Polymeric Nanoparticles of Ketoprofen: Formulation and Characterization

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Abstract: **In the present study polymeric nanoparticles of Ketoprofen were developed using sodium alginate and Chitosan. The nanoparticles were prepared by ionotropic gelation method. The effect of variables like polymer concentration (sodium alginate, chitosan), surfactant (Tween 80) concentration and cross-linking agent (glutaraldehyde) was studied. The influence of the order of addition of chitosan & calcium chloride on particle size, polydispersity index was also investigated. Ketoprofen loaded alginate Nanoparticles displayed a particle size in the range of approximately 219-466 nm. Glutaraldehyde, cross-linking agent was found to have no significant effect on particle size. The entrapment efficiency (EE) of the nanoparticles increased with increase in concentration of polymers (Sodium alginate, Chitosan) while loading capacity (LC) decreased. Glutaraldehyde was found to increase both EE and LC. The result indicated that drug loaded nanoparticles may be effective for sustained delivery of Ketoprofen.**

Key-words: **Nanoparticles, Ionotropic Gelation, Ketoprofen, Sodium Alginate.**

I. INTRODUCTION

Particulate systems like nanoparticles have been used as an important approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules[1]. Nanoparticles are defined as particulate dispersions or solid, sub-micron, colloidal particles with a size in the range of 1-1000nm. The drug is dissolved, entrapped, encapsulated or covalently attached to a nanoparticle matrix. Depending upon the method of preparation, nanospheres or nanocapsules can be obtained. Nanospheres (monolithic/matrix device) are matrix systems in which the drug is physically and uniformly dispersed while nanocapsules(reservoir device) are vesicular systems in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a unique polymeric membrane. In this case the active substances are usually dissolved in the inner core but may also be adsorbed to the capsule surface[2]. Depending on the type of material or carrier used, four broad classes of nanoparticles are recognized:

- Polymeric nanoparticles[3]
  - Lipid based nanoparticles[4,5]
  - Metal (gold, silver and platinum) based nanoparticles[6]
- Biological nanoparticles in which virus based nanoparticles developed for tissuespecific targeting and imaging agent in vivo[7]

The polymers used for the manufacturing of nanoparticles can be classified broadly into two categories[3]:

• Natural hydrophilic polymers which include proteins and polysaccharides
• Synthetic hydrophobic polymers

A. Methods of Preparation of Nanoparticles

To prepare NPs, several methods have been developed. Out of those, most frequently used methods are[1]:

• Dispersion of preformed polymers which includes
- Solvent evaporation method
- Solvent diffusion method
• Polymerization of monomers.
• Ionic gelation or coacervation of hydrophilic polymers.

1. Ionic Gelation Method

Nanoparticles obtained from ionic gelation procedure are synthesized in totally aqueous media. Ionic nanogels can be obtained from aqueous solutions of charged polysaccharides which gel in the presence of small ions of opposite charges. The gelation of the polysaccharide should be performed in very dilute solution using concentrations of the gelling agent below the gel point. This corresponds to the pre-gel phase in which the chains of the polymer reacting with the gelling agent are forming small clusters that can be highlighted by electron microscopy or by a clear reduction of the viscosity of the polysaccharide solution. Clusters formed in the pre-gel phase are stabilized by forming complex with opposite charged polyelectrolytes. Using alginate, the gelation is

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induced with calcium and the pre-gel phase is then stabilized with polycations like polylysine and chitosan[8,9].

B. Separation and Purification Techniques of Nanoparticles

Depending on the method of preparation, potentially toxic impurities can be present in the nanoparticulate suspensions. These impurities are organic solvents, surfactants, residual monomers, polymerization initiators and large polymer aggregates. Separation of the drug entrapped nanoparticles from free polymer and unentrapped drugs is a very critical step in producing pure nanoparticles. The separation can be achieved by: Magnetic fields, chromatography, density radiant centrifugation, electrophoresis, selective precipitation, membrane filteration[10] and Diafiltration[11].

C. Drug Loading of Nanoparticles

Drug loading can be done by two methods[1]:

- Incorporating at the time of nanoparticles production (incorporation method).
- Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption /absorption technique).

