www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1394-1402, Oct-Dec 2009

# Preparation and Characterization of Rofecoxib microspheres using cross-linked starch as novel drug delivery system

**Thombre N. A.[*1], Chaudhari M.R.[2], Kadam S.S. [2]**
**[1] Department of Pharmaceutics, MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik, Maharashtra, India – 422 003.**
**Telephone: +91 253 2303515, Fax: +91 253 2303203**
**[2]Bharati Vidyapeeth's University, Poona College Of Pharmacy, Erandwane, Pune-411038**

***Corresponding author : nilimathombre@gmail.com***

**ABSTRACT:** The objective of the present investigation was to produce microspheres by cross-linking starch with epichlorhydrin, which is a very efficient divalent cross-linking agent for starch. An attempt was made to improve the dissolution of poorly soluble drug, Rofecoxib. Rofecoxib was microencapsulated with developed cross-linked starch, using Solvent Evaporation Technique. The prepared microspheres were white, free-flowing, and almost spherical in shape. The efficiency of the cross-linked starch polymers was evaluated by using different tests like extent of cross-linking of starch by Iodine reaction with it, IR interpretation, Mean particle size, Water regain capacity, Action of α-amylase on native starch and cross-linked starch, Gel filtration chromatography, In vitro drug release. Extent of cross linked starch was found to be highest. Microspheres ranging 91 to 105 µm with narrow size distribution could be obtained. Water regain capacity was found to be 6.09 %w/w. IR studies indicated high stretching of ether linkage of cross-linked starches. α-amylase test revealed less susceptibility to microbial enzymes. Gel filtration chromatography confirmed the higher porosity with entrapment in its matrix. Release in vitro study of Rofecoxib revealed that the microspheres could gives sustained release of drug. The prepared cross-linked polymer system holds good for further drug delivery studies in connection to its super swelling and biodegradability.
**KEYWORDS:** microspheres, solvent evaporation technique, crosslinking, starch, epichlorhydrin

## INTRODUCTION

Naturally occurring polymers are high molecular weight, having hydroxyl, carbonyl, amino and thiol functional group. They are made up from glycosidic, peptide, ester linkages. Modifications are essential to improve the properties of these polymers for desired application. Naturally occurring polymer like starch is very high molecular weight substances.[1]

Starch is homopolysaccharide made from α-D- glucose and consist of two components amylose and amylopectin.The smaller of the two polysaccharides, amylose is a linear molecules comprising of (1-4) linked α-D glucopyranosyl units having a molecular weight of several hundred thousands.Amylose is water-soluble but gives an unstable solution.Amylose stains deep blue with iodine ($\lambda_{max}$ c 660nm).[2]

The larger of the two components, amylopectin, is highly branched with molecular weight of several hundred million. This structure contains α-D- glucopyranosyl units linked mainly by (1-4) linkages (as amylose) but with greater proportion of (1-6) linkages, which gives a large, highly branched structure.Amylopectin yield purple color with iodine ($\lambda_{max}$ c 540nm). Solution of amylopectin is relatively stable.[3]

Starch is polysaccharide, most widely used in foods, pharmaceutical and cosmetic industry for their different applications. The application of starch is based on the purity and cost. Starch is used as an excipient in different pharmaceutical dosage forms as binder, diluent, disintegrant, adsorbent, lubricant etc. It has been used in several industries but starch in its native form has many problems like stability at high temperature and at different pH.4.[7]

Starch is a biodegradable polymer which degrades very fast with the help of enzyme, amylase present in human saliva and also by microorganisms. Starch degrades very

fast to generate α-D glucose. Starch is a very good media for microorganism growth. Therefore, its fast degradation is hurdle in application of starch in controlled drug delivery system. Hence it has been essential to modify the starch for the use. Modification of the starch is done to improve biodegradation. Chemical crosslinking is one of the methods used for the modification of starch in this direction to improve the stability and reduce the degradation. Various degrees of crosslinking can be introduced into starch for the purpose of generating larger molecule aggregates with enhanced viscosity profiles or for the preparation of insoluble products with wide range of swelling characteristics. Some common cross linking agents include epichlorhydrin, acetaldehyde and formaldehyde. Crosslinking of starch develop resistant to action of enzymes and its degradation is also slow down.[4- 5, 7]

Cross liked starch, known as a modified starch have potential applications in the field of medicine, agriculture and food industries, controlled release of pesticide, micronutrients in soil, to retain flavor in food preparations, to increase water retention capacity of soil by putting modified starch in soil. In analytical field modified starch gels are used as a media for separation by molecular exclusion chromatography.[2,4]

Cyclooxygenase-2 (COX-2) inhibitors constitute a new group of non steroidal anti-inflammatory drugs (NSAIDs). Rofecoxib is about 100-1000 times more selective on the COX-2 than on the COX-1 is form with lower incidence of gastric bleeding and other gastro-toxic effect. It is indicated for the treatment of symptoms and signs of osteoarthritis. It is sparingly soluble in acetone, slightly soluble in ethanol, and insoluble in water. [6]

The main objective of the present work is to develop newly synthesized cross linked polymer of starch with a cross-linker epichlorhydrin that could be used as a novel drug delivery device in future.

