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Development and Evaluation of Ketoprofen Loaded Nanoparticles

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Soumen Pattanayek*[1], S.B.Puranik[2]

[1]*Teva Pharmaceuticals 1776 Aspen Ln Weston, FL, 33327*

[2]*Drishti Institute of Distance Learning, Bangalore, Karnataka, India.*

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ABSTRACT

The objective of the present study was to prepare ketoprofen loaded nanoparticles. Drugs with high molecular weight and high lipophilicity show poor permeation and absorption. To overcome this problem, the drug was formulated in the form of nanoparticles using chitosan as a polymer. The prepared drug-loaded nanoparticles were characterized by FT-IR, SEM and particle size analysis. FT-IR studies revealed that there was no drug and polymer interaction. The average particle size for optimized nanoparticle formulation (F4) was found to be 174±6 nm. SEM photograph showed that nanoparticles are roughly spherical in shape and free from cracks. The formulation F4 showed 44.1 % of encapsulation efficiency and 77.85 % of percentage yield. The drug release from in vitro studies was found to be 92.3% at the end of 24 h in pH 7.4 phosphate buffer.

INTRODUCTION:

Today, nanotechnology is found in a wide range of applications in the pharmaceutical industry [1]. Due to new advances in nanotechnology, it is now possible to produce drug nanoparticles that can be utilized in a variety of innovative ways [2].

Chitosan is a polysaccharide derived from naturally occurring chitin. Its unique properties make it attractive for many biomedical applications like controlled drug release, wound healing, nutrition supplements, water purification, removal of toxins, scaffolds for tissue engineering, and semipermeable membranes. Due to its pH-dependent solubility, it forms stable films on various surfaces under neutral and basic pH conditions. Its amine groups serve for the covalent attachment of biomolecules, and it can be co-deposited with other polymers or nanoparticles [3]. Chitosan is a more versatile form of this polysaccharide, which is the second most abundant natural polymer on earth after cellulose. An important application of chitosan is the development of drug delivery systems, with a regulated drug release rate and a reduced frequency of administration of the drug due to its gel-forming ability in low pH range [4]. The ionotropic gelation technique, as the most important technique for ionic cross-linking of chitosan with low molecular weight counterions, hydrophobic counterions, and high molecular weight ions, most importantly involves the use of sodium tripolyphosphate (TPP) [5-7].

Ketoprofen a potent non-steroidal anti-inflammatory (NSAID) drug that is often used for the treatment of acute and chronic arthritic conditions has pH-dependent solubility and permeability. Although ketoprofen is highly permeable through the stomach, its poor water solubility limits its entry into systemic circulation. During gastric emptying, ketoprofen enters the small intestine, where it cannot permeate through the membrane despite being solubilized. Ketoprofen is classified in the Biopharmaceutics Classification Scheme as a class II drug. Since dissolution is the rate-limiting step in drug absorption, the poor water solubility in oral forms of ketoprofen results in low bioavailability due to incomplete absorption. In addition to absorption difficulties, oral formulations of ketoprofen can cause gastric mucosal damage, which may result in ulceration and bleeding [8-10].

In the present work, an attempt was made to enhance the absorption and provide sustained release of drug by incorporating the drug in nanoparticles.

MATERIALS AND METHODS:

MATERIALS:

Ketoconazole was a gift sample from Emcure Pharmaceuticals Ltd., Pune. Chitosan, Poloxamer 188 and Sodium tripolyphosphate were procured from Sigma Aldrich, USA. Acetic acid was purchased from SD fine chemicals, India. Deionized water was obtained from Millipore filtration system, USA.

METHODS:

Preparation of nanoparticles [11-13]:

Nanoparticles were prepared by using chitosan (CS) as polymer and sodium tripolyphosphate (STPP) as a cross linking agent by ionic gelation method, where the positively charged amino group in the CS interacts with negatively charged STPP to form coacervates with a size in the range of nanometer. Initially, various concentrations of chitosan solution in 1% acetic acid was prepared and pH was adjusted to 4. To the chitosan solution, drug solution was added slowly under constant stirring. 1.5 % w/v poloxamer 188 was added as stabilizer. After 15 mins, 0.25% w/v STPP solution was added drop by drop slowly under magnetic stirring (Remi, India) at the constant speed of 1500 rpm for 2 h and finally subjected to high-speed homogenizer (SB1, Thomas Scientific, USA) at 25,000 rpm for 1 h. The suspension of nanoparticles was then centrifuged at 10,000 rpm at 4 °C for 20 min and the separated nanoparticles were resuspended in purified water and then freeze-dried (Bioline Technologies, Thane, India) using 5% mannitol as cryoprotectant. The various formulations developed are shown in table 1.

