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Synthesis and characterization of carboxymethyl chitosan hydrogel: Application as site specific delivery for lercanidipine hydrochloride

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Abstract. **In the present study, carboxymethylchitosan (CMCS) was prepared from chitosan, crosslinked with glutaraldehyde and evaluated *in vitro* as a potential carrier for site specific drug delivery of lercanidipine hydrochloride (LERH). LERH was incorporated at the time of crosslinking of CMCS. The chitosan was evaluated for its degree of deacetylation (*DD*) and average molecular weight, which were found to be 84·6% and 3·5 × 10^4 Da, respectively. The degree of substitution on prepared CMCS was found to be 0·68. All hydrogel formulations showed more than 86% and 77% yield and drug loading, respectively. The swelling behaviour of prepared hydrogels were checked in different pH values, 1·2, 6·8 and 7·4, indicated pH responsive swelling characteristic with very less swelling at pH 1·2 and quick swelling at pH 6·8 followed by linear swelling at pH 7·4 with slight increase. *In vitro* release profile was carried out at the same conditions as in swelling and drug release was found to be dependent on swelling of hydrogels and showed biphasic release pattern with non-fickian diffusion kinetics at higher pH. The carboxymethylation of chitosan, entrapment of drug and its interaction in prepared hydrogels were checked by FTIR, ^{1}H-NMR, DSC and *p*-XRD studies, which confirmed formation of CMCS from chitosan and absence of any significant chemical change in LERH after being entrapped in crosslinked hydrogel formulations. The surface morphology of formulation *S*6 was checked before and after dissolution, revealed open channel like pores formation after dissolution.**

Keywords. **Chitosan; carboxymethyl chitosan; hydrogel; site specific; lercanidipine hydrochloride.**

1. Introduction

Controlled drug delivery technology using natural biodegradable polymers as carriers represents one of the most rapidly advancing areas of science. Chitin and its derivatives have been widely utilized as excipients in pharmaceutics to purposefully delivery drugs or nutriments (Wang *et al* 2008).

Carboxymethylchitosan (CMCS) is an attractive biocompatible and biodegradable polymer which is obtained from the reaction of chitosan with monochloroacetic acid and in alkaline condition (Chen S *et al* 2004). Due to its antimicrobial activity, film-forming ability and capacity to interact with different substances and solubility in wide range of pH, it is used in medical and pharmaceutical areas, mainly for the controlled release of drug (Raimunda-de-Abreu and Campana-Filho 2009). It is also used in tissue engineering and viscosupplementation (Chen S *et al* 2004).

Comparing with commonly used chitosan, CMCS shows several potentials in local drug delivery (Wang *et al* 2008). Local application of drug delivery system could be very advantageous, both in terms of raising drug concentration directly in the action site, and in preventing systemic side effects such as gastrointestinal complaints, depression and tachycardia (Wang *et al* 2008).

Examples of the advantages of site-specific employing CMCS vehicles include pH-sensitivity, bioadhesive ability, solubility and absorbability, controllable biodegradability, nontoxicity of the degradation end products, sustained release potential and ease of administration (Chen S *et al* 2004; Yinsong *et al* 2007). The swelling, drug permeation and release properties of CMCS can be controlled by the pH changes because it contains cationic amine groups and anionic carboxyl groups in matrix (Chen S *et al* 2004).

Hypertension is one of the major risk factors for coronary artery disease and the most important risk factor for cerebrovascular diseases (Jabor *et al* 2003). Antihypertensive drugs diminish cardiovascular risk in hypertensive patients by lowering blood pressure (Jabor *et al* 2003).

Upon oral administration of an immediate release form of LERH, peak plasma level occurs 1–3 h following administration and falls below 1 ng/ml by 24 h. As the modified release dosage forms can be used to prolong pharmacologic action and reduce variability in the plasma concentration of a drug,

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LERH encapsulated CMCS hydrogels are developed to provide the modified release of LERH such that it can provide pH dependant drug release in sustained manner for 24 h.

The objective of the present study was to prepare CMCS from chitosan and use it as carrier for pH specific controlled drug delivery for lercanidipine hydrochloride (LERH), hence its erratic absorption could be reduced with increased oral bioavailability. In this study, chitosan was modified by carboxymethylation and the characteristics of CMCS were studied, and it was used as the controlled release carrier for LERH for pH specific drug delivery.

2. Materials and methods

2.1 *Materials*

Chitosan was a kind gift from Central Marine Fisheries Research Institute, Cochin. LERH was obtained as a gift sample from Zydus Research Centre, Ahmedabad. Monochloroacetic acid, isopropyl alcohol and methanol were purchased from Rankem (India). All other chemicals and reagents used were of analytical grades and used as received.

2.2 *Methods*

2.2a *Characterization of chitosan*: The average molecular weight of chitosan was determined using the Mark-Houwink visometry method in a solvent of 0·1 M acetic acid/0·2 M NaCl maintained at 25 °C (El-Sherbiny 2010). The degree of *N*-deacetylation was determined by FTIR using the following relationship:

$$\%N\text{-deacetylation} = 100\left[1 - \left(\frac{A_{1676}}{A_{3400}}\right)\left(\frac{1}{1{\cdot}33}\right)\right], \quad (1)$$

where A is the absorbance at the given wave number. These two absorption signals at about 1676 and 3400 cm^{-1} correspond to the amide and the primary amino groups of chitosan. The factor (1·33) represents the value of ratio A_{1676}/A_{3400} for the fully *N*-acetylated chitosan (El-Sherbiny 2009).