D. Drug Release from Nanoparticles

The release mechanisms, the diffusion coefficient and biodegradation rate are the key factors governing the release rate. Drug from NPs is released by one or more of the following mechanisms[12]:

• Desorption of surface- bound drug.
• Diffusion through matrix or the polymer well.
• Erosion.
• Combined erosion-diffusion process.

There are several factors that affect the release rate of the entrapped drug. Larger particles have a smaller initial burst release and longer sustained release than smaller particles. In addition the greater is the drug loading, the greater the burst and the faster the release rate.

II. MATERIALS AND METHODS

Ketoprofen was obtained as gift sample from Neon Laboratories Ltd., Mumbai. Sodium Alginate was obtained from Titan Biotech Limited, Bhiwadi. Chitosan was purchased from HIMEDIA. Calcium Chloride and Mannitol was purchased from SD Fine-Chem Ltd, Mumbai. Gluteraldehyde Solution (25%), Potassium Dihydrogen Orthophosphate, Anhydrous and Sodium Hydroxide Pellets were purchased from High Purity Laboratory Chemicals, Mumbai. Acetic Acid Glacial Extrapure and Carrageenan were purchased from SD Fine-Chem Ltd, Mumbai. All other chemicals used were of high grade.

A. Preparation of the Nanoparticles (Nps)

1. Preparation of Blank Sodium Alginate Nanoparticles

The method used to prepare the nanoparticles was a two-step method. Aqueous calciumchloride (2 ml of 30 mM) was added to 10 ml of aqueous sodium alginate (0.1-0.5 %) whilestirring for 30 minutes and then 4 ml of chitosan solution (0.04-0.1 %) was added into theresultant calcium alginate pre-gel and stirred for additional 1.5 hour. The resultant opalescentsuspension was equilibrated overnight to allow nanoparticles to form uniform particle size.After that nanoparticles were separated by two cycle of centrifugation (11000 rpm, 45minutes, 4ºC) by cooling centrifuge. Then the pellets ware washed using distilled water. The pellets were redispersed in 10-20 ml distilled water and mannitol was added as acryoprotectant. Then the nanoparticle suspension was freeze dried and characterized.

2. Preparation of drug loaded sodium alginate nanoparticles

The drug was dispersed into sodium alginate aqueous solution and then it was processed asgiven above for preparation of blank sodium alginate nanoparticles.

TABLE 1
DIFFERENT BATCHES OF NANOPARTICLES

Formulation	Drug(mg)	Sodium Alginate(%)	Chitosan (%)	Calcium Chloride(mM)	Tween 80 %	Gluteraldehyde(%)
S1	300	0.1	0.08	30	-	-
S2	300	0.2	0.08	30	-	-
S3	300	0.3	0.08	30	-	-
S4	300	0.5	0.08	30	-	-
S5	300	0.3	0.08	30	-	-
S6	300	0.3	0.04	30	-	-
S7	300	0.3	0.06	30	-	-
S8	300	0.3	0.1	30	-	-
S9	300	0.3	0.08	30	0.1	-
S10	300	0.3	0.08	30	0.3	-
S11	300	0.3	0.08	30	0.5	-
S12	300	0.3	0.08	30	-	2

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B. Characterization of Nanoparticles

1. Determination of particles size and size distribution of NPs

The size of nanoparticles and polydispersity index were determined by particle size analyser (Malvern Zetasizer).

2. Determination of Zeta Potential of NPs

Zeta potential of suitably diluted nanoparticle suspension was determined using zeta potential analyser based on electrophoretic light scattering and laser Doppler velocimetry method (ZetaplusTM, Brookhaven Instrument Corporation, New York, USA). The temperature was setat 25ºC.

3. Differential Scanning Calorimetory (DSC)

The physical state of drug inside the NPs was investigated by DSC. The thermogram of thedrug loaded NPs were obtained using DSC (TA instruments, Model no. Q10). For this, thesmall amount (2-7 mg) of sample was sealed in the aluminium pan and the temperature wasraised at 100C/min from 40 to 3000 C.

