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PREPARATION AND CHARACTERIZATION OF INDOMETHACIN-LOADED CHITOSAN NANOPARTICLES

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Abstract:

The aim of the present study involves the preparation and characterization of chitosan loaded Indomethacin nanoparticles. The possibility to achieve a therapeutic dose was much higher by using the Prepared Nanoparticles. The prepared Indomethacin chitosan loaded nanoparticles would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional Indomethacin tablets. Out of all the prepared formulations F3 with the Indomethacin concentration of 40mg showed the highest entrapment efficiency of 81.25 % when compared to that of other formulations and the highest extent of release (79.9%). The Chitosan-NPs based controlled delivery system developed in this study would have potential applications and could serve as an optimal model to encapsulate any hydrophilic or hydrophobic therapeutic agent, vitamins, probiotics, enzeymes, flavors, fatty acids, antimicrobial agents and peptides etc.

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INTRODUCTION:

Nanotechnology is getting developed at various levels such as materials, systems and devices. At present in commercial applications and scientific information, the most innovative level is nanomaterials. In nanotechnology, a minor object that acts as a complete unit in terms of its properties and transport is known as a particle. It can be categorized according to sizes as fine particle and ultrafine particle. In terms of diameter, fine particles are sized between 100 and 2500 nanometers, while ultrafine particles cover a rangebetween 1 and 100 nanometers. Nanoparticles are also sized between 1 and 100 nanometers similar to the ultrafine particles. Nanoparticles may or may not demonstrate size- related properties that varyknowingly from those observed in bulk materials and fine particles.[1]Thus nanoparticles are sized less than a few 100 nm. This reduction in size brings about significant changes in their physical properties with respect to those observed in bulk materials. They can be mineral, metallic, polymer-based or a combination of materials.[2]

Nanotechnologies is important in terms of diagnosis, treatment, and prevention of disease. The word nanoparticles come from the Greek word nanus which means dwarf or very small. Nanoparticles (NPs) are the novel invention of modem science in which drug is surrounded by a polymeric membrane where the drugs are dissolved, entrapped, adsorbed, attached and/or encapsulated into or onto a Nano-particulate matrix. The drug delivery vehicles are generally < 100 nm in size with at least one dimension and consist of different biodegradable materials such as natural or synthetic polymers, lipids, or metals. It is composed of three layers i.e. (a) the surface layer, which can be functionalized with a variety of little molecules, metal ions, surfactants, and polymers. (b) The shell layer, which is with chemicals completely different material from the core in all aspects, and (c) The core, that is the central portion of the NPs [3]

MATERIAL AND METHODOLOGY:

1.1. Development of Calibration Curve of Indomethacin:

Selection of solvent: The selection of solvent was done based upon the drug solubility, stability and absorbance maxima of the compound in the particular solvent. 10 mg of Indomethacin was weighed and solubility of this sample was checked in the 0.1N HCL, 0.01N NaoH, Methanol, Ethanol, Phosphate buffer pH6.8 and distilled water. Results showed that the drug indomethacin having highest solubility in methanol.

Preparation of standard stock solution: 10 mg of indomethacin was dissolved in 10ml of methanol to form a solution of 100µg/ml was prepared as a stock solution. From this 0.8ml was taken and the volume was made up to 10 ml to make solution concentration 8µg/ml. The resulting solution was scanned by using UV-Visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). The absorbance was taken in triplicate manner. The absorbance maxima (λ_{max}) were noted

Preparation of sample solution: 10 mg of indomethacin was dissolved in 10ml of methanol to form a solution of 100µg/ml was prepared as a stock solution. From this stock solution 0.1, 0.2, 0.4, 0.6 & 0.8ml was taken and the volume was made up to 10 ml to make solution concentration1,2,4,6 & 8µg/ml. Absorbance of the resulting solution was measured by using UV-Visible spectrophotometer at respective absorbance maxima (λ_{max}). The absorbance of each dilution was taken in triplicate manner and a concentration vs absorbance was plotted for preparation of calibration curve.

1.2. Preparation of indomethacin nanoparticles

Chitosan nanoparticles were prepared spontaneously based on ionic gelation by addition of TPP anions aqueous solution to CS solution according to the procedure first reported by Calvo et al., 1997b. Different amounts of CS were dissolved in acetic aqueous solution togive concentrations of (0.1, o.3, 0.5, 0.7, 1.0, and 2.0 mg/mL). The concentration of acetic acid in aqueous solution was 1.5 times that of CS. Under magnetic stirring at room temperature, 4 mL of TPP aqueous solution with various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) was added into 10 mL CS solution respectively. Three kinds of phenomena were observed: solution, aggregates and opalescent suspension. The zone of opalescent suspension was further examined as nanoparticles. The formation of particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of CS

1.3. EVALUATION STUDIES

1.3.1. Organoleptic properties

Color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology.