2.2b *Preparation of Carboxymethylchitosan (CMCS)*: CMCS was synthesized as per the method described earlier with slight modification. In brief, 2 g of chitosan was added to 50 ml isopropyl alcohol and stirred using magnetic stirrer at room temperature for 2 h. The suspension was then transferred to 500 ml RBF and 80 ml of aqueous NaOH solution (60%) was added and refluxed at 85°C for 4 h. Then, 100 ml of aqueous monochloroacetic acid solution (60% w/v) was added in 5 equal parts over a period of 10 min. The mixture was heated with stirring, at 65°C for further 8 h. The reaction mixture was then neutralized using HCl solution (4 M). After removal of the undissolved residue by filtration, the resulting CMCS were precipitated by adding methanol. The product was filtered, washed several times with a mixture of CH_3OH/H_2O (1:1) and dried under vacuum (El-Sherbiny 2010).

2.2c *Degree of substitution*: Degree of substitution (D_s) which is the relative number of carboxymethylated groups in the chitosan chain was determined using conductimetric method (Zamani *et al* 2010). A solution of 0·1 g of CMCS in 100 ml of 0·05 M HCl was prepared and the pH was increased to 2·0 by adding 0·1 M NaOH solution. It was then titrated with 0·1 M NaOH up to pH 11·5. The titration curve is shown in figure 1, and D_s value was calculated as follows

$$D_s = \frac{(V_2 - V_1) \times DD}{V_3 - V_2}, \quad (2)$$

where D_s is the degree of substitution of prepared CMCS and *DD* the degree of deacetylation of chitosan (Zamani *et al* 2010).

2.2d *HPLC analysis*: HPLC measurements were carried out by using a Shimadzu LC 2010 AHT system equipped with a wavelength detector at 356 nm and a Kromasil C_{18} (250 × 4·6 mm ID, 5 µm pore size) column with auto integrator. The mobile phase consisted of acetonitrile/0·01 M phosphate buffer, pH 4·0 in 65:35 v/v ratio. The flow rate was kept as 1·0 ml/min at 50°C for 15 min run time with a pressure of 1500 PSI. The retention time of LERH was 5·1 min. The calibration curve at concentrations varying from 1 µg/ml to 20 µg/ml was used to evaluate all the samples with 20 µl injection volume. The details of linearity and validation are mentioned in table 1.

2.2e *Formulation of hydrogel, yield and drug entrapment*: For the synthesis of hydrogel, 3% CMCS (w/w) was filtered, degassed and transferred to a cylindrical mould in which different amounts of LERH was added according to the ratios with CMCS as shown in table 2. Glutaraldehyde (2·5% v/v) was added as a crosslinking agent to the above

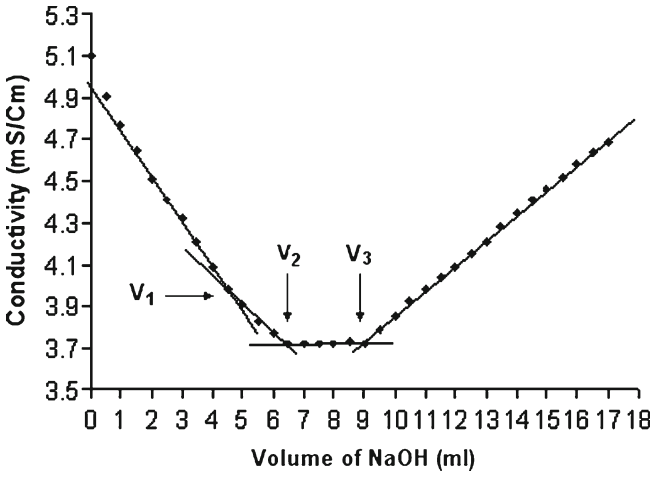

Figure 1. Cunductimetric titration curve of CMCs in HCl titrated with NaOH solution.

suspension and kept at room temperature overnight for gel formation (Chen S *et al* 2004). The crosslinked hydrogels were removed and washed several times with distilled water to remove un-reacted glutaraldeyde and dried under vacuum until used.

The yield of prepared formulations were calculated as per the following equation

$$\%\text{Yield} = (A/B) \times 100, \tag{3}$$

where A is the actual weight of hydrogel and B the theoretical weight of hydrogel.

The entrapment efficiency of LERH in hydrogel was calculated using the following equation

$$\%\text{Entrapment efficiency} = \frac{(\text{Actual amount of drug in hydrogen}) \times 100}{\text{Theoretical amount of drug in hydrogel}}. \tag{4}$$

To calculate entrapment efficiency, the amount of dried hydrogels equivalent to 20 mg of LERH was taken. These hydrogels were stirred at 100 rpm for 48 h in phosphate buffer solution (PBS, pH 6·8), diluted further and analysed at 356 nm using HPLC (Shimadzu LC 2010 AHT, Japan).

2.2f *Swelling studies*: Swelling of the hydrogels was determined by placing hydrogel in 100 ml solutions with pH values of 1·2, 6·8 and 7·4 maintained at 37 ± 0·5°C for 2 h, 3 h and 19 h, respectively (Tavakol *et al* 2009). The hydrogels were collected at regular intervals of time; the excess of moisture was blotted off and weighed. The weight change of wet hydrogels and dry hydrogels were noted using the following expression (Vaghani *et al* 2010a):

$$\%\text{Swelling index} = [(W_t - W_0)/W_0] \times 100, \tag{5}$$

Table 1. Summary of calibration curve and validation of HPLC method used for LERH.

Linearity range (ppm)	0·5–20
R^2	0·9999
Slope	13615
Intercept	−266
LOD (ppm)	0·07
LOQ (ppm)	0·22

where W_t and W_0 are the weight of the hydrogels at time 't' and dry state, respectively.

2.2g *In vitro drug release studies*: The *in vitro* dissolution study of LERH from hydrogels was performed using USP XXIV dissolution rate test apparatus (type II, model TDT-08L, Electrolab, Mumbai, India) fitted with paddle (100 rpm) at 37±0·5°C using 250 ml simulated gastric fluid (SGF, pH 1·2) for 2 h, 900 ml phosphate buffer solution (PBS, pH 6·8) for next 3 h and subsequently in 900 ml PBS (pH 7·4) for 12 h. Weight equivalent to 20 mg of LERH was taken for dissolution. At the predetermined time interval, 10 ml samples were withdrawn, filtered through a 0·45 μm membrane filter, diluted and assayed as per the above mentioned method. Cumulative percentage drug release was calculated using an equation obtained from a calibration curve.