4. FT-IR (Fourier Transform Infrared Spectroscopy) Spectral Analysis

Infrared spectroscopy of the different formulations was studied to confirm the drug loading and drug-excipient interaction by KBr pellet method.

5. X-Ray Diffraction Studies (XRD)

In order to determine change in physical state of the drug in formulation, whether it is incrystalline or amorphous form the XRD studies were carried out.

6. Determination of Encapsulation Efficiency (EE) and Loading capacity (LC) of NPs

The suspension of nanoparticles was centrifuged at 11000 rpm for 45 minute at 4ºC. Theclear supernatant was analysed for free ketoprofen at 258 nm spectrophotometrically.

$$\text{EntrapmentEfficiency} = \frac{\mathbf{Totalamountofdrugadded - amountoffreedrug}}{\mathbf{Totalamountofdrugadded}} \times 100$$

$$\text{LoadingCapacity} = \frac{\mathbf{Totalamountofdrugadded - amountoffreedrug}}{\mathbf{Totalamountofdrugadded}} \times 100$$

III. RESULTS AND DISCUSSION

A. Characterization of Nanoparticles

1. Particle Size

The particle size is dependent upon the concentration of sodium alginate and chitosan. The particle size increased with increase in concentration of both polymers. This may be due to more cross-linking of sodium alginate and chitosan to form polyelectrolyte complex and make the bulk of nanoparticles matrix which cause the increase in size of nanoparticles. The particle size range of the sample was formed to lie between 219-466 nm.

As the concentration of the surfactant (Tween 80) was increased, the size of NPs decreased which could be due to solubilisation of drug (Ketoprofen) that is entrapped or encapsulated in NPs. The cross-linking agent (glutaraldehyde, 0.25%) was found to have no significant effect on size of nanoparticles. The influence of the order of addition of calcium chloride and chitosan on size of alginate nanoparticles was also studied. The observation indicated that the mechanism of nanoparticles formation was probably different when chitosan was first added to sodium alginate solution instead of calcium chloride. The size differences between two types of particles could be explained by the structure of the complexes that was formed either between calcium chloride and alginate or between chitosan and alginate. The interaction between calcium ions and alginate polymer occurred at the level of the oligopolyguluronic sequences. Furthermore, calcium ions induced a parallel packing of the oligopolyguluronic sequences to give egg-box structures which leads to formation of compact domains in the alginate molecules. The addition of chitosan allowed only strengthening of this system to obtain well-defined particles.

2. Polydispersity Index

PDI is a measure of homogeneity in dispersed systems and ranges from 0 to 1. Homogeneous dispersion has PDI value close to zero while PDI values greater than 0.3 suggest high heterogeneity. With increase in polymer concentration, polydispersity index was also found to increase. This could be explained on account of local aggregation of sodium alginate at higher concentrations.

3. Zeta Potential

Zeta potential of the nanoparticles was primarily affected by the chitosan and alginate concentration. Zeta potential describes the stability of NPs in suspension. The NPs having value of zeta potential below or above -30 to +30 are more stable in suspension. The prepared formulations have zeta potential nearly -30 mV as in Table 2 which indicate stability of nanoparticles in suspension.

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TABLE 2
PARTICLE SIZE, ZETA POTENTIAL AND POLYDISPERSITY INDEX OF NANOPARTICLES

Batch	Avg. Diameter (nm)	Polydispersity Index (P.I)	Zeta Potential (mV)
S1	292.1	0.202	-1.01
S2	328.9	0.289	-26.8
S3	397.4	0.206	-37.1
S4	417.5	0.659	-53.5
S5	402.6	0.734	----
S6	330.1	0.147	-26.2
S7	346.6	0.200	-26.8
S8	466.1	0.571	-24.5
S9	318.6	0.509	5.48
S10	276.2	0.401	-44.2
S11	219.4	0.417	-49.8
S12	411.4	0.476	-28.0

4. DSC (Differential Scanning Calorimetory)

Ketoprofen has the melting point of 95.39ºC. Blank nanoparticles were showing a peak at 167.73ºC which could be due to presence of mannitol. Drug loaded nanoparticles showed reduced intensity peak at 94.16ºC, that may be due to free drug adsorbed on the surface of nanoparticles and a peak at 167.73ºC which was due to presence of mannitol. The suppression of endothermic peak of Ketoprofen indicates that drug is in amorphous form rather than crystalline form. The absence of endothermic and exothermic peaks of sodium alginate and chitosan in nanoparticles could be result of a chemical reaction between both polymers. The DSC thermogram of blank and drug loaded NPs are shown in Fig. 1 & Fig. 2.

