RESEARCH ARTICLE

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# DESIGN AND CHARACTERIZATION OF IONOTROPIC CROSS-LINKED CHITOSAN MICROPARTICLES FOR CONTROLLED RELEASE OF BACLOFEN

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**Abstract:** 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 baclofen loaded chitosan microparticles by ionotropic gelation method. Drug loading efficiency (DLE) of microparticles was found to be between 62.20 to 92.93 % and depended on the formulation variables. Increase in the 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 pH of TPP solution and was found between the ranges of 1194.1 to 1568.9 µm. Microparticles slightly swelled and showed biphasic release giving burst effect in phosphate buffer pH 7.4 in first hour followed by sustained release for 8 h. FTIR and DSC showed no interaction of 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 for Higuchi $\leq 0.9805$ suggesting diffusion controlled release process. The result concluded that TPP-chitosan microparticles developed by ionotropic gelation method may become potential delivery system to prolong the release of drug baclofen.

**Key words:** Baclofen; 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].

Baclofen, an analogue of gamma-aminobutyric acid, is a centrally acting muscle relaxant. It interferes with the release of excitatory neurotransmitters at the spinal cord level. It may also act at supraspinal site producing CNS depression. Baclofen is one of the drugs commonly used for the symptomatic relief of severe chronic spasticity associated with a variety of conditions.Baclofen is rapidly and almost completely absorbed from gastrointestinal tract followed on oral dose[3, 4, 5].

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[6, 7].

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[8, 9]. 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[10].

Tripolyphosphate (TPP) is a polyanion, and can interact with cationic chitosan by electrostatic forces[11, 12]. 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 variables such as ; pH of TPP solution, concentration of TPP and cross-linking time were studied and microparticles were evaluated for its particle size, morphology study, loading efficiency and in-vitro release study to investigate and develop an optimized bead (microparticles) properties to release baclofen in a sustained manner.

## MATERIAL AND METHODS

### Materials

Chitosan was obtained as a gift sample from India Sea Foods, Cochin, and Kerala. Baclofen was obtained as gift sample from Hetero Drugs, Hyderabad. Pentasodium tripolyphosphate (TPP) and pectin was purchased from Loba chemie; Mumbai, India and other reagents were all commercially available and used as received.

### Preparation of drug loaded TPP-chitosan microparticles

The beads 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 hr; latter this solution was filtered through muslin cloth to remove insoluble. Required amount of baclofen (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$^{o}$ C for 6 hrs and investigated for optimization of beads properties (Table 1).

### Particle size analysis

Samples of the microparticles were analyzed for particle size by optical microscopy. The instrument was calibrated and 100 microparticle sizes were calculated under magnification.

### Drug loading efficiency (DLE)

Drug loading efficiency was studied by dissolving microparticles in phosphate buffer 7.4 for 24 h. The amount of drug loaded was determined by spectrophotometrically at 266nm. All the experiments were carried out in triplicate.

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

### Morphology observation

Scanning electron microscopy was used to examine the surface morphology of microparticles. Dried microparticles were mounted onto stubs and to investigate the cross-linking process and surface topology, the microparticles were cut into two halves with blades and observed under scanning electron microscope.

**FTIR Study**

IR spectra (KBr pellets; $cm^{-1}$) for drug and drug loaded chitosan microparticles were recorded in a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu-8400, Japan).

**Differential Scanning Calorimetry:**

DSC scans Aceclofenac and drug loaded chitosan microparticles were performed by using an automatic thermal analyzer system (NETZSCH, DSC 200 PC) using Indium as a reference. Sample was run at a scanning rate of $10^{o}$C/min from 30-$300^{o}$C.

***In vitro* drug release studies**

Microparticles (00.01g) were taken for dissolution in phosphate buffer 7.4. Samples were periodically removed and volume of each sample was replaced by the same volume of fresh medium. The amount of drug released was analyzed with a spectrophotometer at 266 nm.