1.3.2 Solubility studies

The drug is to be tested for its solubility because solubility is directly related with the release of the drug from the formulation, hence absorption of the drug into blood stream. Solubility study was carried out by “Shake flask method” as given in Indian pharmacopoeia [6]. The solubility of indomethacin was studied in the solvents of different pH range (1.2-8.0), water, methanol, ethanol & polysorbate 80. The solubility of indomethacin was determined by adding excess amount of drug in vials with respective solvent system and kept under agitated conditions at 25°C in a water bath shaker for 24 hours. The dispersions were filtered through a 0.45 µm pore filter and analyzed for the quantity of drug dissolved. The drug quantity was calculated by taking the absorbance of a known concentration of drug (standard curve) in respective solutions. The solubility was plotted against the pH of the medium & respective solvent systems.

1.3.3.Compatability studies*:*

To know about the interaction between the drug and carriers used in the formulation, the IR analysis was carried out. The IR spectra of pure Indomethacin, Pure Polymer, and Prepared Indomethacin nanoparticles was studied by FTIR It is scanned over the Frequency range of 4000-500 cm-1.

The fourier transform infrared analysis was conducted for the structure characterization. FTIR spectra of Indomethacin, pure polymer and formulated nanoparticles were recorded.

The samples (drug, polymer and mixture of drug and polymers) were mixed with 200- 400 mg of potassium bromide (KBr). The samples were compressed as discs by applying pressure of 5 tons for 5 minutes in a hydraulic press. The prepared pellet was placed in the light path and the spectrum was recorded from 650 to 4000 cm^{-1}.

1.3.4.Differential scanning colorimetry

DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies. A complement to X-ray diffraction, this method is regularly used to determine the extent to which multiple phases exist in the interior or to which the various constituents, including the drug,

interact.

Phase transition behavior of Indomethacin NPs were analyzed by the differential scanning calorimeter. As a control, the pure Indomethacin, Pure Polymer and Prepared Nanoparticles were analyzed by DSC Approximately 5 mg of samples were loaded to aluminium pan, crimped, sealed and analysed at a scanning rate of 10°C/min from 25°C to 200°C under nitrogen atmosphere (flow rate was100 mL/min).

1.3.5. X-Ray Studies:

The XRD studies for analyzing structural nature of Indomethacin , and nanoparticle formulation of Indomethacin. The samples were placed in sample cell and spread evenly. The sample cell was placed in X-ray Diffractometer (BRUKER ECO D8). The samples were scanned over the frequency range of 10-80.

1.4 Characterization of nanoparticles

The optimized nanoparticles containing Indomethacin were characterized by studying various physico-chemical properties.

1.4.1Particle size[4]

Nanoparticle size was determined using Photon Correlation Spectroscopy (PCS). All samples were diluted with ultra-purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25ºC. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate.

1.4.2. Zeta potential and Surface morphology

Nanoparticles were characterized with Zeta potential using a Zeta Sizer. The measurements were performed by diluting the nanoparticles with distilled water and the samples were placed in the electrophoretic cell using an aqueous dip cell in an automatic mode.

The surface morphology of the particles was studied using Scanning Electron Spectroscopy set at from290 nm-632 nm, 200 kV by placing an air dried nanoparticle suspension on copper electron microscopy grids[5-10].

1.4.3 Drug content

The total drug amount in nanosuspension was determined spectrophotomertically A residue was dissolved in water and filtered with a 0.45µm filter, and Indomethacin content was assayed spectrophotomertically at 266 nm.

$$Total\ Drug\ Conent = \frac{Volume\ total}{Volume\ aliquot} \times drug\ amount\ in\ aliquot$$

1.4.4 Drug entrapment efficiency

The entrapment efficiency is also known as Association Efficiency. The drug loaded nanoparticles are centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant is assayed for non-bound drug concentration by UV spectrophotometer. Entrapment efficiency was calculated as follows6[-12.]

$$DEE\% = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

***1.5. In vitro* release studies**

In-vitro diffusion studies (drug release studies) were performed by using diffusion apparatus. A semipermeable membrane was supported on a ring of diffusion cell and the sample was kept on a membrane in such a way backing layer was phased towards donar compartment.[46] The glass beaker was filled with 100ml of phosphate buffer sample of 1ml was withdrawn at regular intervals from glass beaker for analysis.1ml of phosphate buffer was replaced immediately after sampling to maintain volume equal to 100ml.The absorbance of sampling was measured at 266nm by using UV spectrophotometer.[13-15.]

