ORIGINAL ARTICLE

Development of cost effective biodegradable implants of ciprofloxacin hydrochloride in treatment of osteomyelitis

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In the present study, an attempt has been made to formulate and evaluate a sustained release implant of ciprofloxacin HCl with biodegradable, cost-effective polymer chitosan. osteomyelitis is one of the oldest disease, which is still in existence and difficult to treat, the prevalence of which is increasing day-by-day. The treatment of osteomyelitis requires large doses of antibiotics administered by systemic routes for a period of 4-5 weeks, however, the parenteral route suffered from many disadvantages and also some limitations. Ciprofloxacin hydrochloride has been the most widely used fluoroquinolone for multi-bacterial bone infection because the minimal inhibitory concentration of ciprofloxacin HCl is low, and it has good penetration properties in most of the tissues and bone. Biodegradable polymers like poly (lactic-co-glycolic acid) (PLGA) and Polycaprolactone (PCL) were widely studied as a carrier for implant but their use is limited because of high-cost and are not easily available. The cross-linking of chitosan was carried out with sodium citrate and cross-linked chitosan (CC) was used as a carrier. The effect of different proportion of chitosan and effect of drug loading on the drug release kinetics has been studied. An *in vitro* result shows that prolonged release was observed with higher drug loading. The CC5 implant containing 50% w/w polymer retards the drug release for more than 5 weeks. Furthermore, from *in vivo* study it is found that the optimized formulation CC5 is biocompatible and implant is not causing any foreign body reaction or hypersensitivity in the body of animal. The CC was found to have excellent release retarding properties and can be used as cost-effective, biodegradable sustained release matrices for designing of implant.

Key words: *Ciprofloxacin hydrochloride, cross-linked chitosan, implant, osteomyelitis, sodium citrate*

INTRODUCTION

Osteomyelitis is as such an historic infection, which is still remains challenging and difficult to treat, despite of advances in antibiotics, and new operative techniques. Osteomyelitis, either acute or chronic, is an inflammatory bone disease caused by pyrogenic bacteria. Osteomyelitis is defined as progressive infection of bone or bone marrow and surrounding tissues. The root words *osteon* (bone) and *myelo* (marrow) are combined with it is (inflammation) to define the clinical state in which bone is infected with microorganisms. It is mainly characterized by inflammation and swelling of bone tissues. It is also called as multi-bacterial bone infection because it is caused by variety of micro-organisms. *Staphylococcus aureus* (80-85%) is the major organisms associated with osteomyelitis. Infection is more common in the long bones and vertebras of the body, however, it can also affect other bones in the body.[1,2] Osteomyelitis is now a days becoming more common because of increased use of prosthetic devices and increased in a number of accidents resulting in traumatic injuries. Therefore, osteomyelitis is a major health problem for both developed countries and developing counties. The treatment of osteomyelitis requires large doses of antibiotics administered by systemic routes a period of 4-5 weeks. Some of the disadvantages of prolonged parenteral therapy include; atient discomfort, High-cost of treatment, Development of systemic toxicity, Patient compliance problems etc., Osteomyelitis results in

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bone necrosis and destruction of bone resulting in limited vascularity to the site of infection, systemic therapy may fail to produce therapeutic tissue concentrations of the antibiotic at the particular site of infection. Furthermore, such a long parenteral therapy may develop systemic toxicity and to overcome some of these problems with the treatment of osteomyelitis, localized drug therapy using non-biodegradable. Polymethyl methacrylate (PMMA) bone cement implants was introduced[2,3] and the advantages of local therapy include high- local tissue, while simultaneously minimizing high-potentially toxic, and systemic drug levels. However, previous studies on the non-biodegradable carriers have shown that the *in vitro* release of antibiotics from PMMA beads is incomplete and poorly controlled. Biodegradable polymers like PLGA, PCL were widely studied as a carrier for implant but their use is limited because of high-cost and are not easily available.[4,5]

Therefore, to avoid the systemic toxicity, to produce effective drug concentration at the infected site and to develop cost-effective alternative to presently available drug therapy for osteomyelitis, subcutaneous implantable drug delivery of ciprofloxacin hydrochloride (HCl) is developed from which drug slowly releases from implant and high-local tissue concentration can be achieved at the infected site. As, the minimum inhibitory concentration of ciprofloxacin HCl is very low (0.25-2 μg/ml) for most of the pathogens that cause osteomyelitis, the growth of causative micro-organism can be easily inhibited.

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl and chitosan was kindly supplied by Glenmark Pharmaceuticals Ltd., (Sinner). Other chemicals includes 0.2 M Sodium hydroxide (NaOH), Potassium dihydrogen phosphate, Sodium azide, 0.1 N HCl, Citric acid, and epichlorohydrin. All the materials used for the study were of analytical grade.

Material characterization

By Fourier-transform- infrared analysis

FTIR spectra of the ciprofloxacin HCl and chitosan were obtained on a Shimadzu 8400 S FTIR (Tokyo, Japan) in the range of 4000-400 cm^{-1}, using KBr pellet.

Method development

By ultraviolet visible spectroscopy

This study aims to develop Implantable formulation of ciprofloxacin HCl, which is to be placed dip inside the subcutaneous tissues. The pH of this region is 7.4 and hence, to mimic these conditions 7.4 pH phosphate buffer is used for both analytical method development of ciprofloxacin HCl and for dissolution studies of Implants. The analytical method development was also carried out in other solvents also for Identification and Comparison.

Spectrum recording

Wave-length of maximum absorption of ciprofloxacin HCl was determined in 0.1 N HCl, distilled water, 7.4 pH phosphate buffer, and 4 pH Citrate buffer solutions. The spectrum of these solutions was recorded using Shimadzu UV 2450 UV-Visible spectrophotometer.

Construction of beer-lambert's plot

The calibration plot of ciprofloxacin HCl was plotted in different solvent as follows:

1. In 0.1 N HCl
2. In Distilled Water
3. In 7.4 pH phosphate buffer solution
4. In 4 pH Citrate buffer solution.

Compatibility study of drug with polymers

By FTIR analysis

The drug and polymer powders were mixed in 1:1 ratio and were kept in the dried glass vial under normal conditions at room temperature for 7 days. Drug powder as obtained, polymers as obtained and their mixture was then analyzed by FTIR.