Table 1: Formulations of ketoprofen nanoparticles

Formulation code	Chitosan (% w/v)	STPP (% w/v)	Drug (% w/v)	Poloxamer 188 (% w/v)
F1	0.1	0.25	0.2	1.5
F2	0.2	0.25	0.2	1.5
F3	0.3	0.25	0.2	1.5
F4	0.4	0.25	0.2	1.5
F5	0.5	0.25	0.2	1.5
F6	0.6	0.25	0.2	1.5
F7	0.7	0.25	0.2	1.5
F8	0.8	0.25	0.2	1.5

Particle size and PDI measurement

The particle size, size distribution [polydispersity index (PDI)] of particles were measured by Zetasizer (Malvern Instruments, UK), based on the dynamic light scattering (DLS) technique. The dispersion homogeneity of the synthesized nanoparticles is the measure of PDI that ranges from 0 to 1. The homogeneity is indicated by values closer to 0 (zero) and heterogeneity is indicated by values greater than 0.5. All samples were diluted with ultra-purified water & measured at 25°C and 90° scattering angle, recorded for 180s in triplicate.

Surface charge (Zeta potential)

Zeta potential of nanoparticles was characterized using a Zetasizer (Malvern Instruments ltd., Malvern UK). The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples (with ultra-purified water) in the capillary measurement cell. The stability of the synthesized nanoparticle was measured by detecting the surface charge at a scattering angle of 90 degrees at 25°C after dissolving the particles in distilled water.

Compatibility studies

In the nanoparticles, a drug is in intimate contact with one or more excipients, which could affect the stability of the drug. The knowledge of drug excipients interactions is therefore

essential for selecting appropriate excipients. This was studied using FT-IR spectrophotometry and differential scanning calorimetry (DSC).

Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectrophotometer Shimadzu-8400S, Japan was used to study FT-IR spectra by KBr pellet method. The individual drug and the final formulation containing excipients were selected and scanned separately. Both the spectra were compared for confirmation of common peaks.

Transmission electron microscopy (TEM)

The sample for TEM was prepared by placing drops of nanoparticle suspension over carbon coated grid and allowing the solvent to evaporate. TEM was performed by JOEL Model 1200 FX instrument operating at 80 K voltage for the characterization of size and shape of nanoparticles. The photographs were observed for morphological characteristics.

Percentage yield [14]

The yield was determined by weighing the nanoparticles and then finding out the percentage yield with respect to the weight of the input materials, i.e., drug, a weight of polymers and crosslinking agent used. The percentage yield was calculated by the following equation.

$$\% \text{ yield} = \frac{\text{weight of nanoparticles}}{\text{weight of polymers + weight of the drug}} \times 100$$

Percentage Encapsulation Efficiency (%EE) [15]

The drug-loaded nanoparticles were centrifuged at a high speed of 15000 rpm for 30 min and the supernatant was analyzed for drug spectrophotometrically at 254 nm. The % encapsulation efficiency drug was calculated by using the below equation.

$$\%EE = \frac{\text{Total amount of drug – Free drug present in supernatant}}{\text{Total amount of Ketoprofen}} \times 100$$

In vitro drug release study

In vitro drug release study of ketoprofen loaded nanoparticles was carried out in diffusion cell apparatus (Electrolab, India) in phosphate buffer pH 7.4. At predetermined time intervals, the samples were withdrawn and replaced with fresh medium and the absorbance was measured spectrophotometrically at 254 nm. Data obtained from the in vitro drug release for formulation were fitted to various kinetic models. Each experiment was performed in triplicate [16].

RESULTS AND DISCUSSION:

Preparation of nanoparticles:

Drug-loaded chitosan nanoparticles were successfully prepared using ionotropic gelation method. In nanoparticle preparation, chitosan was cross-linked with sodium tripolyphosphate by ionic linkage. It is worth noting that chitosan has a high density of amine groups in its backbone and the amine groups are protonized to form -$NH3^+$ in acidic solution. These positively charged groups in chitosan get physically cross-linked with multivalent anions derived from sodium tripolyphosphate (STPP) as shown in figure 1.

