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Der Pharmacia Lettre, 2012, 4 (1):110-127
(http://scholarsresearchlibrary.com/archive.html)

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ISSN 0975-5071
USA CODEN: DPLEB4

Cross-linked chitosan diclofenac sodium beads for sustained release systems

[1]Ketan Gulabrao Albhar*, [2]Vaibhav Sudhakar Wagh, [1]Bhagwat Babasaheb Chavan

[1]JSPMS Jayawantrao Sawant College of Pharmacy & Research, Hadapsar, Pune
[2]Padamshri Dr Vithalrao Vikhe Patil Foundations College of Pharmacy (PDVVP), Ahmednagar.

ABSTRACT

Cross-linked beads of diclofenac sodium with chitosan like 86.60%DOD, 87.82%DOD, 93.10%DOD and their different ratios with different concentrations of sodium tri-polyphosphate (STPP) were done to develop a sustained release system. Cross-linked beads were formulated using different grades of deacetylated chitosan like 86.60% DOD, 87.82%DOD, 93.10% DOD, and their different ratios with different concentration of sodium tri-polyphosphate. The cross-linking were done by ionotropic gelation method. Beads were characterized by DSC and SEM. The effect of concentration of sodium tri-polyphosphate, entrapment efficiency and percentage of swelling on drug release profiles of beads. Stability studies were conducted as per ICH Guidelines at 40°C/75% RH for 6 months. The diclofenac sodium content within the beads decreased with increasing STPP concentration. Thermal analysis shows there was melting of chitosan and diclofenac sodium respectively indicating molecular dispersion of drug in chitosan beads. SEM showed the beads were spherical in shape and rough in surface. It is also observed that as the drug to polymer ratio decreases, entrapment efficiency increases. It is observed that degree of swelling decreased with increasing concentration of STPP. Increased cross-linking reduced the degree of swelling and drug release. It can be concluded that that utilization of multiparticulate system is an effective system for obtaining sustained release of drugs.

Keywords: Sustained release, chitosan, sodium tri-polyphosphate, drug release, swelling.

INTRODUCTION

Multiparticulate system is thought to be preferable to a single-unit dosage form because the small particles spread out more uniformly in the gastrointestinal tract. This results in a more reproducible drug absorption and reduces the risk of locale irritations (Ehab Hosny A.et.al.).

Recently, the use of complexsation between oppositely charged macromolecules to prepare chitosan beads (or macrosphers) as a drug controlled release formulation, because this process is very simple and mild. In addition, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, is applied to avoided possible toxicity of reagents and other undesirable effects. (X.Z. Shu et.al.)

Biodegradable systems: (Ehab Hosny A. et.al., Leticia Martinez et.al., B.Prasad et. al.)
All of the previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. However, a great deal of attention and research effort is being concentrated on biodegradable polymers. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable, and progressively smaller, compounds. In some cases, for example, polylactides, polyglycolides, and their copolymers - the polymers will eventually break down to lactic acid and glycolic acid, enter the Krebs's cycle, and be further broken down into carbon dioxide and water and excreted through normal processes. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix. For some degradable polymers, most notably the polyanhydrides and polyorthoesters, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system.

Cross-linking of polymers(Ehab Hosny A.et.al., S. Bandari et. al):
Forming cross-links between polymer functional groups decreases its hydrophilicity, thus slowing down the rate of permeation of biological fluids throughout the hydrated matrix and subsequently the rate of drug diffusion from the matrix. The cross-linking can be performed by addition of suitable compounds or by heating. Formation of physical bonds between polymer molecules can be achieved by ion tropic gelation, a process that involves interaction of one polymer with other polymers, electrolytes or polyelectrolyte. Cross-linking by adding agents to form chemical bonds has the major drawback of the toxicity of the chemicals used. These substances (e.g. formaldehyde) are highly toxic on inhalation and ingestion, and act as irritants to the skin and the respiratory tract. Ionic cross-linking has the advantage of avoiding addition of toxic chemicals; hence this method is of particular interest in the pharmaceutical field. Formation of hydro gels by polyelectrolyte complexation is an interesting alternative to covalently cross-linked hydro gels. Polyelectrolyte complexes (PEC) are generally biocompatible networks exhibiting interesting swelling characteristics. They are formed by reacting two oppositely charged polyelectrolyte in aqueous solution forming ionic interactions with a high water content and charge density. However, the main drawback of these systems is their preparation, especially in large-scale processes. PEC's are formed by ionic interactions as with ironically cross-linked networks. Consequently, the distinction between these two types of network is faint.

Indeed, cross-linking is usually considered as a bridge of molecules with much smaller molecular weight than the molecular weight of the chains between two consecutive cross-links. The molecular weight of a small polymer forming a PEC and of a large molecule involved in ionic cross-linking could converge and be of a similar magnitude. Reacting oppositely charged

electrolytes in an aqueous solution forms PEC's. Such networks are formed by ionic interactions/bonds as represented in Figure-1 (a-d).

