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RESEARCH ARTICLE

FORMULATION AND CHARACTERIZATION OF TPP CROSS-LINKED CHITOSAN MICROSPHERES LOADED WITH LORNOXICAM

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ABSTRACT

In the present study, tripolyphosphate (TPP) cross-linked chitosan microspheres loaded with lornoxicam were prepared by ionic gelation technique. The effect of formulation and process variables on physicochemical properties of microspheres was investigated. FT-IR, DSC and XRD studies revealed the absence of any chemical interactions between the drug and excipients used. Scanning electron microscope images revealed that the prepared microspheres were discrete, broadly spherical with rough surface characteristics. The size of the microspheres was in the range of 615.32 ± 3.21µm to 855.14 ± 2.83µm which increased with increase in core: coat ratio; reduced when the pH of the TPP medium was changed from its original alkaline to acidic and also with increased extent of cross-linking. Percentage encapsulation efficiency was found to be within 56.33 ± 1.8 to 96.59 ± 3.62 and it was largely dependent on core: coat ratio, pH of the TPP medium and extent of cross-linking. Higher drug release was observed for the microspheres prepared at lower core: coat ratios and also for those prepared at the original pH of dispersion medium. On the other hand, more controlled drug release was observed with the microspheres containing higher polymeric levels prepared in the acidic medium. The drug release was also reduced with higher cross-linking conditions. Non-Fickian diffusion controlled drug release mechanism was observed for all the microspheres except those prepared at higher cross-linking conditions, which exhibited zero order release kinetics. Overall, formulation and process variables have significantly affected the physicochemical properties of the prepared microspheres.

Key words: Lornoxicam; Chitosan; Tripolyphosphate; Ionic gelation; Micropsheres; Charecterization

INTRODUCTION:

Multiparticulate dosage forms like microspheres have gained popularity as oral drug delivery systems. Compared to non-disintegrating single-unit dosage forms, microspheres exhibit more uniform distribution and absorption of the drug in the gastrointestinal tract, reduced local irritation, higher colonic residence time, more predictable gastric emptying and also eliminates unwanted intestinal retention of polymeric material[1,2] .

Chitosan is a potentially useful and the second most abundant polysaccharide obtained by deacetylation of naturally occurring chitin[3,4]. It is an interesting biopolymer to prepare microspheres owing to its unique polymeric cationic character, good biocompatibility, non-toxicity, biodegradability, mucoadhesiveness and also due to its absorption enhancing effect[5,6]. Chitosan being polycationic in acidic media (Pka 6.5) can readily interact with negatively charged species like tripolyphosphate (TPP). The ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP leads to formation of biocompatible cross-linked chitosan microspheres[7,8]. Moreover, the preparation of ionically cross-linked chitosan microspheres using TPP is found to be simple and mild than chemical cross-linking and this procedure is also found to be useful in the pharmaceutical industry[9].

Lornoxicam, a congener of tenoxicam, is a novel NSAID belonging to the oxicam group[10] with extremely potent anti-inflammatory and analgesic activities[11,12]. It is widely used for the symptomatic treatment of pain and inflammation in patients with rheumatoid arthritis and osteoarthritis[13]. However, lornoxicam's usefulness is limited due to its short half-life that ranges from 3 to 5 h[14,15]. Due to its rapid elimination rate, frequent administration of lornoxicam is needed to achieve long lasting and constant pain relief. Thus, in the present

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study, TPP cross-linked chitosan microspheres were prepared with an aim to control the release of lornoxicam thereby reducing its frequent administration and prolonging its pain relief action. The effect of various formulation and process variables that tailors the drug release from the microspheres has been investigated in the present study.

MATERIALS AND METHODS:

Chitosan (Chitoclear®, 95% deacetylated) was generously supplied Primex Ltd, Iceland. Lornoxicam pure drug was received as gift sample by Sun Pharma Ltd., Vadodhara. TPP was purchased from Molychem Ltd Hyderabed. All other chemicals and reagents were of analytical grade and were purchased from SD fine chemicals, Mumbai.