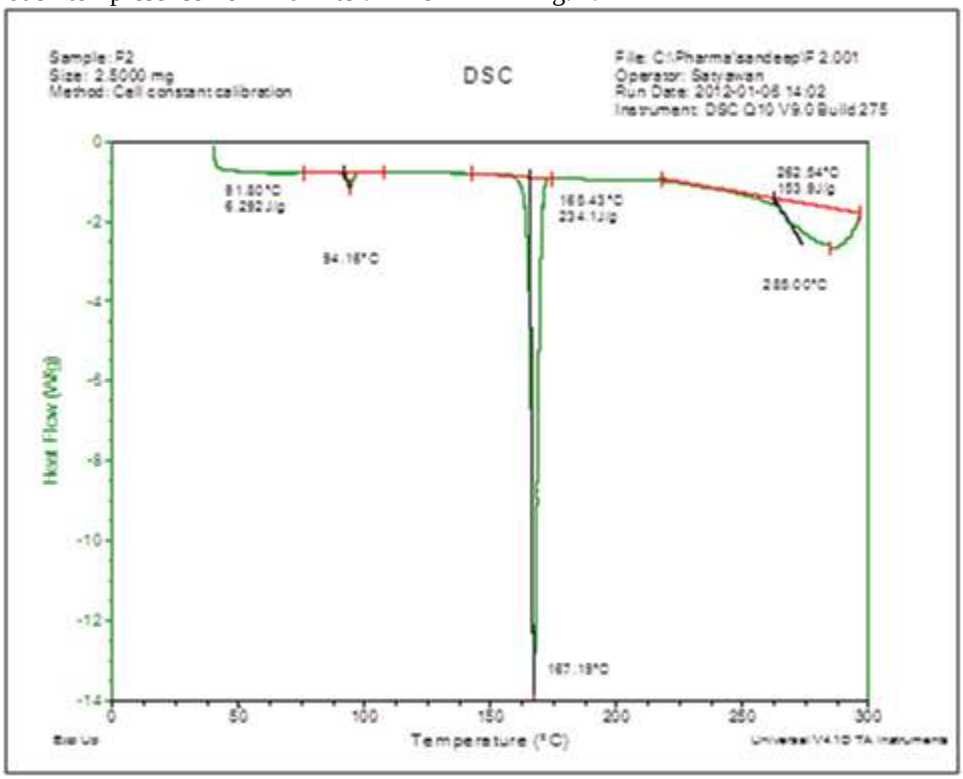

Fig. 1 DSC Thermogram of Blank Nanoparticles

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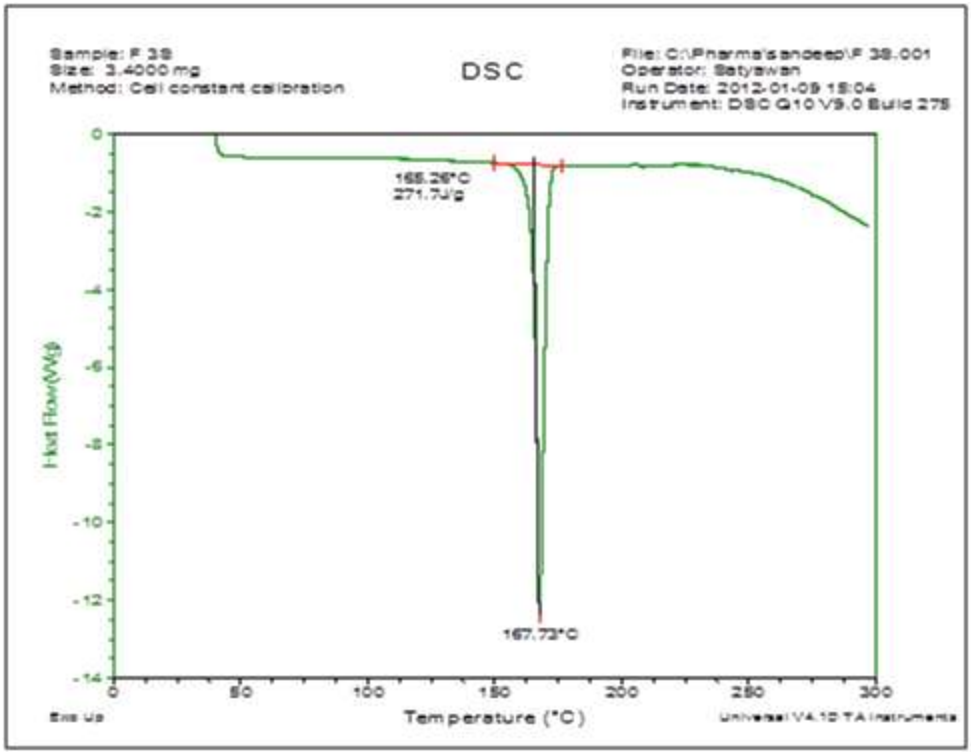

Fig. 2 DSC Thermogram of drug loaded NP.

5. FT-IR Spectral Analysis

FT-IR was used to confirm the incorporation of