By X-ray diffraction study

Drug, polymers, and mixture triturated following compression were analyzed by XRD in order to check effect of compression on crystallinity of ingredients as well as to check any interaction between the excipients. Powder XRD patterns were obtained by a diffractometer (PW 3710, Philips) at the following Time-Conditions per step: 0.400 s, step size: 2 θ =0.020° (θ is incident angle), current: 30 mA at 40 kV, CuK (copper, whose characteristic wavelength for the K radiation) rays (wave-length = 1.542 A°).

Characterization of plain non-cross-linked chitosan

Determination of degree of deacetylation by potentiometry

(Using a modified acid-base titration method, i.e., Potentiometric Titration).

Chitosan was (0.2 g) dissolved in 20.0 ml of 0.1 N HCl and the solution was titrated potentiometrically with a standard solution of 0.1 N NaOH. This gives a titration curve having two inflection points, the difference between two along the abscissa corresponding to the amount of acid required to protonate amino group. The degree of de-acetylation was calculated from the amount of NaOH consumed between two inflection points by the following equation.[6,7]

$$DD = 16.1(Y - X)\, f/w \quad (1)$$

Where Y and X are the consumed NaOH volume of the equivalent points, f is molarity of the NaOH solution and w is the initial chitosan weight (in g).

Preparation of CC with sodium citrate[8-11]

CC was prepared by soaking the 2 g of chitosan in an aqueous solution of 100 ml sodium citrate (10.0% w/v) at 4°C for 3-4 h.

At the same time, the pH of the solution was maintained in the range of pH 4.5-6.5 using HCl and/or NaOH And then the resultant slurry was washed with distilled water, put on a glass plate and oven-dried at 37°C for 48 h, and then dried under vacuum at room temperature until reaching a constant weight.

Mechanism of crosslinking

Sodium citrate interacts with amine groups present in chitosan and the cross-linking is formed due to the electrostatic interaction between NH_3^+ on chitosan and COO^- on citrate [Figure 1].

At the same time, pH of the reacting medium was maintained between pH 4.5 and 6.5 because at low-pH (< 4.1), the ionization of carboxyl groups was normally depressed (the degree of ionization was usually < 0.3), i.e. less than one negative charge was carried by one citrate. For chitosan (a weak polybase), the opposite was the case as the ionization of amine groups decreased greatly when the solution pH increased above 6.0 (around the p*K*a of chitosan 6.3) and at pH higher than 7.5 usually < 10% of amine groups were ionized.

Characterization of sodium citrate CC

By potentiometric titration method: The amounts of protonable (amino) groups in Citrate CC were measured using potentiometric titration as described for simple chitosan

By FTIR: The amounts of protonable (amino) groups in Citrate CC were measured using FTIR.

Compatibility study

Differential scanning calorimeter study

Thermograms of Drug And mixtures of drug and CC were obtained using Shimadzu DSC-60 DSC using aluminum pans to check the compatibility of drug and polymer after cross-linking. Different temperature programming was carried out for different samples. Nitrogen was purged through cooling unit. Indium standard was used to calibrate the DSC temperature. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 20°C/min, over a temperature range of 0-700°C. Inert atmosphere was maintained by purging nitrogen at the flow rate of 30 mL/min.

Formulation development

Experimental design: Citrate CC as a carrier

Formulations were developed in order to establish a controlled release implantable dosage form. The active ingredient (ciprofloxacin HCl) and polymer (Citrate CC) were weighed accurately and passed through 60# sieve. Mixing of powders was carried out by spatulation.

Drug: Polymer ratio (D: P) were the two formulation variables and there effect on *In vitro* release is studied. Weight of implant tablet was kept constant in all the formulations (150 mg).

Preparation of implants

The compression of powder blend (CC1 to CC5) was carried out by direct compression method on rotary compression machine (General machine, India). The compression was carried out using 8 mm flat-faced circular punches. All

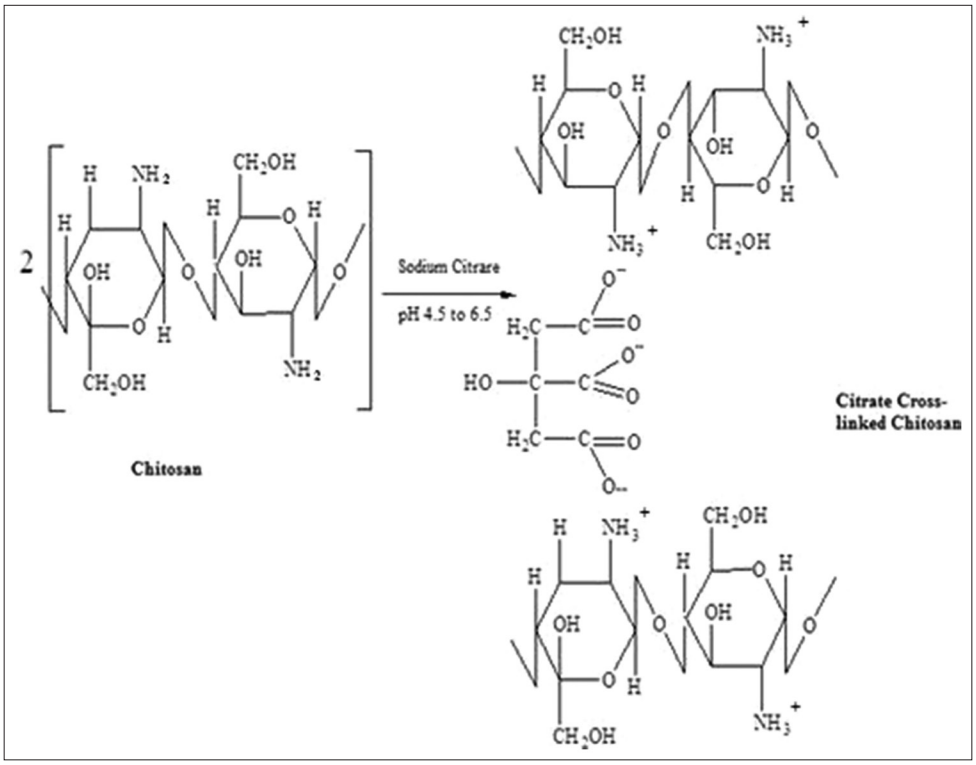

Figure 1: Crosslinking reaction of chitosan with sodium citrate

formulations were compressed at a constant force to achieve the tablet weight of 150 mg and hardness in between 4.5 and 5.5 kg/cm^2 for all formulations.

