

Interpenetrating networks of carboxymethyl tamarind gum and chitosan for sustained delivery of aceclofenac

Kailas Krishnat MALI, Shashikant C DHAWALE, Remeth J DIAS, Vijay D HAVALDAR, Pankaj R KAVITAKE

ABSTRACT

The aim of present investigation was to characterize carboxymethyl tamarind gum (CMTG) based interpenetrating networks (IPNs) of aceclofenac for site specific sustained delivery. The drug loaded IPNs were prepared by using chitosan and CMTG as polymers and glutaraldehyde as crosslinking agent. The IPNs were characterized by Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, thermal analysis, X-ray powder diffraction and solid state ¹³C-nuclear magnetic resonance spectroscopy. The prepared IPNs were evaluated for the drug entrapment efficiency and equilibrium swelling. The drug release from IPNs was studied in 0.1N HCl for 2h followed by phosphate buffer pH 6.8 for further 10h and compared with commercial tablet. The results of ATR-FTIR

and thermal analysis for blank IPNs indicated intercalation of polymeric chains of crosslinked CMTG and chitosan. The results of solid state characterization revealed that the aceclofenac is compatible with IPNs. Entrapment efficiency of IPNs was found to be increased with increase in crosslinker concentration as well as amount of CMTG. The equilibrium swelling study indicated pH dependent swelling of IPNs. The drug release by IPNs showed sustained release of aceclofenac upto 12h while commercial formulation showed fast release within 8h. From the results, it can be concluded that the IPNs of CMTG and chitosan has potential in development of site specific sustained drug delivery.

Key words: Interpenetrating networks; carboxymethyl tamarind gum; chitosan; aceclofenac; crosslinking.

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1. Introduction

Amongst various routes of administration, oral route of drug delivery is widely used for sustained drug delivery because of advantages like ease of administration, improved patient compliance, site specific delivery and reduction in dosing frequency [1]. The multiple dosing is required in case of chronic disorders and may be associated with certain side effects. In order to reduce dosing frequency and associated side effects, sustained and site specific drug delivery approach has been widely applied [2]. The goal of site specific drug delivery system is to maintain a dose of drug at desired target site for specified time. The site specific drug delivery directly targets the dose of drug to certain biological location. It may be preferable in certain situations like drug instability, low solubility, shorter half life, poor absorption, low specificity and low therapeutic index [3].

It has been shown that single polymer is unable to give oral site specific sustained drug release and hence combination of polymers could be better option. One of the promising approach for oral site specific sustained drug delivery is interpenetrating networks (IPNs)[4]. IPNs are polymeric network of two or more polymers that forms a rigid composite network structure by cross-linking of at least one polymer in presence of other. The combination of polymers ensures improved mechanical strength, loading capacity, sustained and controlled drug release[5,6]. IPNs provides space for drug encapsulation in three dimensional structure which may or may not be cross linked [7]. IPNs are prepared to combine individual properties of polymers, suggesting that combination or mixture of polymers can be effectively used for drug delivery system. The natural and synthetic polymers can be widely used to prepare IPNs [8]. Natural polymers combined with other natural, semi-synthetic or synthetic polymers can achieve combined polymer properties for better management of drug delivery.

Chitosan is a natural, pH sensitive biopolymer obtained from deacetylation of chitin. It is biocompatible, biodegradable polysaccharide, which is degraded by human enzymes [9]. Chitosan is widely utilized in various drug delivery systems, tissue engineering and also in food technology [10]. It is not stable in acidic environment due to its fast dissolution leading to fast release of drug in stomach [11]. Hence, alone chitosan is not suitable for development of site specific sustained drug delivery. The combination of chitosan with other polymer may prove effective in such cases. Chitosan, having a amino group shows net positive charge which may be combined or cross-linked with negatively charged COO⁻ to form rigid composite structure of IPNs.

Tamarind gum (TG) is another natural polymer which can be used in formation of IPNs [8,12]. It is cheap and biocompatible polysaccharide used as emulsifier, suspending, gelling and binding agent [6,13]. TG has some disadvantages like unpleasant odor, dull color and fast degradation [14]. To overcome these problems carboxymethylation of TG is done [15]. Carboxymethyl tamarind gum (CMTG) is a semisynthetic derivative of TG which imparts viscosity, enhances shelf life, shows anionic character and controls swelling as well as drug release properties [7,14]. Thus, it may be good release retardant material in drug delivery [16-20]. The CMTG based formulations have been reported in literature for bone tissue engineering which supports adhesion and production of osteoblast precursor cells [21,22]. It has also been used to proliferate human keratinocyte cells in skin

tissue engineering application [23]. Also, it has potential biomedical application suggesting minimal cytotoxicity towards mammalian cells with affinity towards bacterial cell wall [24]. Novel CMTG-g-polyacrylonitrile hydrogel has also been reported as an adsorbant in literature for the diaper application [25]. The interpenetrating hydrogels of CMTG and alginate for delivery of acyclovir has been reported in literature [18].

CMTG has low cost than other semi-synthetic derivatives of polysaccharides. It may be the suitable alternative from biomaterial point of view because the structure involves the backbone as that of cellulose which is hydrophilic in nature. The IPNs of chitosan with tamarind gum was reported in literature for delivery of aceclofenac [6]. However, no study has been reported till date, where combination of semi synthetic polymer CMTG and chitosan has been used for preparation of IPNs. Therefore it was proposed to prepare IPNs using CMTG with chitosan so that site specific extended release of aceclofenac can be warranted.

