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Preparation and Characterization of Ionotropic Cross-Linked Chitosan Microparticles for Controlled Release of Aceclofenac

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ABSTRACT
Aceclofenac, (2-[2-[2-(2, 6-dichlorophenyl) aminophenyl] acety] oxyacetic acid) a non-steroidal anti-inflammatory drug (NSAID), has been indicated for various conditions like post-traumatic pain, rheumatoid arthritis, ankylosing spondylitis. Multiple-unit systems have been reported to avoid the variations in gastric emptying and different transit rates through gastro-intestinal and spread over a large area preventing exposure of the absorbing site to high drug concentration on chronic dosing. The purpose of this study was therefore to develop aceclofenac loaded chitosan microparticles by ionotropic gelation method. Drug loading efficiency (DLE) of microparticles was found between 62.20 to 92.93 % and depended on the formulation variables. Increase in the Tripolyphosphate (TPP) concentration, pH of the TPP solution and cross-linking time decreased the drug release. The particle size decreased with increase in cross-linking time and found between the ranges of 1194.1 to 1568.9 µm. Drug release showed slight burst effect in phosphate buffer pH 7.4 in first hour followed by prolonged release for 8 hrs. FTIR and DSC revealed that there was no interaction between drug and polymer. The release data was fitted into first order, zero order and Higuchi model to find release kinetics. The values of regression coefficient r^2 were found to be greater (≤ 0.9541) for first order than for zero order (≤ 0.8740) and the r^2 value for Higuchi was ≤ 0.9805 suggesting diffusion controlled process. The result concluded that TPP-chitosan microparticles developed by ionotropic gelation method might become potential delivery system to prolonging the release of aceclofenac.

Keywords: Aceclofenac, microparticles, ionotropic gelation, tripolyphosphate, chitosan.

INTRODUCTION

Multiple unit dosage forms such as microspheres or beads have gained in popularity as oral drug delivery systems because of more uniform distribution of the drug in the gastrointestinal tract, more uniform drug absorption, reduced local irritation and elimination of unwanted intestinal retention of polymeric material, when compared to non-disintegrating single unit dosage form. [1-2]

Aceclofenac is a new orally effective NSAID of phenyl acetic acid group. [3] It posses remarkable anti-inflammatory, analgesic and antipyretic properties. The short biological half-life (about 4 hrs) and dosing frequency more than one per day makes aceclofenac an ideal candidate for sustained release. [4] It is a newer derivative of diclofenac and has less gastrointestinal complications. [4-6] However, its adverse effects are seen in patients with active or suspected peptic or duodenal ulcer or history of recurrent peptic or duodenal ulcer or who have gastrointestinal bleeding or other active bleedings or bleeding disorders. [7] These concerns let to the objective of this study are to prepare aceclofenac entrapped microspheres for the controlled release application using natural polymers. Chitosan, the N-deacetylated product of the polysaccharide chitin, is an interesting biopolymer to prepare microspheres owing to its unique polymeric cationic character, good biocompatibility, non-toxicity, biodegradability and its mucoadhesivity and absorption enhancing effect. [8-9] Recently, the use of complexation between oppositely charged macromolecules to prepare chitosan beads (or microspheres) as a drug controlled release formulations, especially for peptide and proteins drug delivery, has attracted much attention, because this process is very simple and mild. [10-11] In addition, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, is applied to avoid possible toxicity of reagents and other undesirable side effects. [12]

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Tripolyphosphate (TPP) is a polyanion, and can interact with cationic chitosan by electrostatic forces. [13-14] Bodmeier et al., 1989 [2] reported that TPP/ chitosan complex could be prepared by dropping chitosan droplets into a TPP solution.

In this present work a number of variables such as pH and concentration of TPP solution, cross-linking time along with particle size, morphology study, loading efficiency and *in vitro* release study were investigated for optimization of bead (microparticles) properties to release aceclofenac in sustained manner in phosphate buffer 7.4.