2.2h *Release kinetics*: Data obtained from *in vitro* release studies were fitted to various kinetics equations to discover the mechanism of drug release from prepared formulations. The kinetic models used were of zero order, first order, Higuchi and Korsemeyer–Peppas models. The rate constants were also calculated for the respective models (Vaghani *et al* 2010a).

Korsmeyer–Peppas model, describing drug release from polymeric system, takes into account that the drug release mechanism often deviates from the Fick's law and follows anomalous behaviour described by the following equation:

$$M_t/M_\infty = kt^n, \tag{6}$$

where M_t is the drug released at time t, M_∞ the quantity of drug released at infinite time, k the kinetic constant and n the release exponent. The value of n is related to the geometrical shape of the delivery systems and determines the release mechanism.

The release data was further treated according to Higuchi equation

$$Q = kt^{1/2}, \tag{7}$$

where Q is the percent of drug released at time, t and k the kinetic constant.

The value of n in (6) determines the mechanism of drug release. When n approximates to 0·5, a Fickian/diffusion

Table 2. Formulae for hydrogel preparation, yield and entrapment efficiency of various hydrogel formulations.

Formulation	Drug:CMCs	Yield ($n = 3 \pm$SD)	% Entrapment efficiency ($n = 3 \pm$SD)
*S*1	1:0·25	86·32 ± 3·01	81·54 ± 4·27
*S*2	1:0·5	87·69 ± 2·12	78·73 ± 3·83
*S*3	1:1	91·34 ± 4·19	77·61 ± 2·97
*S*4	1:1·5	92·51 ± 3·07	82·32 ± 2·34
*S*5	1:2	93·16 ± 2·78	85·73 ± 3·09
*S*6	1:2·5	91·92 ± 3·21	86·95 ± 3·12

controlled release is implied, where $0{\cdot}5 < n < 1{\cdot}0$ non-Fickian transport and for $n = 1$, zero order (case II transport). When n approaches 1·0, phenomenologically one may conclude that the release is approaching zero order (Vaghani *et al* 2010a).

2.2i *FTIR study*: The formation of CMCS and drug-polymer interactions were studied by FTIR spectroscopy. IR spectra for chitosan, CMCS, LERH and formulation *S*6 were recorded in a Fourier transform infrared (FTIR) spectrophotometer (FTIR-8400 S, Shimadzu, Japan) with KBr pellets. The scanning range was 400–4000 cm^{-1} (Vaghani *et al* 2010b).

2.2j *Nuclear magnetic resonance (NMR) study*: NMR study was carried out to confirm the formation of CMCS. The ^1H FT–NMR spectrum of CMCS was acquired at room temperature, using 500 MHz spectrometer (Bruker AVANCE). The polymer solution in D_2O was prepared for acquiring the spectrum.

2.2k *Differential scanning calorimetry (DSC)*: DSC scans of about 10 mg, accurately weighed chitosan, CMCS, LERH and formulation *S*6 were performed by using an automatic thermal analyser system (DSC 60, Shimadzu, Japan) with TDS tread line software. Sealed aluminum–lead pans were used in the experiments for all the samples. All the samples were run at a scanning rate of 10 °C/min from 50–300 °C (Vaghani *et al* 2010b).

2.2l *Powder X-ray diffractometry (PXRD)*: The powder X-ray diffraction study was carried out to characterize the polymorphic forms of chitosan, CMCS, LERH and formulation *S*6. A Philips X'Pert PW 3040/60 (Almelo, the Netherlands) was used as an X-ray generator for CuKα radiation ($\lambda = 1{\cdot}54178$ Å). Data was collected in a continuous scan mode using a step size of 0·01° 2θ. The scanned range was 5–50° (Vaghani *et al* 2010b).

2.2m *Scanning electron microscopy (SEM)*: SEM was used to examine the surface morphology of formulation *S*6 before and after dissolution. Dried films were mounted onto stubs by using double-sided adhesive tape. The films were coated with gold and observed under a scanning electron microscope (JEOL, JSM-5600 LV, Japan) for surface characteristics (Vaghani *et al* 2010b).

3. Results and discussion

3.1 *Characterization of chitosan and preparation of CMCS*

The average molecular weight and %*N*-deacetylation of chitosan were found to be $3{\cdot}5 \times 10^4$ Da and 84·6%, respectively. For the carboxymethylation on chitosan, 2 sites are available, hydroxyl groups bonded to C_3 and C_6 of glucopyranose unit as well as the amino group bonded to C_2. As per the literature (Le-Dung *et al* 1994; Chen *et al* 2002; Chen and Park 2003; Chen L *et al* 2004; Yinsong *et al* 2007; Raimunda-de-Abreu and Campana-Filho 2009), the carboxymethylation occurs at C_2 and C_6 when the reaction is carried out at elevated temperature and room temperature, respectively. In the present study, carboxymethylation was carried out by reaction of chitosan with monochloroacetic acid in isopropyl alcohol/aqueous sodium hydroxide at elevated temperature so carboxymethylation of chitosan would have occurred on amino group bonded to C_2. As shown in figure 1, four linear branches were observed in conductimetric titration curve of CMCS. The first branch was attributed to the volume of NaOH used for neutralization of the excess amount of HCl (0 to V_1). The second branch showed that the NaOH volume reacted with carboxymethyl groups (V_1 to V_2). The third branch showed that the volume of NaOH reacted with NH_3^+ groups. The fourth branch resulted due to the excess amount of NaOH in the solution. Here V_3–V_2 is not dependent on the number of carboxymethylated groups and is proportional to the number of glucosamine residues on chitosan chain, hence, $(V_3–V_2)/DD$ is proportional to the number of building blocks of the polymer chains (Zamani *et al* 2010). So D_s can be calculated as per (1), and found to be 0·68 for the prepared CMCS.

