Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 1, 2013

Research Article

CROSS LINKED HIGH AMYLOSE STARCH BIODEGRADABLE IMPLANTS FOR SUSTAINED DELIVERY OF CIPROFLOXACIN HYDROCHLORIDE.

***PAWAR ASHISH Y.[1], JADHAV KHANDERAO R.[1] & DERLE DEELIP V.[2]**

[1]Department of Pharmaceutical Sciences, MGV's Pharmacy College, Panchavati, Nashik, Maharashtra, India, [2]Department of Pharmaceutical Sciences, N.D.M.V.P.'s, College of Pharmacy, Gangapur Road, Nasik, Maharashtra, India. Email: pawarashish23@gmail.com

Received: 27 Nov 2012, Revised and Accepted: 24 Dec 2012

ABSTRACT

Objective: The main objective of present study is to formulate Ciprofloxacin implants in treatment of Osteomyelitis & other bone infections using Crosslinked high amylose starch matrices as a carrier.

Methods: The High amylose starch (corn starch) is one of the most commonly used excipients in the pharmaceutical industry due to its biodegradability and binding properties. A number of sources of starch are commercially available, with corn starch being the most common. The High amylose starch forms uniform, stable crosslinks with different crosslinking agents which is a major advantage of corn starch over other simple starches. The High amylose starch has reactive hydroxyl groups that can be modified chemically for various biomedical and pharmaceutical applications. Different formulations were developed by varying drug to polymer ratio. Preparation crosslinked high amylose starch based implants was carried out by direct compression technique. The effects of crosslinking agent on water uptake, erosion, drug release rate, release kinetics and drug loading were investigated in this study.

Result: The release from all developed formulations is based on water uptake& the optimized CS3 implant formulation shows a slow zero order kinetics of drug release. The prolonged drug release was observed with higher drug loading.

Conclusion: Therefore from this study it is proved that, the crosslinked starch implant has potential to retard the drug release for more than five weeks in the treatment of osteomyelitis & bone infections.

Keywords: Ciprofloxacin Hydrochloride, Cross-linked high amylose starch, Implant, Osteomyelitis.

INTRODUCTION

Starch is one of the most commonly used excipients in the pharmaceutical industry due to its biodegradability, disintegration and binding properties. A number of sources of starch are commercially available, with corn starch being the most common. Starch is the major carbohydrate reserve in plant tubers and seed endosperm where it is found as granules each typically containing several million amylopectin molecules accompanied by a much larger number of smaller amylose molecules. By far the largest source of starch is corn (maize) with other commonly used sources being wheat, potato, tapioca and rice [1-3].The High amylose starch forms uniform, stable crosslinks with different crosslinking agents which are a major advantage of corn starch over other simple starches. The High amylose starch has reactive hydroxyl groups that can be modified chemically for various biomedical and pharmaceutical applications like implants.

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 *itis*(inflammation) to define the clinical state in which bone is infected with microorganisms. It is mainly characterized by inflammation and swelling of bone tissues .*Staphylococcus aureus*(80-85%)isthe major organisms associated with osteomyelitis. Infection is more common in the long bones and vertebras of the body, but it can also affect other bones in the body [4,5]. 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 four to five weeks. Some of the disadvantages of prolonged parenteral therapy include; Patient discomfort, High cost of treatment, Development of systemic toxicity, Patient compliance problems. Osteomyelitis results in 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. Also such a long parenteral therapy may develop systemic toxicity. To overcome some of these problems with the treatment of osteomyelitis, localized drug therapy using non-biodegradablepolymethacrylate (PMMA) bone cement implants was introduced [6,7]. The advantages of local therapy include high, local, tissue, while simultaneously minimizing high, potentially toxic, systemic drug levels. However, previous studies on the nonbiodegradable 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 as carrier for implant but their use is limited because of high cost and are not easily available [8].

Therefore, to avoid the systemic toxicity, to produce effective drug concentration at the infected site subcutaneous implantable drug delivery of Ciprofloxacin Hydrochloride 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 Hydrochloride HCl is very low (0.25-2 µg/ml) for most of the pathogens that cause osteomyelitis, the growth of causative microorganism can be easily inhibited.

MATERIALS AND METHODS

Materials

Ciprofloxacin Hydrochloride HCl and High amylose starch was kindly supplied by Wockhardt Pharmaceuticals Ltd., (Aurangabad). Other important chemicals includes Sodium hydroxide, Potassium dihydrogen phosphate, Sodium azide, HCL &epichlorohydrin. All the materials used for the study were of analytical grade.

