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Research Article

chitosan based Nanoparticle for better anti-inflammatory therapy

Formulation and Characterization of Chitosan Based Dexibuprofen Nanoparticles Using Ionotropic Gelation Method

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Abstract : Dexibuprofen is a pharmacologically active enantiomer of racemic ibuprofen (NSAID), which is used to treat pain and inflammation. Like common NSAIDs, Dexibuprofen is an active enantiomer of ibuprofen that suppresses the prostanoid synthesis in the inflammatory cells via inhibition of the COX-2 isoform of the arachidonic acid COX. The therapeutic use of Dexibuprofen is limited by the rapidity of the onset of its action and its short biological half-life. Hence, our aim was to develop Dexibuprofen nanoparticles formulation to overcome these disadvantages using optimized concentration of polymers by appropriate methods for nanoparticle preparation. The drug and the nanoparticle formulation of Dexibuprofen F11 were comparatively assessed for FT IR spectrums by using FT-IR method. The DSC study was used as one of the tool to assess the compatibility between drug and the excipients. As per DSC thermograms, the drug as well as drug with mixture of excipients chitosan, sodium tripolyphosphate had shown no interactions with dexibuprofen. The ionotropic gelation method was used to prepare Dexibuprofen nanoparticles. The chitosan and sodium tripolyphosphate (TPP) of different concentrations were used as polymers to prepare Dexibuprofen nanoparticles. Total eleven different formulations were explored with different concentrations of drug : polymer ratios using ionotropic gelation method to identify optimal concentrations of polymer. Among different formulations, F11 formulation with optimized concentration of 5% chitosan and 1% Sodium tripolyphosphate polymers along with Dexibuprofen showed maximum drug release. The objective was to evaluate the developed Dexibuprofen nanoparticles. In-vitro drug release was evaluated in 0.05M phosphate buffer pH7.2 and found that the drug release of F11 formulation of Dexibuprofen nanoparticle had shown release till 24 hours more than that of other trials. Hence, F11 formulation was considered as the optimized nanoparticle formulation to control drug release till 24 hours. The entrapment efficacy of the formulated Nanoparticles was found to be in the range of 75.48%-91.22% respectively.

Keywords: Dexibuprofen, chitosan, sodium TPP, FT-IR, ionotropic gelation method

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1. INTRODUCTION

Oral drug administration is the optimal approach for producing mutually systematic and local therapeutic results. However, there are a number of concerns with conventional oral delivery formulations, including the requirement to take them many times per day to maintain the prescribed medication dose within the therapeutically beneficial range, which results in a fluctuated drug level and, as a consequence, undesirable toxicity and inefficiency. In general, the oral Non Steroidal Anti Inflammatory Drugs have adverse events especially on gastro related issues. Dexibuprofen tablets had shown adverse events related to gastrointestinal issues like diarrhea, constipation, nausea, vomiting, abdominal pain etc., As a result, the principle of controlled drug delivery systems was implemented to alleviate the complications associated with conventional oral dosage types. The real difficulty in designing a controlled drug delivery system is not only controlling drug release, but rather extending the duration of the dosage form's persistence in the absorption site until all of the drug is delivered entirely within the expected time. Dexibuprofen was found to be a water insoluble drug. The researchers had explored different techniques to improve the solubility and the bioavailability of drugs. Development of a controlled drug delivery system to maximise the release of drug from the formulation for longer duration of time, was identified as one of the technique to improve bioavailability of drug as well as to minimize adverse events. To overcome the above-mentioned issues, Nanotechnology was found to be one of the appropriate drug delivery systems to control drug release as well as to improve bioavailability of poorly soluble drugs. There are various techniques followed by the researchers to develop Nanoparticle formulation like desolvation technique, dialysis technique, Nanoprecipitation technique, solvent evaporation technique, spray drying technique, ionotropic gelation technique, supercritical fluid technology etc., Among these techniques, ionotropic gelation method was identified as one of the techniques which uses natural polymers like chitosan instead of toxic chemicals. In Nanoparticle development, the polymer plays a critical role in control of drug release from the formulation, which in turn enhances the permeation effect of the drug. The formulation development of Nanoparticles can be defined as objects ranging in size from 1-1000 nm that due to their size may differ from the bulk material. The size and shape of nanoparticle formulation is one of the important determining factors to achieve controlled release of drugs from the nanoparticle formulation. The Nanoparticles are defined as particulate dispersions or solid particles drug carriers that may or may not be biodegradable. Nanoparticles are having application in various fields of life sciences such as separation technologies, histological studies, clinical diagnostic assays and drug delivery systems. The major challenge in designing nanoparticles drug delivery system is to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. As per published literature information, the researchers have developed dexibuprofen nanoparticle formulation by emulsion droplet coalescence method using polymers like chitosan, wherein the surfactants like Tween were used[1]. Another researcher had used microchannel fluidic rector technique to develop Dexibuprofen nanocrystals[2], wherein Poloxamer 407 was used as surfactant. The use of minimal or no surfactant in Dexibuprofen nanoparticle formulation can help to achieve better in-vivo efficiency. Till now, there is no research work published on Dexibuprofen nanoparticles without surfactant, more specifically by ionotropic gelation technique. The aim of the present research work is to develop Dexibuprofen nanoparticle formulation by ionotropic gelation technique using natural materials like chitosan, sodium alginate etc., without use of toxic chemicals and surfactants like tween etc., which expected to show better in-vivo efficiency.