3.2 *Yield of preparation and entrapment of drug*

As shown in table 2, all the formulations showed more than 86·32% and 77·61% yield and drug loading, respectively. The high yield of the preparation was attributed because no loss step was involved while preparation except washing. Some amount of drug was lost during washing of hydrogels.

High D_s resulted in stronger intermolecular interaction which resulted in higher entrapment of LERH in the formulations. With decreasing D_s value, the isoelectric point (IEP) of CMCS shifted to higher pH value and the minimum degree of swelling value at IEP increased due to less intraionic attractions between opposite charges with decreasing amount of COO^- groups. When pH increased, CMCS gels was due to increase in intermolecular hydrogen bonding with increasing amount of carboxymethyl groups, which counteract the osmotic pressure and clasp the gel (Chen S *et al* 2004).

3.3 *Swelling studies*

The prepared formulations were checked for their swelling in simulated gastric fluid (SGF, pH 1·2) for 2 h, in phosphate buffer solution (PBS, pH 6·8) for 3 h and successively in PBS (pH 7·4) for 7 h (18). Figure 2 shows % SI profiles of various formulations. Results indicated that at pH 1·2 for 2 h, all the formulations showed less than 10·32% swelling while at pH 6·8 for next 3 h showed high swelling up to 100·07%. The swelling was further increased slightly at pH 7·4 and observed at about 135·22%. The swelling ratio was

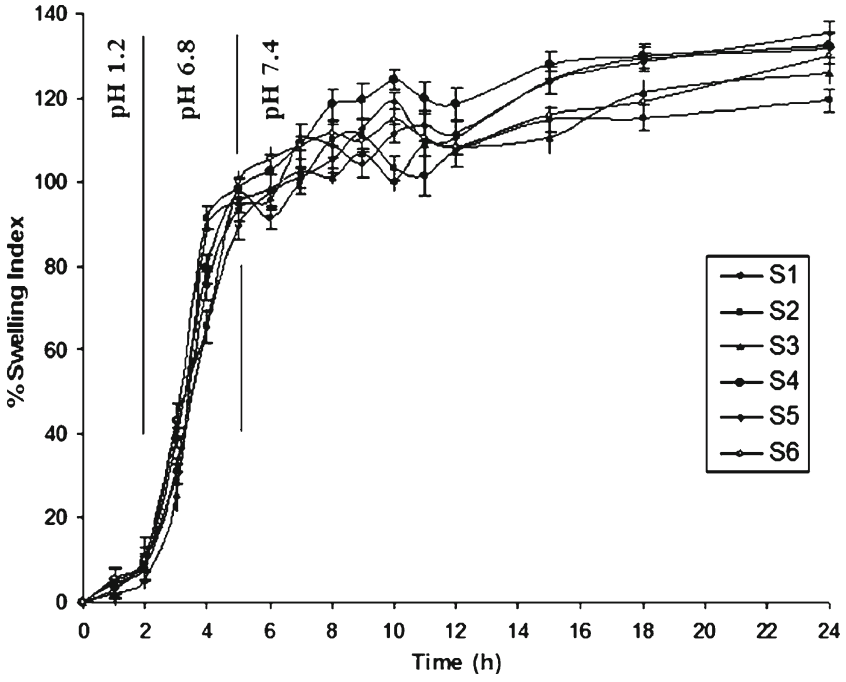

Figure 2. Swelling behaviour of formulations *S*1–*S*6 in different pH conditions.

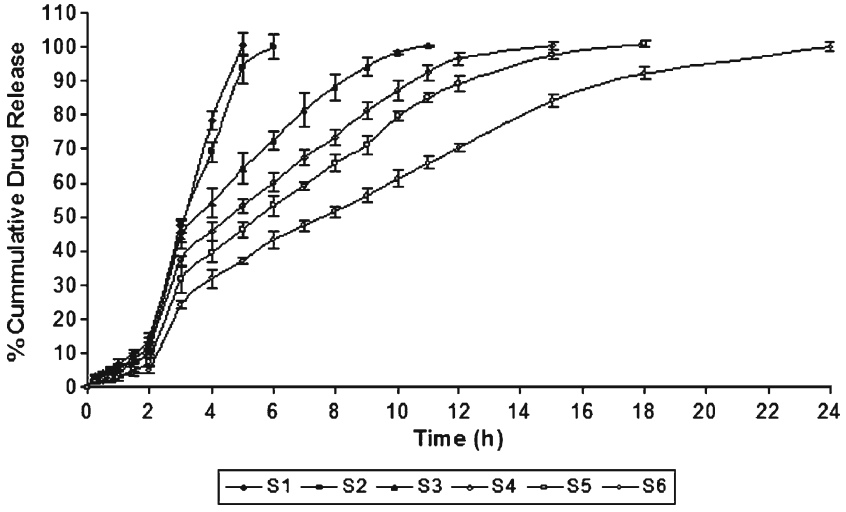

Figure 3. Release profiles of LERH from hydrogel formulations *S*1–*S*6.

varied as the amount of CMCS was varied in various formulations because the swelling was attributed to its amount. The state of the swelling mainly depends on osmotic pressure difference between inside the gel and the surroundings caused by the redistribution of mobile ions (Chen S *et al* 2004). Here pH dependant swelling was observed because prepared CMCS hydrogels contain both carboxyl and amino groups, and thus forms a network with oppositely charged structures which could change the charge state of the ionic groups varying with pH. Further, it also depends on the degree of deacetylation (*DD*) and degree of substitution (D_s) of prepared CMCS (Chen S *et al* 2004). At pH 1·2, the dominant charges in the gels are protonated amino groups. As the *DD* of the obtained chitosan was found to be 84·6% and D_s of prepared CMCS was observed as 0·68, as per ^{1}H NMR study only about 27% amino groups were available on CMCS. Due to the fewer amounts of amino groups, and further crosslinked, which are responsible for swelling at low pH, the swelling was found to be limited up to 10·32%. In case of higher pH (pH 6·8 and pH 7·4), the dominant charges in the gels are the unprotonated carboxyl groups. The swelling at these conditions was higher as compared to low pH (1·2) due to about 58% unprotonated carboxyl ions.