In this study aceclofenac was used as model drug. It is a non-steroidal anti-inflammatory drug (NSAID) with short half-life (4h) used for the symptomatic treatment of pain and inflammation. Due to short half-life of aceclofenac, it is required to administer frequently [26]. Hence, aceclofenac is an ideal candidate for developing a sustained release site specific dosage form.

Considering above facts, an attempt was made to develop pH dependent site specific IPNs of aceclofenac using CMTG and chitosan. The prepared IPNs were characterized by ATR-FTIR, thermal analysis, X-ray diffraction and solid state ¹³C NMR. Also, IPNs were evaluated for equilibrium swelling and drug release.

2. Results and Discussion

2.1 Preparation of IPNs

For the preparation of IPNs at least two polymers are required, from which one polymer should be crosslinked by crosslinker. In present study, chitosan was used as base polymer and was crosslinked using glutaraldehyde to form network while the second polymer, CMTG, gets entangled in crosslinked chains of chitosan. Further, it was proposed that secondary -OH of CMTG may get crosslinked with glutaraldehyde. Also, the intercalation of second polymer in chitosan may be feasible due to formation of polyelectrolyte complex between free -NH₃⁺ of chitosan and -COO⁻ of CMTG. The proposed structure of IPNs is given in Figure 1.

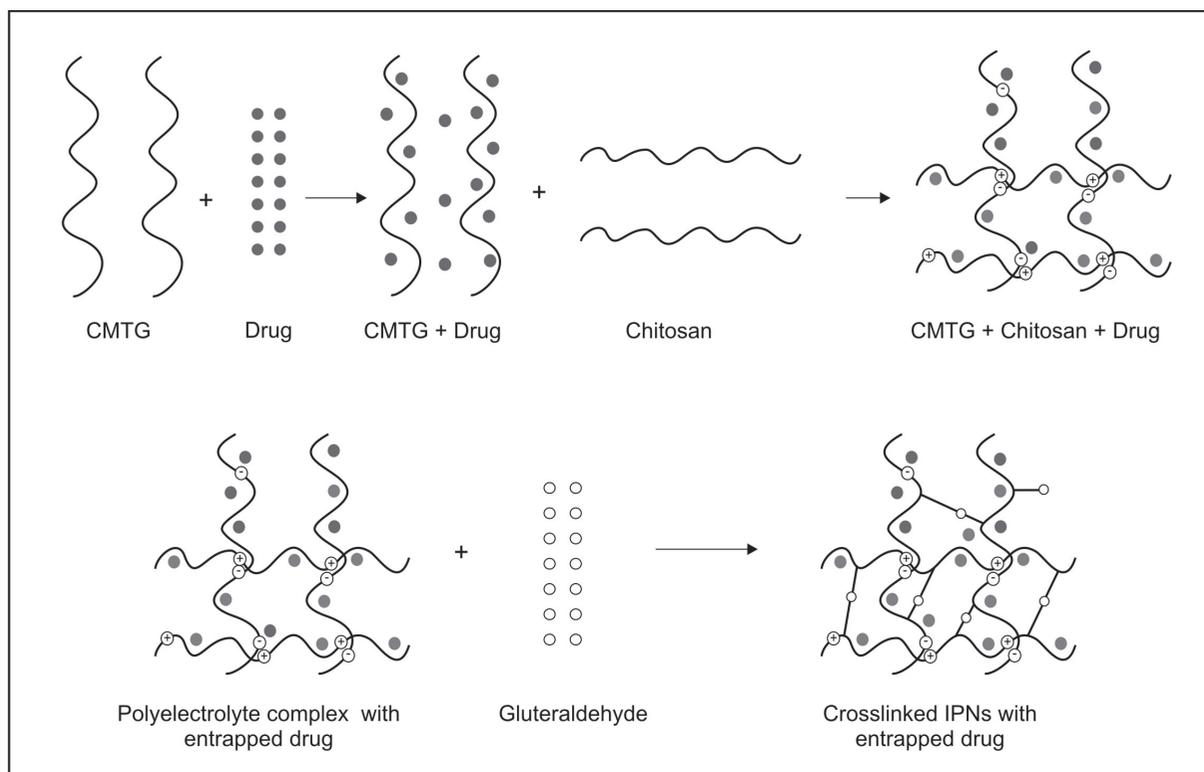


Figure 1. Proposed structure of IPN entrapped with drug

2.2 Drug entrapment efficiency

Drug entrapment within polymeric complex was expressed as percentage ratio of actual aceclofenac entrapped in polymeric complex with that of initial amount of aceclofenac used in formulation. Drug entrapment efficiency of IPNs was observed in the range of 61.3 to 88.5% (see Table 1). In case of batch C3 high drug entrapment efficiency was noted due to high concentration of glutaraldehyde. The drug entrapment efficiency was found to be increased significantly ($p < 0.05$) when concentration of glutaraldehyde was increased. This may be due to increase in cross linking degree of IPNs [27]. Also CMTG concentration had significant effect on

entrapment efficiency. This might be due to formation of thick surface which decreases loss of drug due to the high viscosity of CMTG. The results of entrapment efficiency were in good agreement with earlier studies [6,28].