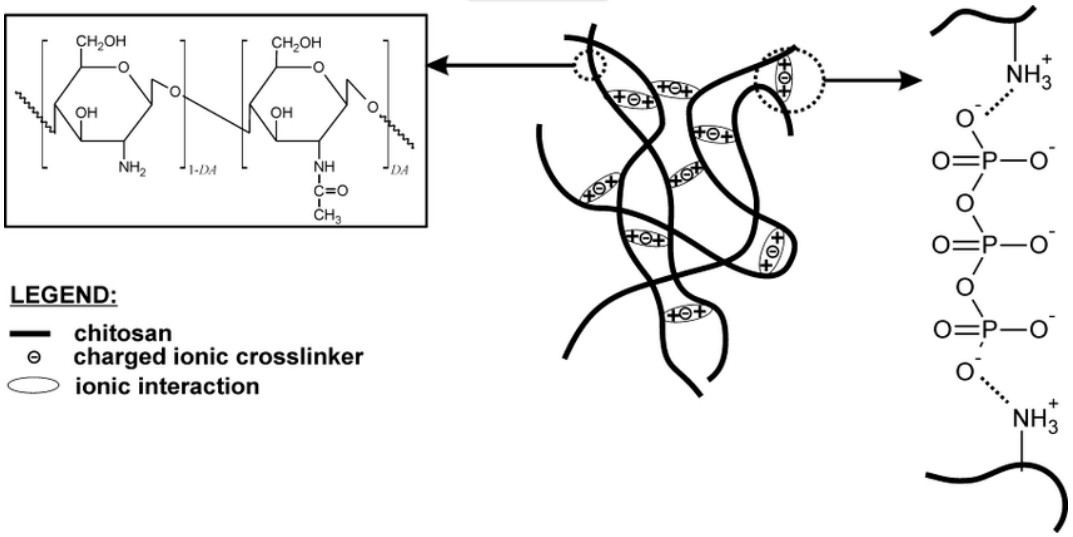

Fig. 1: Chemical structure of chitosan and TPP-crosslinked chitosan [17]

Particle size and PDI measurement

It was observed that the particle size of the nanoparticles was dependent on the concentration of chitosan employed. The particle sizes for the formulations are provided in figure 2. The

lowest particle size was shown by formulation F4 with 0.4% of Chitosan. Theoretically, lesser concentration than 0.4% should have resulted in lesser particle size but concentrations below 0.4 % of chitosan showed higher particle size. This may be attributed to the presence of an excess of anions resulting in a decrease in zeta potential, leading to flocculation that caused larger particle size. When concentration increased beyond 0.4% of chitosan, it resulted in the formation of large size nanoparticles. This increase in size may be due to insufficient sodium tripolyphosphate responsible for poor gelation of chitosan solution [17].

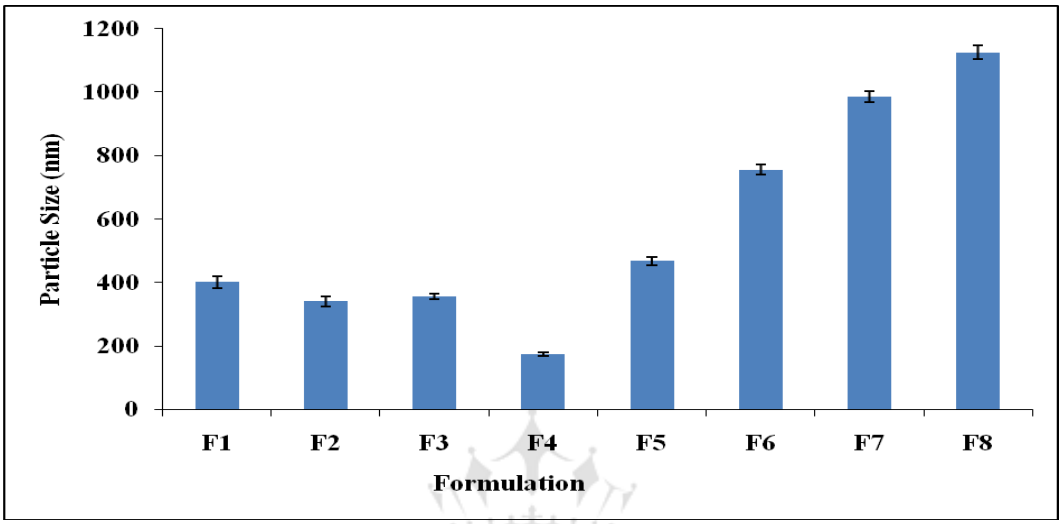

Fig. 2: Particle sizes for various formulations of nanoparticles (mean ± standard deviation, n=3)

The polydispersity index (PDI) is a measure of the width of the dispersion of particles. The polydispersity index values should between 0 to 1. If PDI value is less it indicates the narrow range distribution of particles and if PDI value is more the distribution of particles is in the wide range. Homogeneous dispersion has PDI value close to zero while PDI values greater than 0.3 suggest heterogeneous dispersion. The PDI values for all the formulations are given in Figure 3. All the prepared formulations showed PDI values between 0.21 to 0.68. The Formulations F3 and F4 showed homogenous dispersion whereas all the other six formulations showed heterogeneous dispersion [18].