Preparation of Cross-Linked High Amylose Starch [9-11]

Corn starch containing 70% W/W amylose was dispersed at 16.7% w/v in 1.0 N NaOH at 54^{0}C. After 15 min, epichlorohydrin (0.85%

v/v) was then added and allowed to react for 15 min. The suspension was then neutralized by addition of sodium acetate and washed three times by acetone 40% in a buchner funnel to remove traces of unreacted epichlorohydrin. Cross-linked high amylose starch was then dried using ethanol 30% and exposition to air at room temperature for 72 h.

Mechanism of Crosslinking

Epichlorohydrin interacts with carboxyl groups present in starch. The crosslinking is formed due to the electrostatic interaction between (CH_2O^-) on starch and (CH_2^+) on epichlorohydrin (Fig. 1).

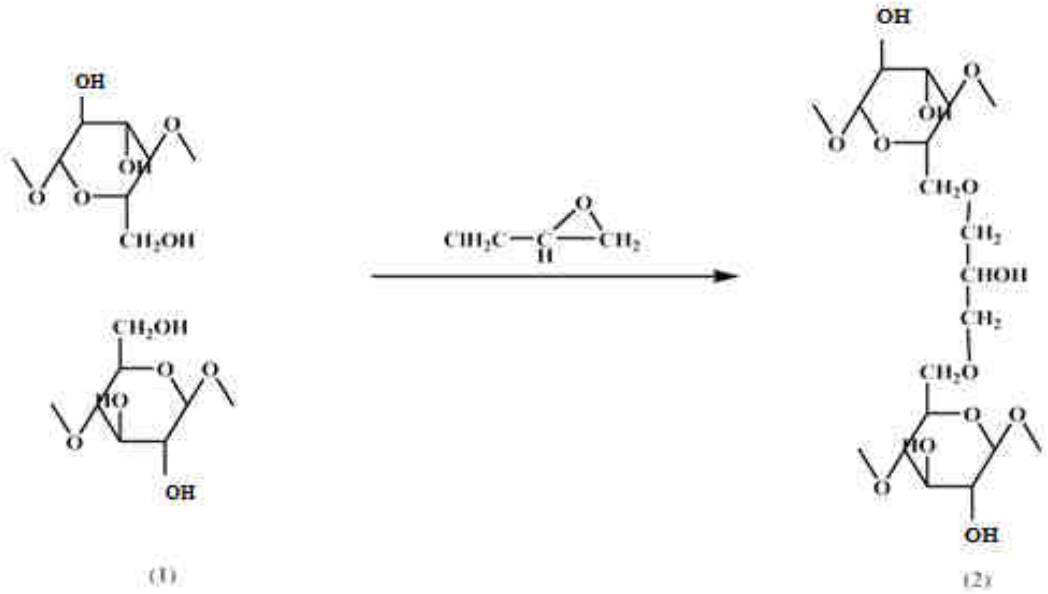

Fig. 1: Reaction of starch (1) with epichlorohydrin gives cross linked starch (2)

Characterization of Cross-linked High Amylose starch [9-12]

BY FTIR Spectroscopy

The dried cross-linked high amylose starch was characterized by FTIR spectroscopy.

Compatibility study of drug with polymers

A) FTIR Analysis

The drug and polymer (Cross-linked High Amylose Starch) powders were mixed in 1:1 ratio and were kept in the dried glass vial under normal conditions at room temperature for 7 days. The mixture was then analysed by FTIR.

B) Thermal Analysis (DSC)

Thermogram of Drug and mixtures of drug & cross-linked chitosan were obtained using Shimadzu DSC-60 Differential Scanning Calorimeter using aluminum pans to check the compatibility of drug & polymer after crosslinking.

Formulation Development

Formulations were developed in order to establish a controlled release implantable dosage form.

Experimental design:Cross-linkedhigh amylose starch as a carrier

The active ingredient (Ciprofloxacin HCl) and polymer (Cross-linked high amylose starch) were weighed accurately and passed through 60# sieve. Mixing of powders was done 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). The formulation code and Drug: Polymer ratio used is as shown in Table 1.

Table 1: Formulation development experiment using cross-linked high amylose starch as a carrier

S. No.	Formulation Code	Ciprofloxacin HCl (%)	Cross-linked high amylose starch (%)	Drug: Polymer	Weight of Implant (mg)
1	CS 1	5	95	1:19	150
2	CS 2	10	90	1:9	150
3	CS 3	15	85	1:5.66	150
4	CS 4	20	80	1:4	150
5	CS 5	25	75	1:3	150

Preparation of Implants

The compression of powder blend (CS1 to CS5) 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.