## *RESULTS & DISCUSSION*

Particle size (Table 2) of various formulations were 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 and concentration 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 surface cross-linked structure of the matrix; it may be explained by the formation of cross-linking gradient during the process, where chitosan when dropped into TPP solution, diffuse out into the surrounding TPP solution. The incoming diffusion of TPP on other hand forms a gradient of cross-linked polymer, leading to decrease in chitosan concentration at the core and increasing its porosity. As the concentration of TPP increases, this gradient attains much faster leading to bigger particle size. However decrease in the size may be attributed to the progressive gelation of chitosan with time. Microparticles decreased in the size with decrease in the pH of TPP and may be attributed to higher solubility of chitosan due to chain cleavage and reversible ionic process in acidic pH, causing lowering the molecular weight and thus the particle size[13].

Drug loading efficiency (Table 2) was found between 62.26 to 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 leading to reverse the ionic bonding and chain cleavage causing dispersed drug to get unavailable for entrapment . Loading efficiency increased slightly with increase in TPP concentration, it may be due to higher availability of counter ion leading to the better cross-linking density of chitosan matrix[14, 15].

SEM photographs (Figure 1) showed the surface morphology of drug loaded TPP-chitosan microparticles. It appeared to be discreet and roughly spherical, slight depression was seen that could be attributed to the process of drying of beads on drying paper. The cross section of TPP-chitosan microparticles indicates that the beads were hollow, strictly conforming the point of existence of cross-linking gradient (between chitosan and TPP) during the preparation of microparticles. The surface topology showed the presence of irregular and rough surface, which was free from cracks.

The drug-excipient study was done by Fourier transform infrared (FT-IR) spectroscopy study, the prominent peaks of Baclofen pure drug were shown at 1100cm$^{-1}$ (due to –C-Cl), 1530 cm$^{-1}$ (due to -COOH), and 1610 cm$^{-1}$ (due to –$NH_2$). These prominent peaks of Drug were also present in the IR spectrum of formulation B.From this it clearly indicates that, the drug was not interacted with the polymers used in the formulations (Figure 2, 3)

DSC thermograms of the drug loaded chitosan microparticles indicated sharp endothermic peak of baclofen at melting point at 196.60$^0$ C for drug loaded chitosan microparticles, indicating negligible change. Result demonstrated that there is no interaction between the drug and the chitosan. (Figure 4, 5)

Figure 6, 7 and 8 shows the *in vitro* release studies of drug loaded chitosan microparticles prepared with TPP solution using three variables. Release studies were carried out in phosphate buffer pH 7.4 for 8 h. All the formulations showed biphasic release giving slight burst in phosphate buffer pH 7.4 in the first hour followed with sustained release for next 7hrs, which may be attributed to first, due to release of surface entrapped drug from matrix. Second due to higher solubility of Baclofen in phosphate buffer pH 7.4 causing a rapid diffusion of surface embedded Baclofen from the TPP-chitosan microparticles in combination to slight swelling of polymer matrix at this pH.

To access the influence of cross-linking time (Figure 4) on release profiles, two different cross-linking times (2 h and 24 h) 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 h.

To evaluate the influence of TPP concentration (Figure 5), 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 density/gradient due to higher availability of counter ion.

To access the influence of TPP solution pH (Figure 6), 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 in spite of more protonation, 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. The values of regression coefficient $r^2$ were found to be greater ($\leq$ 0.9541) for first order than for zero order ($\leq$ 0.8740) indicating the concentration dependent release. Further the $r^2$ values for Higuchi was calculated and found to be $\leq$ 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.

## CONCLUSION

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

***ACKNOWLEDGEMENTS***

Hetero Drugs Ltd, Hyderabad and India Sea Foods, Cochin for providing gift samples of Baclofen and chitosan respectively.