3.4 *In vitro drug release studies and release kinetics*

The release profile of LERH from various hydrogel formulations is shown in figure 3. Formulations *S*1 and *S*2 failed to retard the drug release and found to be disintegrate within 5 h and 6 h, respectively. In these formulations very less amounts of CMCS were present as the ratio of drug:CMCS kept as 1:0·25 and 1:0·5 (table 2) so matrices of CMCS failed to provide adequate retention of drug. Formulations *S*3, *S*4 and *S*5 also failed to retard the drug for 24 h and complete drug releases were observed within 11 h, 15 h and 18 h, respectively. Though the amounts of CMCS were high in these formulations as compared to *S*1 and *S*2, then also it was not sufficient to retard the drug release in controlled manner for 24 h. Formulation *S*6 provided adequate drug retention and controlled release for 24 h with complete drug release at 24 h, so this formulation was considered as an optimized formulation and further study was carried out using this formulation. The differences in drug releases were observed depending upon the swelling behaviour. Though the swelling was completely different, release was also affected by the drug loading in the polymer matrix of respected formulations and the concentration of polymers in the formulations (table 2) (Koutroumanis *et al* 2010). LERH showed biphasic pH dependent release pattern from all the formulations. The release was very less from all the formulations for first 2 h, as the dissolution was carried out at pH 1·2. At pH 1·2, swelling of hydrogels were very less, as discussed in swelling studies, hence the drug was unable to diffuse from the hydrogels. Some amounts of drug release were observed due to swelling and the free drug remaining at the surfaces that was not entrapped efficiently within the crosslinked polymer network (Prabaharan and Gong 2008). Release rate was increased at higher pH as compared to release at pH 1·2 due to the fact that the hydrogels swelled rapidly at pH 6·8 and also the swelling was found to be almost linear with a slight increase at pH 7·4. Drug release was found to be followed by first order kinetics for first 2 h and showed non-fickian diffusion (diffusion coupled with erosion) kinetics with $n > 0{\cdot}5$ value for further release at higher pH (table 3) (Vaghani *et al* 2010a).

3.5 *FTIR study*

As shown in figure 4, FTIR spectrum of chitosan showed a broad absorption band between 3500 cm^{-1} and 3100 cm^{-1}, centred at 3400 cm^{-1}, due to O–H stretching vibration, N–H extension vibration and the intermolecular H-bonds of the polysaccharide moieties (figure 4a) (Raimunda-de-Abreu and Campana-Filho 2009; El-Sherbiny 2009, 2010). A band at 2881 cm^{-1} was observed corresponding to the axial stretching of C–H-bonds. A peak at 1676 cm^{-1} was observed which is attributed to the axial stretching of C=O bonds of the acetamide group which indicated that sample was not fully acetylated. A band at 1557 cm^{-1} was observed, which is attributed to the angular deformation of the N–H bonds of the amino group. A band at 1370 cm^{-1} due to the symmetrical angular deformation of CH_3 and the amide III band at 1320 cm^{-1} were observed. The band corresponding to the polysaccharide skeleton, including vibrations of the glycoside bonds, C–O and C–O–C stretching in range 1156–898 cm^{-1}, was observed.

The carboxymethylation provoked structural changes which were clearly identified by comparing the infrared spectra of chitosan and carboxymethylchitosan. The occurrence of a broader band centred at 3444 cm^{-1} revealed more hydrophilic character of CMCS as compared to the parent chitosan (figure 4b). The introduction of carboxymethyl groups was cofirmed by the occurrence of an intense band at 1593 cm^{-1} and a moderate band at 1423 cm^{-1}. These bands were attributed to symmetric and asymmetric deformation of COO^-, respectively (Zhao *et al* 2002; Chen *et al* 2007; Raimunda-de-Abreu and Campana-Filho 2009).

As shown in figure 4c, FTIR spectrum of LERH exhibited peaks at 3184·25 cm^{-1} due to NH stretching, at 3064·68 cm^{-1} and 2948·96 cm^{-1} attributed to alkyl and phenyl stretching, respectively; 2655·68 cm^{-1} due to

Table 3. Correlation coefficients of different pharmacokinetic models for LERH release profile from *S*1–*S*6.

Sl. No.	Time	Formulation	Zero order	First order	Higuchi	Korsemeyer–Peppas	
			R^2	R^2	R^2	N	R^2
1	Up to 2	*S*1	0·9878	0·9566	0·9945	1·5578	0·9813
2		*S*2	0·9542	0·9269	0·9692	1·1214	0·9681
3		*S*3	0·9694	0·9266	0·9907	0·6511	0·9905
4		*S*4	0·9501	0·8975	0·9818	0·6568	0·9879
5		*S*5	0·938	0·8636	0·9792	0·6872	0·984
6		*S*6	0·9514	0·8508	0·9888	0·6996	0·9902
7	2–12	*S*1	0·9878	0·9566	0·9945	1·5578	0·9813
8		*S*2	0·9542	0·9269	0·9692	1·1214	0·9681
9		*S*3	0·9694	0·9266	0·9907	0·6511	0·9905
10		*S*4	0·9501	0·8975	0·9818	0·6568	0·9879
11		*S*5	0·938	0·8636	0·9792	0·6872	0·984
12		*S*6	0·9514	0·8508	0·9888	0·6996	0·9902