All formulations were compressed at a constant force to achieve the tablet weight of 150 mg and hardness in between 6-6.5 kg/cm^2 for CS1 to CS5 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 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 [13-19]

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[13-21]

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,

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

Where, W_w is the wet weight,

W_i is the initial weight

Mass Loss (% Erosion)[13-19]

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 CFX released after 5 weeks was calculated from UV spectrophotometry assays.

% erosion was determined using following equation,

$$\text{\% Erosion} = \frac{(W_i - W_{CFX\ Released}) - W_d \times 100}{W_i}$$

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)[13-20]

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) (60rpm). 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 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 S.D. was determined.

Stability study

Stability study was conducted as per the ICH Guidelines. Shortly, optimized formulation was subjected to Accelerated stability study (40±2°C/75±5% RH). The selected formulation was stored at above mentioned conditions for a period of six months. At the end of six months the formulations was analyzed for organoleptic characteristics, hardness, drug content and dissolution profiles.

RESULTS & DISCUSSION

Preparation of cross-linked high amylose starch

Cross-linked starch weighing **1.9g** was obtained from **2g** starch powder The % yield was found to be95%. Loss of 5% may be due to loss during collection and drying of the residue.

Characterization of Cross-Linked High Amylose Starch

By FTIR spectroscopy

FTIR spectrum of the cross linked starch (0.85%) (Fig. 2) showed all the characteristic IR peaks as reported in the literature except the peak of O-H Stretching. This peak is not observed when compared to simple high amylose starch. This clearly indicates that at 0.85% concentrationepichlorohydrin forms cross-link with OH group of polymer sample.

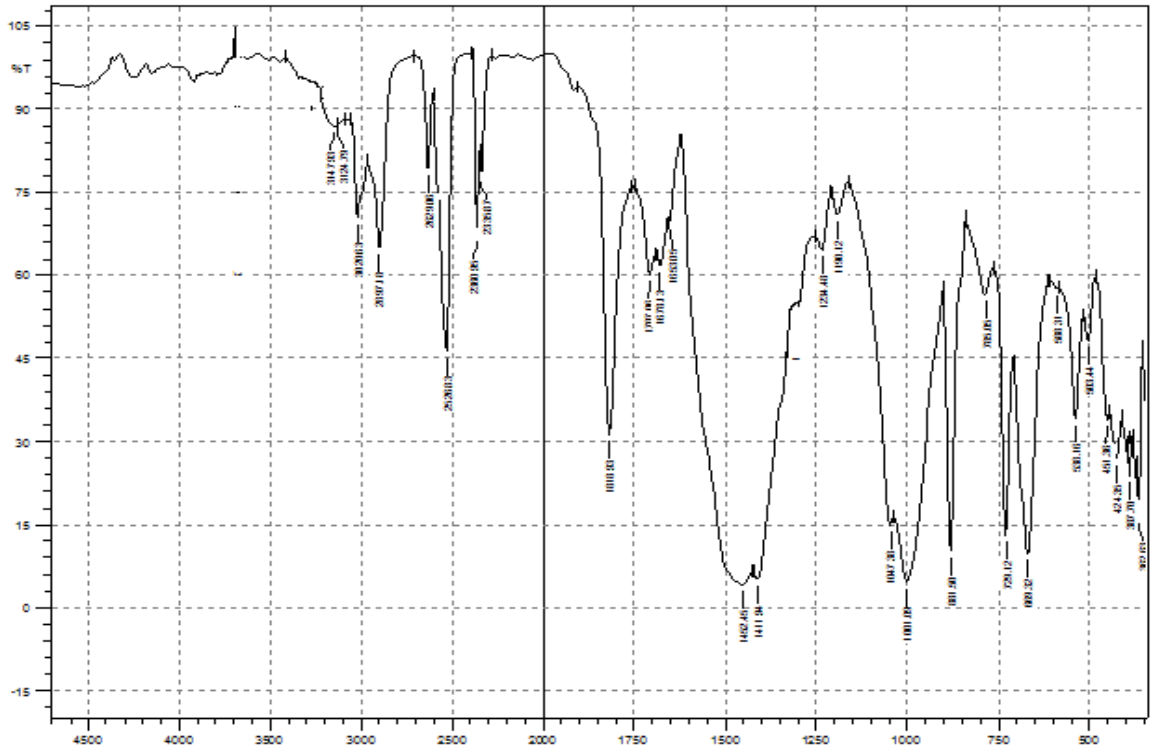

Fig. 2: FTIR spectra of cross-linked high amylose starch

Table 2: Values of major peaks in FTIR spectrum of cross-linked starch (0.85%) sample

Remarks	Peak (Wave number) cm^{-1}(Observed)	Peak (Wave number) cm^{-1} (Reference)
C-H Stretching	2897	2990-2850
C-O-C Stretching	1047	1200-1020
C-O Stretching of hydroxyl	No peak Observed	1350-1260
O-H Stretching	No peak Observed	3600-3400

Compatibility study of drug with polymers

A) FTIR analysis

FTIR can be considered as first line analytical technique to study compatibility of drug with excipients.