## MATERIALS AND METHODS

Rofecoxib (Cipla Ltd., Mumbai, India), Potato starch (Sd fine chemicals Ltd.), Epichlorhydrin (Sisco Research Lab) of commercial purity grade were used. All other chemicals used were of analytical reagent grade. FT/IR spectrophotometer (Jasco 5300, Jasco Inc., Easton, MD, USA) and Dissolution test apparatus USP XXIV (Erweka-DT-1, Commerce Dr., Easton, MD-21601, Germany) were used for drug content estimation and dissolution studies respectively.

### Preparation Of Microspheres Of Cross-Linked Starch:[8]

Defatted starch has been prepared in alkaline solution and air dried it. Cross linked starch have been prepared according to Solvent Evaporation Technique using epichlorhydrin (ECH) as a cross linking agent. For a typical batch defatted potato starch (10g) was dispersed in sodium hydroxide (1M, 100ml) in presence of sodium borohydried (250mg) and was kept for 15 hrs. Liquid paraffin (250ml) was taken in a one liter, 2 necked, round bottom flask equipped with stainless steel mechanical stirrer and heated to $70^0$C over hot water bath. The dispersed starch was added to liquid paraffin under constant stirring (1400 rpm) to obtain a uniform emulsion. Then epichlorhydrin (40ml) was added to the emulsion, drop wise, with continuous stirring for 1 hr. Then emulsion was neutralized with hydrochloric acid (1M) and liquid phase was decanted. The obtained microspheres were washed with toluene. The microspheres were isolated by centrifugation and washed repeatedly with saline solution. Finally Microspheres were dehydrated with alcohol and air-dried which kept in closed containers before use. By following the above mentioned procedure six other batches of microspheres with drug: cross linked starch ratio of 1:1(F1, F2), 1:2 (F3, F4), 1:3 (F5, F6) were prepared. [Figure 1]

### Reaction with Iodine:[3,8]

The extent of cross-linking of starch was determined by Iodine reaction. The prepared cross linked starch was treated with Iodine at pH 4.8 (Acetate buffer, 0.1M)

### Fourier Transform Infra Red spectroscopy studies:

FT-IR spectra of the cross-linked polymer along with homopolymers were recorded to check drug polymer interaction and stability of the drug on a Jasco 5300( Jasco Inc., Easton, MD, USA) spectrophotometer using KBr pellets in the range 400-4000/cm.

### Particle size analysis:[9]

The particle size of microsphere was determined using optical microscopy method; approximately 100 microspheres were counted for particle size using a calibrated optical microscope (Magnus MLX-DX) .

### Micromeritic properties:[9]

1. **Angle of repose**

Angle of repose of different formulations was measured according to fixed funnel standing method [11] ( $n = 3$) [Table 1].

$$\theta = \tan^{-1} h / r$$

where θ is the angle of repose, $r$ is the radius, and $h$ is the height.

2. **Bulk density**

Bulk and tapped densities were measured by using 10 ml of graduated cylinder. The sample poured in cylinder was tapped mechanically for 100 times, then tapped volume was noted down and bulk density and tapped density were calculated. Each experiment for micromeritic properties was performed in triplicate manner and reported in [Table 1]

3. **Carr's index**

Compressibility index (Ci) or Carr's index value of micro particles was computed according to the following equation [Table 1]:

$$\text{Carr (\%)} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

**4. Hausner's ratio**

Hausner's ratio of microparticles was determined by comparing the tapped density to the bulk density using the equation[Table 1]:

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

**Water regain capacity:**

The solvent absorption in the core of microsphere was determined by water regain capacity. Water regain capacity was evaluated by exposing 100mg of microspheres of cross-linked starch in 5 ml of water for 24 hrs. Then the microspheres were centrifuged at 500 rpm and the water is decanted. The water regain capacity of the microspheres was calculated by using the equation.

$$\text{Water Regain Capacity} = \frac{\text{Initial weight of the microspheres}}{\text{Weight of the microspheres after the test}}$$

**Determination of reducing sugars:**[10,11]

3, 5 dinitro salicylic acid (DNSA)reagent was prepared just before use by mixing the stock solutions.1 ml of test reagent was added to 3 ml of the maltose solution of different concentration (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/ml) in a test tube. Blank was prepared by adding 1 ml of the reagent to the 3 ml of distilled water. All the test tubes were covered with a marble & place in a boiling water bath for 5 min, cooled to room temperature. Absorbance was recorded against blank on photoelectric colorimeter at 540nm against the blank.