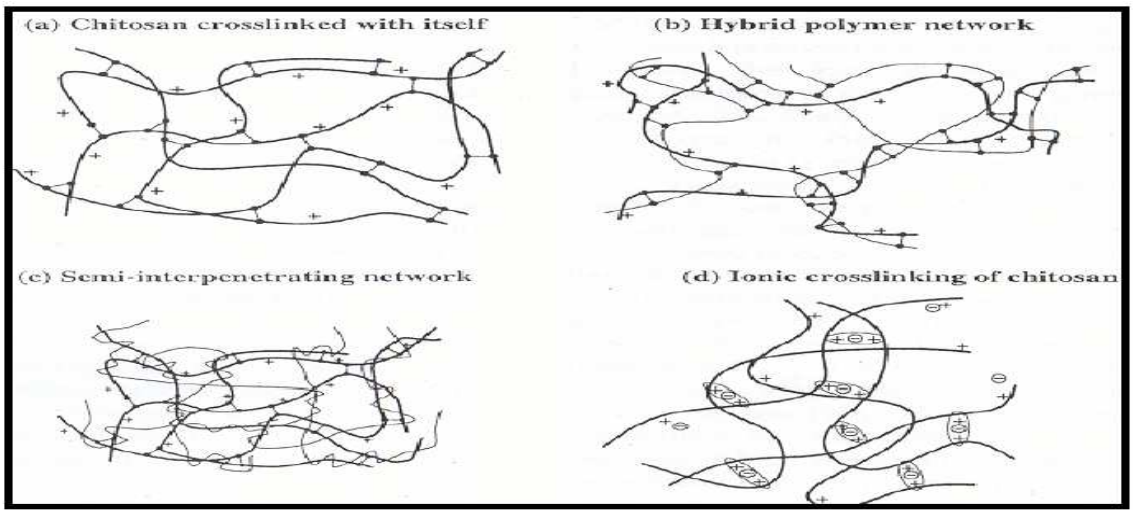


Figure No.-1(a-d): **Formation of cross-linked gels by use of polyelectrolyte complexation.**

In ionic cross-linking, the entities reacting with chitosan are ions or ionic molecules with a well-defined molecular weight. In contrast, polyelectrolyte complexation involves reaction of chitosan and entities, which are polymers with a much broader molecular weight distribution.

Diclofenac sodium(DFS) is a potent non-steroidal ant-inflammatory drug with pronounced antipyretic properties. Its half-life has been reported to be 1-2h.DFS produces side effects in about 20% of patients. Gastrointestinal effects such as bleeding, ulceration or performation of intestinal wall are commonly seen. Due to short biological half-life and associated adverse effects it is considered as an ideal candidate for controlled drug release formulations. Chitosan due to its antacid and antiulcer characteristics prevent or weakens drug irritation in stomach. Therefore chitosan has great potential for use as a suitable carrier in sustained drug delivery systems(Gupta K.C.,et.al.).

The concentration of a drug in the blood fluctuates over successive doses of most conventional single unit oral dosage forms. The main reason for this is that the drug is released immediately after administration (i.e. burst release effect). This causes the drug blood concentration to rise quickly to a high value ("peak") followed by a sudden decrease to a very low level ("trough") as a result of drug elimination. One way of addressing this problem is by means of formulating dosage forms with sustained release profiles over an extended period of time (i.e. usually 8 to 12 hours).The ideal drug delivery system would keep the drug blood plasma level constant over the entire treatment period after administration of a single dose. However, these dosage forms could not address the need for sustained drug release for certain drugs. The challenge remains to find a suitable polymers and compatible cross-linking agents to form beads that can effectively sustain or control the release of drugs across the various pH ranges in the gastrointestinal tract. The drug release properties of chitosan beads systems made with various concentrations of cross-linking agent using a novel ion tropic gelation method were investigated in this research project. In

recent years, multiparticulate systems have received a considerable interest. The influence of gastric emptying time and intestinal motility on intra and inter-subject variation in the rate and the extent of availability can be largely avoided by the use of multiple-unit dosage forms. It is accepted that the size of most multiparticulates enables them to pass through the constricted pyloric sphincter so that they are able to distribute themselves along the entire GI tract.Due to the advantages of multiparticulate dosage forms over single unit preparations, such as more uniform dispersion in the GI tract, more uniform drug absorption, less inter- and intra-individual variability, and more flexible formulation process, interest in multiparticulates as oral drug delivery systems has been growing steadily. Because of their small particle size, multiparticulates can pass through the upper GI tract easily(Clemence Tarirai et.al., Ferna´ndez-Herva´s M.J.et.al., Hua Zhang et.al.).

Chitosan (pronounced ky-toe-san) is derived from a material called chitin, which is an amino polysaccharide, extracted from the powdered shells of crustaceans like shrimps and crabs. Chitosan is prepared by deacetylation of chitin. To prepare chitin, crab and shrimp shells are demineralised in dilute hydrochloric acid (HCl), deproteinated in dilute sodium hydroxide (NaOH), and then decolorized in potassium permanganate ($KMnO_4$). The chitin is then deacetylated to become chitosan by boiling it in a concentrated sodium hydroxide solution as shown in Figure 2-8. Biochemical grade/purified chitosan is prepared by repeating the deacetylation process(Clemence Tarirai et.al., Hua Zhang et.al., Waree Tiyaboonchai et.al., Ruey-Shin Juang et.al., A. Sunil Agnihotri et.al., Vieira R.S.et.al., Leticia Martinez et.al., Yongmei Xu et.al., Anil Anal K.et.al., Sanjay Jain K.et.al.).

Chitosan beads containing diclofenac sodium were prepared by dropping drug containing chitosan solutions into tripolyphosphate solutions.The interaction of the positively charged chitosan molecules with the anionic counterion, tripolyphosphate, caused the formation of gelled spheres(Roland Bodemeier et.al.).

MATERIALS AND METHODS

2.1 Materials

The drug Diclofenac sodium was procured as gift sample from (Glenmark pharmaceutical).Chitosan (86.60%, 87.82% and 93.10% DOD) were procured from (Research-Lab Fine Chem.Industries,Mumbai.400002(India),Crosslinking agents Sodium tripolphosphate(anhydrous)(STPP),Oleic acid(OA) and Polysorbate 80(Tween 80) were procured from(Loba Chem).All other chemicals were purchased and were of analytical grade.

2.2Preparation of beads(X.Z. Shu et.al., Gupta K.C.et.al., Clemence Tariraiet.al., Sinha V.R.et.al., Cenk Aral et.al.)

All the formulations were prepared by using ionotropic gelation method.Diclofenac sodium used in the formulation were initially passed through sieve #40 separately before mixing.