Fig. 3 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.

B) Thermal Analysis (DSC)

Thermogram of mixture of drug & cross-linked starchwas obtained to check the compatibility of drug & polymer after crosslinking, which is as shown in Fig. 4.

In the DSC thermogram of Drug +cross-linked starch mixture, the endothermic peak of Ciprofloxacin HCL was well retained in the mixture as shown in Figure4. The drug thermogram is well retained with slight shifting of peak temperature value to higher temperature this type of phenomenon may be observed due to simple mixing of drug and excipients which lowers the purity of each compound. From above results it is concluded that the drug–excipient combination does not show any major evidence of Drug excipient incompatibility.

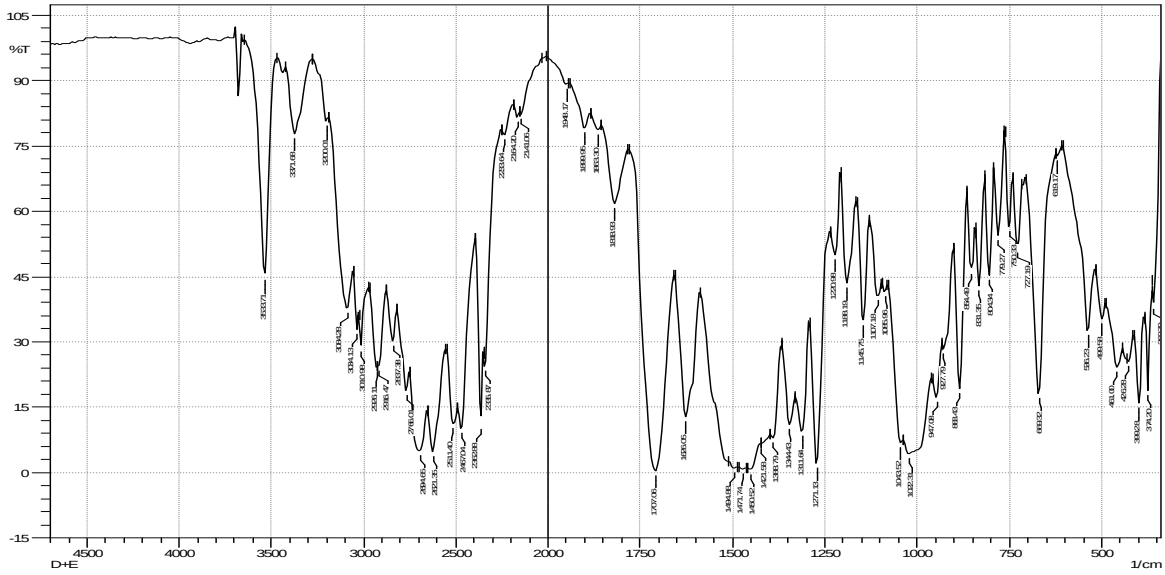

Fig. 3: FTIR Spectrum of mixture of drug and of cross-linked starch

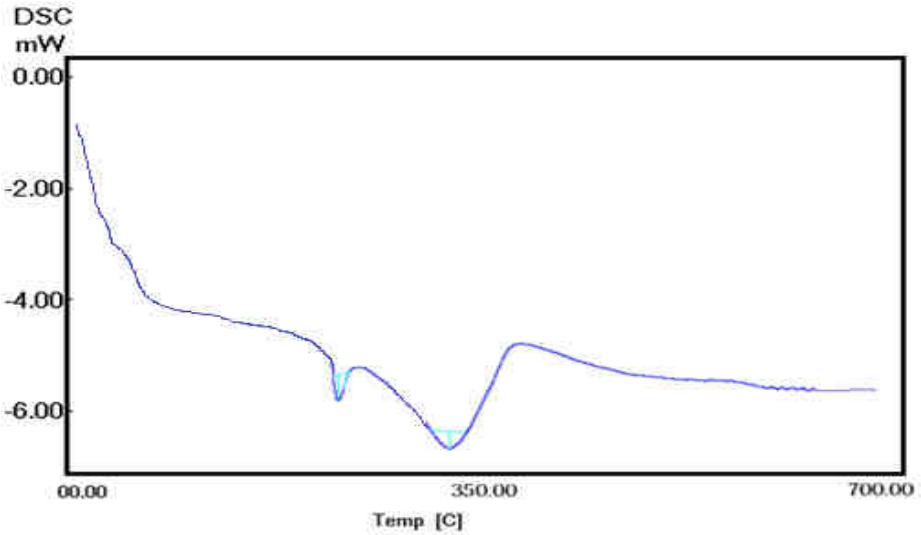

Fig. 4: Thermogram of mixture of drug & cross-linked starch

Table 3: Corresponding peak temperatures values of Ciprofloxacin HCL in drug-excipient physical mixtures.

Sample	Ratio Drug:Excipients	Peak		
		Onset	Peak	Endset
Ciprofloxacin HCL	1:0	309.03℃	317.56℃	325.17℃
Mixture of Drug & cross-linked starch	1:1	312.65℃	323.41℃	331.11℃

Evaluation of Implants

Cross-Linked High-Amylose Starch as a Carrier

Thickness and Diameter variation Test

The implants were evaluated for diameter, thickness and hardness. The results are as in Table 4. 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 5.

Water Uptake Study

Percent water uptake of CS1 to CS5 formulation is as shown in Fig. 5.

Table 4: Diameter, thickness and hardness of CS1 to CS5 formulation

Parameters	Formulation Code				
	CS1	CS2	CS3	CS4	CS5
Diameter (mm)	8.06(+0.016)	8.05(+0.012)	8.08(+0.008)	8.06(+0.012)	8.05(+0.010)
Thickness (mm)	2.34(+0.014)	2.34(+0.018)	2.31(+0.014)	2.32(+0.019)	2.30(+0.016)
Hardness(Kg/cm2)	6.2(+0.026)	6.2(+0.043)	6.1(+0.033)	6.2(+0.068)	6.2(+0.071)

Table 5: Drug content of different CS1 to CS5 formulation

Formulation Code	Drug Content (%)
CS1	98.93(+0.052)
CS2	98.87 (+0.047)
CS3	99.34 (+0.093)
CS4	98.78 (+0.056)
CS5	99.13 (+0.074)