**Action of α-amylase**: [10, 11]

The action of α-amylase was evaluated by dissolving each of 100 mg of starch & cross linked starch in 10 ml of buffered saline in separate container. 1 ml of each solution into a series of 7 test tubes for each was diluted with 3 ml of saline diluted (about 1 in 100 with water). The tubes were incubated at $37^0$C for upto 1 hr. The test tube was removed. 0.5 ml of NaOH was added to it and the reaction was stopped by adding DNSA reagent. The test tubes were heated on boiling water bath for 15 minutes and cooled to the room temperature. Absorbance of different concentrations was recorded on photoelectric colorimeter at 540nm.

**Gel Filtration Chromatography**: [12, 13]

This evalution test was carried out at $4^0$C.A glass column (D 1.5 cm, L 25.0 cm) was packed with micro spheres of cross linked starch and equilibrated with saline (0.145M NaCl).The flow rate was adjusted to 12.0 ml/hr with the help of a peristaltic pump. Void volume and elution volume were determined by using Blue Dextran 2000 and Glucose. A mixture of BSA (Bovine Serum Albumin MW-66000) and Cytochrome c (MW 12400) dissolved in saline was applied to column and the separation was checked on the column. The graph of elution profile for cross linked starch beads CS-100 against Absorbance was obtained.

**Loading Of drug in the micro spheres of cross linked starch**: [14]

The inclusion of the drug, Rofecoxib was carried out in the dark by the evaporation method. Saturated solution of Rofecoxib (10 ml) in acetone was poured over 4 gm of micro spheres, packed in a glass column (D 1.5 cm, L 25.0 CM) and coated with carbon paper to keep it away from light. The column was capped for 2 hrs. Then it was uncapped to evaporate acetone completely.

**In vitro drug release study:**

*In vitro* dissolution studies were carried out on the prepared Rofecoxib microsphere using cross-linked starch at 37°C ± (0.5°C) at 100 rpm with USP dissolution apparatus II; 200-mg Rofecoxib microsphere was placed into the dissolution apparatus. The *in vitro* dissolution studies were performed at 7.4 pH, which is simulated intestinal fluid pH. An accurately weighed sample was responded in dissolution media consisting 900 ml of Phosphate buffer (pH 7.4).The sample (5 ml) was withdrawn at regular intervals and replaced with the same volume of test medium and the withdrawn samples were diluted if required and then estimated for rofecoxib concentration at 219 nm spectrophotometrically (Shimadzu Pharmspec UV-1700 series, Japan). The sample was diluted (0.5 ml up to 10 ml) and absorbance was measured at 219 nm. Finally, the drug content in all fluid was determined from the calibration curve of rofecoxib to determine the release pattern.

## RESULT AND DISCUSSION

Rofecoxib microspheres with varying proportions of rofecoxib and cross-linked starch were prepared by solvent evaporation method.

**Description of the Microspheres**:

Ocular and stage microscopy shows that the microspheres obtained were spherical and free flowing is shown in Figure. The yield obtained in all the batches was good which was above 75% & none of the variables affected the yield. It indicated that rofecoxib microspheres have a discrete spherical structure without aggregation [Figure 2].

**Reaction with Iodine:**
The treatment of iodine with the Microspheres prepared by using cross linked starch does not show any coloration with iodine. It indicates that there was an extensive cross-linking of starch.

**FT-IR Interpretation:**
IR Spectrum indicated O-H stretching at 3333cm$^{-1}$ in native starch which characteristics of polymeric association and C-H at 1645, 858 cm$^{-1}$. Cross-inked starch has shown O-H stretching at 3437 cm$^{-1}$, C-H stretching at 2928 & 1456 cm$^{-1}$. Also it has been shown weak C-O stretching at 1010 cm$^{-1}$, which is characteristic of ether functional moiety [Figure 3 and Figure 4].

**Particle size analysis:**
The particle size of Microspheres of cross linked starch was in the range of 91-105µm, and most microspheres prepared from the different formulation batches are used for the micromeritic studies. [Table1], [Figure 2]

**Micromeritic properties of cross linked starch microspheres:**

***Angle of repose***
All the formulations show angle of repose value in the range of 16-20, i.e., less than 30,which shows free-flowing nature of the formed microspheres [Table 1].

***Bulk density and tapped density***
Bulk and tapped densities showed good packability of the microspheres [Table 1].

***Carr's index (Ci)***
Carr's index ranges from 14% to 17%, $F_5$ had lowest Ci index, indicating excellent compressibility [Table 1].