**Table 1**

**Formula 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 | 30 | 2 | 2 | 4 | 2 |
| F2 | 30 | 2 | 2 | 4 | 24 |
| F3 | 30 | 2 | 10 | 4 | 2 |
| F4 | 30 | 2 | 10 | 4 | 24 |
| F5 | 30 | 2 | 2 | 2 | 2 |
| F6 | 30 | 2 | 2 | 2 | 24 |
| F7 | 30 | 2 | 10 | 2 | 2 |
| F8 | 30 | 2 | 10 | 2 | 24 |

**Table 2**

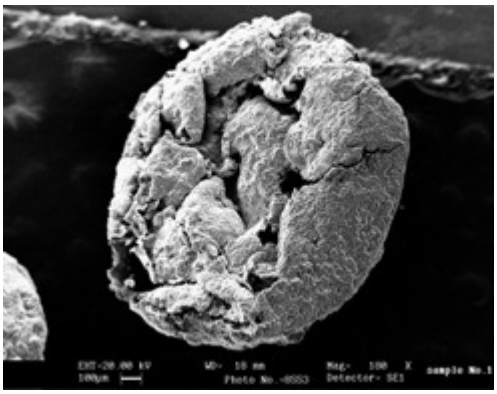
**Particle size analysis, DLE and $t_{50\%}$ in phosphate buffer 7.4**

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

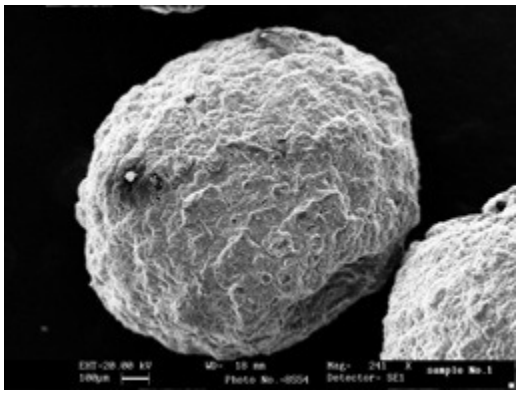
**Table 3**

**Kinetic parameters for all the formulations**

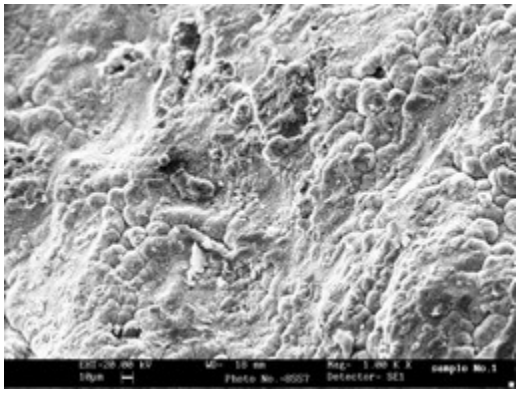
| Formulation | Zero order $r^2$ | First order $r^2$ | Higuchi Model $r^2$ | Release mechanism |
|---|---|---|---|---|
| F1 | 0.8412 | 0.9341 | 0.9750 | Higuchi |
| F2 | 0.8034 | 0.9012 | 0.9532 | Higuchi |
| F3 | 0.8128 | 0.8780 | 0.9588 | Higuchi |
| F4 | 0.7938 | 0.8496 | 0.9457 | Higuchi |
| F5 |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