2.3 Attenuated total reflectance-Fourier transform infrared spectroscopy

The formation of crosslinked IPNs was confirmed by ATR-FTIR analysis (see Figure 2). The CMTG showed peak at 1402 cm^{-1} (C=O), which confirms carboxymethylation of tamarind gum [10] and also peak at 1639 cm^{-1} is due to

Table 1. Formulation of aceclofenac loaded IPNs and entrapment efficiency

Batch	Chitosan (% w/v)	CMTG (% w/v)	Aceclofenac (% w/v)	Glutaraldehyde* (25% w/v) (ml)	Entrapment efficiency (%)
C0	0.4	0.6	00	1	-
C1	0.4	0.6	0.8	1	69.82±2.56
C2	0.4	0.6	0.8	2	78.37±2.63 ^a
C3	0.4	0.6	0.8	3	78.37±2.63 ^a
C4	0.4	0.4	0.8	1	61.30±2.87 ^a
C5	0.4	0.8	0.8	1	73.93±2.84 ^a

CMTG- carboxymethyl tamarind gum; *diluted to 5ml with distilled water; ^a statistically significant ($p < 0.05$) than C1

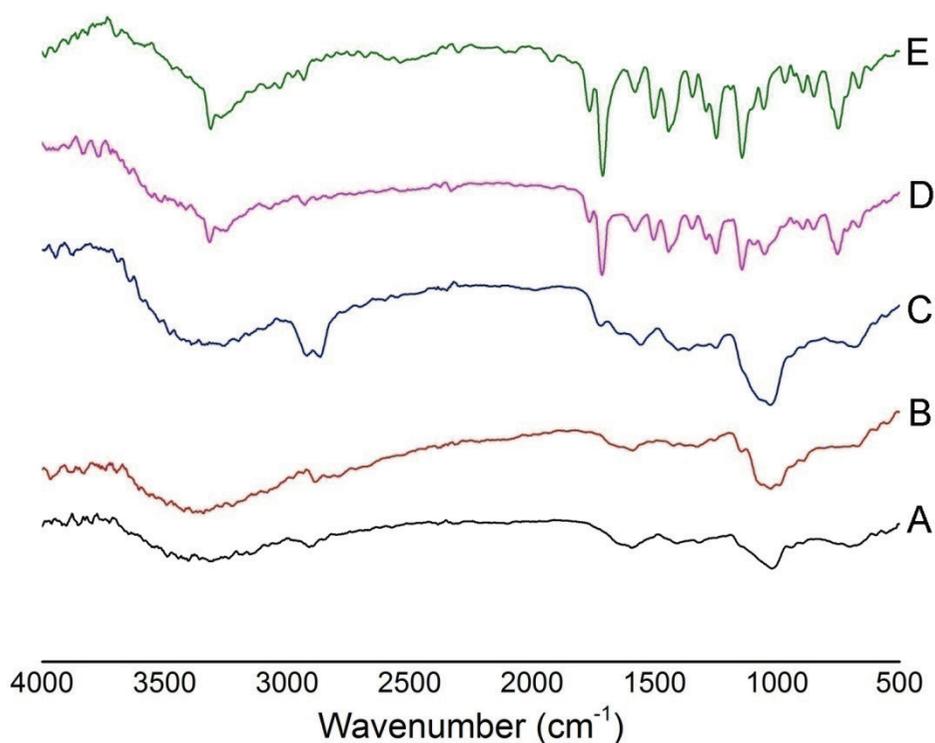


Figure 2. ATR-FTIR of CMTG (A), chitosan (B), blank IPN (C), drug loaded IPN (D), aceclofenac (E)

COO⁻. The broad peak at 3423 cm⁻¹ is of O-H stretching and peak at 1010 cm⁻¹ is indicative of C-O-C stretch of glycosidic linkage [18]. The spectra of chitosan showed characteristic peak at 3421 cm⁻¹ due to N-H and -OH stretching. Three peaks appeared at 1650 cm⁻¹, 1589 cm⁻¹ and 1375 cm⁻¹ due to amide I, amide II, amide III [29].

The spectra of blank IPNs showed all characteristic peaks of both chitosan and CMTG along with emerging new peaks at 1645 cm⁻¹ and 1028 cm⁻¹ due to C-N stretching vibration of imine group of Schiff base formed from NH₂ group of chitosan and -CHO group of glutaraldehyde and an acetal group formed due to OH group of CMTG and -CHO groups of glutaraldehyde respectively. Also, one more peak emerged at 1720 cm⁻¹ of carbonyl group due to formation of linkage between glutaraldehyde and CMTG. This may be attributed to the formation of acetal bridges due to reaction of -OH group of CMTG with aldehyde group of glutaraldehyde [30]. This confirms the crosslinking of chitosan and CMTG by glutaraldehyde. Further, broadening of -OH stretching vibration in case of blank IPN suggests the intercalation of polymeric chains due to strong intermolecular interactions including hydrogen bonding of CMTG and chitosan [18]. Similar findings have been reported in case of CMTG-gelatin

IPNs [7]. A peak at 1404 cm⁻¹ in IPNs indicates presence of C=O stretch of -COO⁻ ions of CMTG and peak at 1556 cm⁻¹ may be for N-H stretch of NH₃⁺ of chitosan leading to formation of interpolymer complex between CMTG and chitosan [10]. From the results of ATR-FTIR study it can be concluded that there exists interpolyelectrolyte complex between -COO⁻ group of CMTG and -NH₃⁺ group of chitosan due to electrostatic attraction and formation of crosslinks between CMTG and chitosan by glutaraldehyde.

The ATR-FTIR spectral analysis was also used to confirm chemical stability of aceclofenac in IPNs. The spectra of pure aceclofenac showed principal characteristic peaks at 2934 cm⁻¹ due to C-H stretching vibrations and a sharp and intense bands at 1712 cm⁻¹ and 1765 cm⁻¹ due to C=O stretching of carboxyl group. A band at 1290 cm⁻¹ is due to -OH in plain bending vibration and peak at 752 cm⁻¹ is of C-Cl stretch. A peak at 3312 cm⁻¹ is due to secondary N-H that confirms aceclofenac [28-31].