Evaluation of implants

The compressed implants were evaluated for thickness, hardness, and drug content.

Thickness and diameter variation test

The thickness and diameter of implants was determined using a Micrometer Screw Gauge (Yamayo classic, Japan). Five implants from each batch of formulation were used and the mean thickness and diameter with respective SD was calculated for each formulation.

Hardness test

For each formulation, the hardness of implants ($n = 5$) was measured using the Monsanto hardness tester (Cadmach, Ahmedabad, India).

Drug content

One milled implant was placed in 100 ml of HCl (0.1 N) and kept under magnetic stirring (50 rpm) at room temperature for 24 h. The solution was filtered using Whatmann filter paper and after filtration the drug content was determined spectrophotometrically at 277 nm.

Water uptake study

Initially, weighed implants (at time = 0) were placed in the 20 ml release medium (Phosphate buffer 7.4 pH) and withdrawn at appropriate intervals, blotting away excess water and weighed again (wet weight)[12-16]. Water uptake was determined using following equation,

$$\text{Water Uptake } (\%) = \frac{W_w - W_i}{W_i} \times 100 \quad (2)$$

Where, W_w is the wet weight,
W_i is the initial weight

Mass loss (% erosion)

Initially, weighed implants (at time = 0) placed in the 20 ml release medium (Phosphate buffer 7.4 pH), withdrawn after 5 weeks, blotting away excess of water and weighed again. The implants were dried at 105°C in an oven and the final weights (dry weight) were recorded. Weight of ciprofloxacin released after 5 weeks was calculated from UV spectrophotometry assays.[12-16]

% erosion was determined using following equation,

$$\%\,\text{Erosion} = \frac{\left(W_i - W_{CFX\ Released}\right) - W_d}{W_i} \times 100 \quad (3)$$

Where,
W_i is the initial weight,
W_d is the dry weight,
$W_{CFX\ Released}$ is the weight of ciprofloxacin HCl released after 5 weeks

In vitro drug release study

Drug release from the prepared formulations was studied by Vial method as described below.

Rotary shaker method (vial method)

In this method, the drug release study was performed in 30 ml screw capped glass vials (diameter = 25 mm) containing 20.0 ml dissolution medium. The implants were immersed with USP phosphate buffer (0.1 M, pH 7.4) containing 0.1% w/v sodium azide as antibacterial agents. Samples from each of formulations were incubated in an oven at 37°C for 5 weeks (or more) with agitation (60 rpm) in orbital shaking incubator (Remi, India) (shaking bath) (60 rpm). At defined time points, whole dissolution medium was withdrawn and replaced with fresh buffer to maintain sink condition. The sample solution was filtered through Whatmann filter paper. Appropriate dilution was prepared using USP phosphate buffer (0.1 M, pH 7.4) and absorbance was measured at 270.8 nm. Drug concentration in the sample was determined using the standard calibration curve. Cumulative percent drug released was found at each point. Release of ciprofloxacin HCl from implants was assayed in triplicate and Mean with SD was determined.[16-17]

Histocompatibility study

All experiments comply with the Ethical Committee Guideline on care and use of animals in experimental procedures. The aim of this study was to evaluate the subcutaneous biocompatibility of optimized implant formulation and the histopathological analysis was performed through histopathologists at Oral Pathology Department of K.B.H. Dental College and Hospital, Nashik. For histocompatibility study the eight animals were allotted into main two groups, each group contains four animals and third group is control group (i.e., muscles on the other side of the same animal, which are not in close/direct contact with implant formulation). Subcutaneous Administration of Implant was carried out by procedure described previously. The third group is the control group. On at 7th and 21st days after post-administration period the animals were killed by cervical dislocation. After removal of residual implant tablet, the surrounding muscles were removed and immersed in 10% phosphate-buffered formalin solution for 48 h. The tissue samples were mounted on glass slide and stained with H and E. Each specimen was analyzed at ×400 magnifications with a light microscope. The samples were evaluated for Cellular inflammatory responses, Necrosis, Capsule formation, Ulcer formation, Cellular infiltration, edema, migration of inflammatory cells at implantation site, and other foreign body tissue.[17]

RESULTS AND DISCUSSION

Material characterization

By FTIR analysis

FTIR spectrum of the drug and polymer sample showed all the characteristic IR peaks as reported in the literature. FTIR spectrums of the ciprofloxacin HCl and chitosan are presented in Figures 2 and 3.

Method development

By UV-visible spectroscopy

Spectrum recording: Absorption maximum of ciprofloxacin HCl in different solvents are presented in Table 1.

Table 1: Absorption maximum of ciprofloxacin hydrochloride in different solvents

Solvent	Observed λ max (nm)	Reported λ max[4,5] (nm)
0.1N HCl	277	277
Distilled water	271.3	271.4
7.4 pH phosphate buffer	270.8	271
4 pH citrate buffer	277	277

Construction of beer-lambert's plot

The calibration plot of ciprofloxacin HCl was plotted in different solvents. Plot of absorbance Versus Concentration by using 7.4 pH phosphate buffer was found to be straight line $R^2 = 0.9977$ and Equation of line was found to be $y = 0.728x + 0.0283$. This was in accordance with Beer-Lambert's law; therefore, this method is used for *In vitro* analysis of ciprofloxacin HCl.

Furthermore, Plot of absorbance Versus Concentration by using pH 4 citrate buffer was found to be straight line $R^2 = 0.9981$ and Equation of line was found to be $y = 0.111x + 0.02$. This was in accordance with Beer-Lambert's law; therefore, this method is used for *In vivo* analysis of ciprofloxacin HCl.

