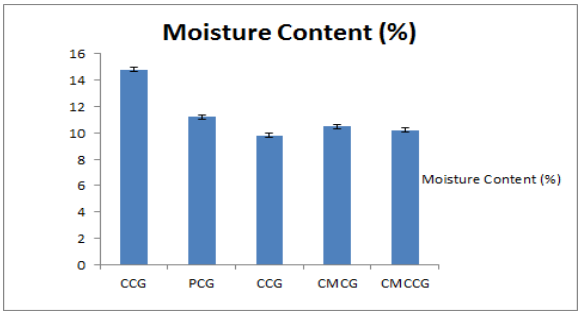


Fig. 5: Moisture content evaluation of the cashew gum.

Table 2: Evaluation of Metallic ion content in the Cashew gum

Metallic content	Cashew gum	
	CCG	PCG
Calcium	1301	662.42
Potassium	0.131	0.061
Sodium	0.089	0.0645
Iron	18.71	2.001
Zinc	8.769	5.234
Magnesium	54.55	52.71

Cross-linking of Cashew gums

Natural gums being hydrophilic swell in the presence of the solution media. Hence, there is a possibility of the entrapped drug leaking out prior to arrival of the drug at its site of absorption. Thus, there is a need to reduce the enormous swelling of the gums by cross linking.

Table 3 shows the product yield and swelling degree of cashew gum gels. It was found that for the cross-linked cashew gum (CG), the crosslinking reaction yield increases with increasing E/G molar ratio up to 0.15 and then decreases. This decrease (for E/G = 0.18, CGgel8 sample) may be due to side chain reactions when excess of epichlorohydrin is present which ultimately resulted in less of the gum molecule been crosslinked. The swelling degree decreases with increasing E/G ratio, for cross linked cashew gum (Table 3). This can be explained by the increasing of crosslinking density which provokes the formation of rigid networks leading to less expanded structures and less water absorption. Carboxymethylated CGgel 7 (CMCGgel 7) has higher Q value (9.8 g H_2O /g gel) than CGgel 7 (7.5 g H_2O/g gel).

The increase of the swelling degree for carboxymethylated gel (30.66 % in relation to CGgel 7) is likely to be due to insertion of hydrophilic groups (-CH_2COO^-) in CMCGgel 7.

Table 3: Crosslinking reaction conditions data

Products	Weight of the gum (gm)	Reaction condition				% yield	Swelling degree
		Volume of alkali used (5 M NaOH in ml)	Volume of epichlorhydrin (ml) E[a]	Volume of water (ml)	E/G[b] molar ratio		
CGgel 1	1.0	1.2	0.1	1.20	0.02	21.9	15.1
CGgel 2	1.0	1.2	0.2	0.91	0.04	22.3	15.1
CGgel 3	1.0	1.2	0.3	0.85	0.06	33.5	14.2
CGgel 4	1.0	1.2	0.4	0.73	0.09	40.8	11.6
CGgel 5	1.0	1.2	0.5	0.65	0.11	66.3	9.7
CGgel 6	1.0	1.2	0.6	0.36	0.13	84.1	8.3
CGgel 7	1.0	1.2	0.7	0.28	0.15	98.4	7.5
CGgel 8	1.0	1.2	0.8	0.26	0.18	81.7	6.2
CGgel 9	1.0	1.2	0.9	0.19	0.20	72.9	5.1
CGgel 10	1.0	1.2	1.0	0.09	0.22	60.0	3.4
CMCG gel 7	1.0	1.2	0.7	0.28	0.15	97.2	9.8

[a]Epichlorhydrin (density = 1.86 gm/ml), [b]E/G = Epichlorhydrin/ Gum = 8.266

Carboxymethylation of Cashew gums

Carboxymethylation of gums increases their hydrophilicityand solution clarity and makes them more soluble in aqueous systems. Modification of tamarind kernel powder, cassiatora gum and guar gum were investigated by [19, 20] the general scheme of carboxymethylation is outlined in fig 6. Regardless of the carboxymethyl content, the aqueous gum solutions were characterised by non-Newtonian pseudo plastic behaviour.