In case of drug loaded IPNs, all characteristic peaks of aceclofenac were observed with insignificant shifting and reduction in intensity. The peak for C=O stretching vibration shifted towards higher frequency indicating hydrogen bonding with IPN network.

2.4 Thermal Analysis

A thermal behavior and physical state of pure aceclofenac in IPN composite was examined through DSC–TGA analysis. Thermal decomposition curve of CMTG shows two main stages of decomposition (Figure 3 TGA). The first stage begins at 35°C and ends at 100°C with 3.91% weight loss. This may be due to the removal of free and bound water from the polymer. The second stage of weight loss was observed at 235°C to 425°C with 54.42% loss of weight. The total weight loss was found to be 90% at 485°C. The weight loss of CMTG in second stage is attributed to the decomposition of the polymer backbone. In case of chitosan first stage started at 35°C and ended at 112°C with 9.64% weight loss. The second stage started at 205°C and ended at 390°C with 52% weight loss which may be due to the degradation of polymer backbone. The total weight loss at the end of 485°C was 81%.

Thermal decomposition curve of IPN shows two main stages of decomposition. The first stage begins at 35°C and

ends at 225°C with 13% loss of weight. In second stage, 42% of weight loss was observed between 225°C to 395°C. The total 72% of weight loss was found at the end of 485°C. The total weight loss in case of IPN network was found to be reduced significantly than the individual polymer indicating formation of polymeric crosslinked structure with intercalation of polymeric chains of CMTG and chitosan.

Thermal decomposition of aceclofenac shows two stages of decomposition. The first stage starts at 35°C and ends at 150°C with 1% weight loss, which may be due to the removal of water. The second stage started at 155°C and ended at 335°C with 46% loss in weight. The total weight loss was found to be 56% at the end of 485°C. In case of drug loaded IPN, first stage started at 35°C and ended at 195°C with 9% weight loss and second stage begins at 195°C and ends at 385°C with 45% loss in weight. The total weight loss at the end of 485°C was 64%. Thermal stability of drug loaded IPNs was found to be improved than the blank IPNs.

The DSC analysis (Figure 3 DSC) for CMTG, chitosan and blank IPNs was in agreement with TGA curve and suggests the formation of crosslinked IPNs. The DSC of aceclofenac shows a sharp melting endothermic peak at 155.92°C [7]. In case of drug loaded IPNs, endotherm of aceclofenac disappeared suggesting the uniform dispersion of aceclofenac at molecular level in the IPNs [6].

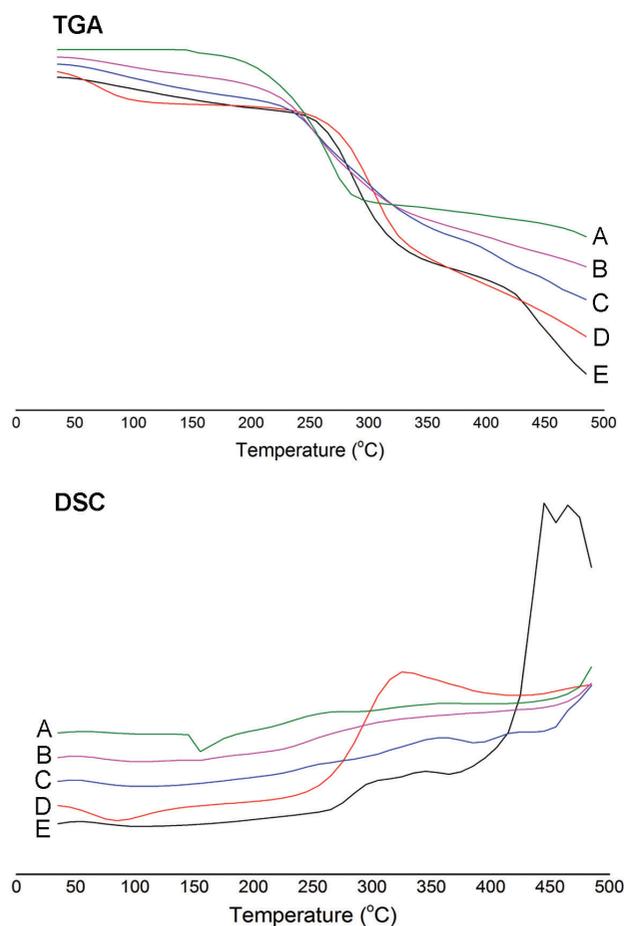


Figure 3. TGA and DSC of aceclofenac (A), drug loaded IPN (B), blank IPN (C), chitosan (D) and CMTG (E)

2.5 X-ray powder diffraction

XRD was performed in order to investigate physical state of drug in IPNs. XRD of aceclofenac, blank IPNs and drug loaded IPNs are given in Figure 4. XRD pattern of pure aceclofenac showed characteristic diffraction peaks at 2θ of 18.46°, 19.42°, 22.26°, 25.94°, 25.96°, 25.98° and 26° with different peak intensity indicating crystalline nature of drug. The intensity of characteristic peaks referred for crystalline nature of aceclofenac was found to be decreased in XRD pattern of aceclofenac loaded IPN batch, which could be due to polymeric interaction. The significant reduction in diffraction pattern of aceclofenac loaded IPNs suggest that the crystalline nature of pure aceclofenac is converted to amorphous form [32].