A 2% v/v acetic acid solution was prepared by dissolving 2 ml of glacial acetic acid in distilled water that was then made up to a volume of 100 ml. A 2% w/v chitosan solution was prepared by accurately weighing an approximate amount of 2 gm of chitosan and dissolving it in 100 ml of the preprepared 2% v/v acetic acid in a 1000 ml size glass beaker. This mixture was stirred using

digital high-speed lab stirrer (Remi Motors LTD) at 30 °C and 50 rpm for 30 minutes and stirrering was continue for overnight so that chitosan get completely swelled in glacial acetic acid media.

After complete dissolution of the chitosan, the solution was labelled (A). Approximately 2 g of the drug (diclofenac sodium), that was accurately weighed, was dispersed into 100 ml of solution A using a high-speed lab stirrer (Remi Motors LTD). This mixer was run at 800 rpm for 10 minutes, and then at 1000 rpm for 15 minutes at 25 °C. This diclofenac sodium suspension was labeled (B) and it was used in the subsequent cross-linking experiments.

By using same method for each type of chitosan grades and prepare chitosan-diclofenac sodium suspension for cross-linking experiments as depicted in Table No.-1.

Table No.1: Composition of chitosan diclofenac sodium beads

Batch No	Chitosan DOD	Drug %	Chitosan %	Sodium tripolyphosphate %
S1	86.60%	1	1	3
S2	86.60%	1	1	6
S3	86.60%	1	1	10
S4	86.60%	1	0.25	3
S5	86.60%	1	0.25	6
S6	86.60%	1	0.25	10
S7	86.60%	1	0.5	3
S8	86.60%	1	0.5	6
S9	86.60%	1	0.5	10
S10	87.82%	1	1	3
S11	87.82%	1	1	6
S12	87.82%	1	1	10
S13	87.82%	1	0.25	3
S14	87.82%	1	0.25	6
S15	87.82%	1	0.25	10
S16	87.82%	1	0.5	3
S17	87.82%	1	0.5	6
S18	87.82%	1	0.5	10
S19	93.10%	1	1	3
S20	93.10%	1	1	6
S21	93.10%	1	1	10
S22	93.10%	1	0.25	3
S23	93.10%	1	0.25	6
S24	93.10%	1	0.25	10
S25	93.10%	1	0.5	3
S26	93.10%	1	0.5	6
S27	93.10%	1	0.5	10

Cross-linking to form gels:

Accurately measured quantities of the different cross-linking agents were dissolved in distilled water to obtain the following concentrations:

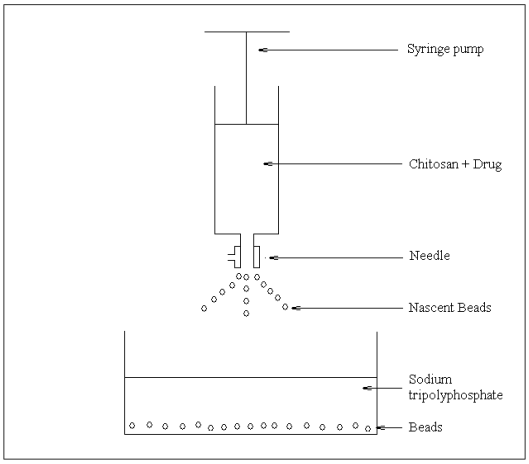
a) Sodium-tri-polyphosphate (STPP, 3, 6, 10% w/v)
b) Oleic acid (OA, 3, 6, 10% v/v)
c) Polysorbate 80 (T80, 3, 6, 10% v/v)

Finally sodium-tri-polyphosphate (STPP, 3, 6, 10% w/v) (C) were finally chosen for cross-linking experiments.

The resultant homogeneous bubble free suspension (B) was dropped using a disposable syringe (No.18) as shown in figure no-2 into 100ml of sodium tri-polyphosphate (STPP) solution for 20 minutes.

The beads were colleted after filtration and washed with deionised water. Beads were dried at room temperature. All batches were prepared in triplicate.

Figure No-2: Schematic spray device used in the preparation of chitosan beads



2.3 Evaluation of beads

2.4 Weight variation, dimensions (Ferna´ndez-Herva´s M.J.etal., British Pharmacopoeia)

A total of 20 beads of optimize formulation were evaluated for weight variation to valuate conformity with standard specifications for solid dosage forms according to the British pharmacopoeia. Twenty beads were randomly selected and weighed one at a time and their average weight was determined. According to the specifications, not more than two of the individual weights were suppose to deviate from the average weight by more than the percentage deviation and none were to deviate by more than twice that percentage. The diameter and height of 20 individual dry beads randomly selected for each formulation were determined using a vernier calliper (Absolute Digimatic No.107S).

2.5 Angle of repose(Mayur Sankalia G.et.al.)

Angle of repose of all batches of beads was determined by fixed funnel free standing cone method.The beads were passed through a funnel having 0.7cm orifice. The distance from the funnel to the base of cone was 2cm.The angle of repose of beads then calculated as

$$Ø=\tan^{-1}(h/r)$$

Where, Ø=Angle of repose (^{0}), h=Height from tip to base of cone(cm)
r=Radius of cone of agglomerate (cm)

2.6 Determination of drug content(Clemence Tarirai et.al., Chambin O.et.al., A. Ehab Hosny et.al.)

Ten beads were crushed and 500 mg of the powder was weighed and stirred for 24 hours in 100 ml of 6.8pH phosphate buffer. The solution was ultrasonicated for 15 minutes and a filtered 5 ml sample was analyzed spectroscopically to determine the quantity of diclofenac sodium present in the sample. The concentration of diclofenac sodium in the sample was calculated by using the equation of the standard curve and the quantity of diclofenac sodium in the powder was then calculated from this concentration.