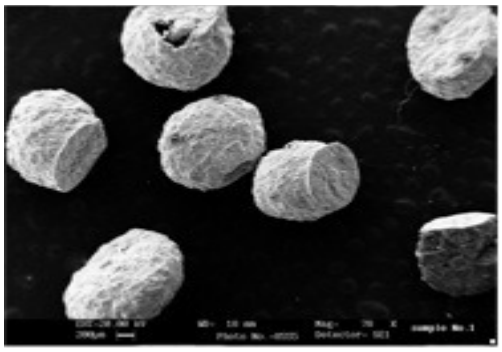
Cross-section



Single microparticle



Surface morphology



Group microparticles

Figure 1: SEM photographs of TPP-chitosan microparticles

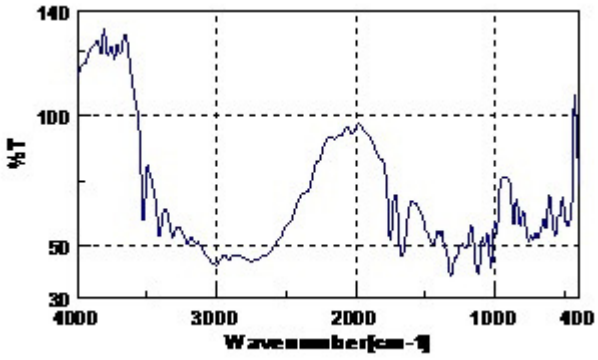



Figure 2: FTIR spectrum of pure Baclofen

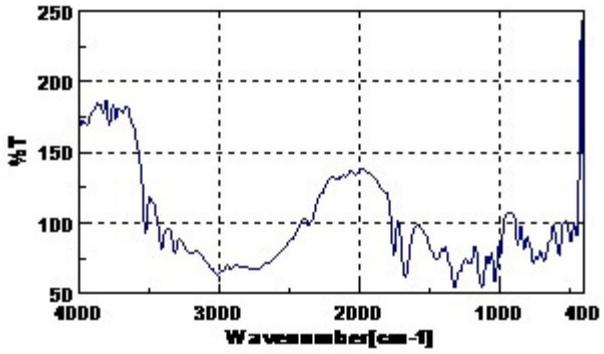



Figure 3: FTIR spectrum of drug loaded chitosan microparticles (Formulation B)