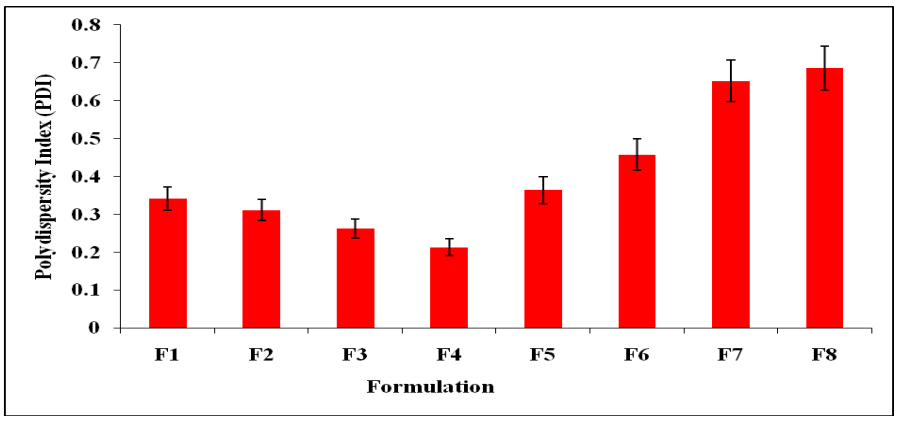

Fig. 3: PDI values for various formulations of nanoparticles (mean ± standard deviation, n=3)

Surface charge (Zeta potential)

The zeta potential value indicates the stability of prepared nanoparticles. The separating line between stable and unstable suspensions is generally taken at either +30mV or - 30mV. Nanoparticles with zeta potentials more positive than +30mV or more negative than -30mV are normally considered as stable. The zeta potential values for prepared formulations are graphically represented in figure 4. The zeta potential for the developed formulations increased with increase in the concentration of chitosan. This may be attributed to more availability of cations with increasing concentrations of chitosan, which in turn increases the zeta potential. Zeta potential of the developed formulations was in the range of +26 mV to + 31 mV, The higher zeta potential value indicates that chitosan nanoparticles were more stable [19].

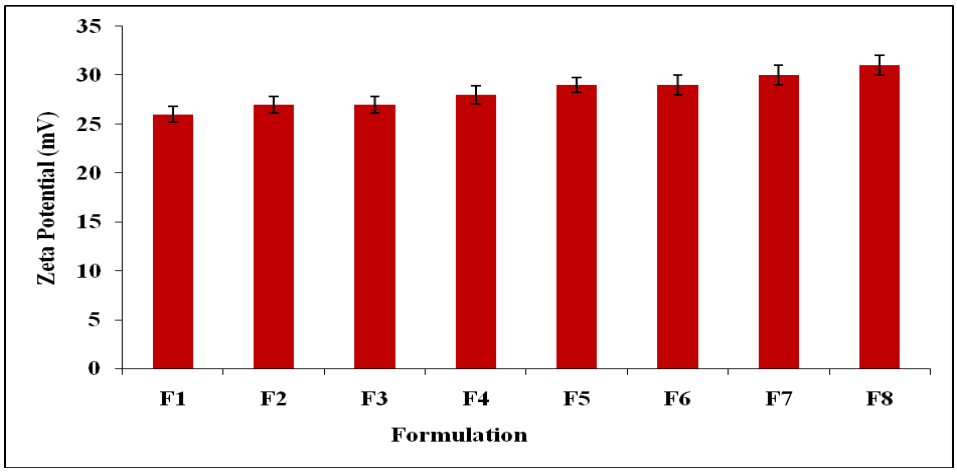

Fig. 4: Zeta potential for various formulations of nanoparticles (mean ± standard deviation, n=3)

Fourier-transform infrared spectroscopy (FT-IR)

Compatibility between the drugs, polymers and other excipients used were studied by using FT-IR spectroscopy. The drug, Verapamil HCl exhibited general characteristic peaks.