Determination of drug content (Entrapment Efficiency)
The diclofenac sodium content of the beads was determined through Eqs. (1) and (2):
entrapment efficiency (drug entrapment ability in %)

$$= \frac{AQ}{TQ} \times 100 \quad \text{----------(1)}$$

in which AQ is the actual quantity of drug present in the beads (drug content) and TQ is the theoretical quantity of drug (initial diclofenac sodium loading dose during the preparation of the beads):

$$\text{Entrapment Percentage} = \frac{AQ}{(\text{Total weight of beads by batch} - AQ)} \times 100 \quad \text{--------(2)}$$

2.7 Percentage yield:
The total amount of dried drug loaded chitosan beads were washed and dried. weight of dried beads was determined and the percentage yield was calculated taking into consideration of initial amount.

$$\text{Percentage yeield} = \frac{\text{Expected Weight}}{\text{Actual Weight}} \times 100$$

2.8 Beads size analysis(Ehab Hosny A.et.al)
Beads size analysis was studied by using a stereomicroscope (Stemi 2000-c,Carl Zeiss,Oberkochem,Germany) and average bead size was calculated.

2.9 Swelling studies(Gupta K.C.et.al., A. Sunil Agnihotri et.al., Anil Anal K.et.al., Oya Sanl et.al.)
The swelling ratio of beads from each formulation was determined. The beads were each weighed and placed in a wire basket of USP dissolution apparatus II .The basket containing beads was put into 750 ml of acidic buffer at pH 1.2 ± 0.1 for 2hrs. and 1000ml phosphate buffer at pH 6.8 ± 0.1 for 5 hrs.respectively, at 37.7°C with continuous stirring at 100 rpm. The beads were removed from the buffer at hourly intervals to a maximum of 7 hours, blotted on a piece of paper to remove excess surface moisture prior to weighing. The degree of swelling (Swt) is based on the difference in weight of the dry and swollen state and was calculated according to equation.

$$Swt = \frac{Ws - Wd}{Wd} X\ 100$$

Where: Ws = weight of swollen beads.
Wd = weight of dry beads.

3. Differential scanning calorimetry(DSC)(Ferna´ndez-Herva´s M.J.et.al., A. Sunil Agnihotri et.al., Mayur Sankalia G.et.al., Oya Sanl et.al., Gonza´lez-Rodrı´guez M.L.et.al., Palomo M.E.et.al., Brahma Singh N.et.al., Maja Simonoska Crcarevskaet.al., Deirdre Corrigan O.et.al.)
Diclofenac sodium, plain chitosan beads and three grades of drug loaded chitosan beads were weighed (4-8mg) into a 40µl standard aluminium crucible and hermetically sealed with perforated aluminium lid. Sample were subjected to thermal study using model mettler Toledo 82 le .The system was purged with N_2 gas at rate of 100 ml/min to maintain inert atmosphere.Heating of samples was done from -30 to 350^0 C at rate of 10^0 C/min.

3.1 Morphological study of chitosan beads(Habib Y.S.et.al.)
Scanning electron microscopy
The samples were investigated with regard to surface morphology. To study the surface morphology a small piece of the surface was removed using a scalpel blade and fixed onto an aluminum button.The samples fixed onto the aluminum buttons were used for electron microscopy.

3.2 In Vitro drug release studies(A. Ehab Hosny et.al.)
The chitosan beads were evaluated for in vitro drug release studies. The release rate of Diclofenac sodium beads was determined using USP Dissolution Testing Apparatus I (Basket type). The dissolution test was carried out at 37 + 0.5”C in 750 ml of 0.1 N HCl (pH 1.2) for 2 h and continued for another 10 h at pH 6.8. The change in pH was achieved by the addition of 250 ml of 0.2 M tribasic sodium phosphate. Dissolution medium samples (5ml) were withdrawn at predetermined time intervals (i.e. 0,1,2,3,4,5,6,7,8,9,10,11 and 12 h), filtered through a whatman filter and suitably diluted and analyzed for diclofenac sodium 281 nm using a double beam UV/VIS Spectrophotometer (JASCO V 530).The sample volume was replaced with fresh dissolution medium and a correction factor was used to account for the change in the dissolution medium volume after the addition of the basic buffer.

3.3 Stability Study(Mathews B.R. et.al.)
Stability studies were conducted on Diclofenac sodium beads to assess their stability with respect to their physical appearance, drug content, and drug release characteristics after storing in them Stability chamber (Thermolab) at 40°C/relative humidity (RH) 75% for 3 months.

RESULTS

4.1 Weight variation:
The average weights of the optimized batches are given in Table No.-2.The weight variation of optimized batches complied with the7.5% standard deviation limit from the average within each type. However, the average weight of the matrix systems varied between the different types to a more pronounced extent. This can possibly be explained as a result of the differences in the densities of the hydrogels formed and concentrations of the polymer used.

Table No.-2: Weight variation, friability, bead size and diameter of formulations S1, S10 and S19 beads:

Sr. No.	Polymer Name	Formulation Code	Weight Variation (mg)	Friability (% loss)	Bead Size (µm)	Diameter
1.	**Chitosan 86.60%DOD**	**S1**	112.090	0.37	0.30	1.529
2.	**Chitosan 87.82%DOD**	**S10**	116.078	0.35	0.29	1.492
3.	**Chitosan 93.10%DOD**	**S19**	109.356	0.41	0.24	1.482

∗ Each value represents the mean (n=3)

4.2. Angle of repose:

The angle of repose of all formulations of beads was depicted in Table No.-3.The smoothness and sphericity of beads is reflected by angle of repose, which were found in the range of 23.32 to 32.5^0.The beads containing higher polymer were more flowable than containing low polymer. Formulations containing drug to polymer ratio is 1:1 gives more spherical beads than ratio 1: 0.50 and 1:0.25 with high degree of angle of repose.