2.6 Solid state ^{13}C Nuclear magnetic resonance spectroscopy

Solid state ^{13}C NMR was performed to investigate compatibility of aceclofenac with IPNs. Aceclofenac shows resonance peaks at 38.05 ppm and 62.57 ppm that are associated with C4 and C2 carbon respectively (see Figure 5). The chemical

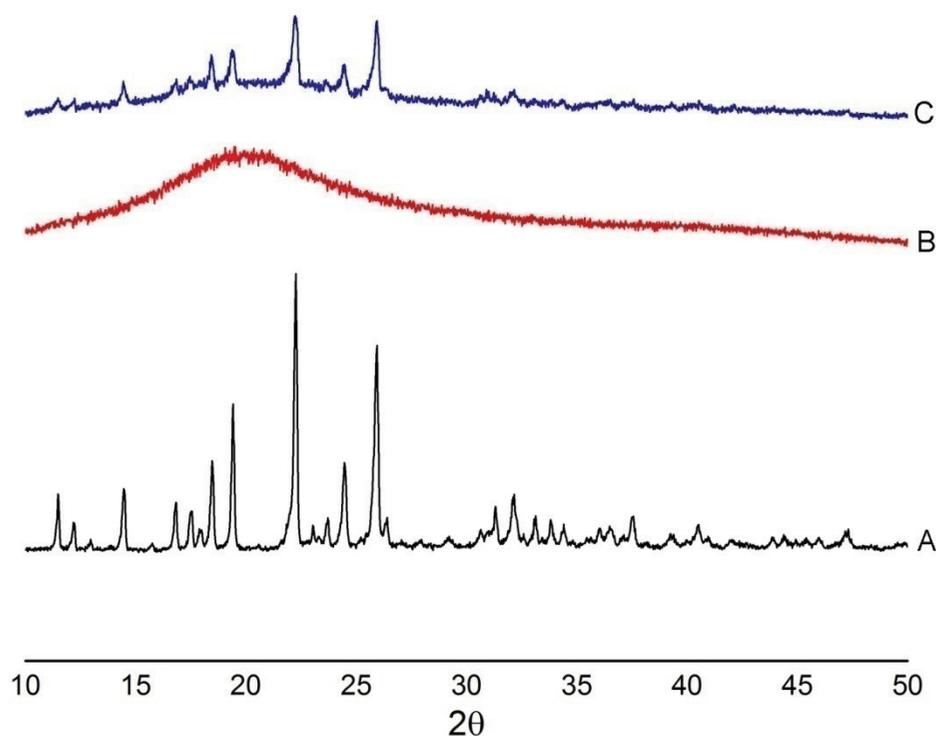


Figure 4. XRD of Aceclofenac (A), blank IPN (B) and aceclofenac loaded IPN (C)

shift from 116.9 ppm to 147.5 ppm is assigned to the benzene carbon atoms (C5 to C10 and C11 to C16). The carbonyl carbon of aceclofenac shows chemical shift at 170.7 ppm for C3 and 175.54 for C1 due to partial ionization of carbonyl group. The benzene ring connected to $-NH$ by carbon C6 and C11 showed chemical shift at 139.3 ppm and 143.5 ppm respectively. The chemical shift at 123.24 ppm is assigned to carbon atom of benzene ring connected to chlorine [31]. In case of aceclofenac loaded IPNs all essential chemical shifts of aceclofenac with slight shifting are seen indicating no any unwanted interaction between drug and IPNs. The peaks of carbonyl carbon for aceclofenac were observed at 170.7 ppm and 175.54 ppm with reduction in intensity, which suggests that there might be formation of hydrogen bonding between aceclofenac and IPNs. Aceclofenac consists of acidic ($-COOH$) group, while chitosan is having $-NH_2$ group. After ionization of $COOH$ into COO^- and NH_2 into NH_3^+ it may be prone to react with each other and able to encapsulate aceclofenac within CMTG - chitosan IPNs. This could be possible reason behind the stabilization of aceclofenac in the IPNs. In case of blank IPNs broad resonance peak was observed at 179.6 ppm, which may be due to free carbonyl group of CMTG and the carbonyl group formed due to crosslinked CMTG [33].

2.7 Equilibrium swelling

The site specific drug delivery is reliant on the pH of medium. Factors which contribute in swelling of ionic polymers like CMTG and chitosan may include polymer charge, concentration and pKa of ionizable group, degree of ionization, crosslinking density, hydrophilicity and hydrophobicity as well as pH of swelling medium. The equilibrium swelling of aceclofenac loaded IPNs was determined in 0.1N HCl and phosphate buffer pH 6.8 (see Figure 6). The variation of equilibrium swelling may be due to the fact that IPNs in contact with swelling medium, prevents expansion of its three dimensional hydrophilic network. The expansion is prevented by the extent of crosslinking and provides an elastic response which affects the swelling of IPNs [34].

IPNs showed significantly low ($P < 0.05$) swelling in 0.1N HCl than the phosphate buffer pH 6.8. CMTG is anionic polymer which is in unionized state in the acidic environment. The pH of 0.1N HCl solution is less than the pKa of the carboxyl group [4-5] present in CMTG, leading to decreased electrostatic repulsion due to protonation of carboxylic groups ($COOH$). This retards swelling owing to reduction in water uptake capacity of IPNs [35]. The chitosan is a cationic hydrophobic

