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Effect of concentration of crosslinked chitosan on ciprofloxacin release from biodegradable implantable matrices

Ashish Y Pawar*, Deelip V Derle

School of Pharmacy, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan.

The purpose of this investigation was to develop and characterize a biodegradable implants containing Ciprofloxacin hydrochloride (HCl) for the localized treatment of osteomyelitis. Chitosan is widely investigated biodegradable polymer and has reactive hydroxyl groups that can be modified chemically for various biomedical and pharmaceutical applications. In this study the reactive hydroxyl groups of Chitosan were modified using epichlorohydrin & crosslinked Chitosan matrices were used as carrier to formulate different Ciprofloxacin implants. The developed formulations were evaluated for different *In vitro* parameters. The formulations having 40% drug loading (EC4) was found to be optimum in terms of various evaluation parameters. The present work concluded that, the concentration of crosslinked chitosan plays crucial role in retardation of drug release from biodegradable implantable matrices.

Keyword: Ciprofloxacin, Osteomyelitis, Degree of deacetylation, Swelling

INTRODUCTION: Ciprofloxacin (CFX) is most widely used fluoroquinolone for bacterial bone infection, since the minimal inhibitory concentration (MIC) of CFX is low (0.25–2 µg/ml). To avoid drawbacks of conventional therapy, subcutaneous implantable drug delivery of Ciprofloxacin Hydrochloride is developed using cross linked chitosan which slowly releases drug from implant and high local tissue concentration can be achieved at the infected site. The biopolymer chitosan is prepared by *N*-deacetylation of chitin, one of the polysaccharides widely distributed in nature as a main component of the shells of crustaceans and insects. Chitosan is reported to be biocompatible and free of inflammatory responses at the same time acting as release retardant in many reports (Balmayor E.R. *et al*., 2011 [2]. Chitosan has reactive hydroxyl groups that can be chemically modified for various biomedical and pharmaceutical applications

Corresponding Author's Contact information:
Ashish Y Pawar *
Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan
E-mail: pawarashish23@gmail.com

Materials And Methods

Ciprofloxacin Hydrochloride HCl and Chitosan (89% deacetylated) was kindly supplied by Glennmark Pharmaceuticals Ltd., (Nashik). Other important chemicals used for the study were of analytical grade.

Material Characterization

Characterization of Simple Chitosan

a) Determination of degree of deacetylation by Potentiometry (Rodrigo SV *et al*., 2006; Avadi MR *et al*., 2003, Xiangping K, 2012) [12, 1, 19]

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 deacetylation was calculated from the amount of NaOH consumed between two inflection points by the following equation.

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

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 gms).

b) Fourier Transformation Infra-red (FTIR) analysis

A Fourier-transform infrared (FTIR) spectrum of the Chitosan was obtained on a Shimadzu 8400 S FTIR (Tokyo, Japan) in the range of 4000-400 cm^{-1}, using KBr pellet.

Preparation of Cross-Linked Chitosan with Epichlorohydrin **(**Wei YC *et al*., 1992; Vanessa LG *et al*., 2005, Shilan Chen *et al*., 2008**)** [18, 17, 14]

The solution of chitosan was prepared with 2 g of chitosan dissolved into 20.0 ml of acetic acid (5% v/v) and added with 50 mL of distilled water. Its pH was adjusted from 3.0 to 11.0 with 1.0M of sodium hydroxide solution. Epichlorohydrin solution (0.85% V/V) was added, and the mixture was stirred (Magnetic Stirrer, Remi) for 24 h at room temperature. Then 50mL of 1.0M sodium hydroxide solution was added into the mixture to form the precipitate. The precipitate was filtered and washed intensively with distilled water to remove any unreacted epichlorohydrin. Subsequently, it was dried on hot air oven for 12 h. The resulting material was grounded and sieved to collect cross linked chitosan particles.

Mechanism of Crosslinking

Epichlorohydrin interacts with carboxyl groups present in chitosan. The crosslinking is formed due to the electrostatic interaction between (CH $_2O^-$) on chitosan and (CH $_2$ $^+$) on Epichlorohydrin (Figure 1).