2. MATERIALS AND METHODS

2.1 Materials

Dexibuprofen was gifted as free sample by Shasun Chemicals & Drugs Ltd., Pondicherry. Chitosan was purchased from Oxford Lab Fine Chem LLP, Mumbai, Tween 80 was purchased from VWR Lab products Pvt. Ltd., Bangalore. Sodium tripoly phosphate was procured from Loba chemical Pvt. Ltd.,

2.2 Methods

2.2.1 Solubility studies

The saturation solubility of Dexibuprofen was determined in Methanol, Ethanol, pH 1.2, pH 6.8 and pH 7.4 phosphate buffers. The Calibration curve was prepared by accurately weighing 100mg of Dexibuprofen and dissolving in small volume of above mentioned solvents and buffer solutions individually in 100 mL volumetric flask and then the volume was adjusted to 100 mL with respective solvents and buffers. A series of dexibuprofen standard solutions in each of above mentioned buffers and solvents in the concentration range of 100 to 600 µg/mL of dexibuprofen solution were prepared separately. The absorbance of each solutions were measured individually at 220 nm in UV spectrophotometer and calibration graph was plotted using concentration versus absorbance. As per published literatures, to assess the saturation solubility, the presence of solid excess in the solution forming a heterogeneous system is necessary to reach equilibrium. Apparent solubility was affected by the amount of solid excess, due to the competition between crystallization and dissolution rates.[1-10] Hence, Dexibuprofen saturation solubility studies were performed by taking an excess amount of Dexibuprofen in different beakers containing measured volume of above mentioned solvents and buffers. The mixtures were shaken for 24 hrs at regular intervals using shake flask method. The solutions were filtered by using Whatman's filter paper grade no. 41. The filtered solutions were analyzed in Shimadzu UV 1900i model UV spectrophotometer at 220 nm.

2.3 FT IR studies

The drug and the optimized nanoparticle formulation of dexibuprofen F11 were studied for FT IR using FT IR spectrophotometer. 2% (w/w) of the sample, with respect to a potassium bromide (KBr; SD Fine Chem. Ltd., Mumbai, India) was mixed with dry KBr. The mixture was ground into a fine powder using mortar and then compressed into KBr discs in a hydraulic press at a pressure of 10000 PSI. Each KBr disc was scanned 10 times at a resolution of 2 cm^{-1} using Happ-Genzel apodization. The characteristic

peaks were recorded. The IR spectra was obtained by the KBr pellet method. (Perkin-Elmer series 1615 FTIR Spectrometer).[11 – 15]

2.4 Drug and Excipients Compatibility studies

The drug-excipient interactions were evaluated for DSC studies in Shimadzu DSC-50 Cell. Approximately weighed drug of 2mg placed in sealed aluminium plate and empty aluminium plan used as control and the DSC was run at 10^0C heating rate/min in temperature range of 25-350^{0}C in presence of Nitrogen at flow rate of 30ml/min. The DSC of drug as well as drug with mixture of excipients evaluated for DSC studies.

2.5 Chitosan loaded Dexibuprofen loaded nanoparticles Preparation Procedure

2.5.1 Ionic gelation method

Drug loaded chitosan nanoparticles were prepared by ionic gelation of Dexibuprofen with TPP anions with some modifications. Chitosan was dissolved in acetic acid solution (2 % v/v) at various concentrations such as 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. 200 mg of drug (Dexibuprofen) was dissolved in methanol and added to 5 ml of 1% w/v tween 80 solutions, which was added to the chitosan solution. Under magnetic stirring at room temperature, 5 ml of 0.5% sodium tripolyphosphate (TPP) aqueous solution was added drop wise into drug and polymeric mixture respectively. The stirring continued for about 30 min. The obtained Nanoparticle suspension was centrifuged at 6000 rpm for 45 min. The supernatant liquid was analyzed by spectrophotometer to calculate the percent drug entrapment and drug loading. Chitosan nanoparticles separated from suspension were dried by a freeze dryer and lyophilized at 0.4 mbar and -40°C for 5 hrs. The lyophilized nanoparticles were stored in a desiccator at 4°C. The lyophilized nanoparticles were resuspended in pH 6.8 phosphate buffer and submitted to characterization experiments[21-23]. The formulation details of nanoparticles preparation of different options are given in Table 1 to Table 5