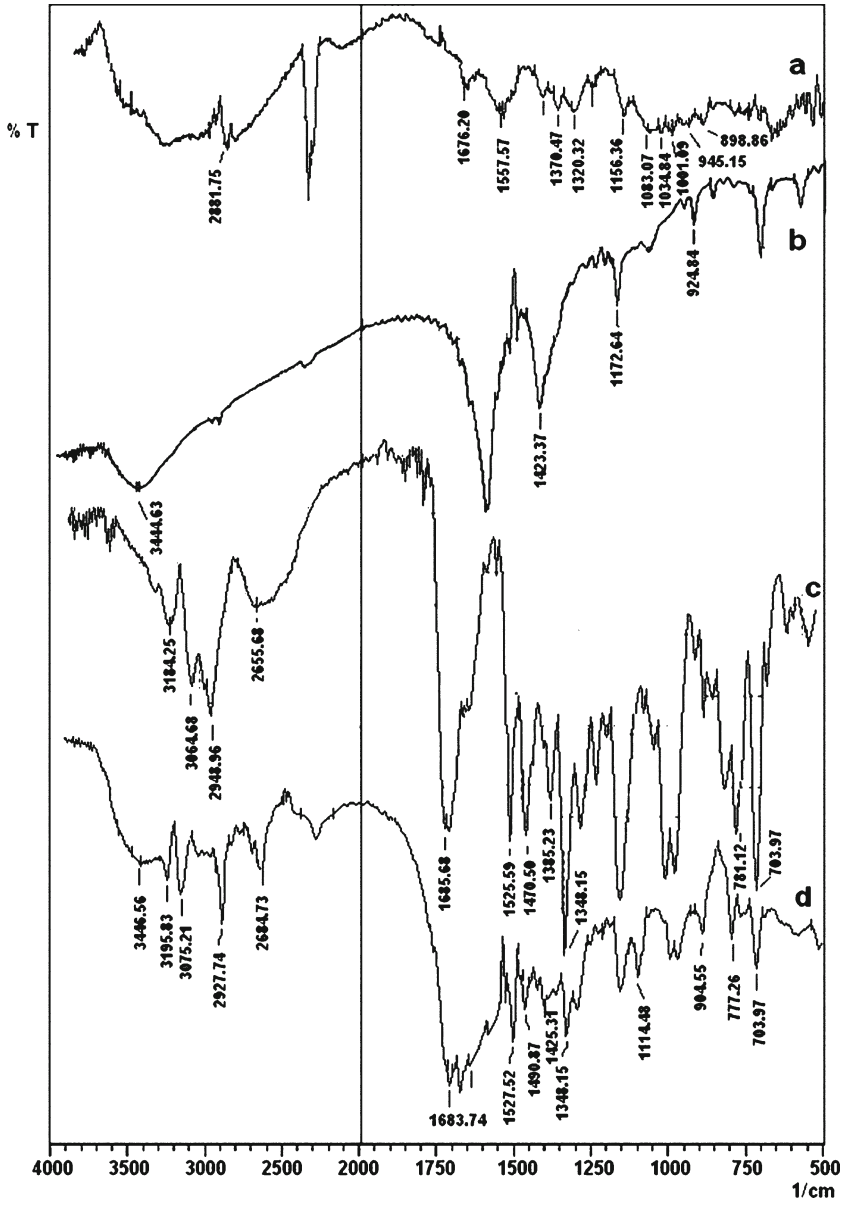

Figure 4. FTIR spectra of chitosan (**a**), prepared CMCs (**b**), LERH (**c**) and formulation *S*6 (**d**).

N^+ stretching, 1685·68 cm^{-1} due to C=O stretching, 1525·59 cm^{-1} and 1385·23 cm^{-1} due to symmetric and asymmetric stretching of NO_2 group; 1470·5 cm^{-1} and 1385·23 cm^{-1} due to bending of geminal methyl group and at 781·12 cm^{-1} and 703·97 cm^{-1} due to out of plane bending of 5 and 3 adjacent hydrogen of aromatic ring (Charde *et al* 2008). All the peaks were maintained in formulation *S*6 also (figure 4d) which confirmed presence of the drug in the formulation without any significant reaction of functional group with other ingredients.

3.6 *Nuclear magnetic resonance (NMR) study*

According to the literature, when carboxymethylation is carried out by reacting chitosan with mono-chloroacetic acid in iso-propanol and aqueous sodium hydroxide, *O*-substitution is favoured if the reaction is carried out at room temperature but *N*-substitution predominates by raising the reaction temperature (Tokura *et al* 1983; Le-Dung *et al* 1994; Chen and Park 2003; Chen *et al* 2002, 2004; Yinsong *et al* 2007; Prabaharan and Gong 2008; Raimunda-de-Abreu and Campana-Filho 2009). Figure 5 shows ^{1}H-NMR spectrum of prepared CMCS. In the ^{1}H-NMR spectrum of CMCS, signal at 3·2 ppm was observed, the evidence of *N*-carboxymethylation at C_2. The appearance of small proton signal at 3·9 ppm was due to occurrence of mono-substitution on some of the primary hydroxyl sites at C_6 of the modified chitosan structure, which indicated that less than 14% hydroxyl groups were also carboxymethylated during occurance of *N*-carboxymethylation. Though in the present study,