Compatibility study of drug with polymers

FTIR can be considered as first line analytical technique to study compatibility of drug with excipients. Figure 4 showed that characteristic IR absorption peaks of drug and polymer. Same peaks were observed in individual samples. This indicates that no chemical interaction between drug and the polymer. This was supported by the XRD spectra of drug, polymer and their mixture, where peaks of drug

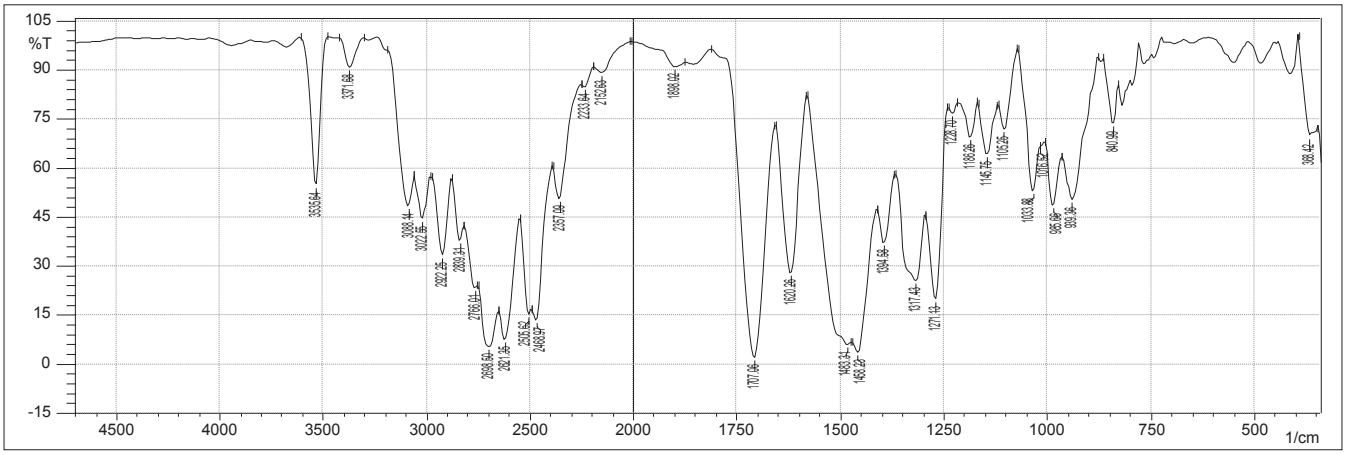

Figure 2: Fourier-transform infrared spectrum of ciprofloxacin hydrochloride

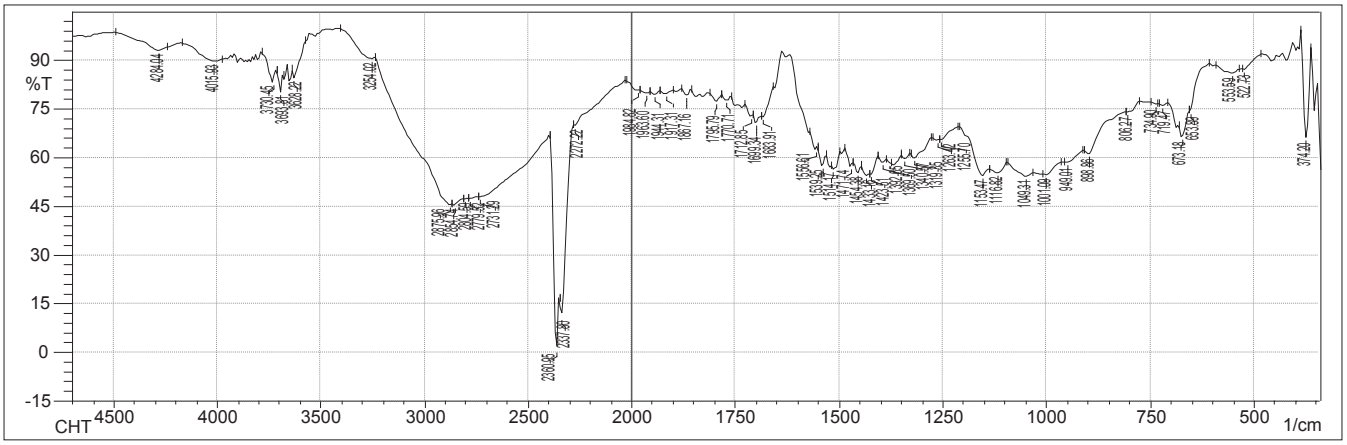

Figure 3: Fourier-transform infrared spectrum of chitosan

sample were observed in the spectrum of the compressed mixture.

The XRD spectrum of drug, polymer and their compressed mixture is as shown in Figures 5-7 respectively. The peaks of pure drug were observed in the spectrum of the compressed mixture, this indicates that there was no chemical interaction between drug and the polymer used. Hence, the drug was found to be compatible with polymer.

Furthermore, the peak intensities of chitosan were found to be reduced in the spectrum of the compressed mixture and this may be due to dilution of the polymer by drug [Table 2].

Characterization of plain non-CC

Determination of degree of deacetylation by potentiometry

The potentiometric plot of chitosan using 0.1 N HCl and 0.1 N NaOH is as shown in Figure 8, which shows two equivalent points first is due to reaction of NaOH with excess of HCl present in the reaction medium and second is due to reaction of NaOH with NH_3^+ group of chitosan polymer. The degree of de-acetylation of chitosan was found to be 88.55% [Table 3].[6,7]

Preparation of CC with sodium citrate

CC weighing 1.76 g was obtained from 2 g chitosan powder (Percent yield = 88.0%). The % yield was found to be 88%. Loss of 12% may be due to loss during collection and drying of the residue.

Characterization of sodium citrate CC

By potentiometric titration method

The amounts of protonable amino groups in polymer were measured using potentiometric titration. In this method, a known amount of HCl solution was added, in excess, into a solution containing a known quantity of chitosan (cross-linked), allowing enough time to charge all protonable groups (such as amino groups). In sequence, the resulting solution is then titrated using a solution of NaOH. The titration curves is obtained, which is as shown in Figure 9 and through the inflections of this curve the amount of amino groups were determined.

The citrate-cross-linked chitosan did not present expressive amount of protonable amino groups. The only inflection observed in this case was that related to the consumption of NaOH to neutralize the added HCl -] Table 4. This indicates that all reactive amino terminals were blocked by citrate groups.