Preparation of microspheres:

Lornoxicam loaded chitosan microspheres were prepared by ionic gelation technique using TPP as cross-linking agent. Briefly, 2% w/v chitosan solution was prepared using 1% aqueous acetic acid by stirring overnight on a magnetic stirrer (Remi Equipments Ltd, Mumbai). Accurately weighed quantity of lornoxicam was dispersed uniformly into the chitosan solution using the magnetic stirrer for 2h and further subjected for ultasonication (15 min) to remove the air bubbles. The drug containing chitosan solution was dropped into the gently agitated aqueous TPP solution with a disposable syringe (22 G) at the rate of 1ml/min using digital over head stirrer (Remi Equipments Ltd, Mumbai). Microspheres were instantaneously formed and after predetermined period of time, microspheres were decanted from the dispersion medium and washed several times with distilled water to remove any surface adhering TPP. The microspheres thus obtained were dried at room temperature for 24h and stored in a desiccator for further use[16,17]. The details of formulation and process variables studied are given Table No: 1

Table No 1: Composition, Yield, Encapsulation Efficiency and Particle size of Lornoxicam loaded chitosan microspheres (n=3)

	Variables		Yield (%)	Actual Drug content(mg)*	Encapsulation Efficiency (%)	Particle Size (µm)
Code	Core: coat	constant:				
C1	1:2	2% TPP,	78.57(2.11)	4.51(0.14)	56.33 (1.8)	762.66 (7.41)
C2	1:4	pH 8.5,	80.3(1.96)	4.84 (0.21)	60.45 (2.65)	794.99(6.47)
C3	1:6	30 min	83.21(1.46)	5.22(0.14)	65.29(1.76)	829.35(5.12)
C4	1:8		85.48(1.59)	5.75(0.14)	71.86(1.84)	855.14(2.83)
	pH of the medium	constant: 1:8,				
H1	6.0	2% TPP,	86.39(1.36)	6.03(0.18)	75.32(2.27)	789.73(7.9)
H2	4.0	30 min	87.25(1.43)	6.45(0.25)	80.59(3.13)	741.24(7.91)
H3	2.0		89.58(1.28)	7.08(0.16)	88.46(2.05)	733.05(6.01)
	TPP % (w/v)	constant:				
P1	4	1:8,	92.28(1.56)	7.3(0.11)	91.29(1.47)	719.22(8.82)
P2	6	pH 2.0,	94.89(1.69)	7.68(0.10)	95.97(1.29)	702.81(9.6)
P3	8	30 min	97.44(1.48)	7.7(0.13)	96.3(1.67)	678.32(5.55)
	Reaction time (min)	constant: 1:8,				
T1	45	8% TPP,	97.58(1.55)	7.73(0.11)	96.59(1.43)	659.25(4.63)
T2	60	pH 2.0	97.19(1.7)	7.63(0.11)	95.39(1.45)	621.67(7.19)
T3	90		96.45(1.41)	7.29(0.29)	91.19(3.62)	615.32(3.21)

Values in the parenthesis represent ± SD

*Actual drug content against theoretic drug content of 8mg of lornoxicam.

CHARACTERIZATION OF MICROSPHERES:

Particle size and surface topography:

The size of the prepared microspheres was analyzed using optical microscopy fitted with a calibrated eye piece micrometer. The mean of 100 microspheres was noted as average particle size[18]. The surface topography of the microspheres was studied using scanning electron microscopy (JEOL, JSM-6360, Japan). Microspheres were mounted on aluminium specimen stubs using double sided adhesive tape and coated with platinum under

vacuum. The morphology of the microspheres was observed at acceleration voltage of 10 kV at different magnifications.