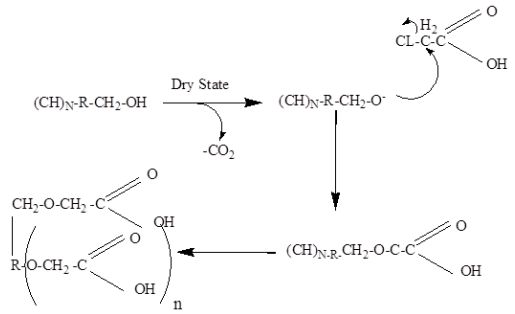


Fig. 6: Carboxymethylation of Gum CharacterizationC^{13} solid-state nuclear magnetic resonance spectroscopy

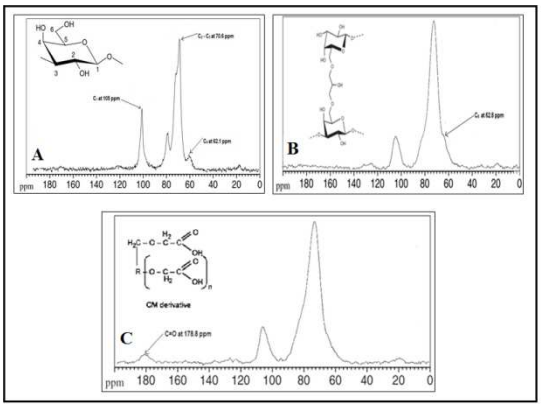


Fig. 7: C^{13} solid-state nuclear magnetic resonance spectra: (A) cashew gum, (B): Crosslinked cashew gum sample CGgel3, (C) Carboxymethylated cross linked cashew gum (CMCGgel3)

Fig. 7 (a) and (b) shows the C-13 CP/MAS spectra for CG and cross linked samples (CGgel7 and CMCGgel7). Although a fairly good resolution can be achieved for CG in solution that is not the case for solid samples. In powder CG spectrum (Fig. 7 (a)) there is a broad overlapped region at 70.6 ppm which is assigned to C-2, C-4 and C-5 carbons of the pyranosic ring. Peaks at 62.3 and 81.0 ppm can be assigned to C-6 and C-3 of galactose in CG. Anomeric carbon resonance was observed at 104.0 ppm. A low intensity peak at 17.3 ppm is due to the CH of rhamnose. The resonance due to C=O of glucuronic acid residues also present in CG was not detected in the solid ^{13}C-NMR. CGgel3 spectrum fig (7 (c)) was found to be similar to the CG spectrum. Carboxymethylation of CGgel3 was confirmed by the presence of signal at 178.8 ppm in CMCGgel3 spectrum (Fig. 7 (C)) which was attributed to C=O resonance of -H_2CCOONa group inserted in CGgel 7.

Fourier transform infrared (FT-IR) spectroscopy studies

Figs. 8 show the FT-IR spectra of cashew gum and its derivatives. The presence of band around 1730 cm^{-1} in Fig. 8 a is characteristic of C=O stretching vibration of glucuronic acid present in the starting polysaccharide (CG). A decrease in peak resolution is observed after the CG crosslinking reaction (Fig. 8 b). The carboxymethylation reaction led to insertion of new carboxylic groups per macromolecule. The observation of a substantial increase in the absorbance of C=O vibration was expected (Figs. 8 c and 8 d) for CMCG and CMCGgel7 samples.

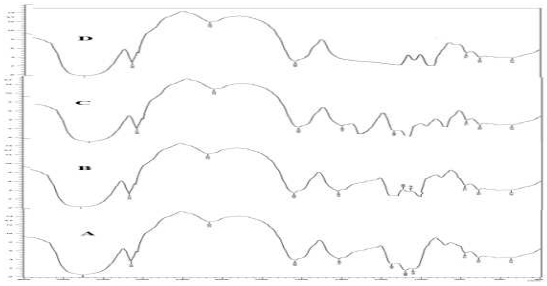


Fig. 8: The FT-IR spectra of cashew gum and its derivatives

Hydration capacity and Erosion

The percentage increase in weight of the hydrated gum matrices at various time intervals up to 8 hr are shown in the table 4. There is a strong degree of water uptake by F 1 (appprox 164.8 % wt increase after 8 hr) and for F 2 (approx184.6 %) at an agitation speed of 50 rpm. The result of F 2 was due to increase in the gum content. In contrast, cross linked cashew gum (F 3 and F 4) displayed a much lowered increase in wet weight over 8 hr period at the similar agitation speed. The rate of erosion of CG gel was less predominant than the cashew gum and shows only 23 % of integrity loss after 8 hr whereas after the same time period CG, the amount of erosion was approx 57%. The carboxymethylated gum depicted increase in wet weight higher than CG but considerably lower than CG gel. The CMCCG showed a 30 % enhancement in the swelling which is likely due to the increase of hydrophyllic group (-CH_2COO^-) in CMCG.