MATERIALS AND METHODS

Chitosan was procured as a gift sample from India Sea Foods, Cochin, Kerala. The viscosity was 179cps (1 % solution at 37°C) and degree of deacetylation was 81.09 %. Aceclofenac was obtained as a gift sample from Rantus Pharma Pvt Ltd., Hyderabad. Penta sodium tripolyphosphate (TPP) and pectin was purchased from Loba Chemie, Mumbai, India and other reagents were all commercially available and used as received.

Methods

Preparation of drug loaded TPP-chitosan microparticles

The Microparticles were prepared by ionotropic cross-linking method. [2] Chitosan solution (2 % w/v) was prepared by dissolving chitosan in dilute acetic acid (0.5 % w/v) adjusted to pH 5.2-5.4 at room temperature. The solution was stirred for 2 hrs; latter this solution was filtered through muslin cloth to remove insoluble. Required amount of aceclofenac and chitosan solution (1:1) was dispersed uniformly and homogenized for 15 min. Bubble free dispersion was dropped through a glass syringe in a gently agitated TPP solution adjusted to desired pH. After cross-linking time, the microparticles were separated by filtration and washed with bidistilled water; air dried overnight and finally vacuum dried at 50°C for 6 hrs and investigated for optimization of beads properties [Table 1].

Evaluation of Microparticles

The prepared microparticles were evaluated for particle size analysis, drug content, drug loading efficiency, *in vitro* release studies, morphological characters, and drug-polymer interactions. Samples of the microparticles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1 unit of eye piece micrometer was equal to 13.83µm nearly about 100 microparticles sizes were calculated under 45x magnification. For the determination of drug content 100 mg of aceclofenac microcapsules were powdered 50 mg of powder was transferred to 100 ml volumetric flask, dissolved in water and made the volume to 100ml. The solution was kept for one hrs with occasional shaking and filtered through the whatman filter paper. The filtrate was collected and diluted with to sufficient amount distilled water maintaining concentration of the drug within the standard plot range. The diluted solution was analyzed for the aceclofenac content by UV-spectrophotometer (UV-1700 Shimadzu Corporation, Japan) at 274 nm.

Drug loading efficiency (DLE) was studied by dissolving microparticles in distilled water for 24 hrs. The amount of drug loaded was determined by spectrophotometrically at 274 nm. All the experiments were carried out in triplicate.

$$DLE = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

In vitro drug release studies were carried out for prepared microparticles. Accurately weighed 100 mg of microparticles was taken for dissolution studies. Dissolution was carried out using USP type-2 apparatus (paddle method) maintained at 37^0 C, 100 rpm; in phosphate buffer pH 7.4. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance spectrophotometrically (UV-1700 Schimadzu Corporation, Japan) for aceclofenac at 274 nm. *In vitro* release studies were performed in triplicate for each of the samples.

In order to understand the mode of release of drug from TPP-chitosan microparticles, kinetic studies were carried out for fitted to the First order kinetics, Zero order kinetics and Higuchi model. [15]

Characterization of aceclofenac microcapsules

FTIR Studies: IR spectra for drug, and powdered tablets were recorded in a Fourier transform infrared spectrophotometer (FTIR 1615, Perkin Elmer, USA) with KBr pellets.

DSC Studies: 10 mg of aceclofenac and microparticles were sealed in perforated aluminium pans for DSC scanning using an automatic thermal analyzer system (Mettler Toledo, USA). Temperature calibrations were performed using indium as standard. An empty pan sealed in the same way as the sample was used as a reference. The entire samples were run at a scanning rate of 10°C/min from 50-300°C.

Morphology observation (Sem analysis): Scanning electron microscopy was used to examine the surface morphology of microparticles. Dried microparticles were mounted onto stubs by using double-sided adhesive tape and vacuum coated with gold film using sputter coater (Edward S-150, UK) and observed under scanning electron microscope(Leica steroscan 430, EDX Oxford ISIS).