Fourier tranform –infrared (FT-IR) spectral studies:

Pure drug, drug-excipients physical mixture and drug loaded microspheres were analyzed using Fourier transformer infrared spectrophotometer (IR Affinity 1 model, Shimazdu Corporation, Japan, with diffuse reflectance scan sampling method). The samples were triturated with KBr and scanned over wave number range of 4000 to 400 cm^{-1}. FT-IR spectra were analyzed for functional groups and drug polymer interactions.

x-ray diffraction (XRD) analysis:

The effect of microencapsulation process on drug crystallinity was studied using XRD analysis. XRD patterns of pure drug, physical mixture and microspheres were recorded on X-ray diffractometer (XRD 6000, Shimadzu corporation, Japan) using Ni-filtered, CuK radiation, a voltage of 40 Kv and current of 30mA. The scanning speed employed was 4˚/min over the 10˚ to 80˚ diffraction angle range. Microspheres were triturated to fine powder before performing the analysis.

Differential scanning calorimetric (DSC) studies:

The pure drug, drug-excipients physical mixture and drug loaded microspheres were subjected to differential scanning calorimeter (DSC, SIECKO, model-6300, Japan). The samples were sealed in aluminium pans and heated at a constant rate of 10^0C/ min over a temperature range of 20-300 ^{0}C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 10 ml/min.

Encapsulation efficiency:

Crushed microspheres equivalent to 8 mg of lornoxicam was accurately weighed and transferred to a 100ml volumetric flask containing 50ml of phosphate buffer of pH 7.4 and the volume was made up to the mark using the same buffer solution. The flask was stirred on a thermostatic water bath (Remi Equipments Ltd., Mumbai) at room temperature for 24h to extract the entrapped drug. The content was filtered and after suitable dilution, the absorbance was noted on a UV spectrophotometer (UV-1700, Shimadzu Corporation, Japan) at 377.0 nm using phosphate buffer of pH 7.4 as blank. Triplicate readings for each batch were noted and the average was determined as drug content of the microspheres[16,19].

In vitro drug release:

The release of lornoxicam from the microspheres was studied using USP type II dissolution apparatus (TDT-08L, Electrolab dissolution tester). Microspheres equivalent to 8mg of lornoxicam were taken into the basket and the release studies were carried out under the following conditions; media: 900 ml of phosphate buffer of pH 7.4; temperature: 37 ±0.5 ˚C; speed: 100 rpm. At fixed interval of time, aliquots were withdrawn and replaced with fresh dissolution media to maintain the constant volume. The concentration of drug released at different time intervals was then determined by measuring the absorbance at 377.0 nm against blank using UV spectrophotometer.

Mechanism of drug release:

To investigate the drug release mechanism from the microspheres, the in-vitro release data was fitted into various kinetics models like zero order, first order, Higuchi's equations. Further, the drug release mechanism was also analysed by Korsemeyer-Peppas equation.

Statistical analysis:

Results were analyzed and expressed as mean ± SD. Effect of various formulation and process variables on particle size, encapsulation efficiency and drug release of TPP cross-linked chitosan microspheres were statistically analyzed by one-way ANOVA (Tukey's Multiple Comparison Test) using GraphPad Prism Software-5.03 (Trial version, Graph Pad Software, San Diego, CA). The differences were considered significant at the level of $P<0.05$.

RESULTS AND DISCUSSION:

Particle size and surface topography:

The average particle size of the microspheres is given in Table No 1. The size of the microspheres was largely influenced by formulation and process variables. Particle size increased with increase in core: coat ratio which could be due to more amount of coat material in same volume of liquid droplet. When the pH of the medium was dropped from its original 8.5 to pH 2.0, significant ($p<0.05$) decrease in the particle size was observed. This could be attributed to the pH dependent ionization of TPP and also as chitosan is a weak polybase, reduction in pH of the solution leads increased ionization of amine group of chitosan thus forming more ionically cross-linked microspheres resulting in reduced particle size. However, microspheres prepared in the original TPP solution (pH 8.5) are dominated by deprotonation and cross-linking is reduced[7] which might have resulted in increased particle size. Another interesting observation is that particle size decreased significantly ($p<0.05$) with an increase in the cross-linking agent concentration as well as extent of cross-linking. This could be possibly due to the formation of more rigid network structures at higher cross-linking extent[20].

SEM photographs revealed that the prepared microspheres were discrete, broadly spherical with rough surface characteristics (Fig. 1). The surface of the microspheres was dense without any porous structures. The morphological studies also revealed the presence of

few cracks on the surface of the microspheres. Our observations are concordant with the findings of other researchers who reported rough surface characteristics of chitosan microspheres prepared by the ionotropic gelation technique using TPP as cross-linking agent[7,9].