Table No.-3: Angle of repose values of chitsan-diclofenac sodium beads

BatchCode	Angle Of Repose($Ø^0$)	BatchCode	Angle Of Repose($Ø^0$)	BatchCode	Angle Of Repose($Ø^0$)
S1	30.24	S10	31.73	S19	31.34
S2	31.34	S11	30.56	S20	32.5
S3	30.12	S12	30.18	S21	30.16
S4	26.34	S13	23.32	S22	-
S5	24.44	S14	22.37	S23	-
S6	26.78	S15	26.41	S24	-
S7	28.41	S16	28.72	S25	-
S8	29.89	S17	27.23	S26	-
S9	27.17	S10	29.02	S27	-

∗ Each value represents the mean (n=3)

4.3 Entrapment efficiency (% E.E.):

Entrapment efficiency of all three grades of formulations is depicted in Table No.-4. The diclofenac sodium content within the beads decreased with increasing sodium tri-poly-phosphate concentration. It is also observed that as the drug to polymer ratio decreases, entrapment efficiency increases, in all chitosan formulations. The release rate of diclofenac sodium from beads decreased with increasing entrapment efficiency.The entrapment of the diclofenac sodium particles within the chitosan beads rendered the crystal surfaces more hydrophilic. This improved the wetability of the crystal by the dissolution media and resulted in the enhancing effect of chitosan on diclofenac sodium release.

4.4 Percentage yield:

Percentage yield of all three grades formulations were depicted in Table No.-4.

4.5 Particle size and shape:

Particle size analysis of optimized batches of drug loaded chitosan beads was analyzed by using a stereomicroscope. Particle size analysis of optimize batches of drug loaded chitosan beads were in the range of 0.24 to 0.30 µm as shown in Table No.-2.

Table No.-4: Entrapment efficiency and percentage yield values of chitosan beads.

Sr. No.	Formulation code	Percentage entrapment efficiency	Percentage yield
1	**S1**	69.45	98.34
2	**S2**	83.21	92.23
3	**S3**	89.75	89.34
4	**S4**	79.46	90.45
5	**S5**	87.12	84.56
6	**S6**	94.36	85.47
7	**S7**	74.56	91.56
8	**S8**	86.34	88.46
9	**S9**	91.87	84.34
10	**S10**	64.53	89.33
11	**S11**	76.54	84.31
12	**S12**	87.90	92.98
13	**S13**	74.23	98.21
14	**S14**	85.34	84.05
15	**S15**	93.26	90.23
16	**S16**	72.89	87.54
17	**S17**	81.78	84.09
18	**S18**	89.49	81.86
19	**S19**	69.12	98.91
20	**S20**	74.61	92.23
21	**S21**	79.56	94.12

∗ Each value represents the mean (n=3)

4.6 Friability:

The friability parameter was tested for all three optimized batches of three grades of deacetylated chitosan beads. The readings were taken in triplicate for each batch and the data is summarized in Table No.-2. Beads of optimized batches show acceptable limits for parameters.

4.7 Moisture content:

Moisture content within the beads was determined by Karl Fischer titrimetric analysis. Formulation S19 gives high percentage of moisture content than S10 and S1.

Table No.-5: Percentage swelling of beads at the end of second and seventh hours dissoluation :

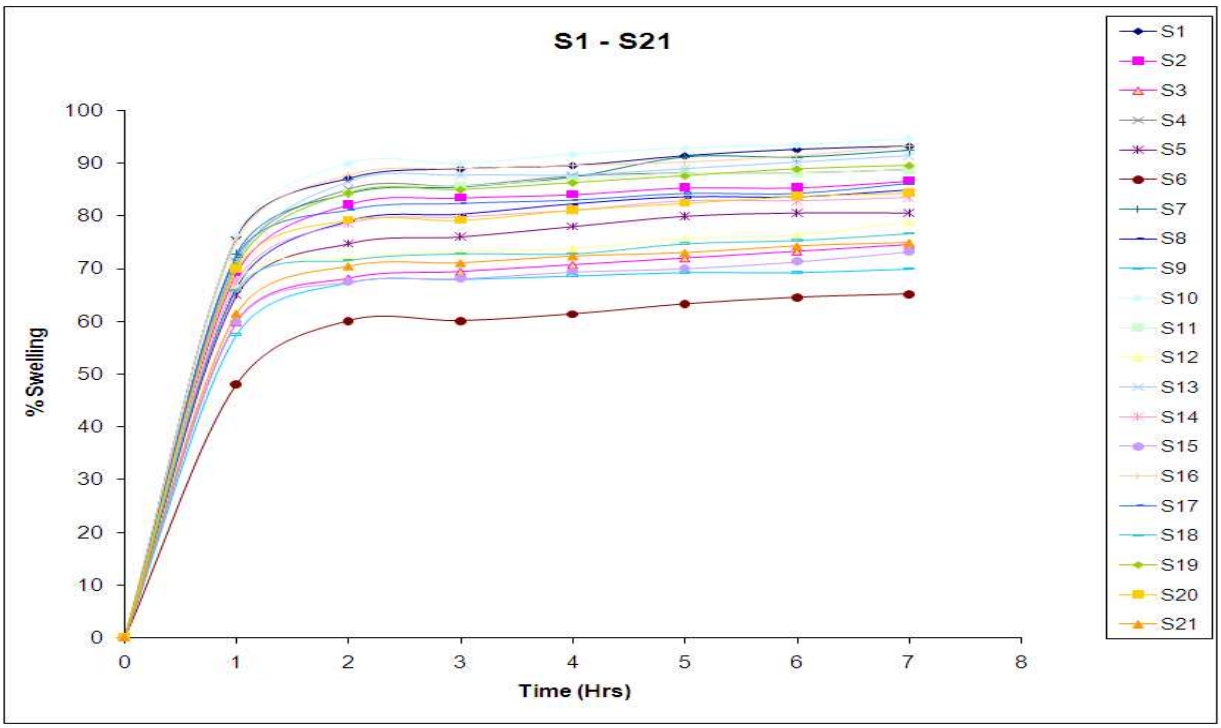
Formulation code	Percentage swelling	
	II Hrs.	VII Hrs.
S1	87.11	93.25
S2	82.16	86.62
S3	68.15	74.52
S4	85.09	88.81
S5	74.67	80.51
S6	60.12	65.18
S7	84.17	92.40
S8	78.94	84.86
S9	67.32	69.93
S10	89.94	94.67
S11	84.37	88.75
S12	72.04	78.88
S13	86.50	91.47
S14	78.52	83.43
S15	67.51	73.24
S16	87.73	93.25
S17	81.13	86.16
S18	71.51	76.58
S19	70.51	89.54
S20	79.22	84.41
S21	84.31	75.0