The characteristic peaks of pure drugs were compared with the peaks obtained for their respective formulations. From the FT-IR peaks, it can be concluded that the peaks of pure drug and formulations were found to be similar indicating that there was no significant interaction between drug and polymer used. The obtained spectra are given in figure 5**.**

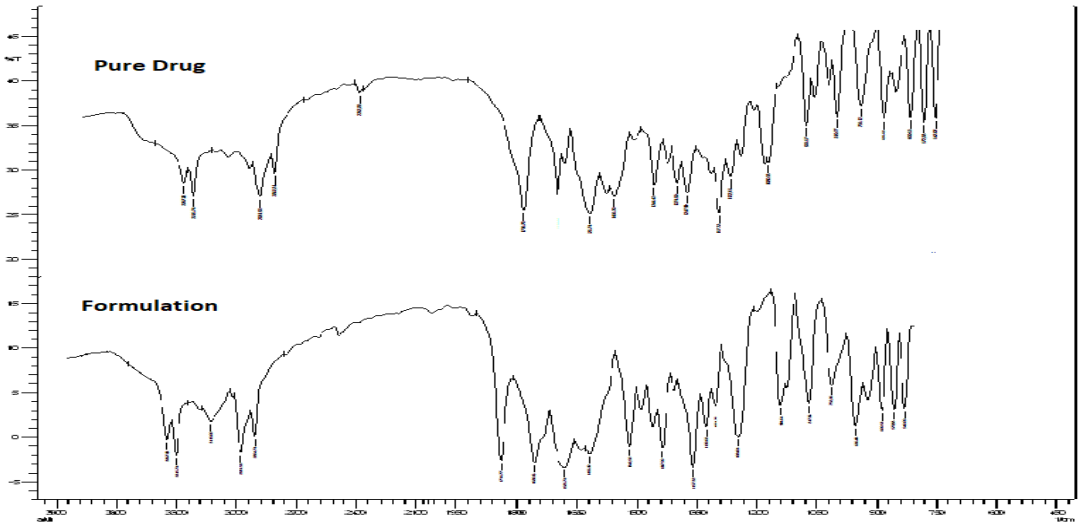

Fig.5: FT-IR spectra of pure drug and formulation F4.

Transmission Electron Microscopy (SEM)

The transmission electron microscopy (SEM) studies were carried out to study the morphology of the nanoparticles and the obtained microphotograph is presented in Figure 6. The TEM photograph showed that the nanoparticles were in nano size. The particles were found to be discreet and spherical in nature. The surface topology showed the presence of particles free from cracks. TEM photographs reveal the absence of drug particles on the surface of nanoparticles showing the uniform distribution of the drug inside the nanoparticles.

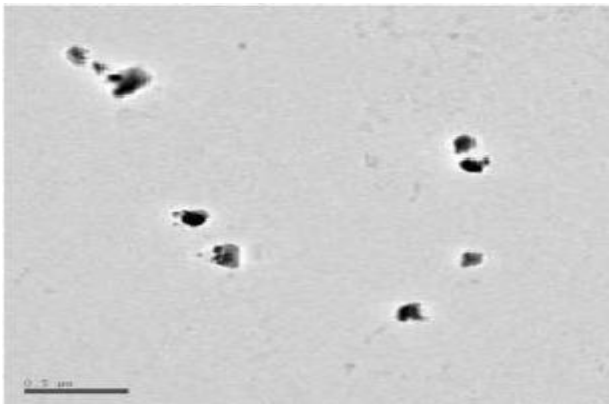

Fig.6: TEM photograph of nanoparticle formulation F4.

Percentage Yield

During the process of nanoencapsulation, the mechanical variables cause loss of final product and hence process yield may not be 100%. Nanoparticles were weighed after freeze-drying and the percentage yield was calculated. The % yield was found to be 73.6% to 76.9% as represented in table 2.

Percentage Encapsulation Efficiency (%EE)

The encapsulation efficiency was found to increase with an increase in the polymer concentration. During the preparation of nanoparticles, encapsulation efficiency is mainly affected by the polymer and the drug ratios. The increase entrapment efficiency may be due to the greater proportion of polymer with respect to the amount of drug. The % Encapsulation efficiency was found to be in the range of 41.3% to 46.23 % for the prepared formulations. The results obtained are given in table 2.