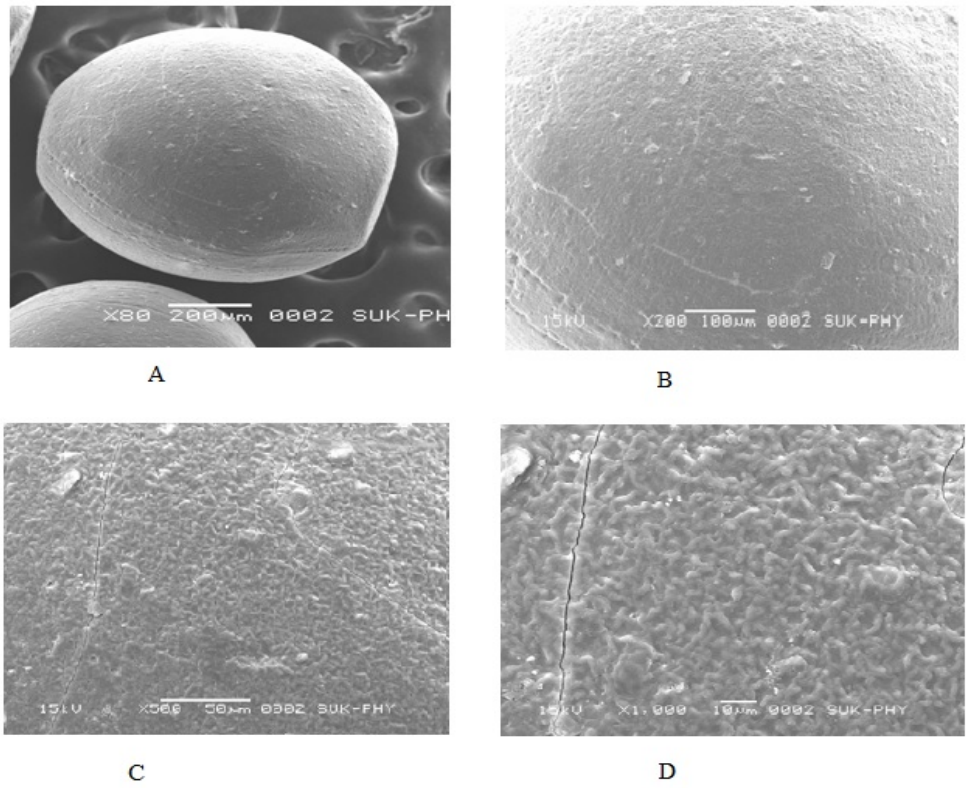

Figure 1: SEM Photographs of TPP cross-linked chitosan microspheres. A. Individual microsphere and its magnified surface topography B. 200X, C. 500X and D. 1000X.

Encapsulation efficiency:

The influence of various formulation and process parameters on encapsulation efficiency is given in Table 1. The percentage of lornoxicam entrapped in the chitosan microspheres was largely dependent on the microsphere fabrication conditions. The encapsulation efficiency increased significantly ($P < 0.05$) with increase in core: coat ratio which could be due to more availability of active TPP binding sites in the polymeric chains leading to higher encapsulation efficiency. When the pH of the dispersion medium was changed from its original alkaline (pH 8.5) to the acidic (pH 2.0), a significant improvement in the lornoxicam entrapment was observed which may be due to pH dependent solubility of lornoxicam, characterized by poor solubility in lower pH which increases with increase in the pH of the medium[21]. Due to the greater gel strength formed at higher TPP levels, encapsulation efficiency in the microspheres was also increased. However, slight reduction in the entrapment of lornoxicam was noted when the curing time was increased from 30 to 90minutes.