∗ Each value represents the mean (n=3)

4.8 Swelling studies:

Swelling studies of drug loaded chitosan beads were carried out in 1.2 pH phosphate buffer (0.1 N HCl) for 2 hrs. and in 6.8 pH phosphate buffer for 5 hrs. In general, swelling ratio increases from acidic medium (1.2 pH HCl buffer) to alkaline (6.8 pH phosphate buffer) medium. Swelling of beads in acidic medium is faster than alkaline medium as depicted in Table No.-5 and Figure no.-3 .It is observed that degree of swelling decreased with increasing concentration of sodium tri poly-phosphate in all formulations of chitosan beads. It is also observed that as the degree of swelling decreases the rate of percent cumulative drug release also decreases.

Figure No-3: Percentage swelling of beads



5. Scanning electron microscopy (SEM):

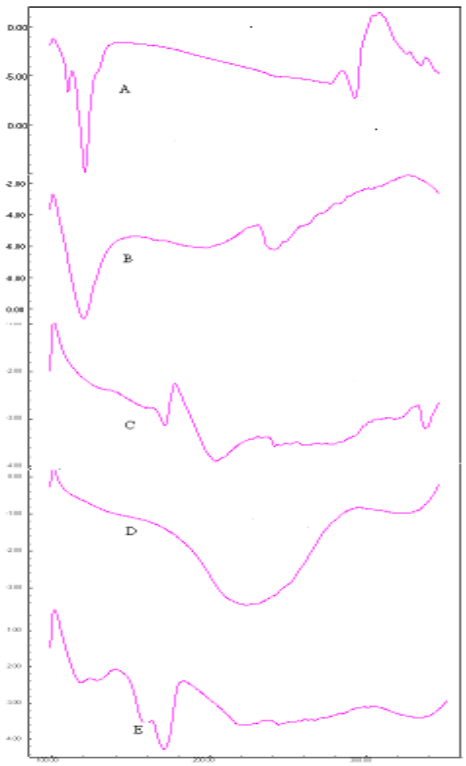
Scanning electron microscopy of drug loaded dried chitosan beads formulations S1, S10 and S19 showed the beads were spherical in shape and rough in surface. At higher magnification, presence of a small pit on the surface were observed.

5.1 In-vitro release study:

The drug release from the chitosan beads depends on the penetration of the dissolution medium into the beads, the eventual swelling and dissolution of the chitosan beads, the dissolution and subsequent diffusion of the drug through the swollen or unswollen chitosan beads. The swelling of chitosan beads was dependent on the pH of the dissolution medium. The beads when wetted by the acidic dissolution medium swelled extensively, and formed a hydrogel matrix before they dissolved completely. Swelling was very less in simulated intestinal fluid. The counterion, STPP acts as an ionic cross-linking agent. Drug release from hydrogels has been controlled by the degree of cross-linking. The degree of swelling and the rate of drug release reduced with increasing degree of cross-linking. The dissolution of the drug crystals and subsequent diffusion

through the beads increased as a result of the swelling which increased the free volume of the matrix, in this case lightly cross-linked (3%) formulations of beads shows high degree of swelling as depicted in Table no.-5 and the rate of percent cumulative drug release also increases. Ionization of the free amino group in 0.1 N HCl caused hydration and swelling of the beads prior to the dissolution of chitosan i.e. degree of swelling is high in acidic 0.1 N HCl medium than intestinal pH as depicted in Table no.-5 and Figure no.-3, on contrary, the beads did not swell or dissolve in simulated intestinal fluid. As the concentration of STPP increased, decrease in degree of swelling and drug release within in 12 hrs. were observed as depicted in Table np.-6 and Figures no.-4**.**Entrapment efficency of drug increased as the drug: polymer ratio decreased with decreased cumulative drug release. S1, S10 and S19 exhibit the 94.75%, 97.48% and 92.41% drug release. Due to the low viscosity of chitosan DOD 93.10% only 1:1 ratio formulations were prepared. It was found that percentage drug release decreased with decrease in the viscosity and hence chitosan beads with DOD 87.82% were more efficient in retarding the drug release as compared to other two grades. On the basis of the results obtained from drug release studies, the formulations S1, S10 and S19 were selected for weight variation, friability, size, diameter, moisture content, DSC, SEM and stability study.

Figure No-4: Differential scanning calorimetry(DSC)