Table 1: Formulae for aceclofenac loaded TPP-chitosan microparticles

Formulation	Drug (mg)	Chitosan (% w/v)	TPP conc. (% w/v)	TPP solution pH	Cross-linking time in TPP (hrs)
F1	200	2	2	4	2
F2	200	2	2	4	24
F3	200	2	10	4	2
F4	200	2	10	4	24
F5	200	2	2	2	2
F6	200	2	2	2	24
F7	200	2	10	2	2
F8	200	2	10	2	24

TPP - Tripolyphosphate

RESULTS AND DISCUSSION

The particle size, drug content and microencapsulation efficiency results were compiled in the Table 2. Particle size of various formulations was affected by preparation variables. The size of microparticles ranged between 1168.2 - 1405.6 µm. Microparticle size tended to increase with increase in the pH of the TPP solution and decreased with increase in the cross-linking time. The increase in the size may be attributed to the formation of more porous and loosely cross-linked structure of the matrix. However decrease in the size may be attributed to the progressive gelation of chitosan with time. Microparticles decreased in the size with increase in the concentration of TPP and may be attributed to higher density of TPP-chitosan matrix formation due to increased availability of counter ion TPP during the preparation of microparticles. [16] The overall drug content was uniform and reproducible in each batch of microcapsules prepared. The microencapsulation efficiency was found

Table 2: Particle size analysis, DLE, drug content and $t_{50\%}$ in phosphate buffer 7.4

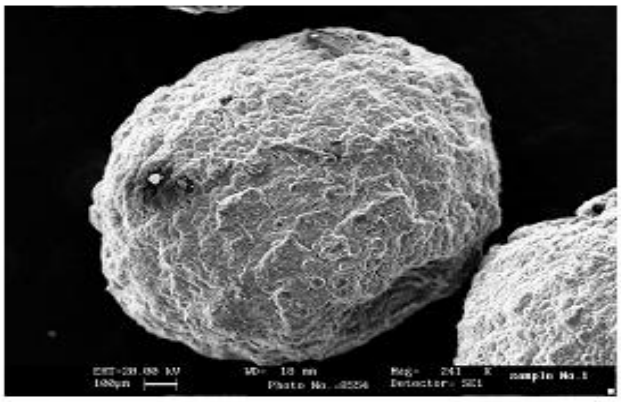
Formulation	DLE (%w/v) (± SD), n=3	Particle size (μm) (± SD), n=100	$t_{50\%}$ (min) (± SD), n=3	Drug content (mg %) (± SD), n=10
F1	92.36 ± (2.33)	1212.0 ± (2.52)	181.58 ± (3.00)	99.22 ± 1.4
F2	87.21 ± (1.39)	1194.1 ± (3.99)	227.12 ± (6.50)	99.41 ± 1.1
F3	92.93 ± (3.12)	1405.6 ± (2.36)	461.36 ± (5.56)	99.37 ± 0.9
F4	89.50 ± (2.11)	1348.9 ± (2.55)	-	99.69 ± 1.5
F5	71.37 ± (1.56)	1191.7 ± (3.15)	85.10 ± (2.06)	99.11 ± 1.2
F6	68.69 ± (1.19)	1168.2 ± (3.85)	82.22 ± (1.61)	99.46 ± 1.4
F7	64.10 ± (2.33)	1277.9 ± (3.25)	64.03 ± (4.10)	99.82 ± 1.2
F8	62.20 ± (1.45)	1247.3 ± (2.26)	62.20 ± (6.17)	99.74 ± 1.1

Note: Values in parenthesis are standard deviation (± SD).

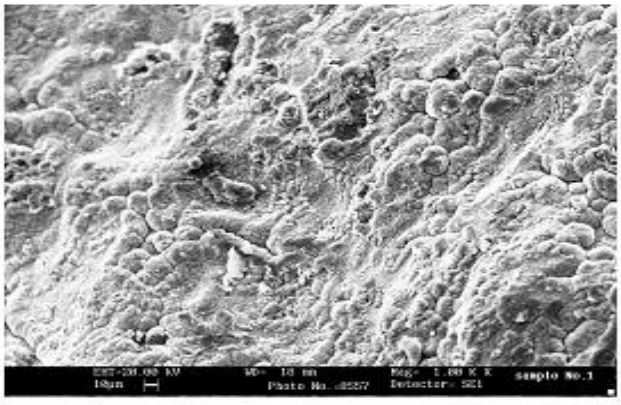
Table 3: Kinetic parameters for all the formulations