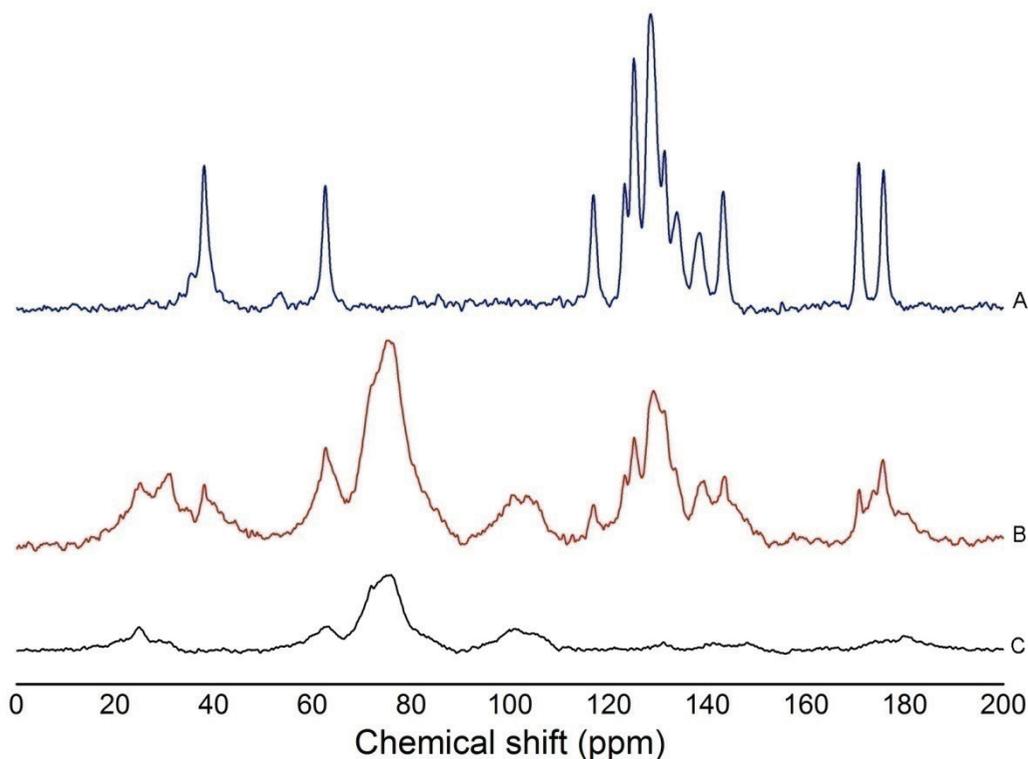


Figure 5. Solid state ^{13}C NMR of Aceclofenac (A), drug loaded IPN (B) and blank IPN (C)

polymer. The most of the amino groups of chitosan are crosslinked by the glutaraldehyde and remaining may be associated with the CMTG. This could be due to formation of polyelectrolyte complex between the CMTG (COO^-) and chitosan (NH_3^+) due to $\text{NH}_3^+\text{COO}^-$ linkage. These crosslinks and polymeric complex were stable in the acidic environment [7, 10]. These factors may be responsible for the decrease in the swelling of IPNs at acidic pH. When concentration of CMTG was increased in the IPNs, the equilibrium swelling was found to be increased. This might be due to hydrophilic nature of the CMTG [16]. The results were in agreement with the earlier reports by Kaur *et al* [10].

In case of C5 batch, high values of swelling in phosphate buffer pH 6.8 may be attributed to electrostatic repulsion of ionized carbonyl (COO^-) groups due to deprotonation of CMTG which increases the polymer mobility and enhances mesh size of network there by increasing the equilibrium swelling. Also, crosslinks formed by glutaraldehyde and the $\text{NH}_3^+\text{COO}^-$ links may be unstable at pH 6.8 and contributes to enhancement in the equilibrium swelling of IPN [7, 10]. When the concentration of glutaraldehyde was increased in IPNs (C1-C3) equilibrium swelling was found to be decreased. The batch C3 showed very low swelling because

of high crosslinking density of IPNs due to reduction in mobility, relaxation and expansion of the polymer chains.

The results of swelling study in 0.1N HCl and phosphate buffer clearly indicates the pH dependent swelling of CMTG-chitosan IPNs. It suggests the suitability of IPNs for oral site specific delivery of drug in order to avoid exposure of drug to erratic gastric environment and drug release in the intestine.

2.8 *In vitro* drug release

The *in vitro* drug release of formulated IPNs was determined by using dialysis bag diffusion technique. The *in vitro* drug release was carried out in 0.1N HCl for 2h followed by phosphate buffer pH 6.8 up to 12h [7]. The IPNs showed less than 5% of drug release in 0.1N HCl at the end of 2h followed by controlled release in phosphate buffer pH 6.8 over period of 12h (see Figure 7). The results of *in vitro* release indicated pH dependent sustained release of aceclofenac from IPNs.

The results also indicated that, when the concentration of crosslinker (C1-C3) is increased from 1 to 3ml the drug release was found to be decreased in phosphate buffer, pH 6.8. This may be due to increased degree of crosslinking