1.6. Modeling of dissolution kinetics

We have determined the release mechanism by fitting the dissolution data to the various kinetic equations: Zero order, First order, Higuchi and model Korsmeyer-Peppas Linearization of dissolution data allows a more direct comparison by referring to classical parameters (Intersection-slopes)

Zero order: Qt = Q0 + K0t

First order: Log Qt = Log Q0 – $\frac{Kt}{2,303}$

Higuchi model; Qt = KH t ^{½}

Korsmeyer and Peppas model: Log ($\frac{Mt}{M\infty}$) = log k + n log t

1.7. Stability Studies

To evaluate the stability of drug, Indomethacin, and the effect of polymer after storing at different Temperature and Relative Humidity for 30 days stability studies were carried out .About 100mg of equivalent of Indomethacin formulations were taken in well closed containers from ideal batches and stored separately at 400C+ 20C/75% RH + 6% (Accelerated testing) and 300C+ 20C/60% RH + 5% (Alternate testing). From these, sample equivalent to 20 mg of Indomethacin was removed at the interval if 10, 20, 30 days and analyzed the drug content by spectrophotometrically at 266 nm1[6-18].

RESULTS AND DISCUSSION:

2.1 Development of Calibration Curve of Indomethacin:

Table No.1: Results of Calibration curve at indomethacin at 266nm.

Concentration (µg/ml)	Absorbance at 266 nm
1.0	0.141 ± 0.002
2.0	0.285 ± 0.004
4.0	0.491 ± 0.03
6.0	0.720 ± 0.024
8.0	0.995 ± 0.055

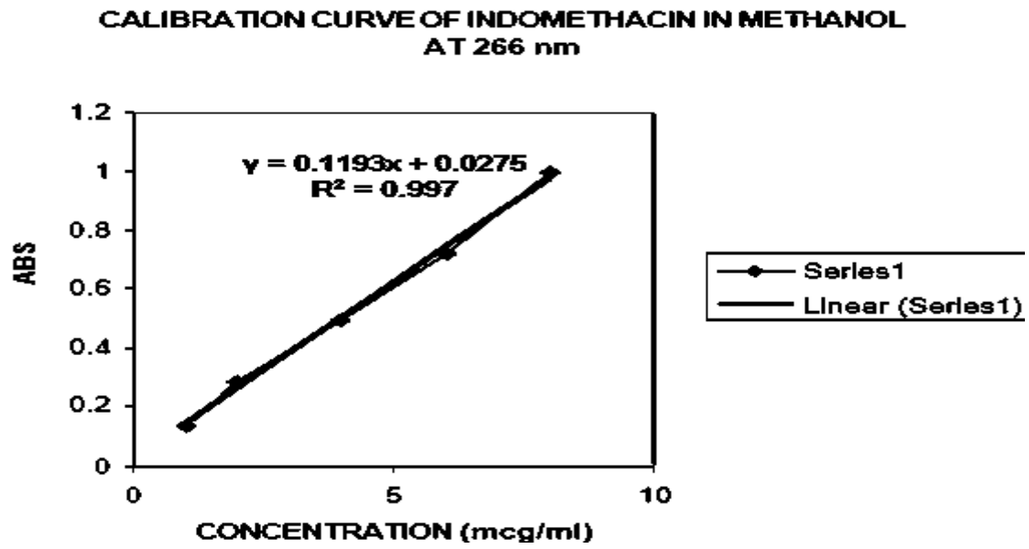


Fig 1: Calibration Curve of Indomethacin In methanol.

2.2 Preparation of Indomethacin Nanoparticles

Chitosan nanoparticles were prepared by ionic gelation method. Briefly, by the addition of TPP aqueous solution to the diluted acetic acid-chitosan solution according to the method [47]. Chitosan was dissolved in acetic acid aqueous solution to reach the concentrations 0.1, 0.3, 0.5, 0.7, 1.0, 2.0mg/mL. Indomethacin was dissolved in 0.25 mL of dichloromethane and mixed with the chitosan solution. Thereafter, 5 mL TPP of different concentrations (0.1, 0.3, 0.5, 0.7, 1.0, 2.0mg/mL) were added to 10 mL chitosan solution at the addition rate of 1.5 mL/min.