2.5.2 Conditions of Nanoparticles freeze drying

Table 1: Formulation of Dexibuprofen Nanoparticles

Condition	Shelf temperature(^{0}c)	Sample temperature(^{0}c)	Cooling rate(^{0}c)	Condenser temp.(^{0}c)	Time(hours)	Pressure (pascals)
Freezing step	-45	-42	1	-	2	-
Primary drying	-30	-32	-	80	12	10
Secondary Drying	+20	+18	-	80	6	5

Table 2 Formulations based on polymer concentration

Trail No	Drug (mg)	Chitosan (%)	Drug: Polymer ratio	0.5% TPP (ml)	Stirring time (mins)	Centrifugation (RPM)6000
F1	200	1.0	1:0.25	5	30	45
F2	200	2.0	1:0.5	5	30	45
F3	200	3.0	1:0.75	5	30	45
F4	200	4.0	1:1	5	30	45
F5	200	5.0	1:1.25	5	30	45

Table 3 :Formulations based on cross linking agent concentration

Trail No	Drug (mg)	Chitosan (%)	Drug: Polymer ratio	TPP (%)	TPP (ml)	Stirring time (mins)	Centrifugation (RPM)6000
F6	200	5.0	1:1.25	0.25	5	30	45
F7	200	5.0	1:1.25	1.0	5	30	45
F8	200	5.0	1:1.25	1.25	5	30	45

Table 4:Formulations based on stirring time

Trail No	Drug (mg)	Chitosan (%)	Drug: Polymer ratio	TPP (%)	TPP (ml)	Stirring time (mins)	Centrifugation (RPM)6000
F9	200	5.0	1:1.25	1.0	5	15	45
F10	200	5.0	1:1.25	1.0	5	45	45

Table 5 :Optimized formulation concentration

Trail No	Drug (mg)	Chitosan (%)	Drug: Polymer ratio	TPP (%)	TPP (ml)	Stirring time (mins)	Centrifugation (RPM)6000
F11	200	1.0	1:1.25	1.0	10	30	45

2.6 Characterization of prepared Nanoparticles

2.6.1 Particle size, Surface Morphology and Zeta Potential.

The surface morphology (roundness, smoothness, and formation of aggregates) and particle size were studied by scanning electron microscopy (SEM) {Model: S 4700-1, Make: Hitachi}. Zeta potential of the best formulation (F11) was measured by Zeta-sizer (Model: Zeta-sizer IV, Make: Malvern instruments). [16-18, 23, 44-45]

2.7 Percentage Drug Entrapment Efficiency

The freshly prepared Nanoparticles were kept in 10ml of water & centrifuged at 10,000 rpm for 20 min using ultracentrifuge. The amount of un-incorporated drug was measured by taking the absorbance of the appropriately diluted supernatant solution at 220 nm using a UV spectrophotometer against blank/controlled Nanoparticles. The amount of drug entrapped into nanoparticles was calculated by using drug entrapment efficiency by subtracting the amount of free drug in the supernatant liquid from that of the initial amount of drug taken [17-20, 44-45].

The entrapment efficiency (EE %) could be achieved by the following equation

$$\%\text{Entrapment efficiency} = \frac{\text{Total amount of drug added} - \text{Non bound drug}}{\text{Total amount of drug added}} \times 100$$

2.8 Invitro Drug Release studies

Drug release were carried out by using dialysis tubes with Dialysis membrane-60 (HI MEDIA, Mumbai). In to the dialysis tube, prepared nanoparticles and 10mL of pH 7.4 phosphate buffer were placed and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 250 ml of phosphate buffer pH 6.8. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37±0.5° C. The sample of the receptor compartment (5ml) was taken at various intervals of time over a period of 24h and each time a fresh buffer was replaced. The amount of drug released was determined spectrophotometrically at 220 nm.[25-28]

2.9 Invitro Kinetic Studies

The results of in vitro release profiles obtained for the Nanoparticles formulations were fitted into four models of data treatment as follows:[23-31]