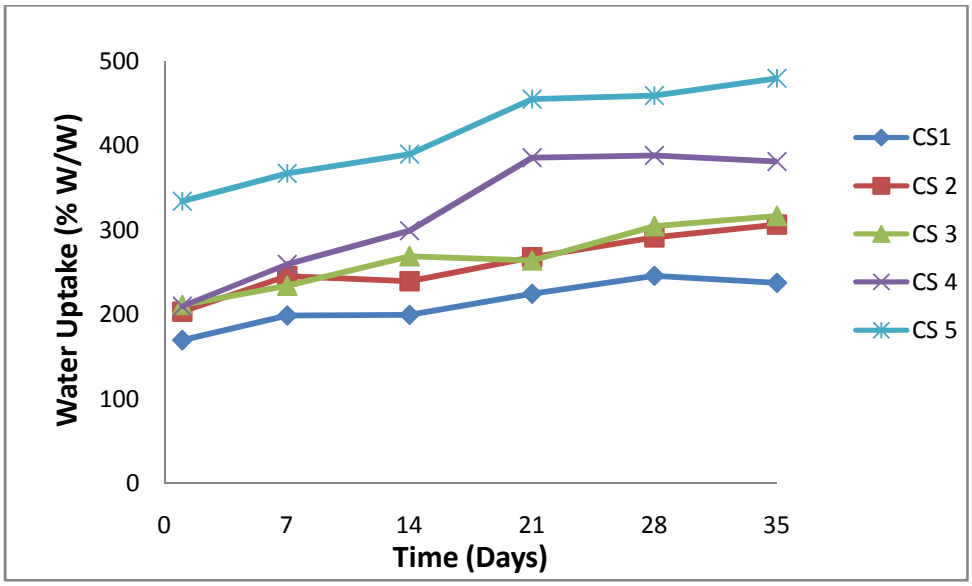

Fig. 5: Water uptake study of CS1 to CS2 formulations

Table 6: Water uptake study of CS1 to CS5 formulations

Time(Days)	Water uptake (% w/w)				
	CS1	CS 2	CS 3	CS 4	CS 5
1	169.43 (+2.81)	203.33 (+3.11)	211.32 (+2.42)	209.60 (+1.57)	334.11 (+2.39)
7	198.87 (+2.22)	245.24(+2.82)	234.02 (+2.70)	259.29(+2.74)	367.24(+2.72)
14	199.70(+1.83)	239.47(+2.57)	268.85 (+3.28)	299.35 (+2.50)	389.87(+2.41)
21	224.56(+3.04)	267.89(+1.63)	264.18 (+2.06)	385.71(+1.32)	455.12(+1.09)
28	245.76(+2.76)	291.26(+1.54)	304.41(+1.68)	388.45(+2.96)	459.23 (+3.45)
35	237.48(+0.87)	306.53(+2.33)	316.52(+2.76)	381.23 (+1.34)	479.87(+3.59)

It is observed that CS1 has comparatively less water uptake capacity than other formulations. This difference in water uptake can be attributed to difference in Cross-linked Starch proportion in different formulations. In CS1 amount of Cross-linked Starch is much higher than other formulations.

As Crosslinked Starch has less water uptake capacity and proportion of Cross-linked Starch is very high in CS1, which results in less water uptake than other formulations. This also indicates hydrophobic characteristics of starch.

The CS4 & CS5 formulation have highest water uptake capacity as compared to others because the amount of Cross-linked Starch is much lower than others. The CS2 & CS3 have moderate water uptake capacity.

Following Fig. 5reveals the ware uptake of different formulations,

Percent Erosion