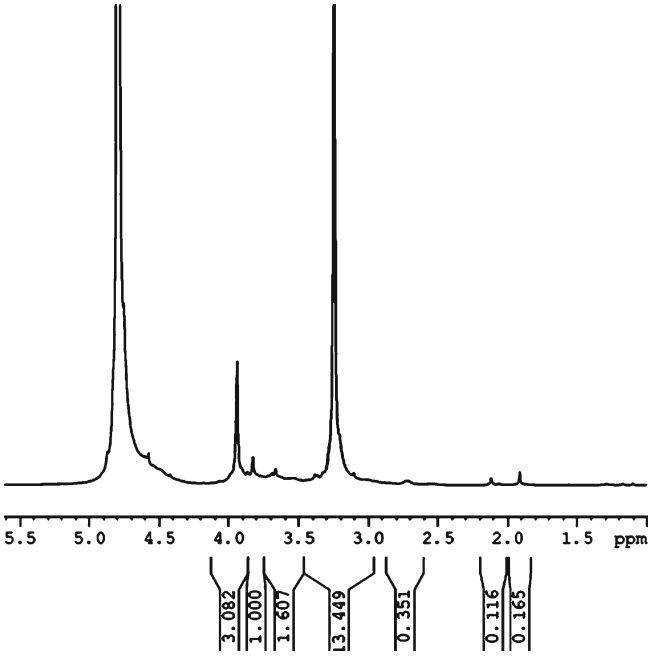

Figure 5. ^{1}H NMR spectrum of prepared CMCs.

reaction was carried out at elevated temperature of <14% *O*-carboxymethylation also occurred. The signal assigned at 4·8 ppm was due to the glucopyranose residue (Prabaharan and Gong 2008).

3.7 *DSC study*

DSC analysis was used to characterize thermal behaviour of chitosan and carboxymethylchitosan; as well as thermal behaviour of LERH in the prepared hydrogel formulation. In the present investigation, DSC thermograms of chitosan, CMCS, LERH and formulation *S*6 were taken (figure 6). The thermogram of pure chitosan showed a broad melting endotherm at 104·02°C (figure 6a) and thermogram of prepared CMCS showed a broad melting endotherm at 73·51°C (figure 6b). This transition may be due to loss of bound water. The decomposition of CMCS would have resulted in exothermic transition at around 287·39°C (figure 6b). The change in the pattern of thermal transition in the CMCS compared to chitosan confirms the carboxymethylation of

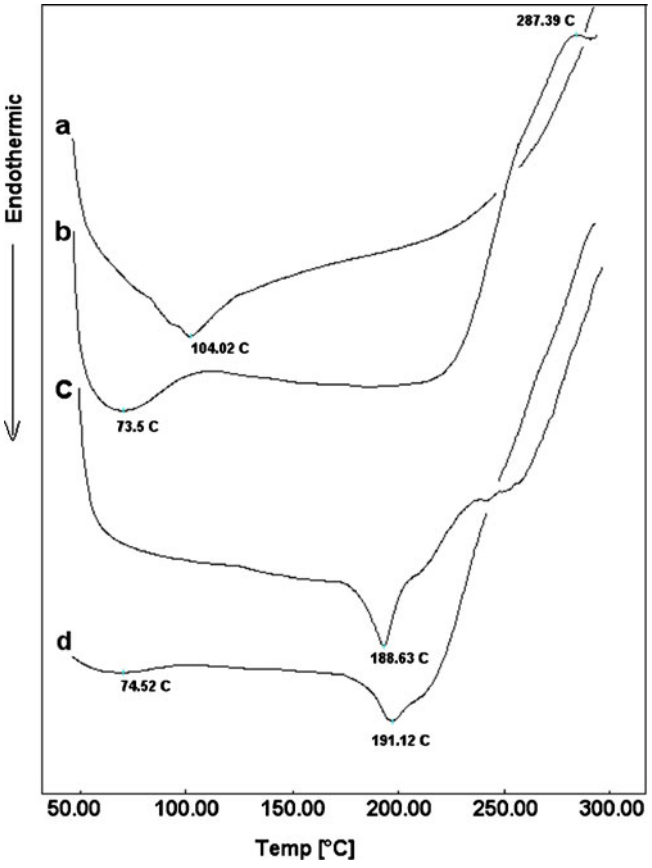

Figure 6. DSC thermograms of chitosan (**a**), CMCs (**b**), LERH (**c**) and formulation *S*6 (**d**).

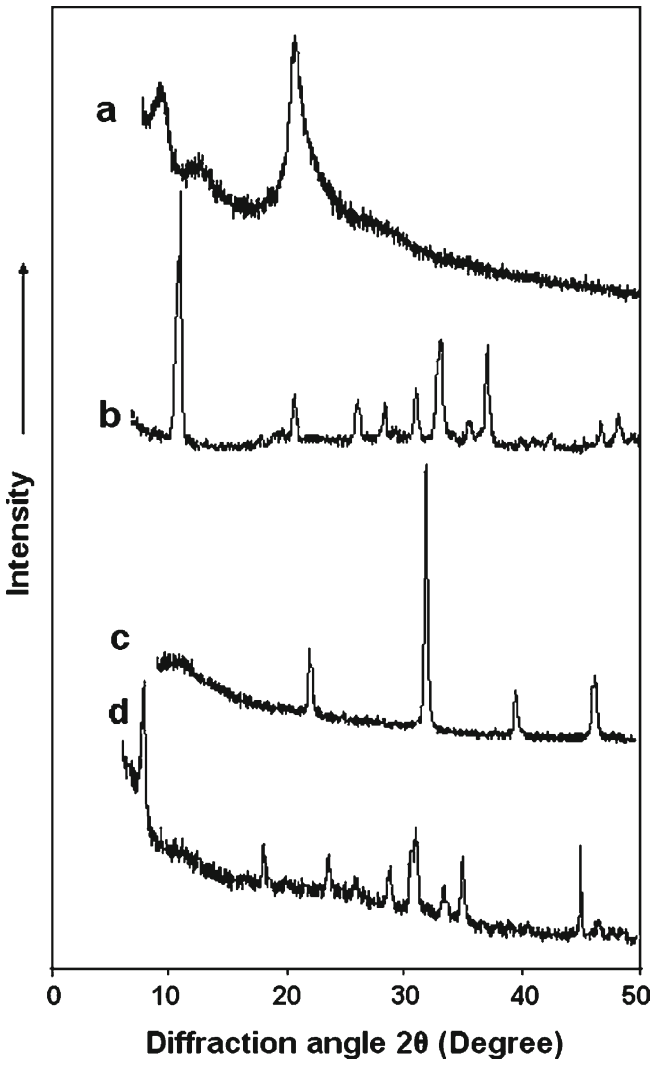

Figure 7. XRD pattern of pure chitosan (**a**), CMCs (**b**), LERH (**c**) and formulation *S*6 (**d**).