By FTIR: The amounts of protonable (amino) groups in FTIR Spectrum of Citrate CC were measured using FTIR. The

Table 3: Determination of degree of de-acetylation of non-cross-linked chitosan

First equivalent point	Second equivalent point	Molarity of NaOH	% DD of chitosan
6.0 ml	17 ml	0.1	88.55

DD: Degree of deacetylation, NaOH: Sodium hydroxide

Table 2: Formulation development experiment using citrate cross-linked chitosan

Formulation code	Ciprofloxacin hydrochloride (%)	Citrate cross-linked chitosan (%)	Drug:Polymer ratio	Weight of implant (mg)
CC1	10	90	1:9	150
CC2	20	80	1:4	150
CC3	30	70	1:2.33	150
CC4	40	60	1:1.5	150
CC5	50	50	1:1	150

CC: Cross-linked chitosan

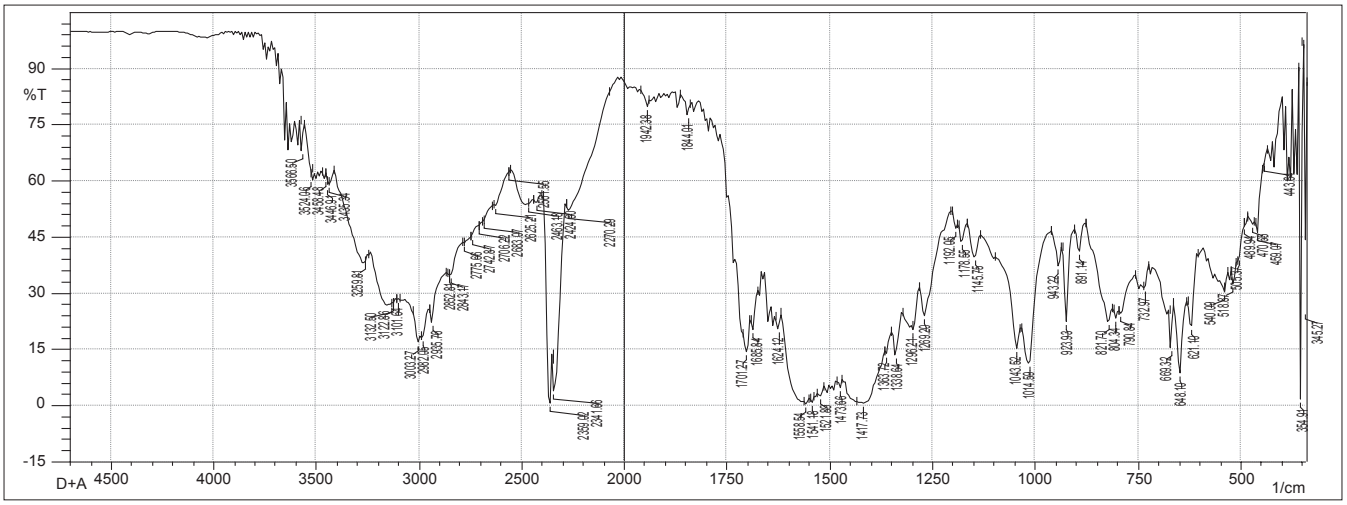

Figure 4: Fourier-transform infrared spectrum of mixture of drug and chitosan

peak of N-H stretching of simple chitosan (3254 cm^{-1}) was not observed in FTIR Spectrum of Citrate CC as shown in Figure 10. This clearly indicates that most of reactive amino terminals were blocked by citrate groups, i.e., cross-links were formed between NH_3^+ on chitosan and COO^- on citrate.

Compatibility study

Thermal analysis DSC: Thermograms of Drug And mixture of drug and CC were obtained to check the compatibility of drug and polymer after cross-linking, which are as shown in Figure 11. In the DSC thermogram of Drug + All excipient mixture, the endothermic peak of ciprofloxacin HCl was well-retained in the mixture. Thus, from results it can be concluded that the drug excipient combination does not show any major evidence of Drug excipient incompatibility.

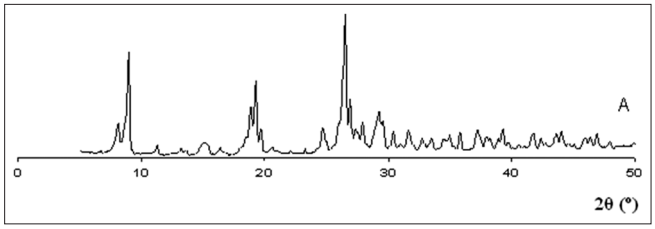

Figure 5: X-ray diffraction spectra of ciprofloxacin hydrochloride sample

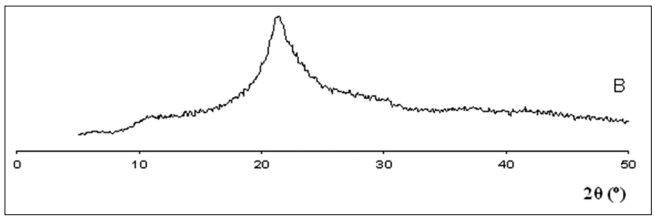

Figure 6: X-ray diffraction spectra of chitosan

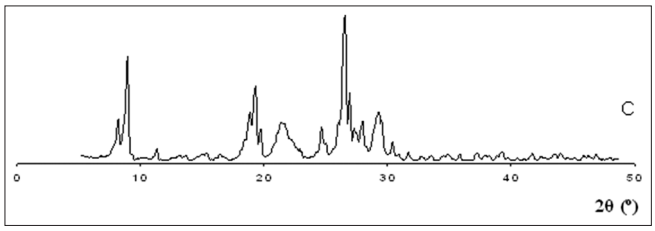

Figure 7: X-ray diffraction (XRD) spectra of mixture of XRD spectra of ciprofloxacin hydrochloride sample with chitosan

Evaluation of implants

Thickness and diameter variation test

The implants were evaluated for diameter, thickness, and hardness. The results are as in Table 5. All the formulations had uniform hardness, thickness, and diameter.

Drug content

All the implants had uniform distribution of drug in all the formulations. Drug content of all formulations were determined and reported in Table 6.