Table 2 : Formulation of Indomethacin nanoparticles

S.NO	Ingredients	F1	F2	F3	F4	F5	F6
1	Indomethacin	40mg	40mg	40mg	40mg	40mg	40mg
2	Chitosan	0.1%	0.3%	0.5%	0.7%	1.0%	1.2%
3	Tripoly phosphate	40ml	40ml	40ml	40ml	40ml	40ml
4	0.1% Acetic acid solution	100ml	100ml	100ml	100ml	100ml	100ml
5	Dichloro methane	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

2.3.1. Organoleptic properties

Color, odor, taste, and appearance play an important role in the identification of the sample and hence they were recorded in a descriptive terminology.

Table 3: Organoleptic properties.

Properties	Results
Description	Crystalline
Taste	Tasteless
Odour	Odourless
Colour	Pale to Yellow Coloured powder

2. 3.2 Solubility studies

Solubility of Indomethacin was performed in various solvents like water, 0.1 N HCL, methanol, ethanol. From the above solvent Indomethacin was freely soluble in methanol, whereas remaining solvents shows insoluble particle sediment in the bottom of test tube. The Results of Solubility Studies were shown in Table 4

Table 4: Solubility studies of Indomethacin in various solvent systems.

S.NO.	Solvent system	Solubility of indomethacin (mg/mL) at 35 °C
1	Water	0.800±0.060
2	0.1 (N) HCl	0.031±0.004
8	Methanol	30.010±1.640

2.3.3. Compatibility studies

The FTIR peaks of indomethacin responsible for characteristic functional group were identified and interpreted & it was also compared with the earlier reported data. FTIR peaks and spectra of indomethacin are exposed in table 5 and figure 2. Wherein the FTIR spectrum of Chitosan Polymer and were shown in Figure 3.

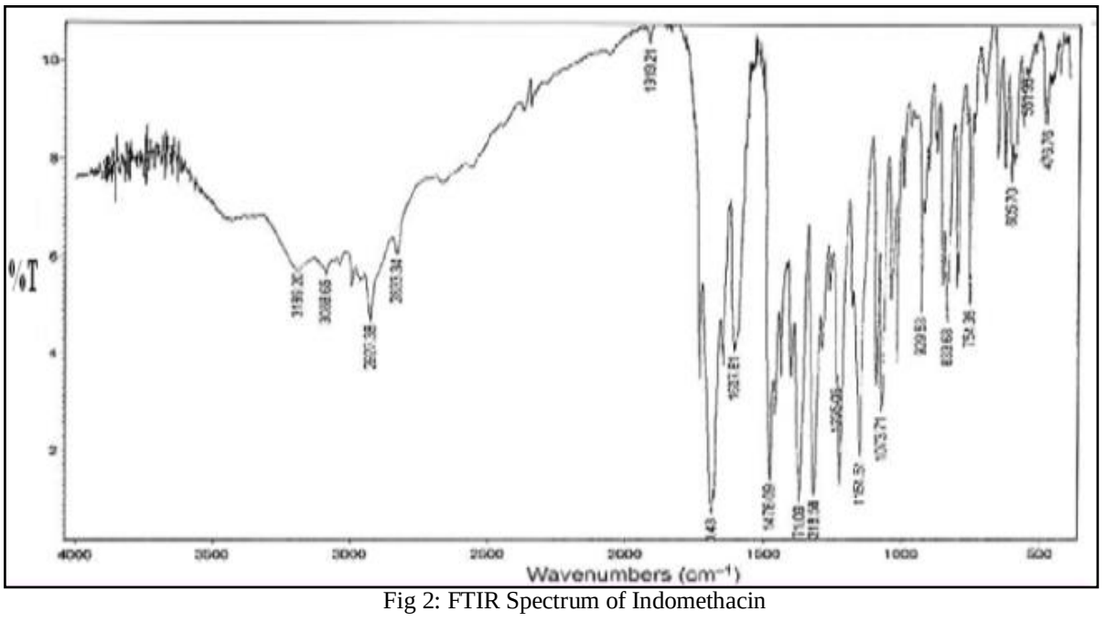


Fig 2: FTIR Spectrum of Indomethacin

Table 5: Interpretation of FTIR Spectra of Indomethacin.