calcium alginate into chitosan matrix and loading of Ketoprofen in the prepared nanoparticles. The FT-IR spectra of blank (Fig. 3) and drug loaded nanoparticles (Fig. 4) were analysed to find out the interpretation.

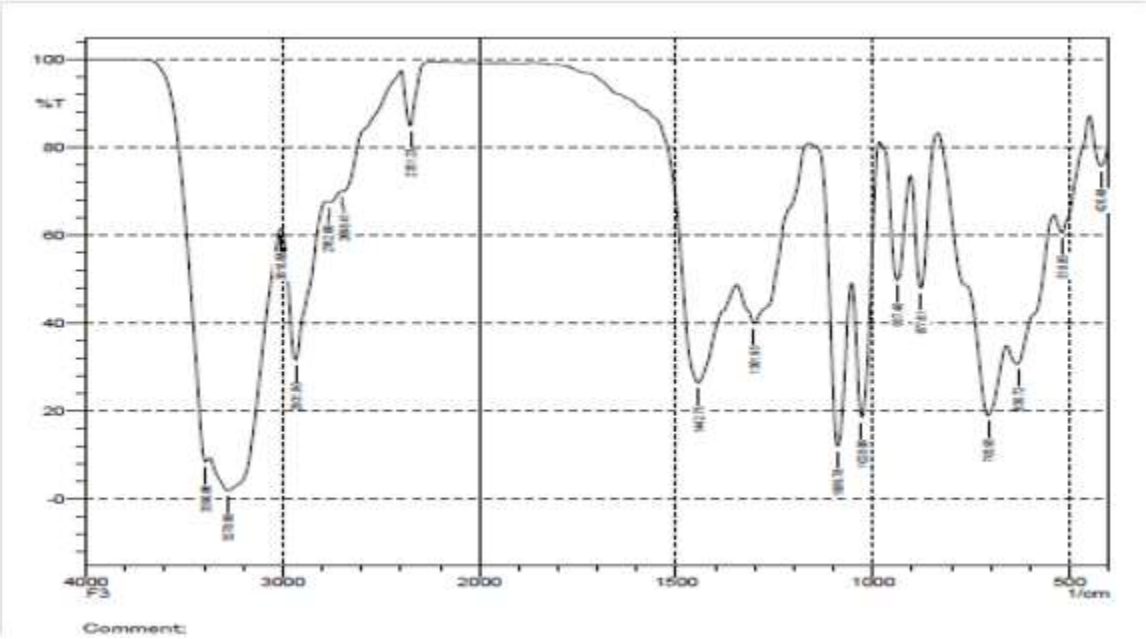

Fig.3 FT-IR Spectrum of Blank Nanoparticles.