Water uptake study

Percent water uptake of CC1 to CC5 formulation is as shown

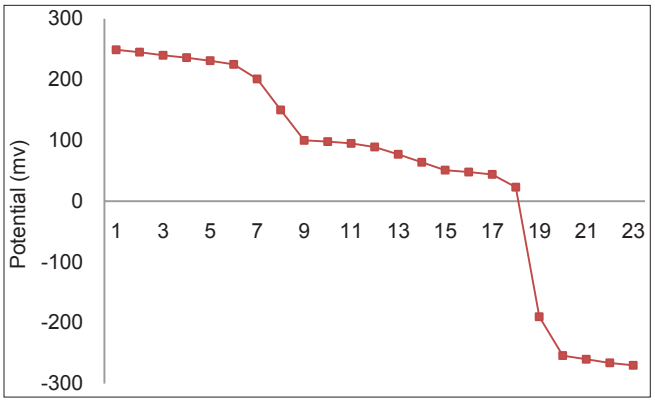

Figure 8: Potentiometric titration curve of simple (non-cross-linked) chitosan

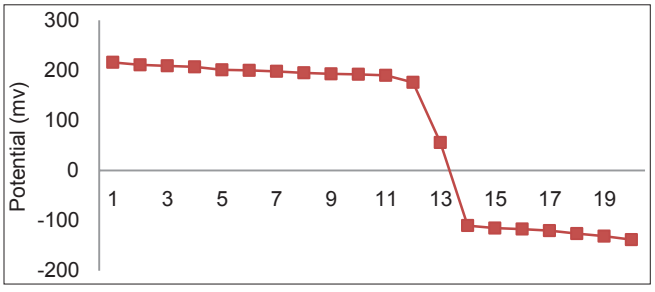

Figure 9: Potentiometric titration curve of cross-linked chitosan

Table 4: Determination of degree of de-acetylation of citrate cross-linked chitosan

First equivalent point	Second equivalent point	Molarity of sodium hydroxide	% DD of chitosan
11 ml	-	0.1 m	88.55

DD: Degree of deacetylation

Table 5: Diameter, thickness and hardness of CC1 to CC5 formulations

Parameters	Formulation code				
	CC1	CC2	CC3	CC4	aCC5
Diameter (mm)	8.08 (0.016)	8.06 (0.011)	8.06 (0.008)	8.05 (0.010)	8.06 (0.014)
Thickness (mm)	2.32 (0.014)	2.36 (0.013)	2.32 (0.016)	2.28 (0.014)	2.31 (0.010)
Hardness (Kg/cm^2)	4.8 (0.024)	4.7 (0.056)	4.8 (0.043)	4.9 (0.057)	4.9 (0.053)

CC: Cross-linked chitosan

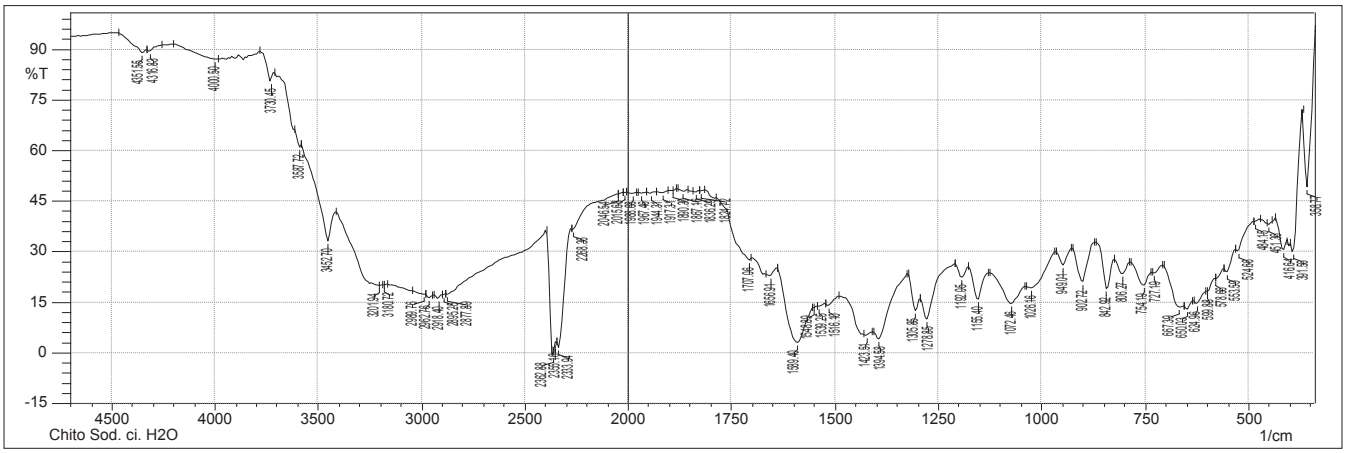

Figure 10: Fourier-transform infrared spectrum of citrate cross-linked chitosan

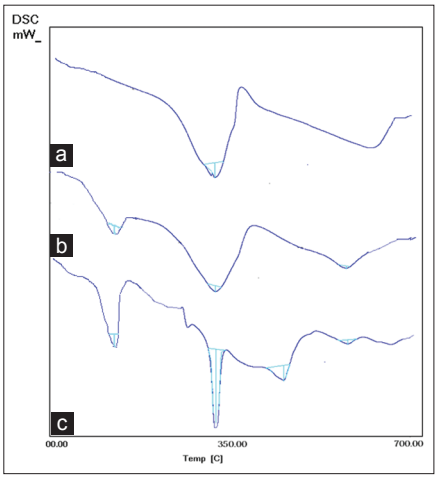

Figure 11: Thermograms of drug (a) and mixture of drug and citrate cross-linked chitosan (b) and mixture of drug and citrare cross-linked chitosan (c)

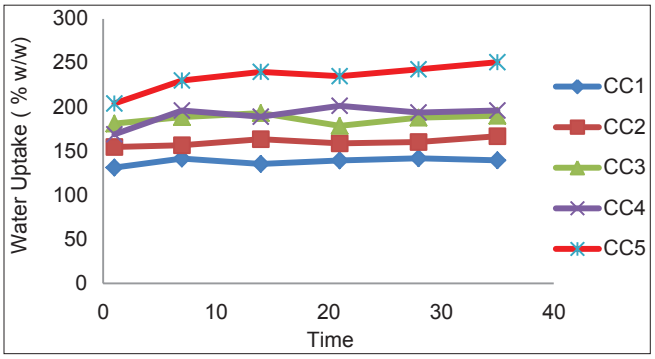

Figure 12: Water uptake study of CC1 to CC5 formulations

in Figure 12. It is observed that CC1 has comparatively less water uptake capacity than other formulations. This difference in water uptake can be attributed to difference in chitosan proportion in different formulations. As chitosan has less water uptake capacity and proportion of cross linked chitosan is very high in CC1, which results in less water uptake than other formulations.