FT-IR studies:

The FT-IR spectra of physical mixture, drug loaded microspheres were compared with the FT-IR spectrum of pure drug (Fig: 2). The pure drug lornoxicam exhibited its characteristic absorption bands at 3099.61 cm^{-1} due to aromatic/heterocyclic C-H stretching and at 2926.01 cm^{-1} due to C-H stretching of CH_3 group. The other prominent absorption bands appeared at 1647.21 cm^{-1} due to stretching vibrations of C=O of CONH, 1618.28 due to aromatic C=C and C=N stretching. Bending vibrations of NH group in the secondary amine were observed at 1593.20 and 1544.96 cm^{-1} whereas aromatic C=C skeleton stretching vibrations were observed at 1500.62 cm^{-1}. The peaks at 1423.47 cm^{-1} and 1327.03 cm^{-1} corresponds to O=S=O group, 790 cm^{-1} due to substituted aromatic rings, 765.74 cm^{-1} due to C-Cl stretching vibrations. The IR spectra of physical mixture and drug loaded microsphere formulation exhibited all the characteristic absorption bands as that of pure drug lornoxicam without any significant variations. It is clear from the spectra that, the drug has not undergone any kind of interactions with the excipients used and retained its identity both in its physical mixture as well as in its formulation.

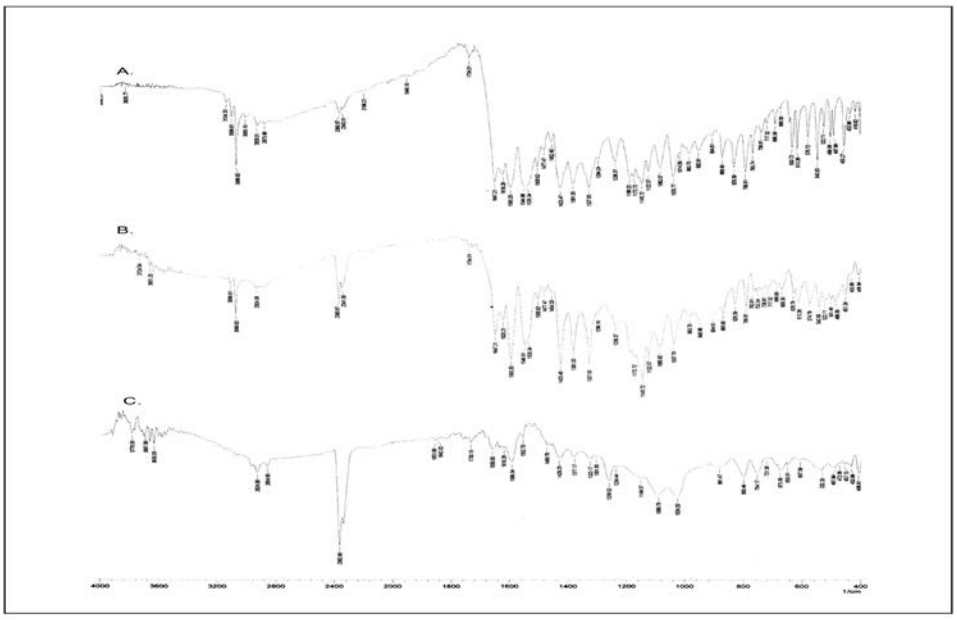

Figure 2: FT-IR Spectra of A. Pure drug lornoxicam, B. Physical Mixture, C. Drug loaded microspheres.

XRD studies:

The XRD patterns of lornoxicam, its physical mixture and drug loaded microspheres are shown in Fig: 3. The X-Ray powder diffractogram of pure drug exhibited a series of intense peaks which is indicative of its crystalline nature. The typical crystalline peaks of the drug in the physical mixture were clearly visible without any significant changes in the positions and relative intensities, thereby ruling out any interaction between the drug and the excipients. However, the diffractogram of the lornoxicam loaded microspheres showed peaks of diminished intensities indicating that the drug is molecularly dispersed in the polymeric matrix or might have undergone amorphization during the microsphere preparation[20].