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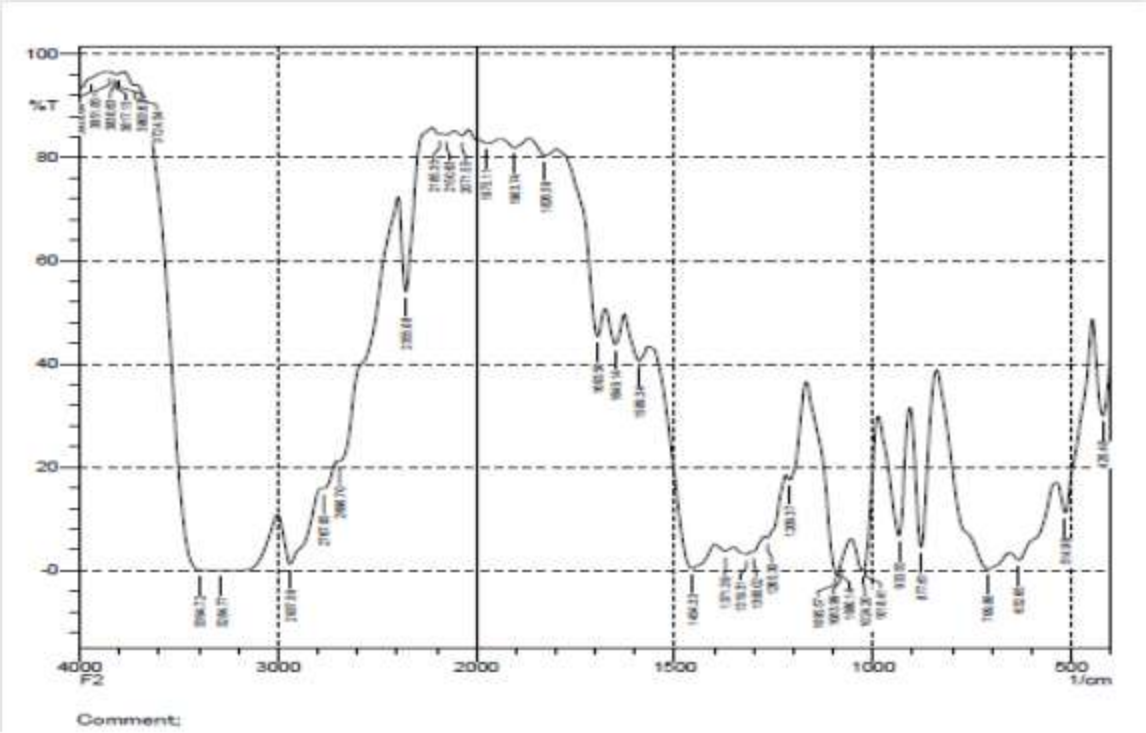

Fig. 4 FT-IR Spectrum of Drug Loaded NPs.

The broad and strong peaks at 3394 cm^{-1} indicated the molecular interaction between sodium alginate and chitosan. Peak at 1319 cm^{-1} indicated the presence of unreacted COOH group of sodium alginate. Peak at 1454 cm^{-1} showed the ionic interaction between NH_2 and COOH group. The peaks of ketoprofen were also present which indicated that there was no molecular interaction between drug and polymers.

6. Entrapment Efficiency (EE) and Loading Capacity (LC) of Nanoparticles

Drug entrapment efficiency (EE) and loading capacity of the prepared NPs were determined using the method described earlier. The EE, LC and percentage yield is given in Table 3.

TABLE 3
THE EE, LC AND PERCENTAGE YIELD OF NANOPARTICLES

Batch	Entrapment Efficiency	Loading Capacity	% Yield
S1	36.6	19.3	70.5
S2	47.7	18.8	71.5
S3	64.5	17.3	69
S4	68.9	14.8	49.6
S5	76.6	40.8	64.4
S6	52.4	17.8	72.5
S7	61.4	18.6	68.9
S8	70.3	13.2	83.9
S9	64.2	16.3	69.6
S10	69.22	15.9	77
S11	65.0	12.6	81.4
S12	74.6	22.7	68

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i) Effect of Sodium Alginate concentration on EE & LC

As evident in Fig. 5, the EE increases as the sodium alginate concentration (0.1-0.5%) increases. The increase in the concentration of sodium alginate enables the entrapment of large amount o f drug which leads to increased EE. As there was opposite effect of drug loading on EE, loading capacity decreased with increase in sodium alginate concentration.