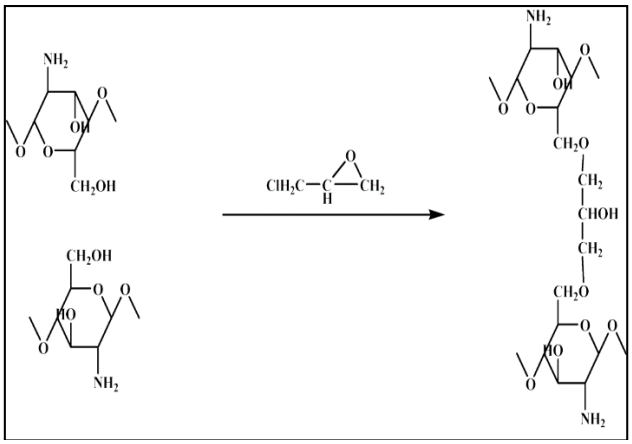


Fig 1: Crosslinking reaction of chitosan with epichlorohydrin

Characterization of Epichlorohydrin Cross-linked chitosan

a) By Potentiometric Titration method

The amounts of protonable (amino) groups in Epichlorohydrin Cross-linked chitosan were measured using potentiometric titration as described for Simple Chitosan.

b) By FTIR

The amounts of protonable (amino) groups in Epichlorohydrin Cross-linked chitosan were measured using FTIR.

Formulation development

Epichlorohydrin Cross-linked Chitosan as a carrier

Formulations were developed in order to establish a controlled release implantable dosage form. The active ingredient (Ciprofloxacin HCl) and polymer (Epichlorohydrin Cross-linked Chitosan) were weighed accurately and passed through 60# sieve. Weight of implant tablet was kept constant in all the formulations (150 mg). The formulation code and Drug: Polymer ratio used is as shown in Table 1.

Table 1: Formulation Development Experiment using Epichlorohydrin Cross-linked Chitosan

Sr. No.	Formulation Code	Ciprofloxacin HCl (%)	Epichlorohydrin Cross-linked Chitosan (%)	Drug: Polymer	Weight of Implant (mg)
1	EC1	10	90	1:9	150
2	EC2	20	80	1:4	150
3	EC3	30	70	1:2.33	150
4	EC4	40	60	1:1.5	150
5	EC5	50	50	1:1	150

Preparation of implants

The compression of powder blend (EC1 to EC5) was done by direct compression method on rotary compression machine (General machine, India). The compression was carried out using 8 mm flat-faced circular punches.

Evaluation of implants

The compressed implants were evaluated for different parameters as-.

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 S.D was calculated for each formulation.

Hardness Test

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

Drug Content (Cyril D 2002; Castro C 2003) [6, 5]:
One milled implant was placed in 100 ml of HCl (0.1N) 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 (Cyril D *et al*., 2002; Castro C *et al*., 2003) [6, 5]:
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). Water uptake was determined using following equation,

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

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

In Vitro Drug Release Study

Rotary Shaker Method (Vial method) (Cyril D *et al*., 2002; Castro C *et al*., 2003; Schliecke G *et al*., 2004; Siewert M *et al*., 2003) [6, 5, 13, 15]:
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 37^0C for 5 weeks (Or more) with agitation (60 rpm) in orbital shaking incubator (Remi, India) (shaking bath) (60rpm) (Fig.2.3). At defined time points, whole dissolution medium was withdrawn and replaced with fresh buffer to maintain sink condition.

Results & Discussion

Material Characterization

Characterization of Simple (Non Cross-Linked) Chitosan

a) Determination of degree of deacetylation

The potentiometric plot of chitosan using 0.1 N HCl and 0.1 N NaOH is as shown in Figure 2, which shows two equivalent points. The first one is due to reaction of NaOH with excess of HCl

present in the reaction medium and the second one is due to reaction of NaOH with NH_3^+ group of chitosan polymer. The degree of deacetylation of Chitosan was found to be 88.55%.