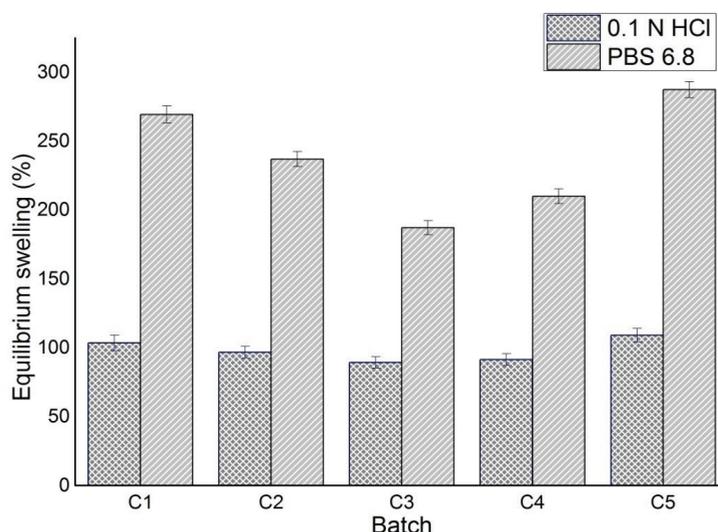


Figure 6. Equilibrium swelling (%) of IPNs in 0.1N HCl and phosphate buffer pH 6.8

within polymeric network resulting in retardation of drug release from IPNs [6]. In case of batch C5, high concentration of CMTG in IPNs also retarded drug release. This may be due to high swelling of CMTG in phosphate buffer which increased the path length to diffuse out the drug into bulk of solution. Drug release from commercial tablet was found to be faster than IPNs and 100% of drug was released at the end of 8h while IPNs extended release upto 12h. The fast release of drug from the commercial tablet in PBS pH 6.8 may be attributed to the dispersion of drug into the polymer matrix. In case of IPNs, drug gets entrapped in the network matrix at molecular level from which it gets released at slower rate than the commercial formulation.

The release data was fitted into the zero order, Higuchi and Korsmeyer-Peppas equation to investigate release mechanism from IPNs [36]. In vitro release parameters like mean dissolution time (MDT), dissolution efficiency (DE) and release kinetics is given in Table 2. The value of MDT was found to be in the range of 4.08 to 4.76h where as dissolution efficiency was found to be in the range of 40.46 to 59.02%. All IPN batches followed the zero order kinetics while commercial formulation followed Higuchi kinetics model. The 'n' is the diffusional coefficient obtained from Korsmeyer-Peppas equation which depends upon the interaction in between drug and the components of IPN matrix. The result of diffusion coefficient (n) and release

Table 2. *In vitro* release parameters of aceclofenac from IPNs

Batch	Drug release at 12h (%)	DE (%)	MDT (h)	Zero order	Higuchi	Korsmeyer-Peppas	
				r	r	r	n
C1	93.64	52.98	4.34	0.998	0.989	0.995	0.75
C2	84.47	45.95	4.55	0.999	0.987	0.997	0.80
C3	70.11	36.71	4.76	0.999	0.981	0.995	0.86
C4	99.71	59.02	4.08	0.997	0.995	0.997	0.68
C5	74.13	40.46	4.54	0.999	0.988	0.998	0.79
CF	99.79	60.93	2.33	0.974	0.991	0.991	0.66

DE- dissolution efficiency; MDT-mean dissolution time; CF- commercial formulation
r- correlation coefficient; n-release exponent

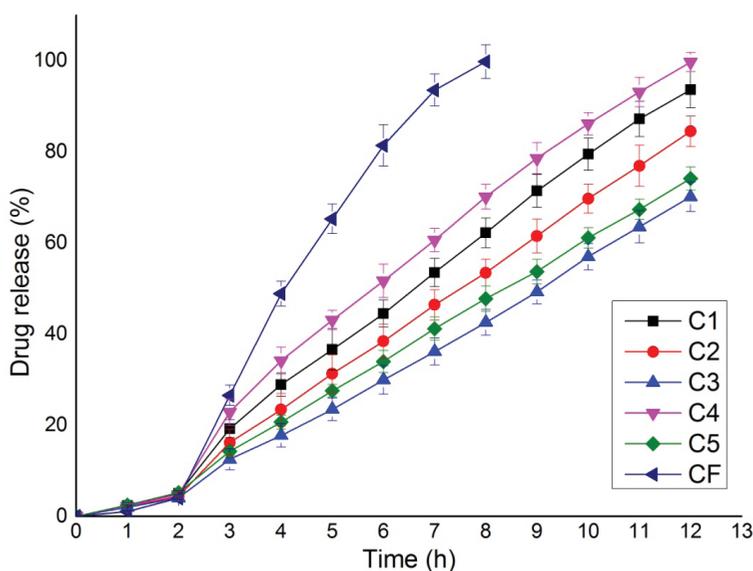


Figure 7. Drug release of aceclofenac from IPNs and commercial formulation (CF)

mechanism is given in Table 2. All batches of IPNs showed Non-Fickian release behavior. The value of diffusion coefficient was found to be greater than 0.5 indicating high interaction between drug and IPNs and drug was released by diffusion coupled with erosion mechanism. The value of 'n' was found to be increased when concentration of crosslinker and CMTG was increased.

3. Conclusion

The IPNs of CMTG and chitosan were successfully developed. CMTG and chitosan has ability to form the polyelectrolyte complex due to electrostatic attraction between $-\text{COO}^-$ group of CMTG and NH_3^+ of chitosan. ATR-FTIR and thermal analysis confirmed the formation of glutaraldehyde crosslinked IPNs. DSC and XRD study revealed the conversion of crystalline aceclofenac to the amorphous form and formation of molecular dispersion of aceclofenac into IPNs. The aceclofenac was incorporated into the IPNs with high entrapment efficiency. The equilibrium swelling study showed the pH dependent swelling of IPNs. Aceclofenac showed the site specific sustained drug release in phosphate buffer pH 6.8 with non-Fickian drug release. From results, it can be concluded that the drug loaded crosslinked IPNs

of CMTG and chitosan can be used for the oral site specific sustained delivery of drugs. Further, *in vivo* study can be addressed to confirm the suitability of IPNs for oral sustained delivery of drugs.