***Hausner's ratio***
It was ranging from 1.14 to 1.35, i.e., all the preparation showed that they had good flow properties. Upon considering the micromeritic properties of all the formulations, B2 had the best flow property, as it had high angle of repose value (20.250), the lowest Carr's index (14.04%), and Hausner's ratio (1.14) [Table 1].

**Water Regain Capacity:**
Water regain capacity is calculated to determine the capacity of micro spheres for solvent absorption in the core. Water regain capacity of Microspheres of cross-linked starch was found to be 6.09 % w/w. From this, Microspheres can retain 6.09 % w/w of the solvents.

**Determination of reducing sugars:**
Standard curve of the maltose was used them to estimate the concentration of the native starch & cross-linked starch [Figure5].

**Action of α-Amylase:**
A-Amylase catalyses the specific hydrolysis of the α-1-4 glycosidic bonds of starch in a random manner with the formation of series of intermediates, determines the power of polymer to resist microbial attack. In present study, the action of amylase on native starch and microspheres of cross-linked starch was determined by DNSA (dinitro salicylic acid) method. The action of α-Amylase on native starch and microspheres of cross-linked is shown in [Figure 6].
From the graph, it was shown that the native starch is more susceptible for microbial growth than cross-linked starch. Native starch is a good nutrient media for microbial growth therefore microbial contamination was readily found in native starch. Present study showed that the microcapsules of cross-linked starch inhibit microbial contamination to far extent than native starch.

**Gel Filtration Chromatography:**
The molecular sieve effect and porosity of micro spheres of cross linked starch was determined by Gel Filtration Chromatography. The study also determined that the micro spheres binds drug reversibly in its core for its controlled delivery in a body system. Gel Filtration Chromatography study showed that the microcapsules are porous, rigid with high mechanical strength and permits inclusion of drug in the microcapsules. Figure shows the gel filtration profile for a mixture of BSA and Cytochrome c.[Figure 7]

**In vitro drug release study:**
*In vitro* dissolution studies revealed that the release of plain rofecoxib tablet was found to be 99 % in less than 50 min while release of rofecoxib from microspheres of cross linked starch was found to be 40% in the same time.
The influence of different processing condition was evaluated on *in vitro* drug release and percentage drug release was found in the range of 77.48 % to 96.44 % at period of 08 hours. A biphasic in-vitro drug release profile was observed with initial burst effect for all the formulation prepared. The initial burst release is due to the presence of drug on the surface prepared microspheres. The initial burst release can be attributed as desired effect, which ensures the quick initial plasma therapeutic concentration of drug. All the formulated microspheres retained their shape and size even after dissolution, which indicated the release of drug diffusion through the polymer wall of microspheres. Through dissolution profiles it was observed that the decrease in drug to polymer ratio from 1:1 to 1:3 resulted a decrease in release rate. It is considered that the higher drug to polymer ratio in the microspheres, result in increase in coat thickness surrounding the drug particles thereby increasing the distance travelled by the drug through coat.[Figure8]

**Table 1: Micromeritic properties of microspheres prepared by using cross-linked starch**

| Formulation Code | Angle of repose(θ) | | Bulk Density (g/ml) | | Tapped Density (g/ml | | Carr's Index (Ci) (%) | | Hausner's ratio | | Particle size (μm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean* | SD*(±) | Mean* | SD*(±) | Mean* | SD*(±) | Mean* | SD*(±) | Mean* | SD*(±) | Mean* | SD*(±) |
| $F_1$ | 16.79 | 0.681 | 0.555 | 0.007 | 0.649 | 0.009 | 14.46 | 1.385 | 1.16 | 0.02 | 105.9 | 23.3 |
| $F_2$ | 20.78 | 0.586 | 0.494 | 0.005 | 0.673 | 0.014 | 15.52 | 0.83 | 1.35 | 0.015 | 93.4 | 29.5 |
| $F_3$ | 16.5 | 0.202 | 0.445 | 0.006 | 0.373 | 0.01 | 16.48 | 0.61 | 1.19 | 0.005 | 91.6 | 22.3 |
| $F_4$ | 17.01 | 0.59 | 0.562 | 0.012 | 0.735 | 0.02 | 17.55 | 1.248 | 1.28 | 0.02 | 95.64 | 46.28 |
| $F_5$ | 18.2 | 0.017 | 0.426 | 0.008 | 0.497 | 0.001 | 14.05 | 1.71 | 1.14 | 0.026 | 99.42 | 29.75 |
| $F_6$ | 19.54 | 0.251 | 0.462 | 0.015 | 0.841 | 0.031 | 16.23 | 1.268 | 1.14 | 0.026 | 104.32 | 17.8 |