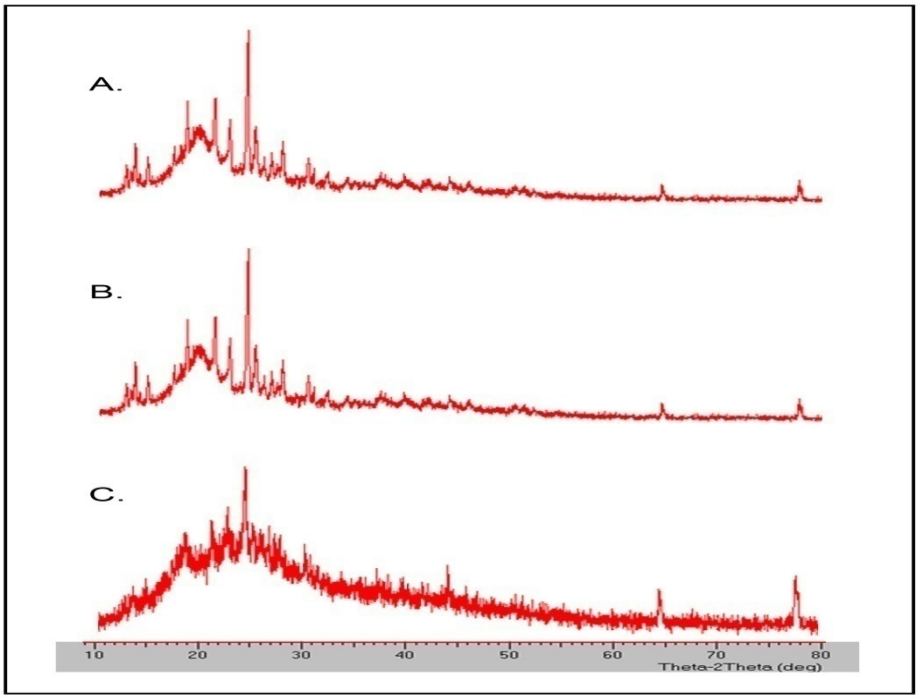

Figure 3: XRD Patterns of A. Pure drug lornoxicam, B. Physical Mixture, C. Drug loaded microspheres.

DSC Studies:

Differential Scanning Calorimetric analysis was also performed in order to establish the identity and integrity of drug in its pure form, physical mixture and also in the microsphere formulation. The thermogram of the pure drug lornoxicam showed an exothermic peak exhibiting its sharp melting point at 231.8 ˚C which corresponds to its reported range[22]. The thermogram of the physical mixture revealed the existence of the lornoxicam exothermic peak at 228.6 ˚C indicating the absence of interaction between the drug and the excipients. However, noticeable broadening of the peak intensity was observed which could be due to the presence of the formulation excipients. The thermogram of drug loaded microspheres revealed the disappearance of the characteristic exothermic peak of lornoxicam. This suggests that, the drug was molecularly dispersed in the polymeric matrix of the microspheres or existed in an amorphous form. This fact was also well supported by our XRD studies. From the study, it can be concluded that there is no interaction of the drug with the polymer and other excipients used in the formulation.