Formulation	Zero order r^2	First order r^2	Higuchi Model r^2
F1	0.8412	0.9341	0.9750
F2	0.8034	0.9012	0.9532
F3	0.8128	0.8780	0.9588
F4	0.7938	0.8496	0.9457
F5	0.8661	0.9307	0.9792
F6	0.7346	0.8772	0.9213
F7	0.8720	0.9549	0.9805
F8	0.7451	0.8246	0.9230

Note: All the values are mean of three observations.



(A) Single microparticle



(B) Surface morphology

Fig. 1: SEM photographs of TPP-Chitosan Microparticles

between 62.26- 92.93 %, depending upon formulation variables. The loading efficiency decreased with increase in cross-linking time, possibly could be due to higher contact time in TPP solution. Whereas decreased with decrease in pH of TPP solution and could be attributed to higher solubility of chitosan in acidic pH. Loading efficiency increased slightly with increase in TPP concentration, indicating the better cross-linking density of chitosan matrix. [16]

Fig. 4-6 shows the *in vitro* release studies of drug loaded chitosan microparticles prepared with TPP solution using three variables (cross-linking time, TPP concentration and TPP solution pH). Release studies were carried out in phosphate buffer pH 7.4 for 8 hrs. All the formulations showed slight burst release in phosphate buffer pH 7.4 in the first hour with sustained release for next 7 hrs, which may be attributed to higher solubility of aceclofenac in phosphate buffer pH 7.4 causing a rapid diffusion of surface embedded aceclofenac from the TPP-chitosan microparticles. To access the influence of cross-linking time [Fig 2] on release profiles, two different cross-linking times (2 hrs and 24 hrs) were evaluated.$t_{50\%}$ [Table 2] increased with increase in the cross-linking time; it may be attributed to the formation of higher cross-linking density of TPP-chitosan matrix cross-linked for 24 hrs. This result resembles the result found by Ko et al. [16]
To evaluate the influence of TPP concentration [Fig 3], two concentrations; 2 % and 10 % were selected. Result reveled that increase in the TPP concentration decreases the release. The $t_{50\%}$ [Table 2] was found to be delayed as concentration increased (10 %), which may be attributed to the increased cross-linking due to higher availability of counter ion. Similar results were reported by Remunan-Lopez [8] and Bodmeier et al [2]. To access the influence of TPP solution pH [Fig 4], two different pH (2 and 4) were investigated. The release study suggested that the release rate was less for microparticles prepared at pH 4 [$t_{50\%}$ in Table 2]. This could be attributed to the higher solubility and chain cleavage of chitosan at lower pH causing less stable ionically cross-linked microparticles.
In order to understand the mode of release of drug from TPP-chitosan microparticles, the data [Table 3] were fitted to the First order kinetics, Zero order kinetics and Higuchi model. [15] The values of regression coefficient r^2 were found to be greater (≤ 0.9541) for first order than for zero order (≤ 0.8740) indicating the concentration dependent release. Further the r^2 values for Higuchi was calculated and found to be ≤ 0.9805, strictly suggesting the drug release as diffusion controlled process based on the Fick's law, in which diffusion coefficient depends upon both the concentration and time.
SEM photographs [Fig 1] showed the surface morphology of drug loaded TPP-chitosan microparticles. It appeared to be discreet and roughly spherical. The surface topology showed the presence of rough surface, which was free from cracks.
FTIR spectrum [Fig 5] of aceclofenac (A) showed the prominent peaks at 3316 along with a small broad peak attached; it may be due to OH hydrogen bonding. 3060 is NH aromatic stretching; Peaks near 2900 including 2933 may be due to CH stretching of CH_2 groups. Carbonyl group vibration at 1769 and 1718. Peak at 1578, 1505 and 1445 indicates the presence of C=C ring stretching. Chitosan

shows a broad peak between 2924-3439, for hydrogen bonding of OH and NH_2. Peak 1658 may be due to NH bending and sharp peak around 1151 of O. Formulation (B) showed all characteristic peaks in the range of 2930-3400 for pure drug as well for polymer without any variations. It indicates that drug polymer interaction was absent.