**Table 2: Different parameters of cross linked starch**

| Sr.No. | Parameters | Micro spheres of cross linked starch |
|---|---|---|
| 1 | Practical Yield (% w/w) | 70 to 75 |
| 2 | Particle size (μm) | 91 to 105 |
| 3 | Water regain capacity (% w/w) | 6.09 |
| 4 | Flow rate under gravity (ml/hr.) | 12.0 ±0.5 |
| 5 | Void volume (ml) | 25.00 |
| 6 | Total elusion volume (ml) | 110.00 |

**Figure 1: Reaction of Amylose with Epichlorhydrin**

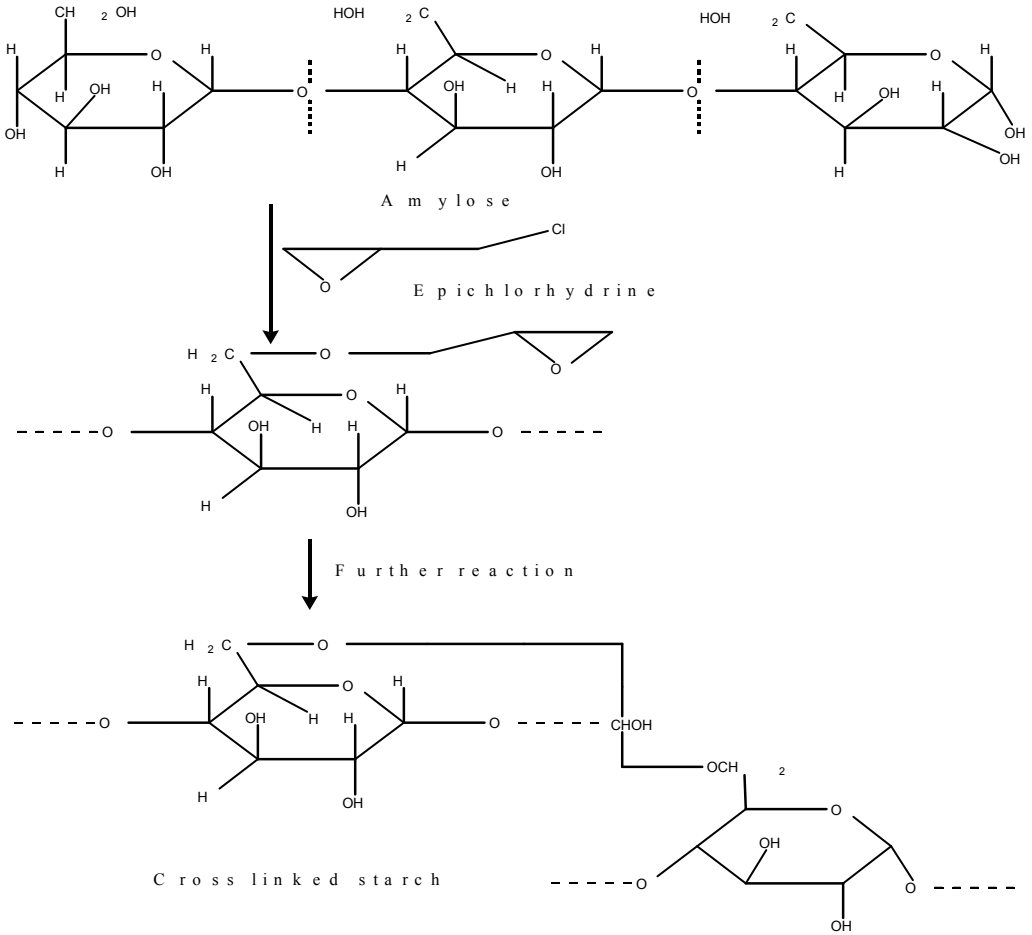