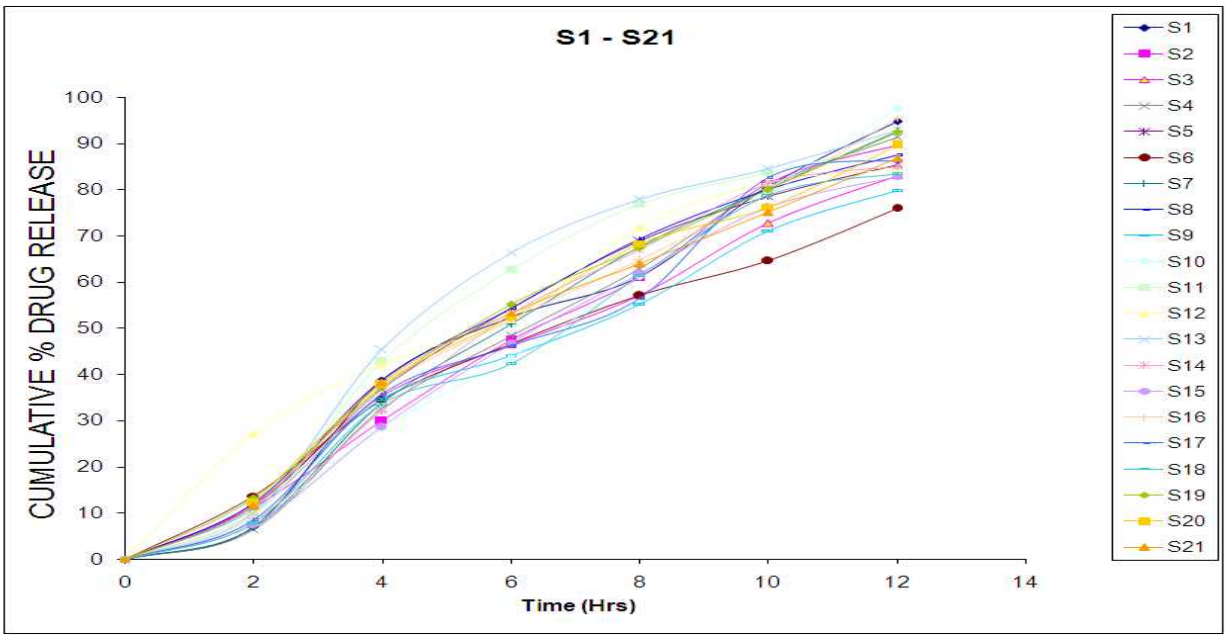
A) Drug B) Plain chitosan beads C) Formulation S1 D) Formulation S10 E) Formulation S19.

Table No. -6 Cumulative percentage drug release from chitosan beads:

Sr. No.	Formulation code	Cumulative Percentage Release of the Drug					
		2 Hrs.	4 Hrs.	6 Hrs.	8 Hrs.	10 Hrs.	12 Hrs.
1	**S1**	6.96	38.60	52.49	61.11	80.58	94.75
2	**S2**	11.27	29.86	47.46	60.91	81.37	89.68
3	**S3**	11.89	35.35	46.45	57.13	72.70	83.05
4	**S4**	6.34	32.50	48.27	62.90	80.58	91.44
5	**S5**	11.48	36.77	54.30	68.88	78.41	85.39
6	**S6**	13.71	34.33	46.65	57.13	64.62	75.84
7	**S7**	6.75	33.72	50.88	67.68	80.19	92.61
8	**S8**	12.09	38.60	54.30	69.28	79.99	87.54
9	**S9**	10.86	34.53	44.04	55.14	71.12	79.74
10	**S10**	8.81	28.23	43.64	61.11	79.69	97.48
11	**S11**	9.42	42.66	62.75	76.84	83.73	89.88
12	**S12**	27.15	42.24	51.82	71.89	81.53	84.22
13	**S13**	10.04	45.3	66.37	77.85	84.52	92.8
14	**S14**	8.19	32.09	53.09	66.89	81.37	85.0
15	**S15**	7.37	28.64	46.86	61.71	76.05	82.86
16	**S16**	10.04	36.77	51.69	64.70	79.00	95.53
17	**S17**	8.40	35.75	46.25	56.53	82.5	86.27
18	**S18**	7.78	33.52	42.23	61.31	78.81	83.44
19	**S19**	13.12	37.28	55.11	67.48	79.99	92.41
20	**S20**	12.09	37.78	52.49	68.28	76.24	89.68
21	**S21**	11.48	38.19	53.30	63.90	75.06	86.76

∗ Each value represents the mean (n=3)

Figure No-4: Drug Release in 12 Hrs



DISCUSSION

A pilot study showed that tween 80 and oleic acid have relatively low cross-linking capabilities on chitosan at 3,6 and 10% v/v concentration range. This could probably be explained by their non-ionic nature leading to little or no bonding (physical or ionic) with the chitosan cationic chains. Unfortunately, the oily hydrogels that had been formed from this cross-linking agent

were difficult to dry. Based on these results and the lack of producing viable matrix systems, tween 80 and oleic acid were excluded as cross-linking agents from further investigations in this research project. In contrast to this, sodium tri-polyphosphate (STPP) exhibited remarkable cross-linking properties starting at a relatively low concentration of 1% w/v up to a concentration of 10% w/v. The concentrations 3% w/v, 6% w/v and 10%w/v form hard, bead-like structures within the hydro gel, therefore these concentrations are selected. TPP is a well-known cross-linking agent used to form chitosan hydro gels and have been included in this study. In this study various formulations were prepared at different concentration (3, 6 and 10%) of sodium tri-polyphosphate (STPP) with three grades of degree deacetylated chitosan polymer (86.60%, 87.82% and 93.10%DOD).

The polycationic polysaccharide chitosan forms gels with suitable multivalent counter ions. The ionic interaction between the positive charged amino groups and the negative charged counterions, tri-polyphosphate, was used in this study to prepare chitosan beads. The protonation of amino groups enables the dissolution of chitosan by large number of strong and weak acids. Solutions of chitosan in 2%acetic acid were dropped into TPP solutions and gelled sphere formed instantaneously by ion tropic gelation. The beads were easily manufactured without any sophisticated equipment(Oya Sanli,Nuran Aya et.al.).

Chitosan is characterized by its degree of deacetylation (DOD) and its viscosity in 2% acetic acid solution. The shape and preparation of beads were critically depends on the viscosity of the chitosan solutions. Three chitosan samples with different degrees of deacetylation, viscosities were evaluated for bead formation. Only the high viscosity chitosan samples resulted in spherical and sufficient strong beads at concentrations of 2%(v/v) in dilute acetic acid. Beads could be prepared from chitosan polymer(86.60,87.82% DOD) with concentration in between 1 & 0.5% w/v in acetic acid except in 92.10% DOD chitosan polymer we could not prepare beads below concentration of 2% w/v of polymer in acetic acid.

The anionic counter ion, TPP, can form either intermolecular or intramolecular linkages with the positively charged amino groups. The intermolecular linkages, which are responsible for the successful formation of the beads.