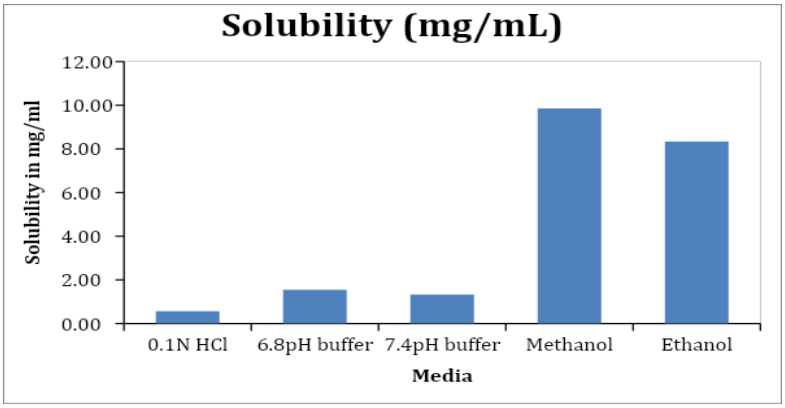
- Cumulative percent drug released versus time (zero order kinetic model).
- Log cumulative percent drug remaining versus time (first-order kinetic model).
- Cumulative percent drug released versus square root of time (higuchi's model).
- Log cumulative percent drug released versus log time (korsmeyer - Peppas equation).

The zero order model describes drug release from a system at constant rate regardless of its concentration and the release is only a function of time. In first order model, drug release rate from system is only a function of the remaining drug concentration. The Higuchi model implies that the amount of drug liberated form the dosage form is a function of the square root of time. Korsmeyer-Peppas is another comprehensive semi-empirical model, that could generally describe the distinct release phenomena involving either of diffusion or swelling. In this study, the best fitted mathematical model (among the four equations evaluated) illustrating drug release pattern from nanoparticles is figured out that is the Higuchi model. It can also be inferred that drug release phenomenon for nanoparticles is dominantly polymer dependent rather than drug dependent. The drug release was found to be best fitted by Higuchi square root model (r2 =0.9931 for Dexibuprofen) which implies that release of drug from matrix as a square root of time dependent process and diffusion controlled. Although more investigations might be necessary to evaluate release patterns of different drugs from nanoparticles.

3. RESULTS AND DISCUSSION

3.1 Solubility study

The solubility studies revealed that Dexibuprofen has highest solubility in 6.8pH buffer and methanol when compared with other solvents. (Fig 1)

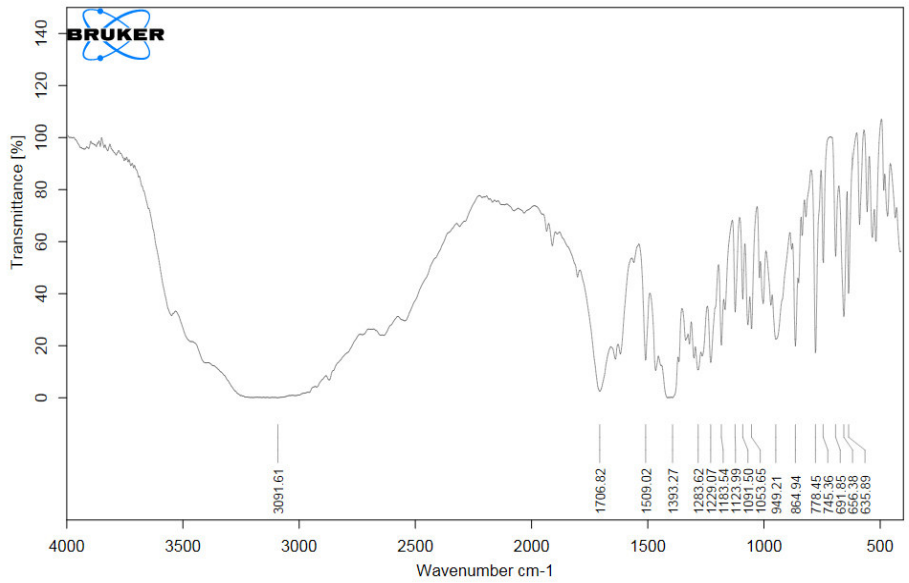


The results were expressed as mg/ml of Dexibuprofen solubilized in different solvents