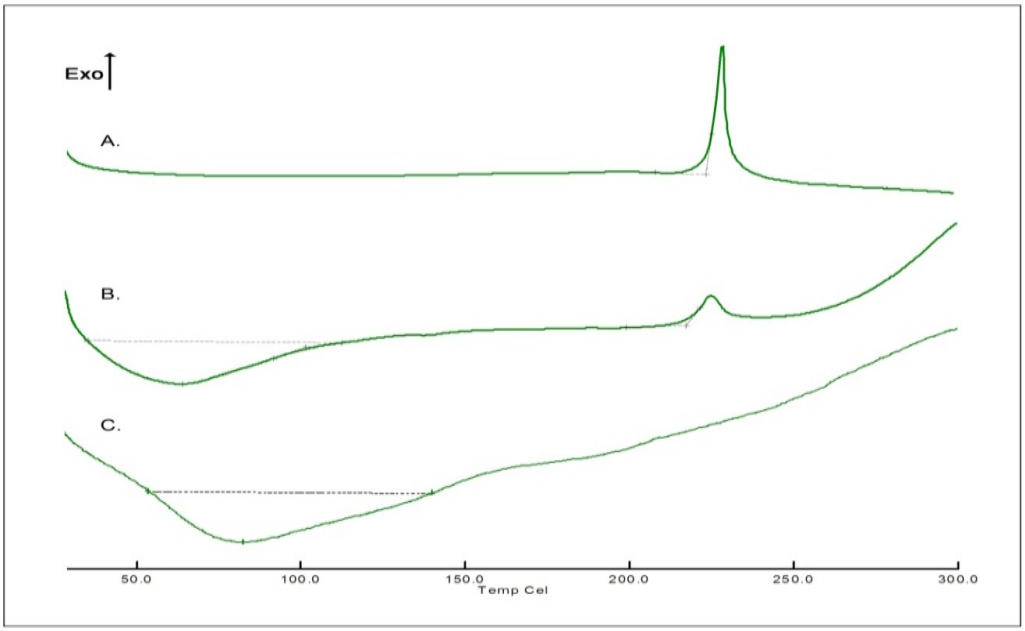

Figure 4: DSC Thermograms of A. Pure drug lornoxicam, B. Physical Mixture, C. Drug loaded microspheres.

In vitro drug release studies:

Drug release from the TPP cross-linked chitosan microspheres was largely influenced by formulation (core: coat ratio, TPP concentration) and process (pH of the dispersion medium, reaction time) variables. The plots of cumulative percentage drug release versus time for different formulations are shown in Fig No 5 and 6. Initially, the microspheres prepared with various core: coat ratios and at fixed TPP concentration (2%w/v), pH of the dispersion medium (original pH 8.5) and reaction time (30 min) were subjected for dissolution studies. Dissolution studies revealed the decrease in drug release with increasing core: coat ratio in the microspheres. This could be due to more efficient entanglement of the drug within the polymeric matrix of the microspheres composed of higher levels of coat material.

The results of the dissolution study of the microspheres prepared at different pH conditions revealed that, as the pH of the dispersion medium was lowered from its original pH 8.5 to pH 2.0, the drug release from the microspheres drastically reduced. On the other hand, microspheres prepared at higher pH exhibited faster drug release. This could be due to the fact that, at lower pH, ionization degree of TPP and chitosan are high and complete cross-linking occurs without deprotonation[7,] thereby leading to more controlled drug release. The drug release from the microspheres proportionately decreased with increase in cross-linking agent concentration and duration of cross-linking. This could be due to more cross-linking of the polymeric chains with increased TPP concentration and also increased duration of microsphere exposure to the coagulant medium would provide more reaction time which leads to more efficient cross-linking and thus reducing the drug release. Overall, the results of dissolution studies revealed that, drug release from the microspheres can be efficiently tailored by altering the formulation and process parameters.