Percent erosion

Percent Erosion of CC1 to CC5 formulation is as shown in Table 7. The percent erosion of CC4 and CC5 formulations is comparatively more than CC1 formulation.

In vitro drug release study

The cumulative percent release from all the formulations (triplicate readings) is determined and is as shown in Figure 13.

The cumulative percent release from implants is mainly depends on D:P ratio. The implants with various D:B ratios retarded the drug release for different time period. The CC1 formulation shows only 31.58% release whereas, CC5 formulation shows 97.28% release in 5 weeks. This effect may be attributed to proportion of CC in different formulation. In CC1 formulation proportion of CC is very high (D:P ratios (1:9)), which results in more retardation of drug release. In CC5 proportion of CC is low (D:P ratio (1:1)), which results in comparatively less retardation of drug release therefore, cumulative percent release from CC5 formulation is increased. In case of CC2 to CC4 formulations D:P ratio is also higher than CC5 formulation (1:4, 1:2.33 and 1:1.5 respectively) therefore shows only 51.55%, 68.64% and 80.56% release in 5 weeks respectively.

The primary reason for this observation is, increasing the proportion of CC reduces swelling of the implants hindering drug release. As the amount of CC increases, the water uptake decreases. The implants produced using higher CC concentrations were more rigid and showed less swelling in phosphate buffer. These results demonstrate that ionic cross-linking is a viable strategy for controlling release of ciprofloxacin from CC matrices. From this study, drug release from Citrate CC matrices was found to be decreasing with increasing proportion of CC.

Effect of drug loading

Similarity factor (f_2) tests were applied to study the effect of

Table 6: Drug content of CC1 to CC5 formulations of ciprofloxacin hydrochloride

Formulation code	Drug content (%)
CC1	98.83 (0.024)
CC2	98.57 (0.031)
CC3	99.21 (0.012)
CC4	98.97 (0.028)
CC5	99.12 (0.021)

CC: Cross-linked chitosan

Table 7: Percent erosion of CC1 to CC5 formulations

Formulation code	% Erosion (w/w)
CC1	14.65 (0.80)
CC2	18.39 (1.04)
CC3	22.63 (0.77)
CC4	24.98 (0.61)
CC5	27.55 (0.56)

CC: Cross-linked chitosan

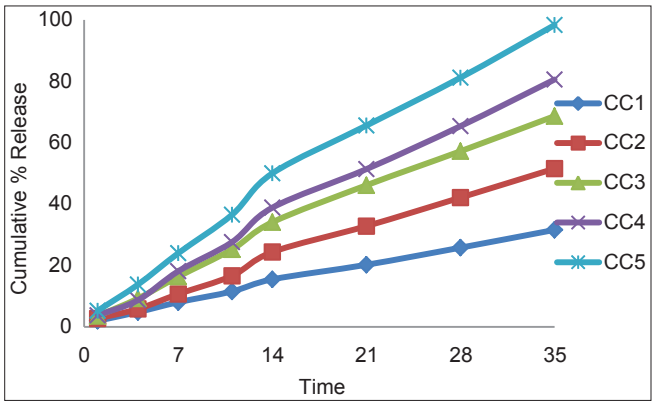

Figure 13: Cumulative percent dug release profile of CC1 to CC5 formulations

drug loading on percent cumulative CFX release from CC1 to CC5 formulations.

The drug loading affects the release profile of ciprofloxacin HCl. As shown in Table 5, the cumulative percent release from implants made with 10% CFX is significantly lower than from implants with 50% CFX for the two formulations CC1 versus CC5. The Similarity factor is calculated and is found to be,

$$f_2 = 33.51 \quad (4)$$

i.e., ($f_2 < 50$), this indicates dissimilarity of dissolution profile and it arises because of drug loading. The cumulative percent release of ciprofloxacin HCl increased with increasing drug loading.

This effect on release profile could be attributed to difference in water uptake capacity of both formulations and the water uptake results indicate that CC1 has a comparatively less water uptake capacity than CC5 because the amount of CC is increased in CC1 (D:P ratio [1:9]) and as CC has minimal water uptake capacity, which results in excess retardation of CFX release and only 31.58% amount of ciprofloxacin HCl is released in 5 weeks. However, in case of CC5 as the dug loading increases the water uptake capacity also increases results in comparatively more hydration and porosity; therefore, total amount (97.28%) of ciprofloxacin HCl is released in 5 weeks.

Drug release kinetics

The *In vitro* data were analyzed using model-independent and model-dependent methods. Since the diffusion, dissolution and erosion influence the drug release in most cases a simple kinetic model is unlikely to explain the overall *In vitro* as well as *In vivo* drug release behavior. The release data obtained were fitted to Zero order, First order, Higuchi, and Korsmayer-Peppas equations to determine the corresponding release rate and mechanism of drug release from the implants. The model that fits to the release data was evaluated by correlation co-efficient (R). For CC1 to CC4 formulation the R values were high for Hixon Crowel equation, indicating that the drug release from these formulations follows Hixon Crowel's kinetics of drug release (i.e., the drug release from these formulation is depend on dissolution of drug rather than diffusion through polymeric matrices), whereas in CC5 formulation R values were high for Korsmayer-Peppas equations indicating that the drug release from these formulations follows Korsmayer-Peppas kinetics of drug release (i.e., CC5 formulation shows biphasic release pattern). The value of Release Exponent '*n*' is also determined for each formulation. The value of '*n*' in Korsmeyer's-Peppas equation indicates the drug release mechanism. With respect to CC1 to CC5 formulations, the value of 'n' is in the range of 0.50-1.0 indicting that drug release from these formulations is controlled by diffusion of drug as well as erosion of polymer chains (non-Fickian diffusion or anomalous diffusion).

Histocompatibility study

The biocompatibility study was carried out according to process given in experimental section. The cumulative % release of CC5 formulation is more than 99% in 5 weeks; therefore, based on release pattern and cumulative percent release, the CC5 formulation of CC based matrices was considered as an optimized formulation and used further for *In vivo* study.