Fig 1: Solubility data of Dexibuprofen in various solvents

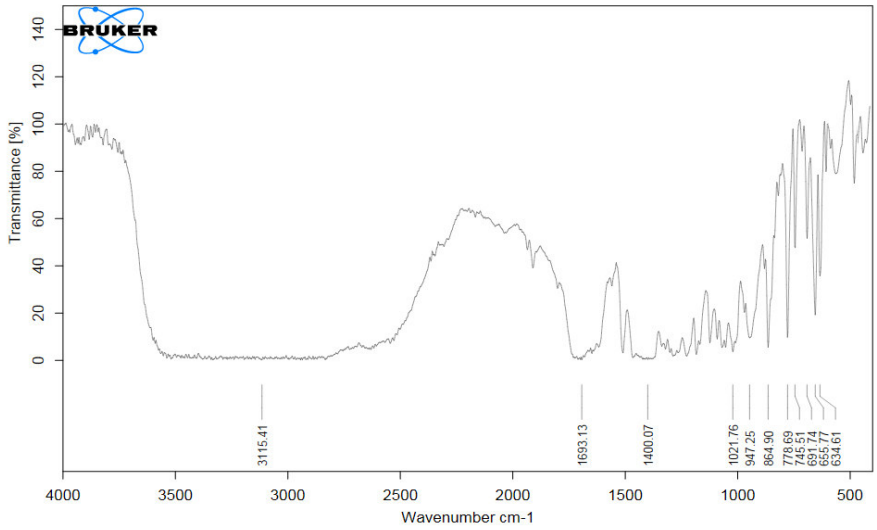
3.2 FT IR Studies

In general, Fourier transform infrared spectroscopy (FTIR) was used widely for quantitative analysis, quality control, and supervision of the manufacturing process of pharmaceutical products[32-33]. In our FT IR evaluation study, the comparative evaluation of drugs and the optimized Dexibuprofen nanoparticle formulation F11 were assessed. It was concluded that the functional groups that were presented in the pure drug were present in the optimized formulation with very minute changes, from this we can conclude that the Dexibuprofen optimized nanoparticle formulation F11 is comparable to that of Dexibuprofen drug (Fig 2, 3)



The FT IR spectrum of Dexibuprofen is expressed as percentage transmittance vs wave number

Fig 2 FT IR spectrum of Dexibuprofen

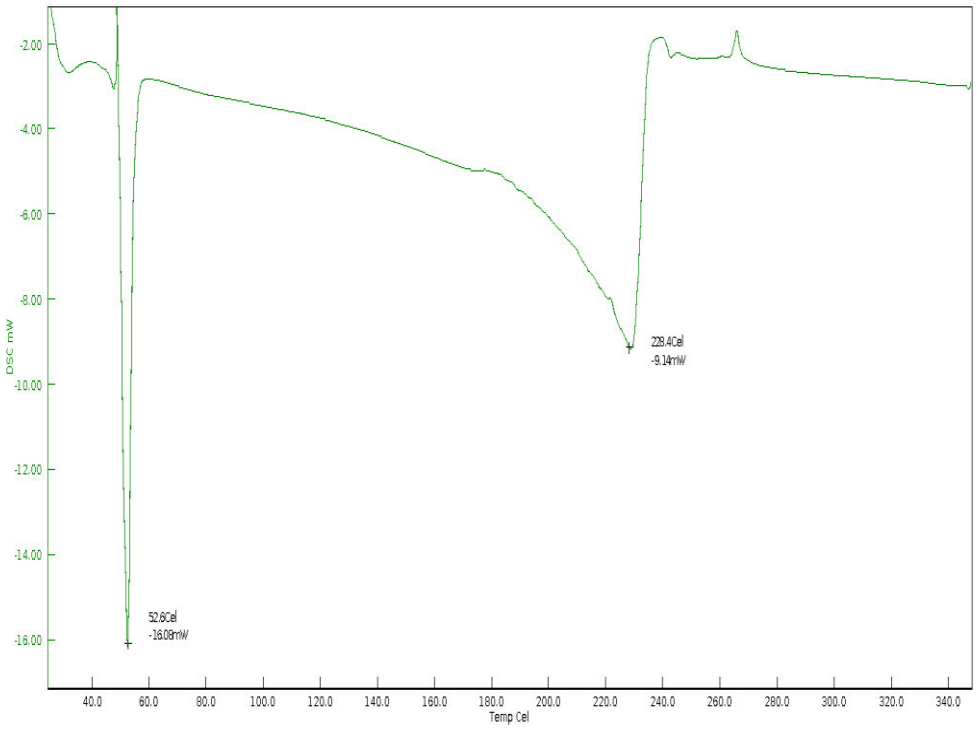


The FT IR spectrum of Dexibuprofen optimized formulation (F11) is expressed as percentage transmittance vs wave number

Fig 3: FT IR spectrum of Dexibuprofen Optimised Formulation(F11)