Table 4: Hydration capacity and Erosion

Time (h)	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8
2	35.5	48.8	12.5	22.0	57.8	44.3	33.6	39.6
4	58.0	73.6	25.7	32.4	75.4	82.7	47.5	57.2
6	104.4	128.8	38.9	48.5	93.8	108.5	88.7	86.4
8	164.8	184.6	53.4	66.5	164.3	179.1	102.4	125.9

***In vitro* release studies**

On contact with an aqueous medium, the hydrophilic polymer matrix gradually begins to hydrate from the periphery toward the centre, forming a gelatinous swollen mass, which controls the diffusion of drug molecule through the polymeric material into the aqueous medium (fig 10). Penetration by the solvent produces a clearly define front (solvent penetration front) at the interface between the dry and hydrated polymer. The hydrated gel layer thickness determines the diffusional path length of the drug.

The *In vitro* drug release profiles of TH from the various formulations have begun as shown in the figure 10. After 2 hr the initial pH of 1.2 was changed to 6.8. It is known that the drug release rate is dependent on the equilibrium solubility of the drug, which, in turn, is dependent upon the pH of its solution, however, TH dissolve in acidic and basic medium. As other authors have demonstrated, the solubility of TH remains almost constant between pH 1. 2 and pH 6.8. In both system, the drug release rate decrease when the proportion of gum increased. It was reasoned that, as the amount of gum in the matrix increase, there would be a greater degree of gum

hydration with simultaneous swelling. This would result in a corresponding lengthening of the drug diffusional pathway and reduction in drug releaser rate (F 2, F 4, F 6, F 8). Drug release was generally linear for most of the formulation. But a linear drug release from hydrophilic matrixes has been attributed to synchronization between swelling and erosion of the polymer in maintaining constant gel layer (Lee and Peppas, 1987) and this was seen in formulation F 3 and F 4. Since TH is soluble in the pH range tested its release from the hydrogel matrix is controlled by the swelling the matrix and the dissolution / erosion in the periphery of the matrix. The glucuronic acid has very least negative charge and hence their hydration process is independent of pH.

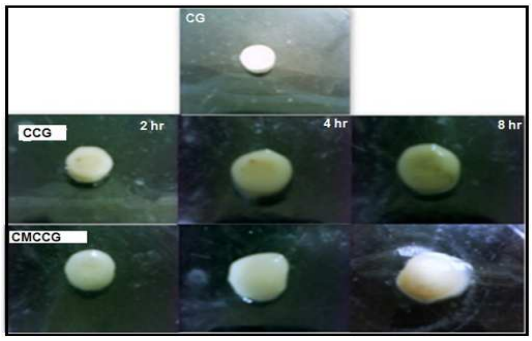


Fig. 9: Comparison of swelling of CCG and CMCCG tablets

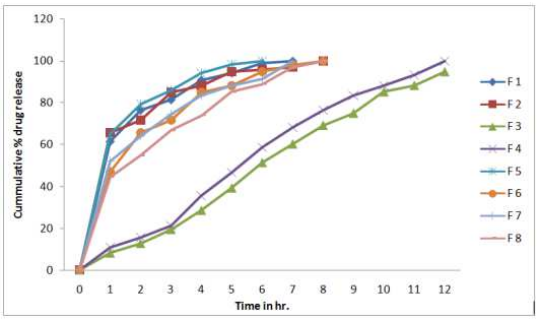


Fig. 10: *In vitro* release pattern for the prepared formulations.

But its charge enhancement may affect their hydration process which may be a scope of future study. During the test, all the formulation swelled and the outer layer of most of the tablets appeared to be hydrated after been placed in dissolution medium with the progressive increase in the size of this hydrated layer specially visualized for the matrices containing CCG (F 3 and F 4) and CMCCG (F 7 and F 8), followed by the gradual loss of integrity, resulting from the hydro dynamic stress induced by the dissolution apparatus. Therefore it remained more or less unchanged until the final stages of dissolution test, when the dry inner core becomes wet. The CG tablets show the higher tendency to loss its integrity than the CCG and CMCCG, probably due to cross linking of the CG, different hydration rate and distribution of this polymer than the former. In case of F 1 a rapid erosion of the hydrated layer was readily noticeable, releasing almost the drug content after 7 h of the experiment while F 2 depicted complete release by 8 h.