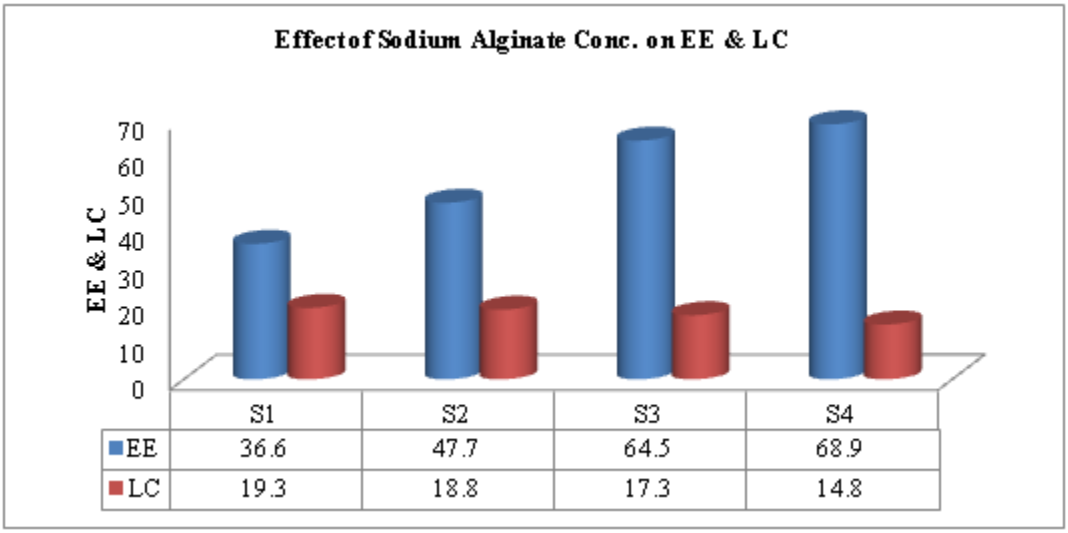

Fig. 5 Effect of Sodium Alginate Concentration on EE & LC of Ketoprofen loaded Nanoparticles

ii) Effect of Chitosan concentration on EE & LC

As shown in Fig. 6, the EE was found to increase with the increase in the chitosan concentration (0.04-0.1%). Increase in the concentration of chitosan leads to the presence of more molecules of chitosan to react with alginate to form polyelectrolyte complex resulting in greater drug entrapped and increase in EE. The loading capacity decreased with increase in chitosan concentration.