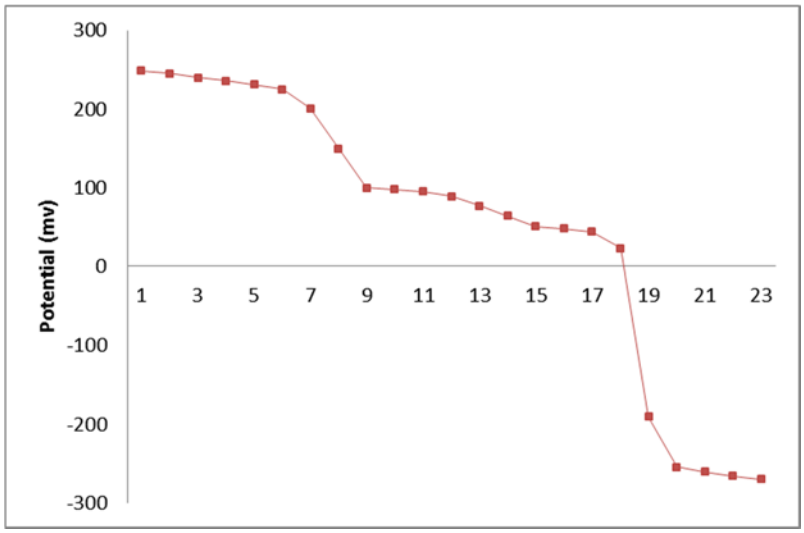
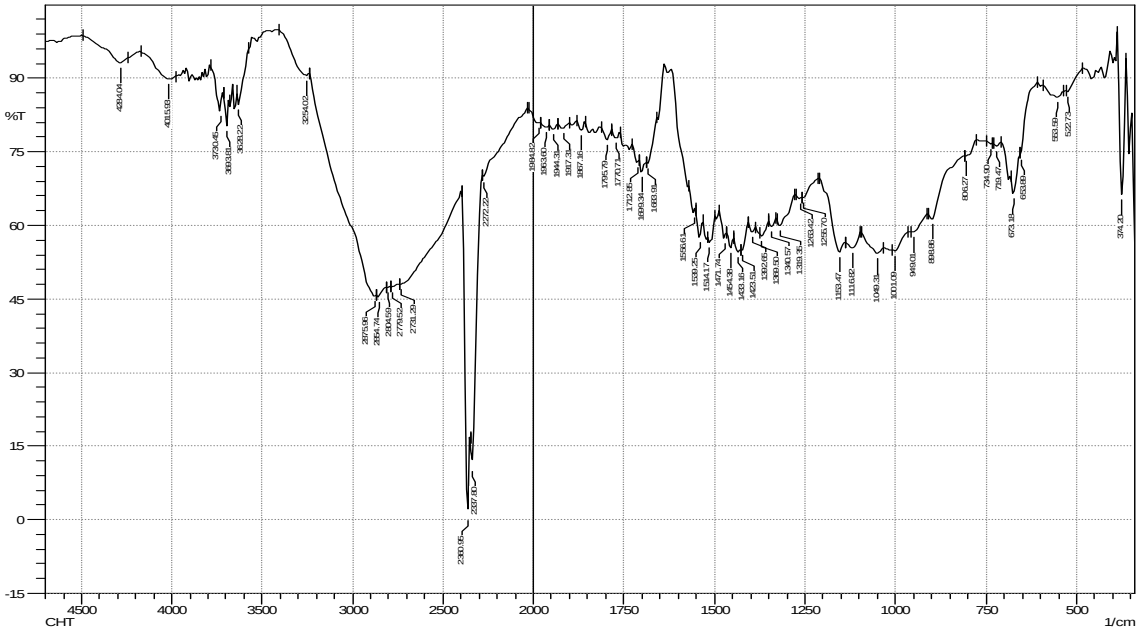