Optimization of the formulation using DoE software

Based on the results of studies carried out to select a suitable polymer and its concentration, different formulations were prepared to optimize polymer concentration. The quantity of the drug was constant in all these formulations. The experiments were designed by using an experimental design software (Design Expert®, v 8.0.1, Stat-Ease, USA).

An I-Optimal Response Surface historical data Design was employed to obtain 8 different factor combinations (Stat-Ease, 2010). Different factor combinations that were obtained and experimentally run to measure the responses R1 (Hydration and erosion), R2 (Release 6hr) are given in Table 7.

The formulations prepared according to the design were analyzed by using Design Expert ® ver 8.0.1 software package. The effect of formulation variables on the response variables was statistically evaluated by one way ANOVA at 0.05 levels [21, 22]. The design was evaluated by response surface method using following polynomial equation:

$$\mathbf{Y= B_o + b1*A + b2*B + b3*C + b4*D - b5*A^2 + b6*B^2 - b7*C^2}$$

Where Y is the response variable, β_0 the constant and b1, b2, b3, b4, b5, b6 and b7 are the regression coefficients. A, B, C and D stand for the main effect [20]. The equation for each response parameter was generated using one-way ANOVA and historical data design [21]. The predictor equation containing only significant terms were generated by backward elimination procedure.

Formula optimization of prepared tablet by design of experiment (DoE) method

Table 7 shows the values of response variables, R1 (Release), R2 (Hydration) obtained from batches prepared according to I-optimal response surface historical data design. In order to determine the significant design terms, their interactions and their effect on the response variables R1 and R2 the design was evaluated by response surface quadratic model that was selected on the basis of model p-values, lack of fit test, adjusted R^2 and predicted R^2.

Linear polynomial model equations were generated by ANOVA. Being close to 1.000, the values of R^2 for linear model indicated excellent fit of response surface polynomials to the response variable data. All the lack of fit values was found to be significant ($p > 0.05$) thus, indicating the validity of selected models.

The closeness of adjusted R^2 and predicted R^2 to actual model R^2also indicated the goodness of fit to the data. [23, 24] Final first order polynomial equations for each response variable obtained from significant coefficient terms are given below:

Release =+54.284-1.15 * A+3.72 * B-2.55 * C-1.00 * D-2.38 * A2 +14.84 B2 -3.48 * C2

Hydration =+277.35+9.90 * A+6.55 * B+7.45 * C+11.75 * D -21.42 * A2+14.60 * B2-22.03 * C2.

Table 5: Design of the prepared formulation

Std	Runs	A	B	C	D	R1 (Release)	R2 (Hydration)
1	1	1	0	0	0	98.1	164.8
2	2	3	0	0	0	95.8	184.6
3	3	0	1	0	0	51.3	53.4
4	4	0	3	0	0	58.73	66.5
5	5	0	0	1	0	100	164.2
6	6	0	0	3	0	94.9	179.1
7	7	0	0	0	1	91.1	102.4
8	8	0	0	0	3	89.1	125.9

Overall, the above data obtained by model analysis indicates the suitability and significance of the selected design, factors, levels and responses.

Effect of polymer concentration on hydration and drug release of the optimized formulation

From the figure 11, 12 it can be seen as the polymer concentration increases the hydration of the polymer increases at 8 and the % drug release from the polymer decreases

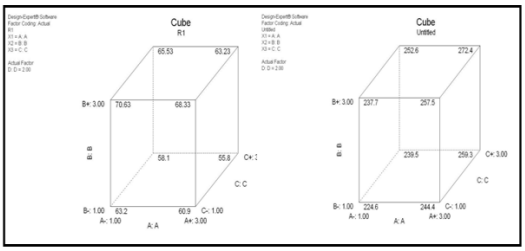


Fig. 11: cube diagram of the prepared formulation.

Optimization of the prepared formulation

The optimal formulation was searched within the studied experimental domain by numerical optimization technique. The constraints were set to the desired goals and entire experimental domain was searched for the compositions where the set constraints were met to the maximum.

Table 8 presents the constraints set for numeric optimization, the resulting optimized solutions, formulation compositions with corresponding response and desirability values. The solutions fulfill the target criteria of minimum release and optimum hydration were selected. Finally, the formulation corresponding to solution 1 was selected as the optimum formulation that had the desirability of 1.0 and the values of response variables were in the desired range.