Percent Erosion of CS1 to CS5 formulation is as shown in Table 7.The percent erosion of CS1 and CS2 formulations is comparatively more than CS5 formulation.

Table 7: Percent erosion of CS1 to CS5 formulations

Formulation Code	% Erosion (w/w)
CS1	28.56 (+1.56)
CS2	26.04(+1.76)
CS3	24.82(+0.89)
CS4	20.29 (+1.66)
CS5	16.88 (+1.43)

***In vitro* drug release study**

The cumulative percent release from all the formulations (triplicate readings) is determined and is as shown in Fig. 6. The cumulative percent release from implants is mainly depends on drug: polymer ratio. The implants with various drug: polymer ratios retarded the drug release for different time period. The CS1 formulation shows 62% release whereas CS2 formulation shows 89.77% release in five weeks. The CS3 formulation shows 98.45% release in five weeks.

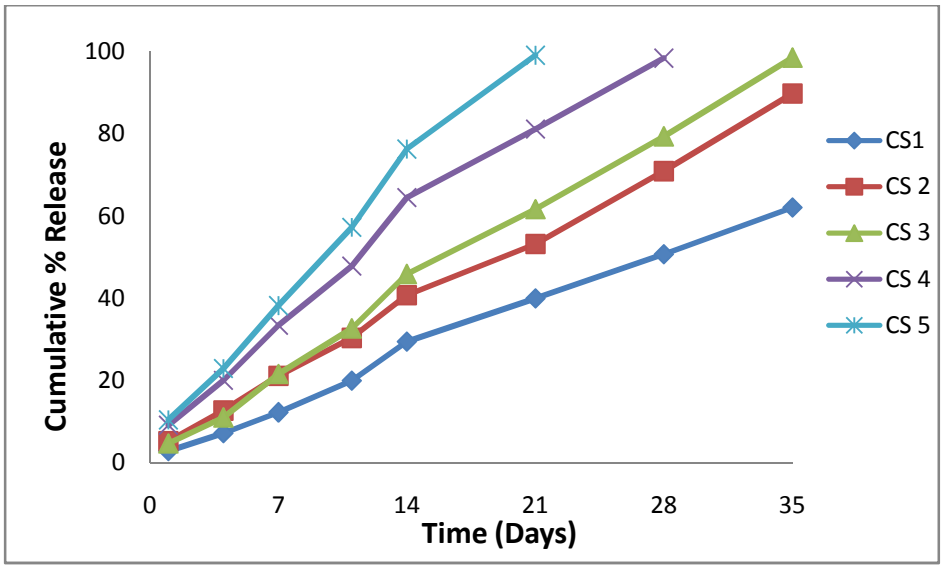

Fig. 6: Dug release profile of CS1 to CS5 formulations

This effect may be attributed to proportion of Cross-linked Starch in different formulation. In CS1& CS2 formulations proportion of Cross-linked starch is very high which results in more retardation of drug release. In CS3 proportion of Cross-linked Starch is lower which results in comparatively less retardation of drug release therefore cumulative percent release from CS3 formulation is increased& 99% drugrelease is observed. Also because of crosslinking of starch water affinity is decreased & hydrophobicity is increased. This is also important reason behind more retardation of drug release in CS1 & CS2.