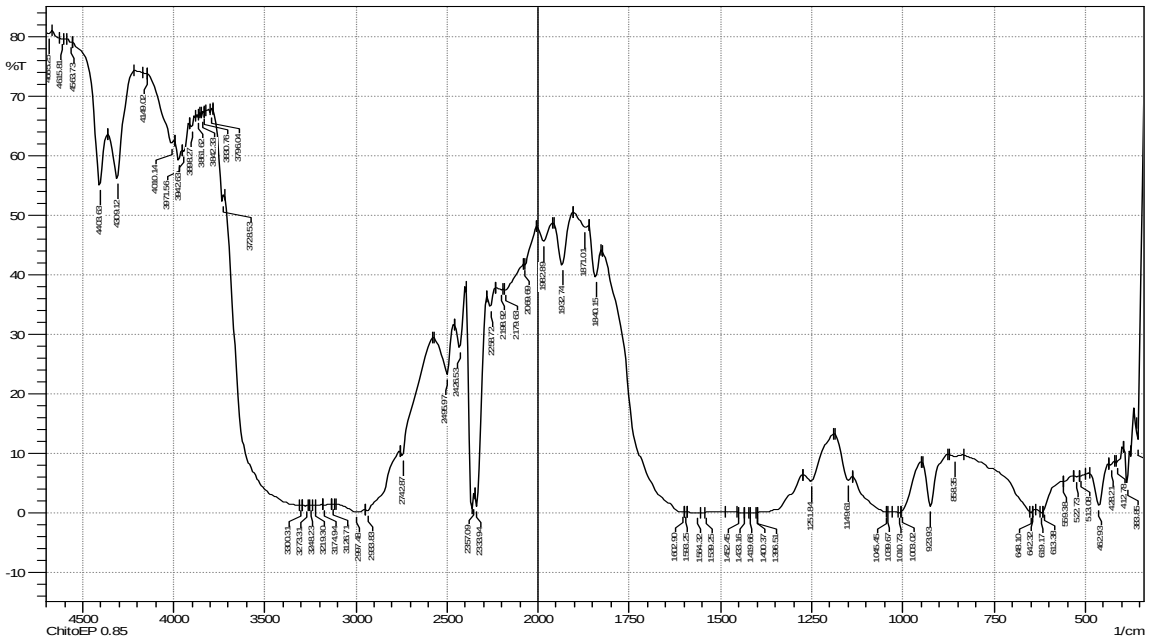
Fig 2: Potentiometric titration curve of Simple (Non Cross-linked) Chitosan showing two equivalent points (The first one is due to reaction of NaOH with excess of HCl present in the reaction medium and the second one is due to reaction of NaOH with NH_3^+ group of chitosan polymer)

b) Fourier Transformation Infra-red (FTIR) analysis

FTIR spectrum of the chitosan sample showed all the characteristic IR peaks as reported in the literature. A Fourier-transform infrared (FTIR) spectrum of the Chitosan is presented in Figure 3.



A



B

Fig 3: FTIR Spectrum of Chitosan (A) & Epichlorohydrin crosslinked chitosan (B)

Preparation of epichlorohydrin cross-linked chitosan

Cross-linked chitosan weighting 1.83 g was obtained from 2g chitosan powder (Percent yield = 91.50 %). The % yield was found to be 91.5%. Loss of 8.5% may be due to loss during collection and drying of the residue.

Characterization of Epichlorohydrin Cross-Linked Chitosan

By potentiometric Titration method

The amounts of protonable (amino) groups in polymer were measured using potentiometric titration. Epichlorohydrin-cross-linked chitosan presents same amount of protonable amino groups close to the value found in natural (simple) chitosan. This indicates that the amino terminals were not blocked by Epichlorohydrin groups & this crosslinking agent is attacking on other proton binding structures besides the original amino groups i.e. hydroxyl groups.

Evaluation of Implants

Thickness and Diameter variation Test

The implants were evaluated for diameter, thickness and hardness. The results are as in Table 2. All the formulations had uniform hardness, thickness and diameter.

Table 2: Diameter, Thickness and Hardness of EC1 to EC5 formulations

Parameters	Formulation Code				
	EC1	EC2	EC3	EC4	EC5
Diameter (mm)	8.01 (±0.011)	8.08 (±0.009)	8.04 (±0.014)	8.08 (±0.010)	8.06 (±0.011)
Thickness (mm)	2.28 (±0.011)	2.30 (±0.018)	2.33 (±0.022)	2.30 (±0.017)	2.32 (±0.010)
Hardness (Kg/cm2)	5.2 (±0.034)	5.2 (±0.041)	5.1 (±0.048)	5.3 (±0.056)	5.3 (±0.053)

Drug Content

All the implants had uniform distribution of drug in all the formulations. Drug content of all formulations were determined and found to be uniform in the range of 98.8 to 99.3 %.

Water Uptake Study

Percent water uptake of EC1 to EC5 formulation is as shown in Figure 4. It is observed that EC1 has comparatively less water uptake capacity than other formulations.