The formulation contained 51.70% minimum release at 6 h, and 53.40 % hydration i. e. cross-linked cashew gum with 1:1 % polymer: drug ratio.

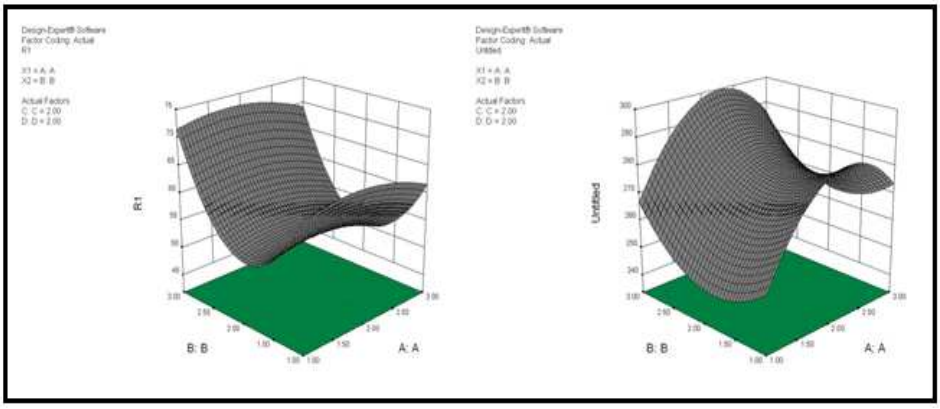
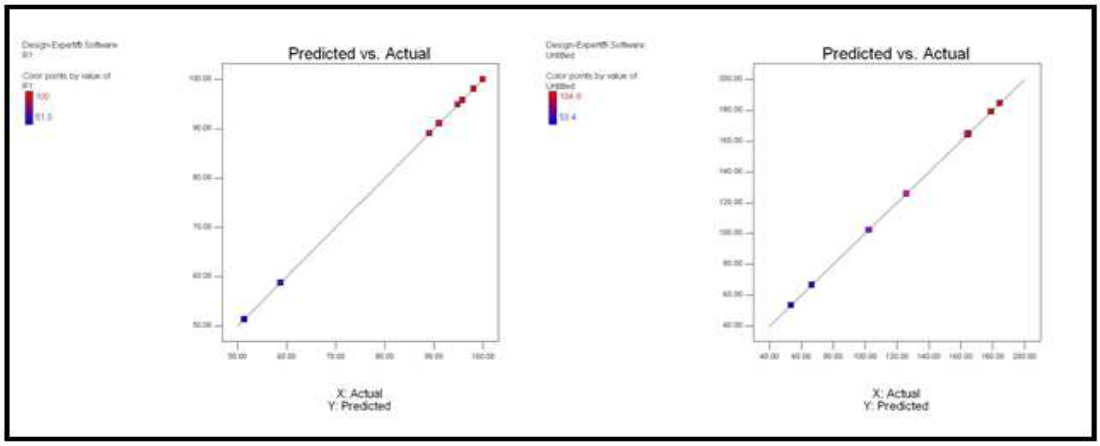


Fig. 12: 3D surface graph of the prepared formulation

Table 6:- Constraints set for numeric optimization

Constraints						
Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
A	is in range	-1	1	1	1	3
B	is in range	-1	1	1	1	3
C	is in range	-1	1	1	1	3
D	is in range	-1	1	1	1	3
Release	is target = 51.7	51.3	100	1	1	3
Hydration	is target = 53.4	53.4	184.6	1	1	3



3.12.4 Comparison of the experimental value and the predicted value using DoE software.

Fig. 13: Graph showing the comparison of the experimental value and the predicted value using DoE software.

Solutions								
Number	A	B	C	D	Release	Hydration	Desirability	
1	0	1	0	0	51.70005539	53.40008103	1	Selected

The result was analyzed using ANNOVA and the result was significant. Actual and predicted in this graph are very close to each other for all three responses. It shows that the variance is very less between predicted an actual values. So the result is significant.

Validation of the optimized formulation

The optimized formulation which was selected using DoE was validated using *In vitro* drug release studies.

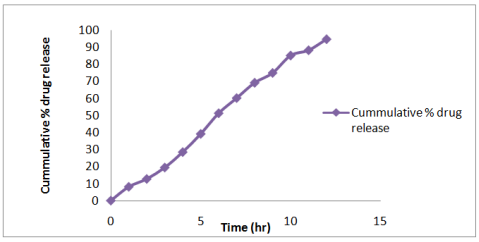


Fig. 14: The % drug release of the optimized formulation

Characterization of the Optimized Formulation

Fourier transform infrared (FT-IR) spectroscopy studies of optimized formulation

As evident from the release studies, the formulation F 3 was found to be optimized and hence FT-IR studies were carried out to rule out any interactions.