In case of CS4 to CS5 formulations drug: polymer ratio is much lower thanother formulations therefore complete drug release is observed in 28 days & 21 days respectively.

The primary reason for this observation is as the proportion of cross-linked starch increases in matrices, swelling of the implants decreases hindering drug release. The implants produced using higher cross-linked starch concentrations were more rigid and showed less swelling in phosphate buffer.

From this study, drug release from Cross-linked Starch matrices was found to be increasing with increasing proportion of drug& vice a versa.

Drug release kinetics

The correlation coefficient (R) value was used as criteria to choose best fit model to describe the drug release from Implantable drug delivery system.For CS1 to CS3 formulations the R values were high for Zero order equation, indicating that the drug release from these formulations follows Zero order kinetics of drug release, in CS4 & CS5 formulations, R values were high for Korsmayer-Peppas equation, indicating that the drug release from these formulations follows Korsmayer-Peppas kinetics of drug release i.e. 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 CS1 to CC5 formulations, the value of 'n' is in the range of 0.50 to 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). The value of Release Exponent 'n' is higher in CS1 indicating that erosion of polymeric chains plays a crucial role in drug release mechanism. This is also proved from erosion study of all formulations.

Stability study

Stability study was conducted as per the ICH Guidelines. Optimized formulations from crosslinked high amylose starch based implants were subjected to Accelerated stability study (40±2°C/75±5% RH). The Implants were analyzed for hardness, thickness, diameter, drug content and drug release after keeping for 6 months at 40± 2^{0}C. The results obtained were compared with that of initial sample reading from the same formulation which was evaluated at room temperature (0 month readings).

The optimised CS3 formulation was subjected to stability study. The Results are shown in Table 8.

Table 8: Stability study of optimized CS3 Formulation

S. No.	Parameters	Initial (0 month)	After 6 months
1.	Physical appearance	Disc shaped implants	No change
2.	Hardness (kg/cm^2, mean±SD)	6.1±0.033	6.1±0.054
3.	Thickness (mm, mean±SD)	2.31± 0.014	2.30± 0.022
4.	Diameter (mm, mean±SD)	8.08± 0.008	8.10± 0.014
5.	Drug content (%)	99.34± 0.093	99.28± 0.065

From the above results it was found that CS3 implants shows good stability profile throughout the 6 months storage period. The implants exhibited no changes in the physical appearance, hardness, thickness, diameter and drug content at 40±2°C/75±5% RH during the whole testing period. All the implants maintained initial physical properties like colour, texture and diameter during stability testing period. The dissolution profile is as shown in Fig 7. Similar dissolution profile was obtained after 6 months of storage at accelerated stability conditions. Thus from above results it is evident that the formulation CS3 having good stability in terms of physical characteristics, drug content and release pattern.