DSC thermograms [Fig 6] of pure drug aceclofenac (A) shows a endothermic peak at melting point at 154^0 C, which is slightly increased to 155°C for drug loaded chitosan microparticles, indicating negligible change. DSC thermogram of the drug and formulation shows all most same melting point it can be concluded no interaction between drug and chitosan.

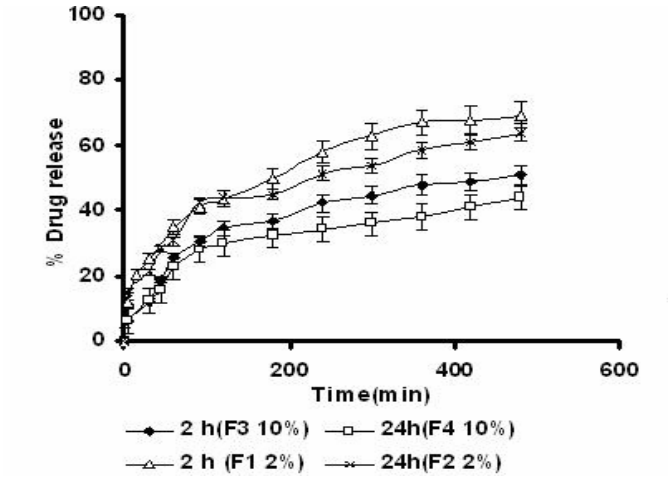


Fig. 2: Influence of cross-linking time on the Aceclofenac release behavior

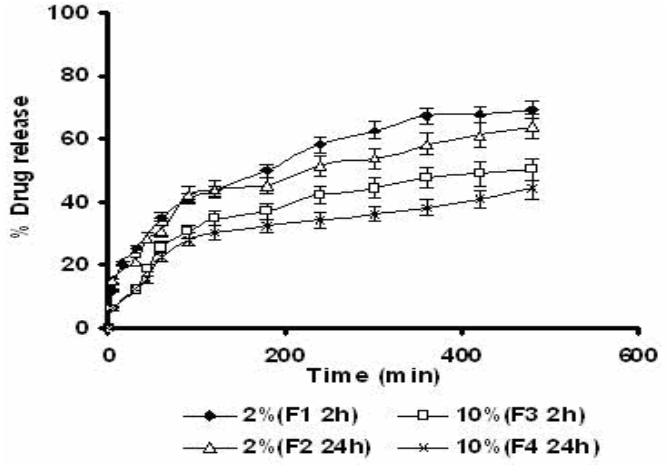


Fig. 3: Influence of TPP concentration on the Aceclofenac release behavior

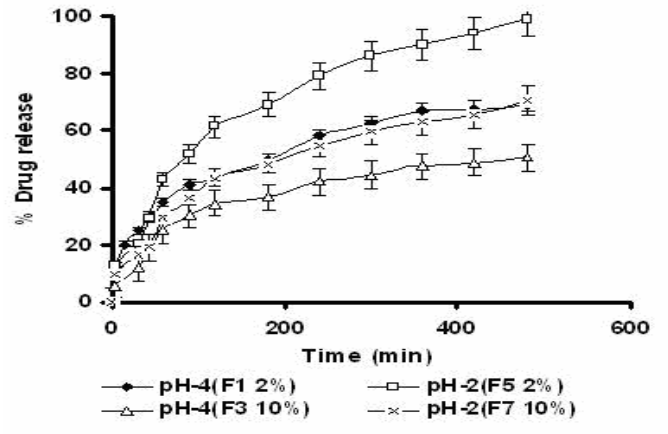


Fig. 4: Influence of TPP solution pH on the Aceclofenac release behavior

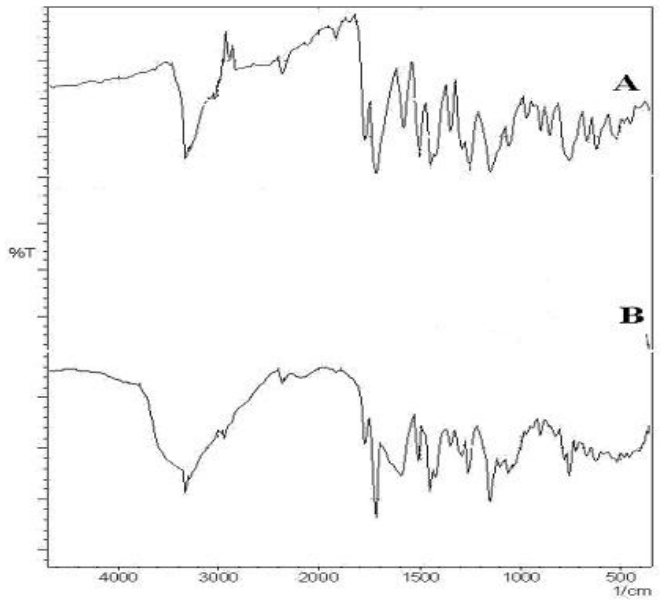


Fig. 5: FTIR spectrum of pure Aceclofenac (A) and drug loaded Chitosan Microparticles (B)

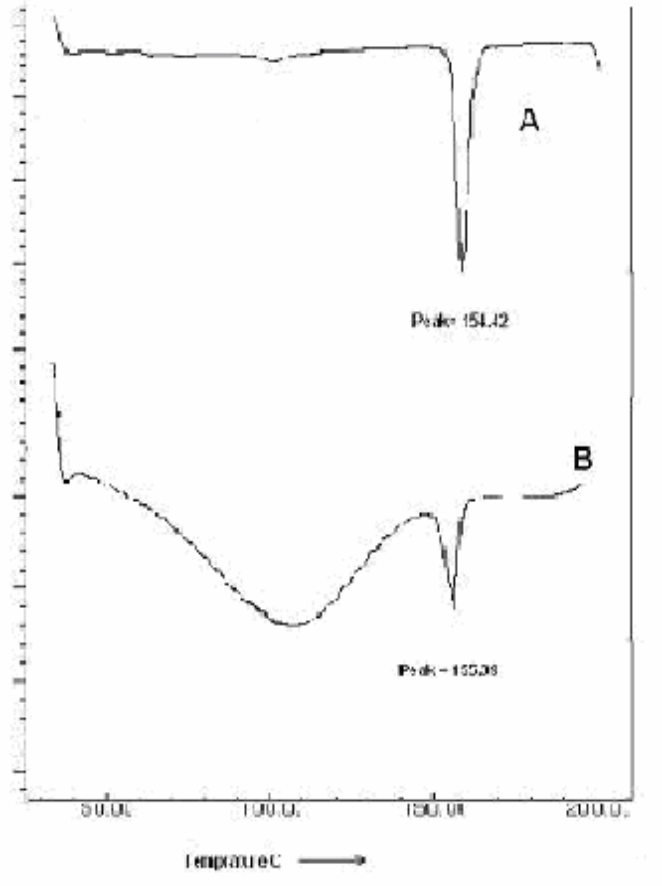


Fig. 6: DSC thermograms of pure Aceclofenac (A) and drug loaded Chitosan Microparticles (B)

The present research work results concluded that TPP-chitosan microparticles were modified by various factors to control the release of aceclofenac. The result showed that the pH and concentration of TPP and cross-linking time play major roles on the TPP-chitosan matrix density; as the cross-linking time and concentration of TPP increased, the release behavior of aceclofenac decreased significantly, whereas decrease in pH increased in the release of aceclofenac.

Application of kinetics showed the Higuchi's diffusion controlled release behavior. Therefore the TPP-chitosan microparticles may be an interesting candidate for maximizing the therapeutic effectiveness and to control the release of drug.

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