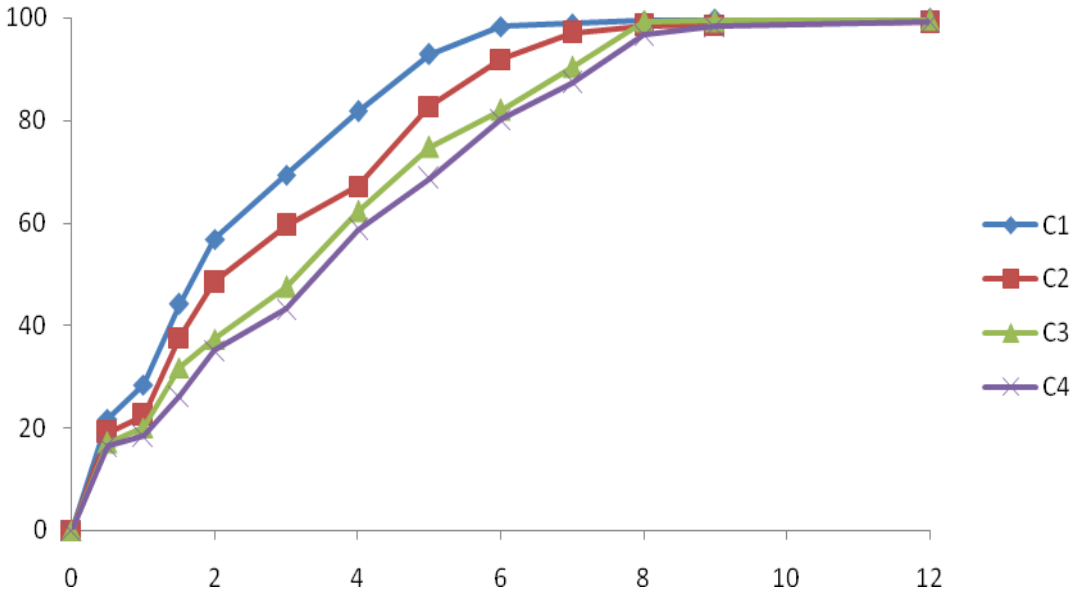

Figure 5: Effect of core: coat ratio on in vitro release of lornoxicam from TPP cross-linked chitosan microspheres.

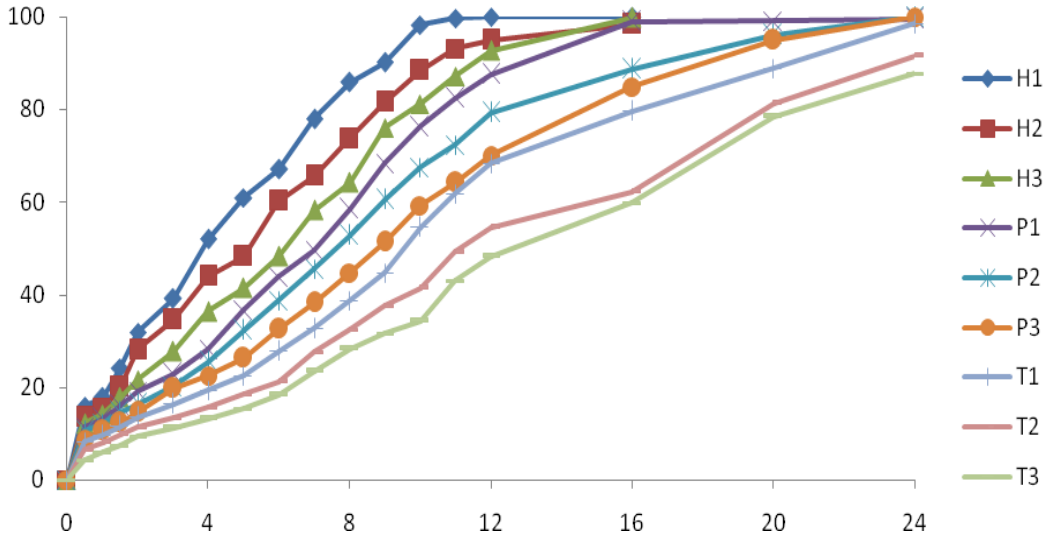

Figure 6: Effect of fabrication conditions on in vitro release of lornoxicam from TPP cross-linked chitosan microspheres.

Mechanism of drug release:

The drug release mechanism was studied with respect to zero order, first order, Higuchi model and Korsemayer and Peppas models. The coefficient values were found to be higher for Higuchi model (0.9952-0.9666) than Peppas model (0.9899-0.8903) followed by first order (0.9668-0.8517) and zero order (0.9907-0.8848) which is an indicative of diffusion controlled drug release. The n values of Peppas equation were in the range of 0.4031-0.8938 suggesting the anomalous (non-Fickian) release mechanism.

CONCLUSION:

The TPP cross-linked chitosan microspheres were found to be discrete, nearly spherical with rough surface characteristics. Particle size increased with higher polymeric levels in the microspheres where as it decreased with enhanced cross-linking conditions in the acidic medium. Encapsulation efficiency was found to be higher in the microspheres prepared in the acidic medium compared to those prepared in the original pH of the TPP solution. Microspheres prepared in the acidic conditions and with increased TPP concentration and cross-linking time showed more controlled drug release compared to those prepared in the higher pH conditions.

Overall, the study demonstrated that, physicochemical characteristics of the microspheres largely depended on the formulation variables as well as fabrication conditions.

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