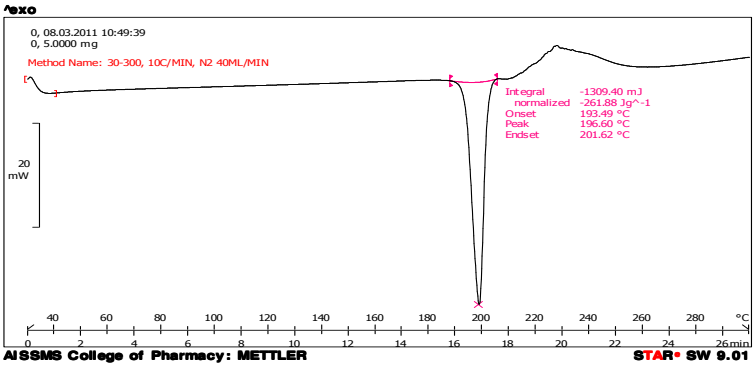



Figure 4: DSC thermo gram of pure drug Baclofen

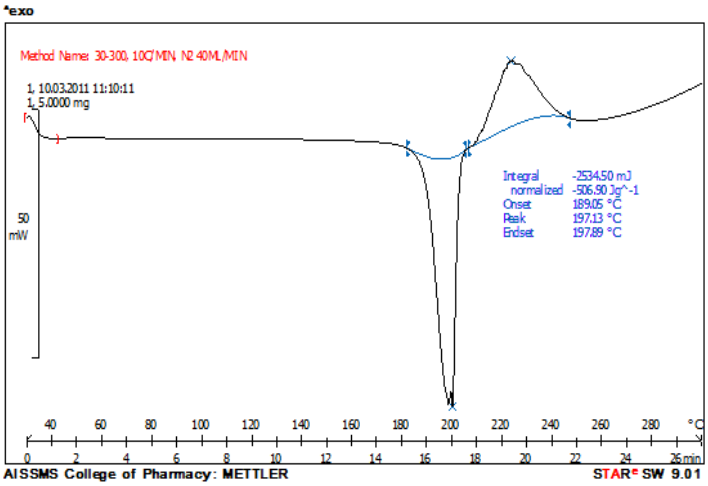



Figure 5: DSC thermo gram drug loaded Chitosan microparticles

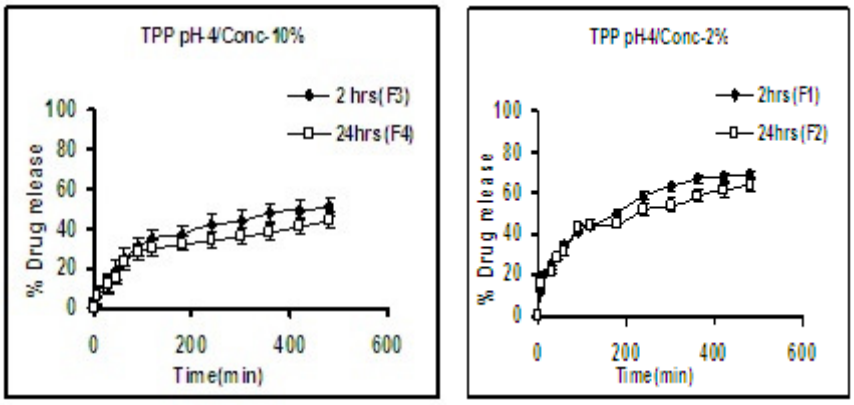



Figure 6: Influence of cross-linking time on the Baclofen release behavior

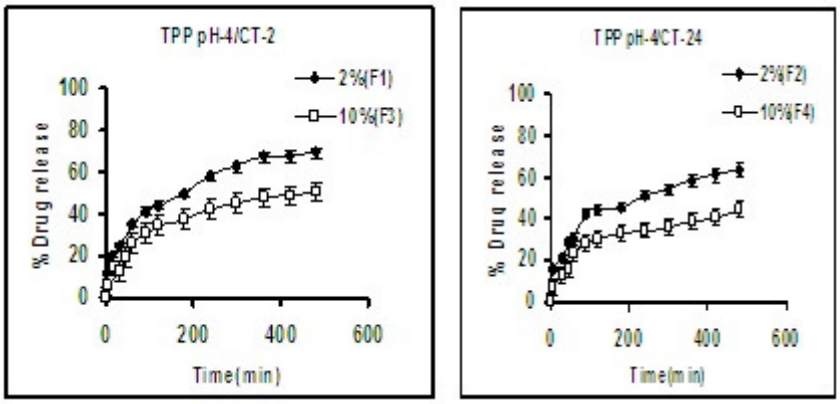



Figure 7: Influence of TPP concentration on the Baclofen release behavior

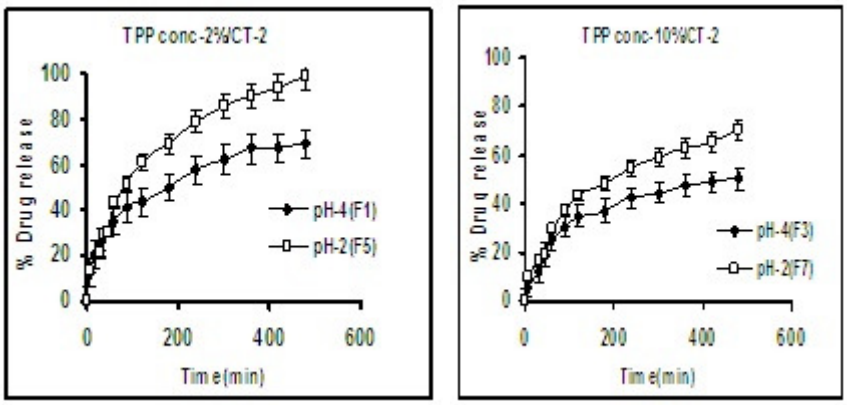



Figure 8: Influence of TPP solution pH on the Baclofen release behavior

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