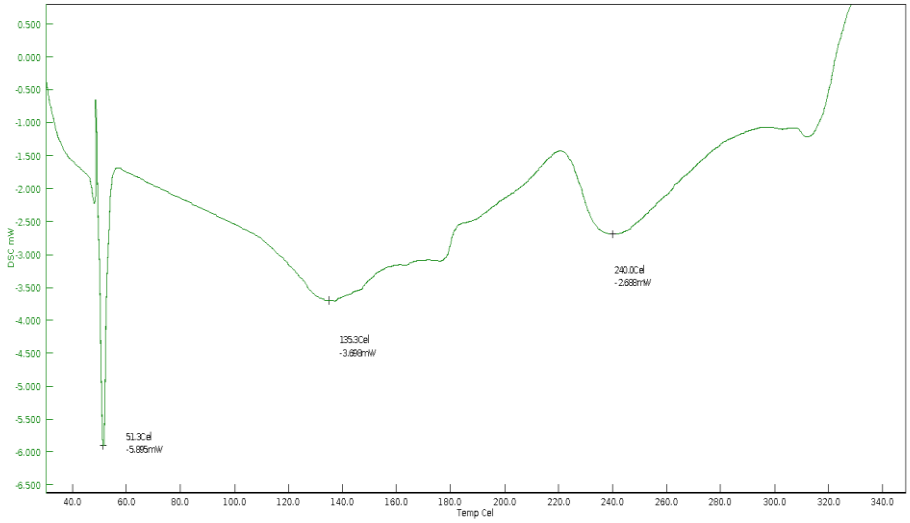
3.3 DSC Studies

The DSC studies are used as a tool to evaluate compatibility of drugs with other excipients by measuring thermal transitions [34-37]. In our DSC study, it showed that the melting point of Dexibuprofen is similar to that of the as mentioned in the official monograph. There was no incompatibility between drug and mixture of polymers. This explains that there are no chemical species present in the drug molecule which degrades the original compound and we can go forward with this in our formulation part. The DSC of Dexibuprofen as well as Dexibuprofen along with mixture of excipients is presented in Figure 4 to 5.



The Thermogram of Dexibuprofen is expressed as temperature vs DSC mW

Fig 4: DSC of Dexibuprofen

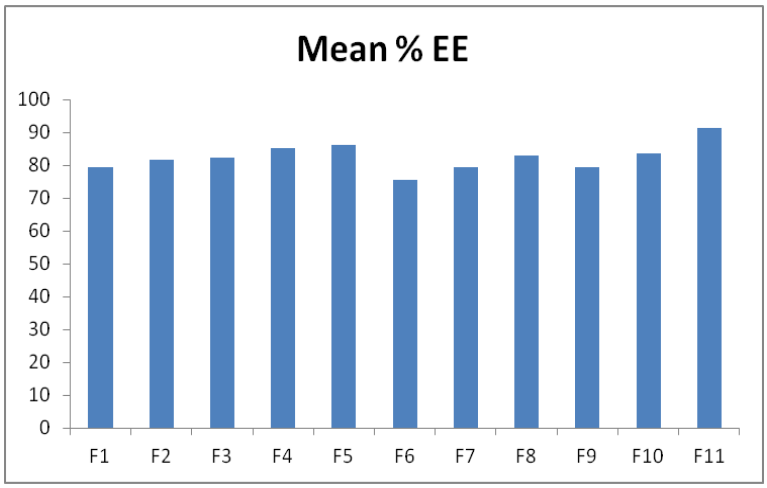


The Thermogram of Dexibuprofen with mixture of excipients is expressed as temperature vs DSC mW

Fig 5: DSC of Dexibuprofen with excipients mixture

3.4 Percentage Entrapment Efficiency

The percentage entrapment efficiency of different formulations used as a tool to assess the efficiency of entrapment of drug into the nanoparticles[39-40]. The performance of ultrafiltration (UF), ultracentrifugation (UC), and microdialysis (MD) for determining the entrapment efficiency was evaluated. Micro dialysis was not suitable for Entrapment Efficiency measurements, which could only determine the total Entrapment Efficiency of submicron oil droplet and liposomes micelles, but it could be applied to determine the amount of free drug in dispersion. Although Ultra Centrifugation was the fastest and simplest to use, its results were the least reliable. Ultra Filtration was still the relatively accurate method for Entrapment Efficiency determination of Dexibuprofen nanoparticle dispersion. The percentage entrapment efficiency of different formulations of F1 to F11 developed by us is presented in Fig 6.

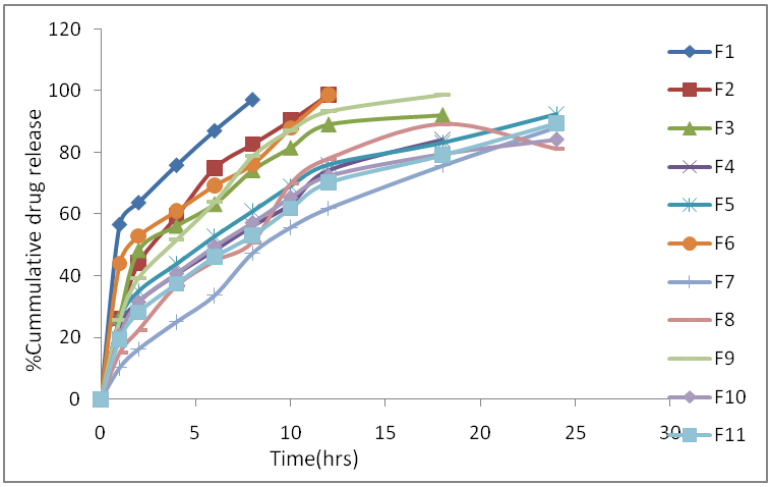


The mean percentage efficiency of different nanoparticle formulations of F1 to F11 is expressed as percentage