**Figure 2: Microspheres of cross linked starch**

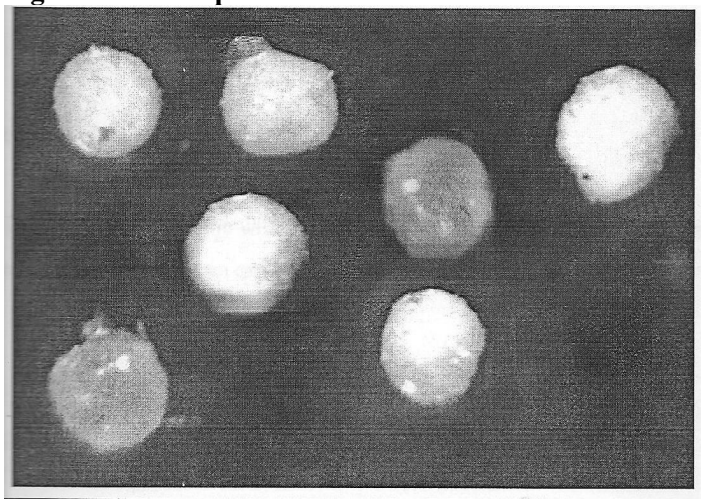


**Figure3: IR Spectra of native starch**

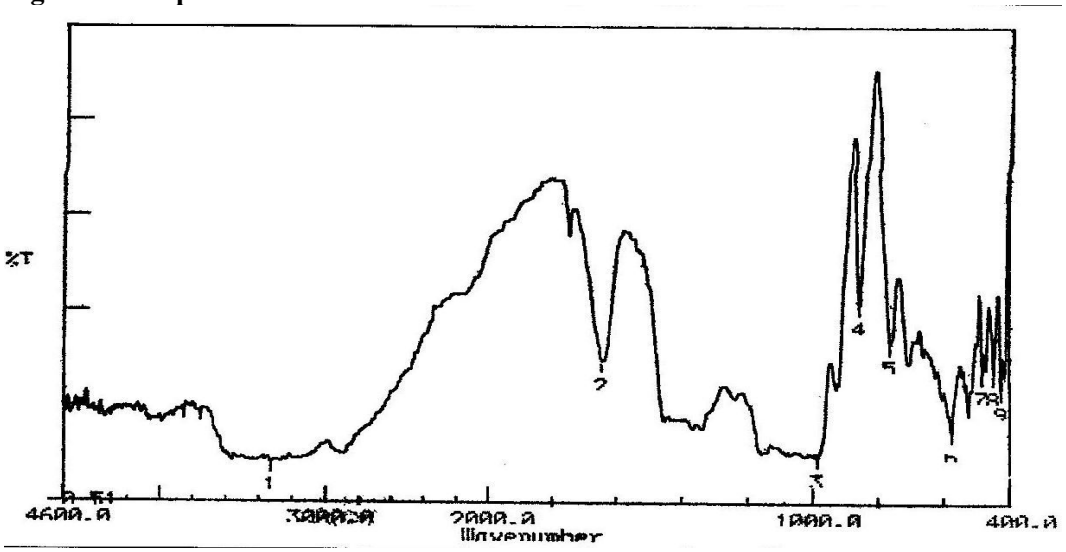



**Figure4: IR Spectra of Cross-linked starch**

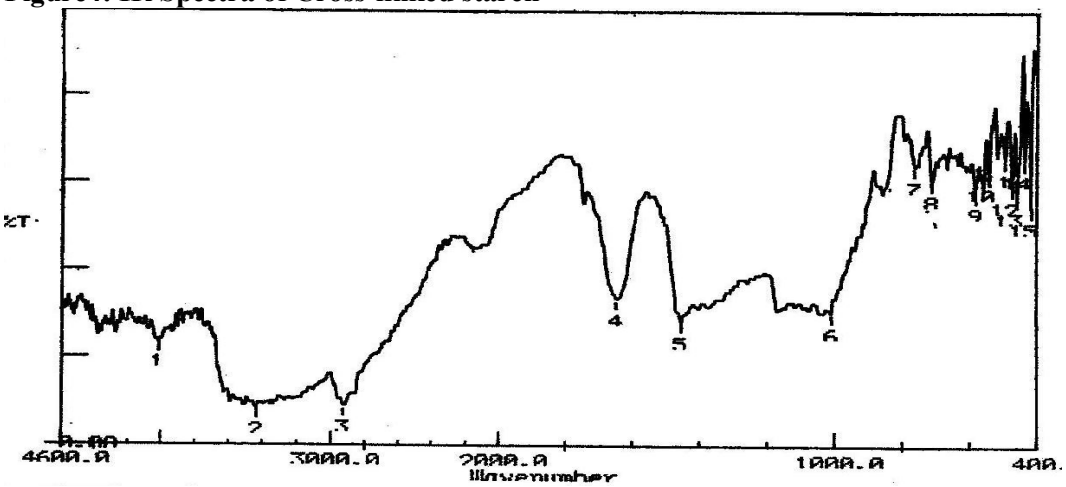


**Figure 5: Calibration curve for maltose**

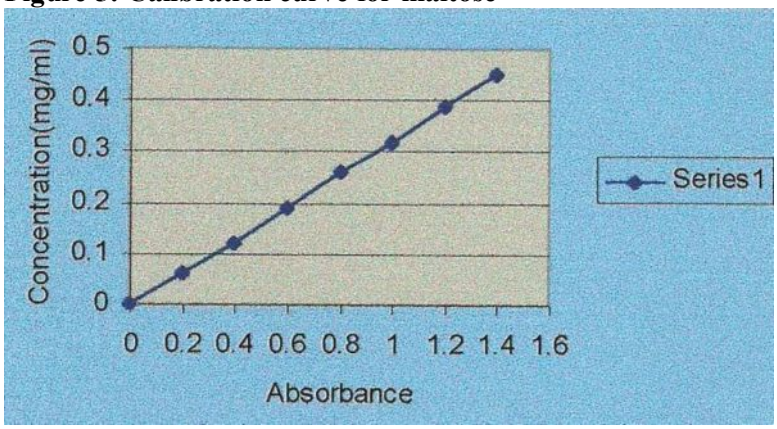



**Figure 6: Comparison between action of Amylase on starch and cross-linked starch**

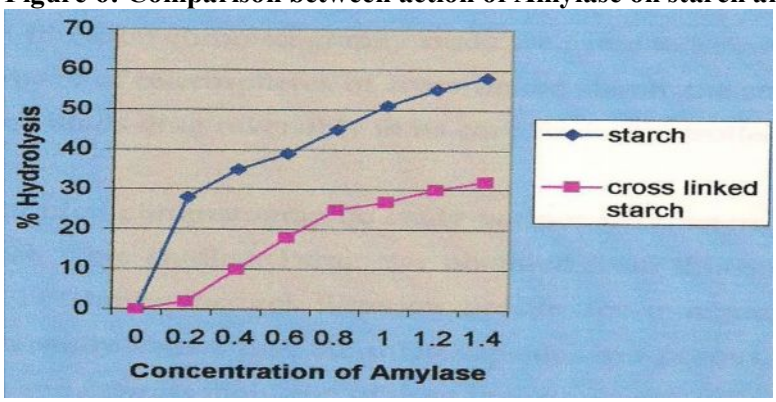
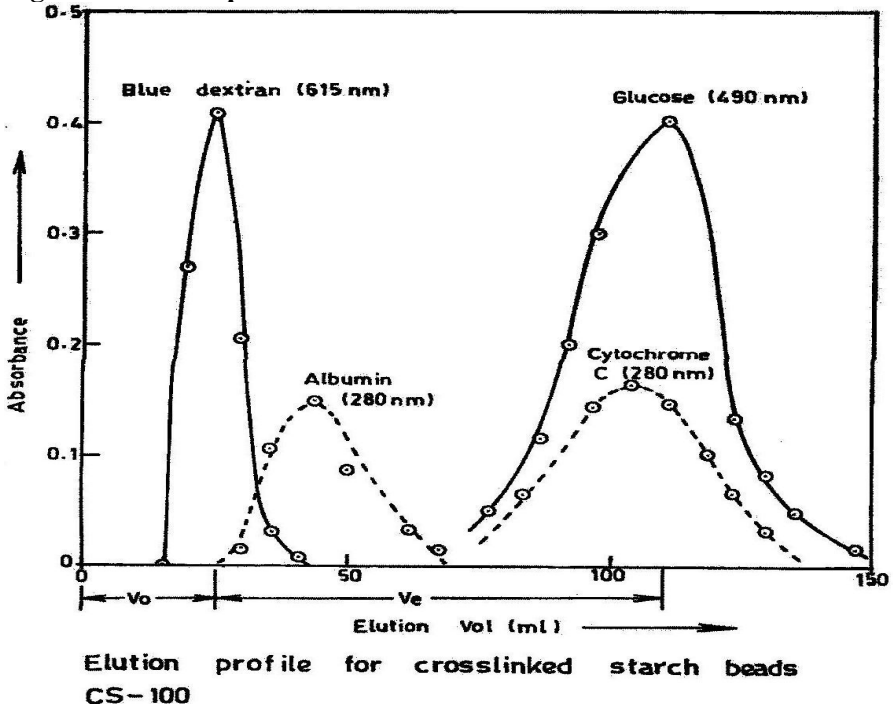
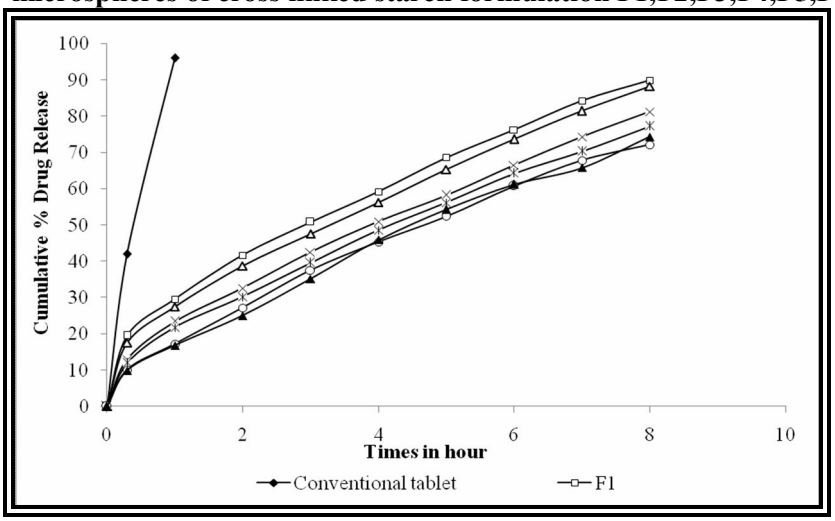



**Figure 7: Elution profile for cross-linked starch beads CS-100**




Elution profile for crosslinked starch beads CS-100

**Figure 8: In vitro drug release of Rofecoxib conventional tablet with rofecoxib microspheres of cross linked starch formulation F1,F2,F3,F4,F5,F6**




## CONCLUSION

The cross linked starch with epichlorhydrin resulted in microspheres with good yield independent of any variables. The extent of cross linking of native starch and stretching of ether linkage was found to be high with Epichlorhydrin.