4. Materials and Methods

Carboxymethyl tamarind gum (Degree of substitution ~0.16) was kindly gifted by Tamarind Magic, Hyderabad, India. Chitosan (degree of acetylation- 83%) was obtained from SD Fine Lab, Mumbai, India. Aceclofenac was obtained as a gift sample from Micro Labs., Bangalore, India. The glutaraldehyde (25% w/v) was supplied by Loba Chemie, Mumbai, Maharashtra (India). All other chemicals were of analytical grade and used as received. Commercial tablet of aceclofenac (Dolokind SR) was purchased from local market.

4.1 Preparation of interpenetrating networks (IPNs)

Aceclofenac IPNs were prepared by slight modification in earlier reported method [6, 8]. In brief, a mixture of carboxymethyl tamarind gum (0.6%w/v) and aceclofenac (0.8%w/vs) was magnetically stirred (Remi, India) at 500rpm for 30 minutes in distilled water. This mixture was added to

a chitosan solution (0.4%w/v) in 1% aqueous acetic acid with 0.5ml of Tween 80 and stirred to homogenize for one hour on mechanical stirrer (RQG126D, Remi, India). A measured quantity of aqueous glutaraldehyde (25%w/v diluted up to 5ml) was added at two minute time interval for 10min and stirred for further 10min. The mixture was kept for 1h to remove entrapped air bubbles and then filtered. The filtrate was washed with water to remove unreacted content. Washed IPNs were transferred to petriplate and subjected to drying for complete removal of water at 40°C in hot air oven (BioTechnic, Mumbai, India). The detailed composition of IPNs batches is given in Table 1. Blank batch of IPNs was also prepared.

4.2 Attenuated total reflectance-Fourier transform infrared spectroscopy

The infrared spectra of CMTG, chitosan, physical mixtures, blank IPNs and aceclofenac loaded IPNs were obtained using ATR-FTIR spectrophotometer (Shimadzu, Miracle 10, IR Affinity, Japan). The samples to be analyzed were placed onto the ATR and spectra were recorded in the range of 600–4000 cm^{-1} at an average of 25 scans and resolution of 4 cm^{-1} .

4.3 Thermal analysis

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) of CMTG, chitosan, blank IPNs, drug loaded IPNs and aceclofenac was performed using Mettler-Toledo TGA/DSC1 thermogravimetric analyzer (Mettler-Toledo, Switzerland). Samples were heated from 30°C–500°C at the rate of 10°C/min, under nitrogen atmosphere (flow rate: 10 ml/min).

4.4 X-ray powder diffraction

X-ray diffraction (XRD) patterns of aceclofenac, blank IPNs and drug loaded IPNs were recorded using X-ray diffractometer (PW1729, Philips, The Netherlands) with a copper target, operated at voltage of 30 kV, 30 mA current, at 2°C/min scanning speed and scanning angle ranging from 0 to 50° (2 θ).

4.5 Solid state ¹³C Nuclear magnetic resonance spectroscopy

Solid state ¹³C cross-polarization-magic angle spinning (CP-MAS) NMR spectra of aceclofenac, blank IPNs and

drug loaded IPNs was measured using JEOL-ECX400 spectrometer operating at 400 MHz (contact time of 3.5 ms, relaxation delay of 5s, sweep width of 35 kHz and spinning speed of 10KHz). The chemical shifts were calibrated with the external hexamethylbenzene standard methyl resonance at 17.3ppm.

4.6 Drug entrapment efficiency

Accurately weighed quantity (100mg) of aceclofenac loaded IPNs were transferred to 100ml volumetric flask and volume was adjusted to 100ml with phosphate buffer, pH 6.8. Resultant mixture was stirred for 24h using magnetic stirrer (Remi, India) at 100rpm and sonicated for 30min. The solution was filtered through 0.44 μ membrane filter paper and drug content in filtrate was determined using UV-Vis spectrophotometer (UV 1800, Shimadzu, Japan) at 273 nm. The drug entrapment efficiency of IPNs was calculated using following formula:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Drug content in IPNs}}{\text{Theoretical drug content in IPNs}} \times 100 \quad (1)$$

4.7 Equilibrium swelling

Known amount (100mg) of IPNs were transferred to 100ml of 0.1N HCl and phosphate buffer pH 6.8 separately and allowed to swell for 24h at room temperature. IPNs were separated after 24h and excess water was blotted with filter paper and reweighed again [7]. Finally equilibrium swelling index of formulated batches was calculated by using formula:

$$\text{Equilibrium swelling (\%)} = \frac{\text{Swollen weight 6 IPNs} - \text{Dry weight 6 IPNs}}{\text{Dry weight 6 IPNs}} \times 100 \quad (2)$$

4.8 In vitro drug release study

In vitro drug release from formulated IPNs was determined by using dialysis bag diffusion technique. Accurately weighed IPNs equivalent to ~200mg of aceclofenac were placed in dialysis bag which was tied and immersed in dissolution vessel containing phosphate buffer pH 6.8, using Type II USP Apparatus (Electrolab, Mumbai, India). The system was maintained at 37°C with stirring speed of 50 rpm. The aliquots (2ml diluted up to 10 ml) were collected from dissolution vessel at regular time interval and replenished with the fresh dissolution media to maintain the sink condition. Collected samples were filtered, diluted and absorbance was measured at 273nm by using UV spectrophotometer (UV 1800, Shimadzu, Japan) [6].

4.9 Statistical analysis

Data was expressed in mean with standard deviation. Statistical data analysis was performed using one-way ANOVA. The *P* value less than 0.05 were considered statistically significant.

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Conflict of interest statement

All authors approve the final manuscript and declare that there are no conflicts of interests.

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