Table 2: Percentage yield and Encapsulation efficiency (%) of formulations F1 to F8

Formulation code	Percentage Yield (mean±SD*)	Percentage Encapsulation Efficiency (mean±SD*)
F1	74.6±1.05	41.3±0.23
F2	74.8±1.03	42.1±0.18
F3	76.4±1.22	43.2±0.21
F4	77.85±1.10	44.1±0.21
F5	74.23±0.86	44.4±0.20
F6	75.6±1.33	44.9±0.23
F7	76.5±1.13	45.1±0.20
F8	74.2±1.20	46.23±0.32

* Standard deviation, n=3

***In vitro* drug release study**

The *in vitro* release studies were carried out for all formulations in pH 7.4 phosphate buffer. The *in vitro* release graph for ketoprofen from nanoparticles is graphically represented in figure 7. The formulation F4 showed the highest amount of drug release 92.3% and F8 showed the least amount of drug release 58.5 % at the end of 24 hrs. The polymer concentration was found to affect the drug entrapment, particle size and ultimately the drug release characteristics of the prepared nanoparticles. It was also observed that size of nanoparticles affected the drug release from the formulations. From the release data, it was observed that formulations with less particle size showed highest drug release This may be due to drug-loaded nanoparticles with larger size are not completely diffused through the surface into the diffusion medium. The formulation F4 was found to be the best formulation [16, 20].

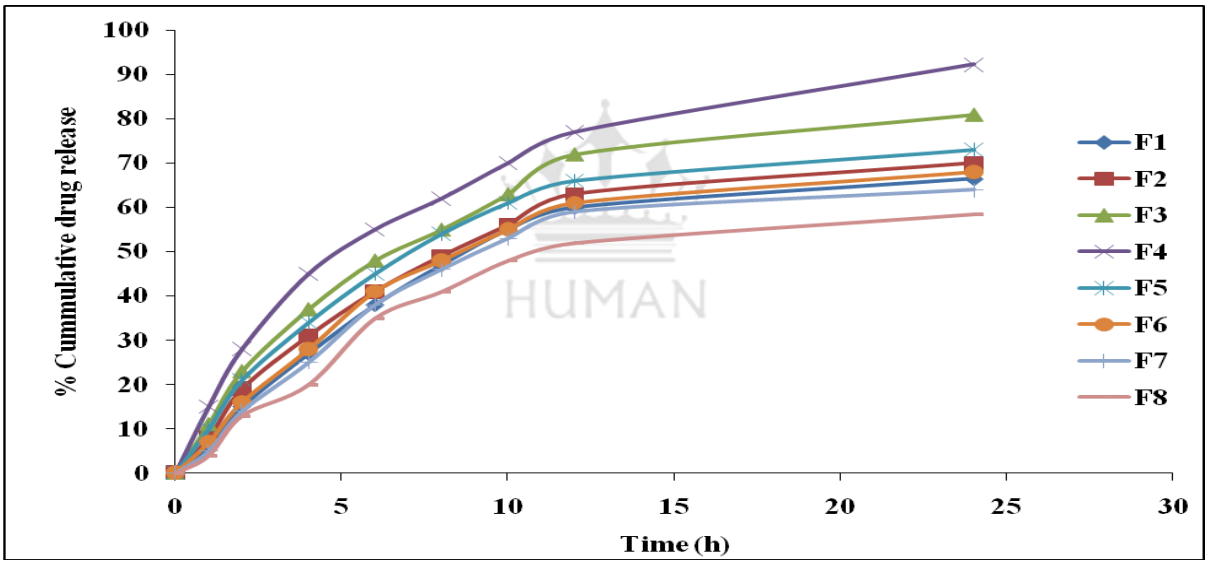

Fig.7: *In-vitro* release ketoprofen from formulations F1 - F8 (mean ± standard deviation, n=3)

CONCLUSION:

In conclusion, an efficient method using the ionic gelation technique for the development of cross-linked chitosan nanoparticles has been described in the present investigation. The synthesized nanoparticle was characterized by FTIR, TEM, Particle size, Zeta potential, and polydispersity index. The synthesized nanoparticles were found to be spherical in shape with a smooth surface. The zeta potential results indicated stable nanoparticles and the

homogeneous distribution at low concentrations as proved by polydispersity index. All the formulations showed excellent homogeneity without any aggregates and fibers. The in vitro drug release of formulations showed sustained release. From the results of the experimental work carried out, it can be concluded that the ketoprofen loaded nanoparticles can be employed for effective sustained delivery of lipophilic drugs.

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