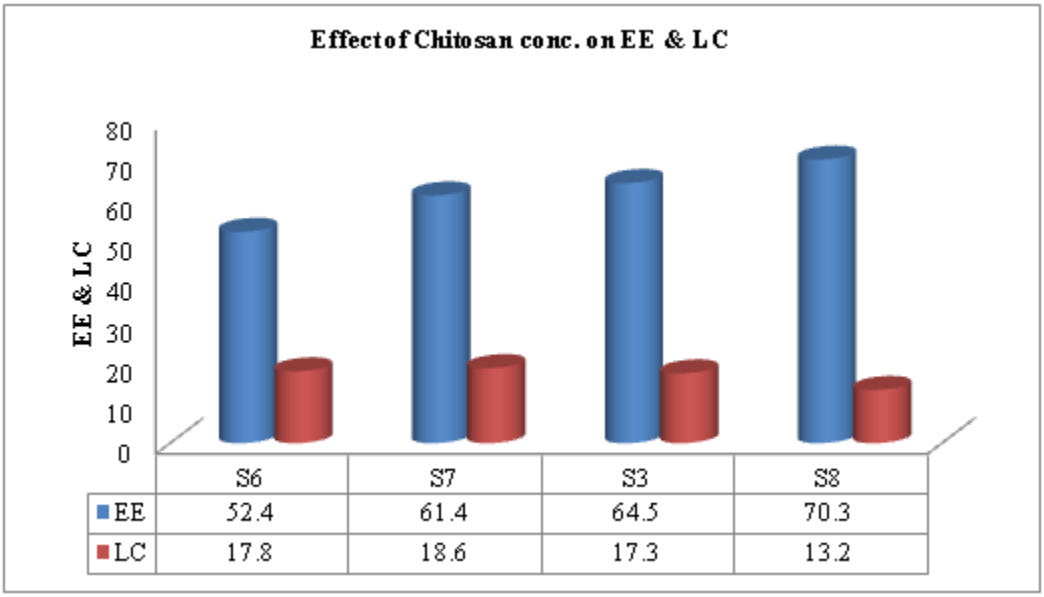

Fig. 6 Effect of Chitosan concentration on EE & LC

iii) Effect of Surfactant and Cross-linking agent on EE & LC

The surfactant (Tween 80) was used to solubilize the ketoprofen into sodium alginate solution. The entrapment efficiency increased with increase in surfactant concentration. This may be due to solubilisation of drug which caused more entrapment of drug into sodium alginate-chitosan complex. The loading capacity decreased with increase in surfactant concentration. Glutaraldehyde was used as cross-linking agent, the EE and loading capacity increased

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by using the cross-linking agent during preparation of nanoparticles.

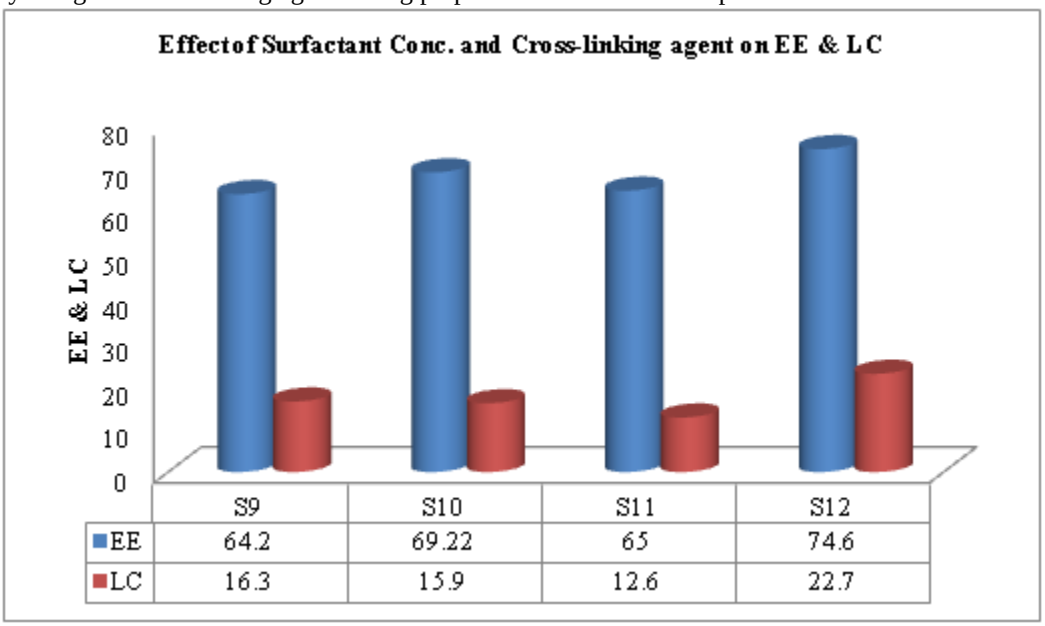

Fig. 7: Effect of Surfactant and Cross-linking agent on EE & LC

IV. CONCLUSION

From the present investigation it can be concluded thatas the sodium alginate concentration increased, the particle size of nanoparticles alsoincreased and slow down the drug release rate.The entrapment efficiency of nanoparticles increased with increase in sodium alginateand chitosan concentration.As the surfactant (Tween 80) concentration increased the drug entrapment efficiency ofthe formulation did not significantly increase. There was small change in entrapmentefficiency.There was burst release of Ketoprofen from nanoparticles initially which may be due toadsorbed drug on surface of NPs. after that drug released at a slower and sustained ratefrom the nanoparticles.

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