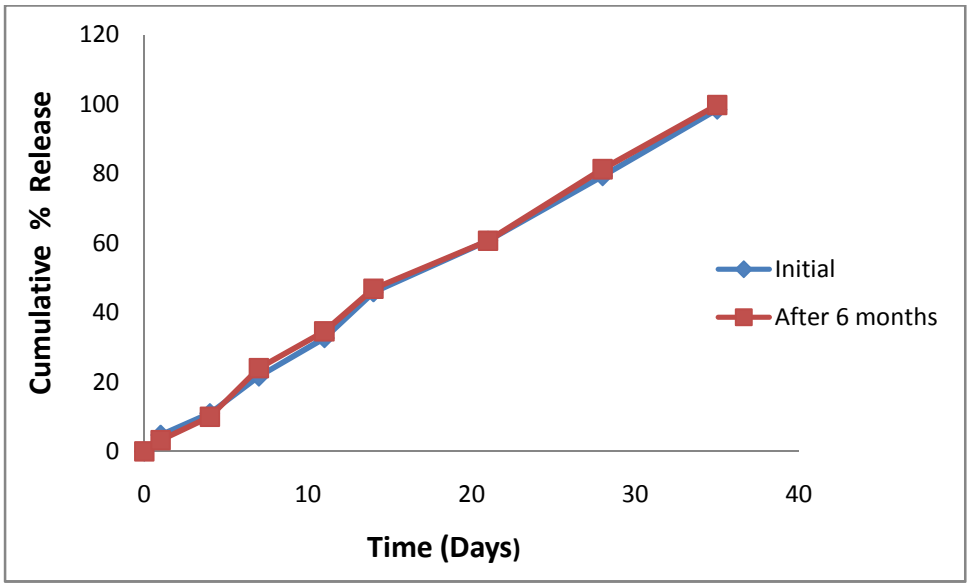

Fig. 7: Effect of temperature on drug release profile of CS3 formulation

CONCLUSION

Crosslinked starch implants are characterized by minimal swelling, erosion and water uptake. Increasing drug loading in starch implants results in prolonged drug release & 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 & the formulations having low water uptake shows lesser cumulative % release. The optimized epichlorohydrin crosslinked starch basedCS3 implant formulationshows a slow zero order kinetics of drug release. Therefore from this study it is proved that, the crosslinked starchimplant have potential to retard the drug release for more than five weeks in the treatment of osteomyelitis & bone infections.

REFERENCES

1. Sheskey P.J.Handbook of Pharmaceutical Excipients. In: Rowe R.C., Weller P.J. Editors. 5th Ed. London Pharmaceutical Press; 2003. p. 1920 –1928.
2. http://en.wikipedia.org/wiki/Corn_starch
3. http://www.elmhurst.edu/~chm/vchembook/547starch.html
4. Tanaka K. Prevention of osteomyelitis. Bioorganic Med. Chem.2008; 16:9217-29.
5. Werner Z., Andrez T., Peter E.O. Prosthetic-Joint Infections. N. Engl. J. Med.2004; 351: 1645 –1654.
6. Luca L., Jon T.M., Jason H.C. Osteomyelitis in Long Bones. J. Bone Joint Surg.2004; 86 : 2305 –2318.
7. Wolter K. Professional Guide to Diseases. Lippincott Williams and Wilkins. 2005.Manouche T. Diagnosis and Management of Osteomyelitis.Pharmacoeconomics.1999; 16(6): 627–647.
8. Lenaerts V., Ronald H., Elsie V. Cross-linked high amylose starch for use in controlled release pharmaceutical formulations & process for its manufacture, United States Patent- 6607748, 2003.
9. Lenaerts V., Ronald H., Elsie V. Cross linked high amylose starch for controlled release of drugs: recent advances, J. Control. Release. 53, 1998, 225-234.
10. Fernanda O. Effects of structure & modification on sustained release properties of starches, Carbohydrate Polymers. 2009;76: 541-547.
11. Guo-xiu Xing, Shu-fen Zhang, Ben-zhiJu, Jin-zong Yang. Study on adsorption behavior of cross-linked cationic starch maleate for chromium (VI).Carbohydrate Polymers.2006; 66: 246–251.
12. Cyril D., Pascal D, Vincent L.Characterization of crosslinked high amylose starch matrix implants 1. *In vitro* release of ciprofloxacin, J. Control. Release.2002; 82: 83–93.
13. Castro C., Sa´ncheza E., Delgadoa A. Soriano I. Ciprofloxacin implants for bone infection. *In vitro–in vivo* characterization.J. Control. Release.2003; 93: 341– 354
14. Ramana V., Nagada C., Himanja M.. Design and evaluation of mucoadhesive buccal drug delivery systems containing Metoprolol. Ind. J. Pharm. Sci.2007; 69: 515 –518.
15. Nakhat P. D. Studies on buccoadhesive tablets of terbutalinesulphate.Ind. J. Pharm. Sci.2007; 69: 505 –510.
16. Schliecke G., Carsten S., Stefan F., Andreas E., Sandow J.*In vitro* and *in vivo* correlation of buserelin release from biodegradable implants using statistical moment analysis. J. Control. Release. 2004; 94: 25– 37.
17. Siewert M., Shah V.P. FIP/AAPS Guidelines to Dissolution/*in vitro* Release Testing of Novel/Special Dosage Forms, AAPS Pharm. Sci. Tech.2003; 4(1): 5 – 15.
18. Patrick L. Drug release mechanism of paclitaxel from a chitosan–lipid implant system: Effect of swelling, degradation and morphology. Euro. J. Pharm. Biopharm.2008; 69: 149–157.
19. Adriana F.M. et al. Development and validation of a high performance liquid chromatographic method for determination of triamcinolone acetonide from polyurethaneintraocular implants, Int. J. Pharm. Pharm. Sci. 2012; 4 (4):132-136.
20. BritoR. S., Harsha V. G., Wasim Raja., Bhaskar Reddy K., Mohanambal E. Design, characterization and *in vitro* evaluation of dental implants of aceclofenac sodium for periodontitis, Int. J. Pharm. Pharm. Sci. 2012; 4 (2):142-144.