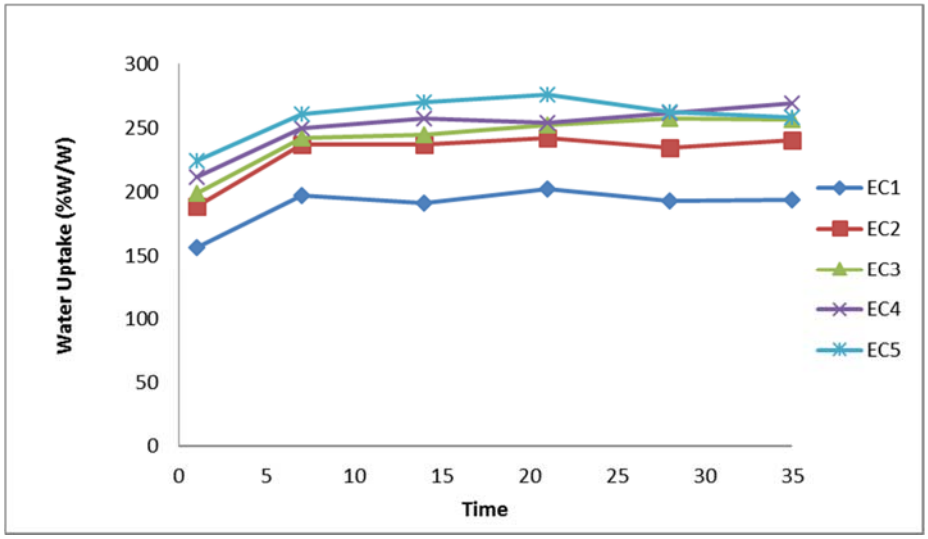


Fig 4: Water uptake study of EC1 to EC5 formulations

In vitro drug release study

The cumulative percent release from all the formulations (triplicate readings) is determined and is as shown in Figure 5. The EC1 formulation shows only 46.07% release whereas EC4 formulation shows 99.46% release in five weeks. This effect may be attributed to proportion of Cross-linked Chitosan in different formulation. In EC1 formulation proportion of Cross-linked Chitosan is very high (drug: polymer ratios (1:9)) which results in more retardation of drug release.

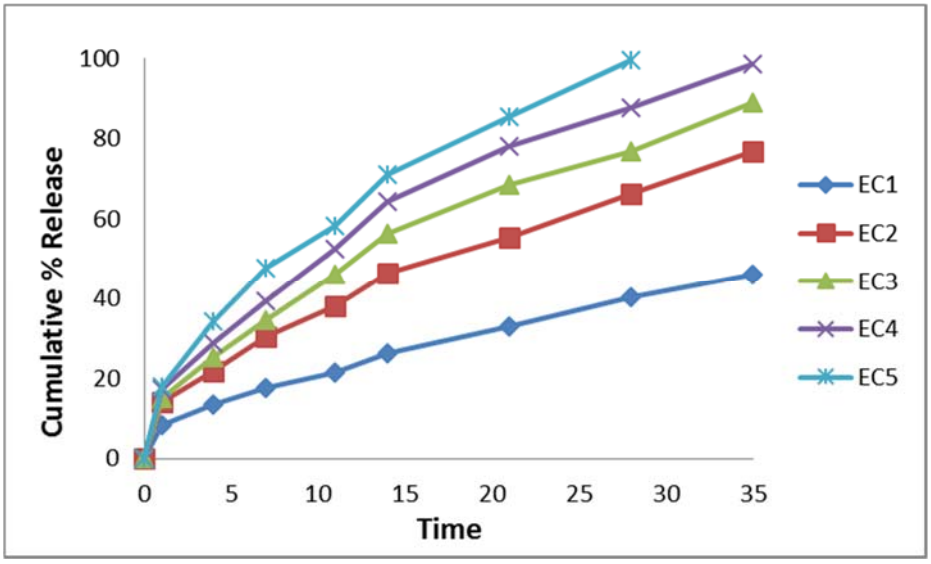


Fig 5: Cumulative percent drug release from EC1 to EC5 formulations

Drug Release Kinetics

For EC1 to EC5 formulation the R values were high for Higuchi equation, indicating that the drug release from these formulations follows Higuchi (matrix diffusion) kinetics of drug release.

Conclusion

By increasing the proportion of cross-linked chitosan reduces swelling of the implants hindering drug release. As the amount of cross-linked chitosan increases, the water uptake decreases. The implants produced using higher cross-linked chitosan concentrations were more rigid and showed less swelling in phosphate buffer. These results demonstrate that ionic crosslinking is a viable strategy for controlling release of ciprofloxacin from cross-linked chitosan matrices.

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