The diclofenc sodium crystal initially dissolves from the surface of the beads and then with increasing time periods, more drugs dissolved from the central regions. In all three types of chitosan formulations, there was decrease in diclofenac sodium content (%E.E) within the beads with increasing TPP concentration. After complete drying, diclofenac sodium crystals were visible on the exterior of the beads(Roland Bodemeier et.al.).

Hydrogel beads of chitosan can be prepared without a tedious process for drug delivery system.In chitosan beads, the polyelectrolyte complex occurs between chitosan and sodium tri-polyphosphate and it protects the gel matrix from environmental conditions.By using this ionotropic gelation technique, roughly spherical and regular shaped chitosan beads were obtained.The weight distribution of beads were found to be change within comparatively narrow ranges(Cenk Aral et.al.).

Angle of repose of drug loaded beads of all batches were in the range of 23.32 to 32.5^0.The beads containing higher polymer were more flow able than containing low polymer. Formulations containing drug to polymer ratio is 1:1 gives more spherical beads than ratio 1: 0.50 and 1:0.25 with high degree of angle of repose.

The entrapment efficiency of the beads varied from 69.12% to 94.36%.It appears that entrapment efficiency of Diclofenac sodium into chitosan beads was significantly affected by the drug to polymer ratio. Drug to polymer ratio decreases, entrapment efficiency increases, in all **c**hitosan formulations. The diclofenac sodium content within the beads decreased with increasing sodium tri-poly-phosphate concentration(Roland Bodemeier et.al., Cenk Aral et.al.).

Swelling ratio of beads in acidic medium is higher than alkaline medium. It is observed that degree of swelling decreased with increasing concentration of sodium tri poly-phosphate in all formulations of chitosan beads. It is also observed that as the degree of swelling decreases, rate of percent cumulative drug release also decreases(Roland Bodemeier et.al.).

In the DSC thermogram of drug, blank chitosan beads and drug loaded chitosan beads formulations shows sharp and broad melting peaks. Drug shows three sharp peaks at 110^0C,118^0C and 290^0C. These degradation exotherm of diclofenac sodium was absent in blank chitosan bead and an additional endothermic peak at 175^0C,220^0C,175^0C corresponding to chitosan-diclofenac sodium interaction was observed. A chemical reaction occurred in solution, modifying the starting reagents and that a strong interaction is present among all the compounds inside the formulation; this fact additionally acts as a stabilizer, since degradation endotherms both for chitosan and diclofenac sodium are absent at the highest temperatures of the thermogram(Gonza´lez-Rodrı´guez M.L. et.al.).

The drug release from the chitosan beads depended on the penetration of the dissolution medium into the beads, the eventual swelling and dissolution of the chitosan beads and the dissolution and subsequent diffusion of the drug through the swollen or unswollen chitosan beads. The swelling of chitosan beads was dependent on the pH of the dissolution medium. The beads when wetted by the acidic dissolution medium swelled extensively, and formed a hydrogel matrix before they dissolved completely. Swelling was very less in simulated intestinal fluid. The anionic counter ion, STPP acts as an ionic cross-linking agent. Drug release from hydrogels has been controlled by the degree of cross-linking. The degree of swelling and the rate of drug release reduced with increasing degree of cross-linking. The dissolution of the drug crystals and subsequent diffusion through the beads increased as a result of the swelling which increased the free volume of the matrix in this case, lightly cross-linked (3%) formulations beads shows high degree of swelling and the rate of percent cumulative drug release also increases. Ionization of the free amino group in 0.1 N HCl caused hydration and swelling of the beads prior to the dissolution of chitosan i.e. degree of swelling is high in acidic 0.1 N HCl medium than intestinal pH, on contrary, the beads did not swell or dissolve in simulated intestinal fluid.

Drug release behavior of chitosan(86.60% DOD) beads formulations S1 to S9 shows percent cumulative drug release, as the concentration of cross-linking STPP increased, decrease in degree of swelling and drug release within in 12 hrs. Entrapment efficiency of drug increased as the drug **:**polymer ratio decreased with decreased cumulative drug release. Formulation S1

shows 94.75% drug release within 12 hrs. This polymer shows high viscosity than 93.10% DOD polymer.

Drug release behavior of chitosan(87.82% DOD) beads formulations S10 to S18 shows percent cumulative drug release, as the concentration of cross-linking STPP increased, decrease in degree of swelling and drug release within in 12 hrs.Entrapment efficiency of drug increased as the drug **:**polymer ratio decreased with decreased cumulative drug release. Formulation S10 shows 97.48% drug release within 12 hrs. This polymer shows high viscosity than other two grades of polymer.

Drug release behavior of chitosan(93.10% DOD) beads formulations S19 to S21 shows percent cumulative drug release, as the concentration of cross-linking STPP increased, decrease in degree of swelling and drug release within in 12 hrs. Only 1:1 ratio formulations were prepared due to very low viscosity polymer. Formulation S19 shows 92.41 % drug release within 12 hrs.

CONCLUSION

With increased cross-linking there was decrease in swelling of beads. Swelling ratio of beads in acidic medium was higher than alkaline medium. Entrapment efficiency of drug increased, with decrease in drug to polymer ratio. The diclofenac sodium content within the beads decreased with increasing sodium tri-poly-phosphate concentration. DSC results shows the melting of chitosan and diclofenac sodium respectively indicating molecular dispersion of drug in chitosan beads**.** SEM of beads showed beads are spherical and rough in surface. Significant differences were observed in the drug release profile of the beads prepared by chitosan of different DOD and the beads with 1:1 ratio exhibited better results. Further no significant change in entrapment efficiency and drug release was observed after short term accelerated stability study after storing them in Stability chamber (Thermolab) at 40°C/relative humidity (RH) 75% for 6 months beads will be stable as per ICH guideline.

Acknowledgement
The authors are gratefully thankful to JSPM's Jayawantrao Sawant college of Pharmacy & Research for giving a lot of unforgettable support in the research work.

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