Fig 6: Percentage entrapment efficiency of Nanoparticle formulation (F1 to F11)

3.5 Invitro Drug Release studies

In-vitro drug release studies were used to assess the drug release pattern of drug from the formulation[42-44]. From in vitro diffusion studies we can say that at low polymer concentrations of chitosan the drug release was rapid and it didn't maintain constant drug release. When the concentration of chitosan was increased 5%, it was observed that the drug release time was increased. So the concentrations were further increased to decrease the drug release time from the nanoparticles which decreased rapidly due to high polymer concentration. By comparing all diffusion profiles it was concluded that the drug release from the F5 formulation containing 5% chitosan, released 92.16% of drug at the end of 24hours. To optimize the best formulation the F5 formulation was further modified by using different ratios of cross linking agent and sodium alginate concentration from F6-F8 formulations. Among them F6 and F8 were formulated by changing the sodium alginate concentrations from lower to higher ratios, and F9 and F10 formulations were prepared by changing the stirring time. Among the F6-F11 formulations it was found to be the formulation containing 5% chitosan with changing cross linking concentration and stirring time, so F11 showed maximum drug release at the end of 24 hrs when compared with the F5 trail. So the F11 trial was considered as the optimized formulation. So further drug release kinetics were performed for the F11 formulation. In-vitro release data of different formulations of F1 to F11 is presented in Fig 7.

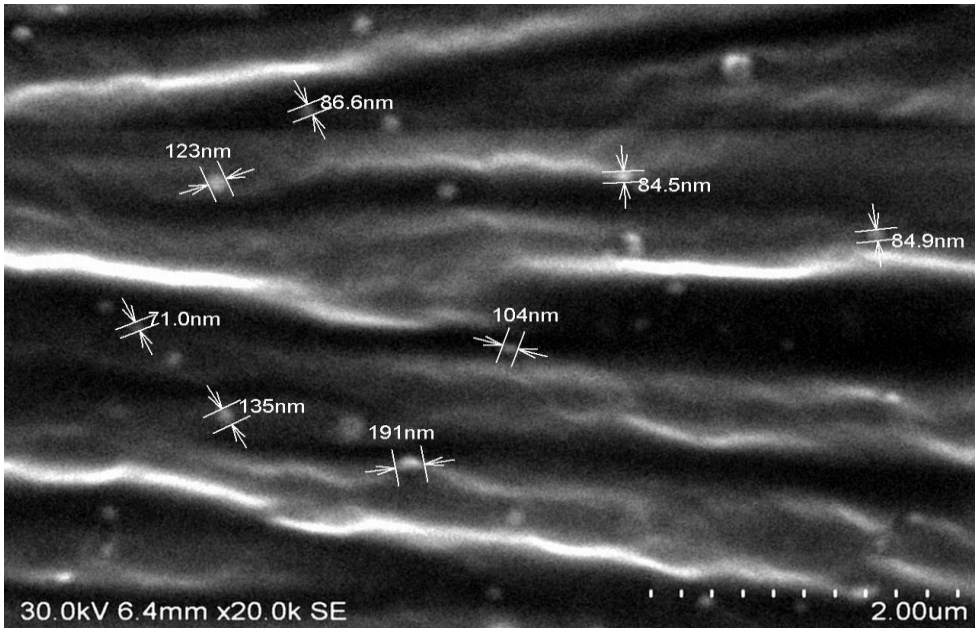


The cumulative percentage drug release of different nanoparticle formulations of F1 to F11 is expressed against time in mins

Fig 7: Invitro drug release of formulations F1 to F11

3.6 SEM

Scanning Electron Microscopic data of optimized nanoparticle formulation F11 is presented in Fig 8.