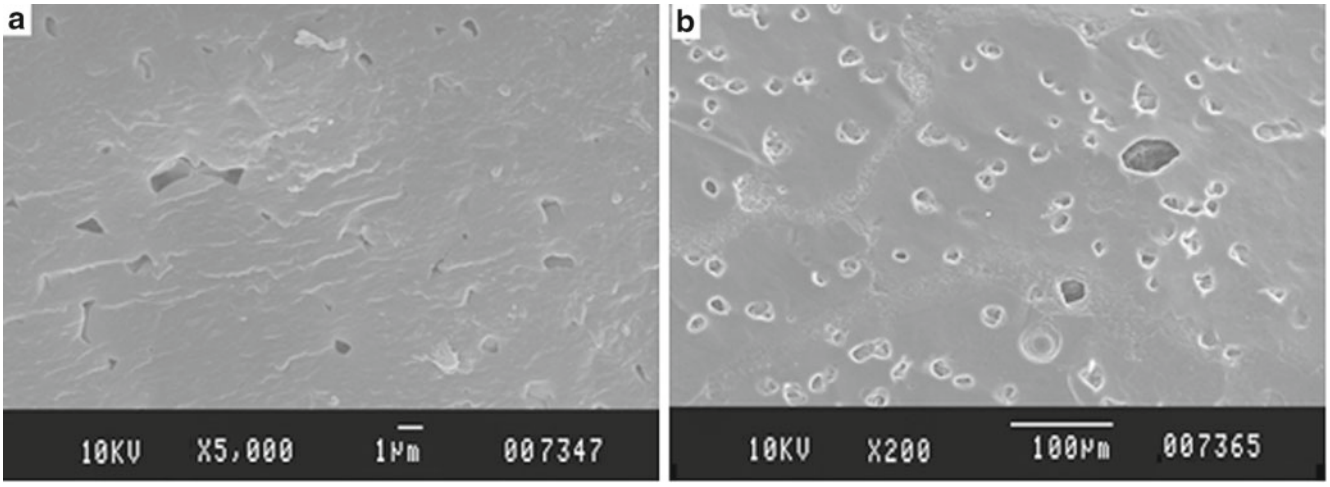

Figure 8. Scanning electron microscopy of formulation *S*6 before dissolution (**a**) and after dissolution (**b**).

chitosan (El-Sherbiny 2009). Pure LERH showed sharp melting endotherm at 188·63°C (figure 6c) which corresponds to its melting point (Charde *et al* 2008). Drug may have been dispersed in the crystalline or amorphous form or dissolved in the polymer matrix during formation of hydrogel (Vaghani and Patel 2011). There is no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymeric matrix but in the formulation *S*6 a sharp peak at 191·12°C as shown in figure 6d was observed which indicated that the drug was not dispersed nor in the solid solution form in hydrogel. The study also revealed that the thermal behaviour and physical state of drug was not changed after being incorporated in formulation and also confirmed the presence of drug in the formulation.

3.8 *PXRD analysis*

Figure 7a shows XRD patterns of the chitosan. It can be seen that the strongest diffraction intensity is the broad peak around $2\theta = 17–23°$ due to substrate chitosan. It indicated semicrystalline nature of chitosan (Pandit and Patil 2009). Figure 7b shows XRD patterns of CMCS which indicated some sharp peaks at 9·34°, 19·1°, 26·9°, 29·66°, 31·68°, 34·22° and 35·77° which revealed the crystalline nature of prepared CMCS. It indicates that the carboxymethylation of chitosan resulted into crystalline form (El-Sherbiny 2009, 2010). In the spectra of LERH (figure 7c), strong characteristic peaks at 22·84°, 32·52°, 40·1° and 46·68° were observed, indicating its crystalline nature (Pandit and Patil 2009). Figure 7d shows spectrum of formulation *S*6, which indicated well maintained characteristic peaks of LERH, e.g., 24·26°, 31·48°, 33·9°, 35·5° and 45·4° along with peaks of CMCS. The results indicated that during the formulation of hydrogels crystallinity of LERH was well maintained.

3.9 *Scanning electron microscopy (SEM)*

The surface morphology of prepared hydrogels was studied before and after dissolution. The surface morphology of formulation *S*6 revealed non-porous translucent membrane (figure 8a) with some crystals of drug present on the surface. The surface morphology was again checked after dissolution which revealed open channel-like structure. Due to ionization of the carboxylic groups of CMCS at higher pH, the chain relaxation occurs which leads to efficient solvent diffusion hence open channel-like structure (figure 8b) was generated in the hydrogel after dissolution.

4. Conclusions

In conclusion, the carboxymethylation of chitosan was successfully achieved and the occurrence of *N*-carboxymethylation was evidenced by FTIR and ^{1}H FT–NMR spectroscopy which revealed that *N*, *O*-carboxymethylchitosan was produced. The aforementioned results indicated the prepared CMCS hydrogels showed excellent pH sensitivity which indicated higher pH dependent swelling and drug release at pH 6·8 and 7·4. The prepared formulations were analysed by FTIR, DSC and pXRD showed absence of any significant chemical reaction of LERH in the formulation and found to be in crystalline form in final formulation also. SEM revealed channel like pore formation after dissolution. The results indicated the prepared hydrogels can be used as promising carriers for the administration of colon specific drug delivery of LERH.

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