Rofecoxib microspheres were prepared successfully by solvent evaporation method using the cross linked starch as polymers in the ratio of 1:1(F1, F2), 1:2 (F3, F4), 1:3 (F5, F6). It was found that the prepared microspheres were spherical, free flowing, high percentage entrapment efficiency and high percentage yielding capacity. It can be concluded from this study that rofecoxib could be made into controlled-release drug delivery system using as cross linked starch polymers retardant materials in the drug to polymer ratios of 1:1, 1:2, 1:3, stirring speed of 1400 rpm and volume of continuous phase of 100 ml as optimum process parameter. The *in-vitro* controlled release of rofecoxib from the prepared microspheres formulations have been reported in this study. The extent of cross linking of native starch and stretching of ether linkage was found to be high with Epichlorhydrin. However, the *in-vitro* release characteristics of drug from the microspheres are subject to confirmation in animal and human studies for coming into conclusion of enhanced bioavailability and reduced dose frequency to improve patient compliance.

The prepared cross-linked starch was found to have high swelling property, biodegradability, and also having comparatively higher stability. Therefore, from these studies, it can be concluded that the prepared polymer system can be used in drug delivery studies in future.

## REFERENCES

**1)** Martin Scott Cardinali and Tak Yu (Fiona)Lam; New Advances in Starch-based Particle Technologies for Aesthetic Modification, National Starch Personal Care,PCIA,Manila-2003

**2)** Fanta G.F. and Doane ; in Modified Starches:Properties and Uses; Edn O.B, CRC Press, Boca Raton,FI. Wurzburg; (1986),149

3) Roberts H.J.;In starch:Chemistry and Technology;vol 1;Edn1.Whistler and E.F.Pashall;Academic Press;New York; 1965,.484.

4) Foster J.F.;In starch:Chemistry and Technology; Edn 1, volume 1,Whistler and E.F.Paschall,Acdemic Press;New York, 1965,484-490

5) G.Hamdi,G.Ponchel et al., Formulation of epichlorhydrin cross-linked starch microspheres, Journal of Microencapsulation, 2001, Vol. 18, No.3, 373-383.

6) MV Ramana, M Himaja, Kamal Dua, VK Sharma, A new approach: Enhancement of solubility of rofecoxib, Asian Journal of Pharmaceutics, Volume 2, Issue2-2, 2008,96-101.

7) Textbook of Pharmaceutical Excipient:522

8) Chaudhari M.R.;Kamath N.D.;Bhide S.V.;Kale N.R.;Preparation and Crosslinked starch beads as a medium for Gel Filtration,Starch/Starke,Vol.41,Issue 11,1989,415-416.

9) Parul Trivedi, AML Verma, N Garud,Preparation and characterization of aceclofenac microspheres, Asian Journal of Pharmaceutics, Volume 2, Issue2-2, 2008,110-115.

10) David T Plumber, An Introduction to practical biochemistry, Edn 3; Tata McGraw-Hill Publishing Company Limited, New Delhi,2006,180-181.

11) Godkar P.B., Godkar D.P., Textbook of Medical Laboratory Technology, Edn 2,Bhalani Publishing House,Mumbai, 2003,176-217.

12) Nandedkar U.N.;Bhide S.V.;Kale N.R., Sephacryl S-300- an affinity matrix which distinguishes concanavallin A from other D-mannose/D-glucose-specific lectins; Journal of Chromatography; Vol. 396,Jun 1987, 363-8.
13) Dubois M.K.;A.Gilles; J.K.Hamilton; P.A.Smith ;F.Smith;Analytical Chemistry ;28(1956):350
14) Rita Cortesi, Gheorghe Fundueanu**,**Paolo Ascenzi,Enea Menegatti, Preparation and characterization of Starch/Cyclodextrin Bioadhesive microspheres as platform for nasal administration of Gabexate Mesylate(Foy$^{(R)}$ ) in allergic rhinitis treatment,Biomaterials, Volume 25, Issue 1,2004,159-170.
15) United State Pharmacopoeia XXI, Asian Edition, 711,2673-2681

*****