The tissue samples were evaluated for Cellular inflammatory responses, Necrosis, Ulcer formation, Cellular infiltration, Edema, Migration of inflammatory cells at implantation site and other foreign body tissue. The microscopical view of tissue specimen is shown in Figure 14 and results are shown in Table 8.

From histocompatibility study - it is observed that the implantation site was free from any signs of macroscopic changes such as reddening, local necrosis, infection, abscess, edema, ulcer formation etc., and during the post-implantation period. Microscopic changes such as migration of blood cells after 7 days and 21 days at the site of implantation were

Table 8: Details of histocompatibility study

Group	Necrosis	Cellular infiltration	Edema hyperemia	Thickness of capsule	Ulceration
Group 1 (control)	–	–	–	–	–
Group 2 (day 7)	–	+	+	–	–
Group 3 (day 21)	–	+	–	–	–

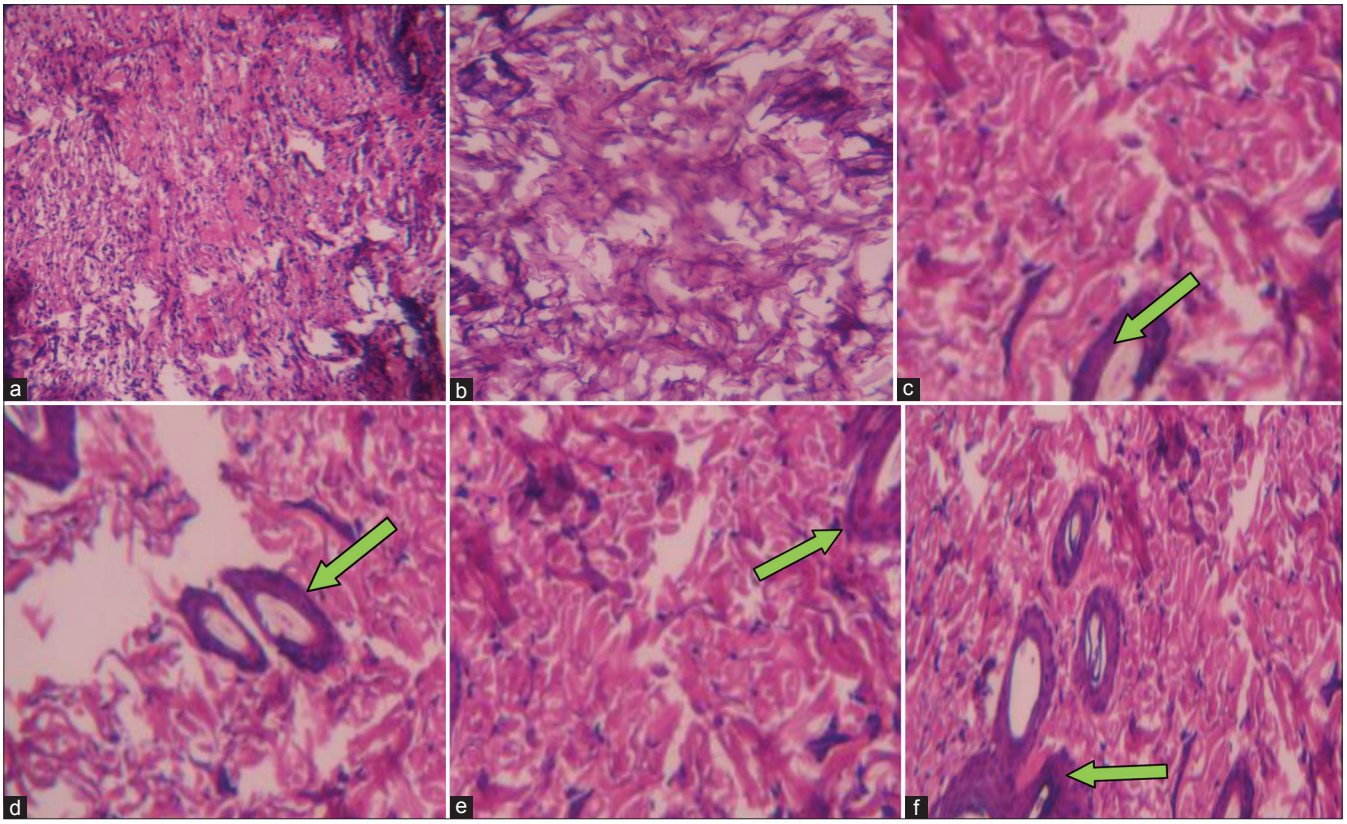

Figure 14: Microscopic view of histological sample of rat subcutaneous tissue implanted with EC4 formulation (chitosan based implant)–(i) Group 1 - micrograph of rat subcutaneous tissue sample (control group) (a),(b) (ii) Group 2 - histological response after 7 days (c),(d), (iii) Group 3 - histological response after 21 days

observed. Migration of blood cells is a feature of normal immune system function i.e., wound healing and the body's response to a foreign material (implant).[14,15] It is a normal for blood cells to migrate around any foreign object implanted in the body and hence, from this study it is proved that Chitosan based implant formulation is biocompatible and implant is not causing any foreign body reaction or hypersensitivity in the body of animal.

CONCLUSION

CC implants are characterized by minimal swelling, erosion and water uptake. By increasing the drug loading in chitosan implants results in prolonged drug release and well-controlled burst release. The release from all developed formulations is based on water uptake, i.e., the formulations having more water uptake shows higher cumulative % release and the formulations having low water uptake shows lesser cumulative % release. Furthermore, from histocompatibility study it is found that the chitosan based implant formulation CC5 is biocompatible and implant is not causing any foreign body reaction or hypersensitivity in the body of animal. The implantation site was free from any signs of macroscopic changes such as reddening, local necrosis, infection etc., and during the post-implantation period. In addition to an excellent biocompatibility and bioresorption, the Citrate CC implants have potential to retard the drug release for more than five weeks in the treatment of osteomyelitis and bone infections. This type of implantable drug delivery system using CC can be a cost- effective alternative to the presently available drug delivery systems of ciprofloxacin HCl in the treatment of Osteomyelitis.

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