The FT-IR spectra of the optimized formulation confirmed that the formulation F 3 had no significant interaction with the cross-linked CG (1:1).

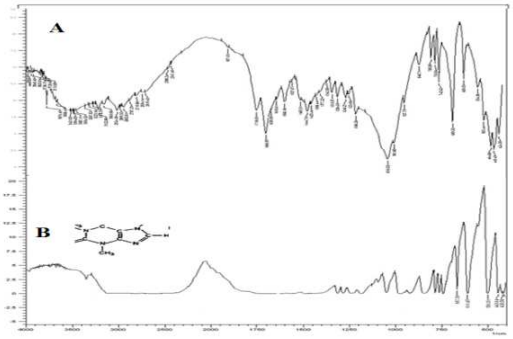


Fig. 15: (A) FT-IR of optimized formulation F 3, (B) FT-IR of theophylline.

Scanning Electron Microscopy (SEM)

Figure 16 displays the scanning electron micrographs showing the surface morphology of optimized formulation F 3. The SEM photographs of the tablets after 2 h, 4 h, 6 h and 8 h showed that the surface of the matrix tablet was highly porous and this would facilitate diffusion of drug from the tablet core to the surface. Since, the gel layer undergoes surface erosion; it was possible that the inner porous network may be exposed after the dissolution of the outer layer of the matrix. The formation of both pores and gel structure on the tablet surface indicates involvement of both erosion and diffusion mechanisms for sustained drug release.

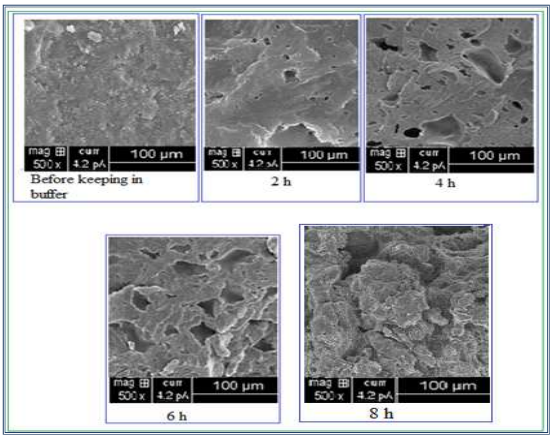


Fig. 16: Optimized tablet surface before and after *In vitro* dissolution

Kinetic studies

The results of kinetic analysis of the dissolution data showed a good fit in the various mathematical equations. Based on the R^2 value, it was found that the release of the drug from the optimized formulation F 3 matrix was independent of the drug concentration i. e it followed zero order kinetics. The release mechanism of the drug was not by single mechanism as the data showed a good fit in peppas equation obtaining near linearity and based on the diffusion exponent value (n), it was characteristic of anomalous non-fickian kinetics which refers to a combination of both diffusion and erosion controlled release.

Stability studies

Stability studies of the drug formulations are performed to ascertain whether the drug undergoes any physical/ chemical change or degradation during its shelf life. In the present study, the optimized formulations F3 offered better sustained release dosage form and hence were selected for stability studies. The stability studies were carried out by exposing the tablets kept in a glassed vial at 40 ± 1°C and at 75% RH for 6 weeks. After the end of each week, 3 tablets were taken and the total drug content was estimated spectrophotometrically. The obtained results of the stability studies are given in Table. From the stability study data, it was concluded that the formulations were stable for the study period.

Table 7: Stability study data of optimized formulations F3

Sampling Interval	% Drug content		
	25 °C/60% RH	30 °C/65% RH	40 °C/75% RH
0 Days	98.9 ± 0.36	98.9 ± 0.36	98.9 ± 0.36
15 Days	98.4 ± 0.21	98.1 ± 0.78	98.2 ± 0.64
45 Days	97.8 ± 0.24	97.6 ± 1.04	97.4 ± 0.51
60 Days	97.5 ± 0.54	96.9 ± 0.48	96.7 ± 0.83

CONCLUSION

The research findings obtained from the studies were found to be satisfactory and it can be concluded that modified cashew gum can be effectively used as the sustained release polymer.

CONFLICT OF INTERESTS

Declared none

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