The SEM image of optimized dexibuprofen nanoparticle formulation F11 shows particle size in nanometer

Fig 8: SEM of optimized dexibuprofen nanoparticle formulation F11

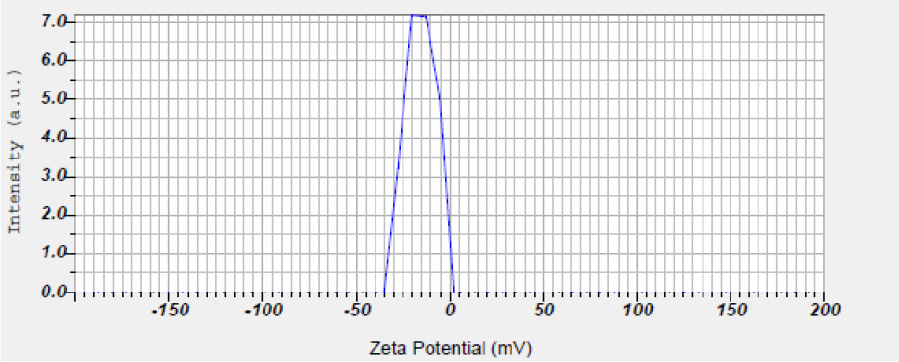
3.7 Zeta potential

Zeta Potential data of optimized nanoparticle formulation F11 is presented in Figure 9.

Measurement Type	:	Zeta Potential
Sample Name	:	DIF7
Temperature of the Holder	:	25.0 °C
Dispersion Medium Viscosity	:	0.894 mPa·s
Conductivity	:	0.109 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-15.8 mV	-0.000122 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs



The Zeta Potential image of optimized dexibuprofen nanoparticle formulation F11 shows percentage intensity Vs Zeta potential in mV

Fig 8: Zeta Potential of optimized dexibuprofen nanoparticle formulation F11

4. DISCUSSION

The saturation solubility of Dexibuprofen had shown that, the drug substance was found to be freely soluble in organic solvents like methanol and ethanol. Dexibuprofen had shown pH dependent solubility in different pH. Dexibuprofen was found to be very slightly soluble in 0.1N HCl and slightly soluble in in pH6.8 and pH7.4 phosphate buffers. The solubility data of Dexibuprofen had shown that, there is a need to design optimum formulation to improve solubility and bioavailability of Dexibuprofen. The FT IR spectrum was used as a tool to conform identification of Dexibuprofen loaded nanoparticle formulation F11 by comparing with that of Dexibuprofen drug substance FT IR spectrum[21-23]. The DSC studies of Dexibuprofen drug substance and the sample of drug with mixture of excipients had shown no significant differences in thermogram, which confirmed that, there are no interactions between drug and excipients[24-27]. Based on DSC studies, it is inferred that, the proposed excipients chitosan, sodium alginate and calcium chloride were found to be compatible with that of dexibuprofen drug substance and found appropriate to use in nanoparticle formulation of dexibuprofen. The percentage entrapment efficiency of different nanoparticles confirmed to the efficiency of entrapment of drug in the nanoparticle formulation[28-31]. The optimized dexibuprofen loaded

nanoparticle formulation of F11 had shown mean entrapment efficiency percentage of more than 90%. This conformed that, the composition and the process optimized for F11 formulation had shown better entrapment efficiency. The *in-vitro* drug release of different nanoparticle formulations was used as a tool to identify suitable formulations to achieve maximum drug release[32 - 34]. The optimized formulation F11 had shown in-vitro drug release till 24 hours. The SEM of nanoparticle formulation had shown the dimension of nanoparticle formulation and it's particle dimension which ranged between 71 to 191nm. The Zetapotential of optimized formulation F11 had shown that, the formulation is negatively charged. Based on the data, it is inferred that, the optimized formulation is stable.

5. CONCLUSION

The Dexibuprofen nanoparticle of different formulations were evaluated. Based on assessment of the data generated, it was found that, the Dexibuprofen loaded nanoparticle formulation F11 had shown maximum in-vitro drug release of 92.59% at the end of 24hours. This conforms that, the F11 formulation of dexibuprofen loaded nanoparticles developed by us was found to achieve maximum in-vitro drug release for maximum duration of 24 hours. This overcomes the rapid onset of action concern of short biological half life drug Dexibuprofen. Hence, the frequency of dosage of Dexibuprofen can be reduced using the invention of Dexibuprofen loaded nanoparticle formulation F11. From the drug release studies it was clearly observed that the chitosan concentration and sodium TPP concentration plays a vital role in nanoparticles formation and strength. From the studies it was concluded that the Dexibuprofen Nanoparticles were successfully formulated by using chitosan(5%), sodium TPP (1%) for sustained drug delivery of Dexibuprofen without use of surfactants like tween 80 or poloxomer 457 etc.,

6. AUTHORS CONTRIBUTION STATEMENT

S.Vivekanandan conceptualized and designed the study and Dr.Venkatesan guided and reviewed the study. Berit Lindholm and Dr.Raghunandan Reddy had reviewed and approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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