S.NO.	Reported values (cm^{-1})	Observed values (cm^{-1})	Responsible Functional groups
1	750	754.38	C-Cl
2	925	929.53	-COOH out of plane
3	1230	1235.05	C-O Stretch
4	1450	1451.09	O-CH3
5	1640	1635.81	Aromatic C=C Stretch
6	1715,1695	1716,1696.43	C=O Stretch
3	3400-2500	3400-2500	Aromatic C-H Stretch,-COOH (s)

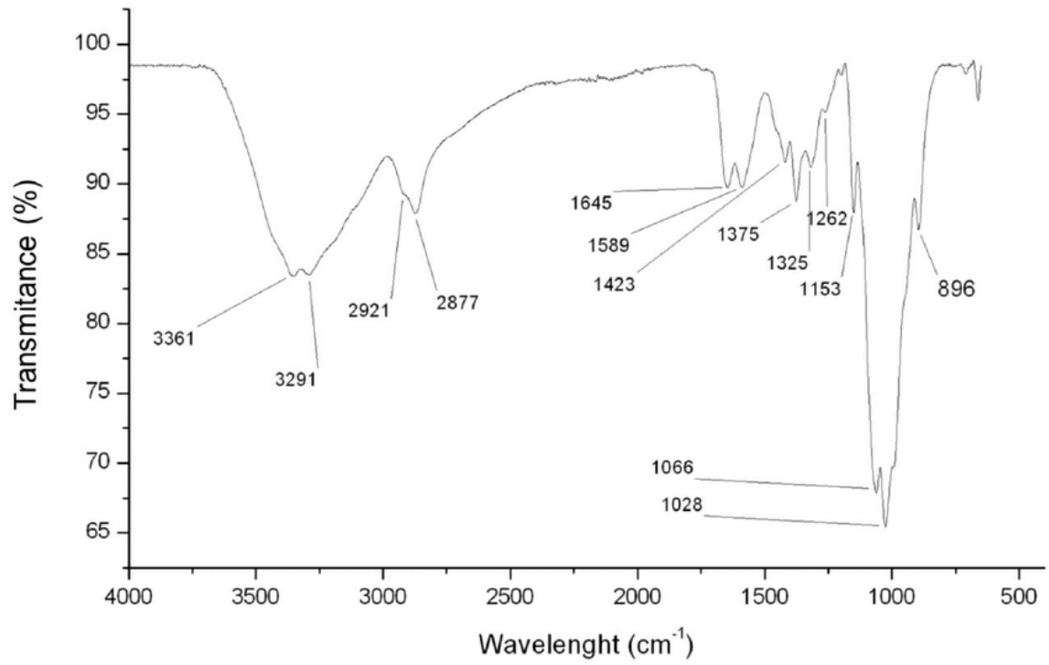


Fig 3: FTIR Spectrum of Chitosan Polymer

2.3.4. Differential scanning colorimetry:

The DSC analysis was carried out for standard indomethacin, chitosan. The DSC studies performed in order to determine the compatibility studies. The peak that is obtained for indomethacin in nanoparticles was compared with standard indomethacin and its ensure there was no interaction between drug and drug with polymer.

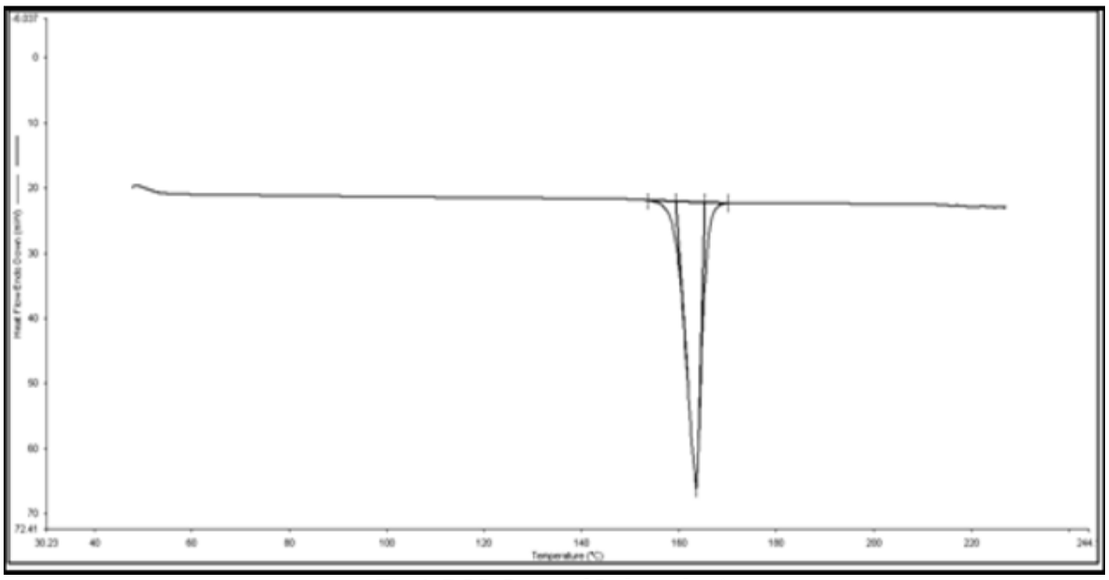


Fig 4: DSC Curve of Indomethacin.

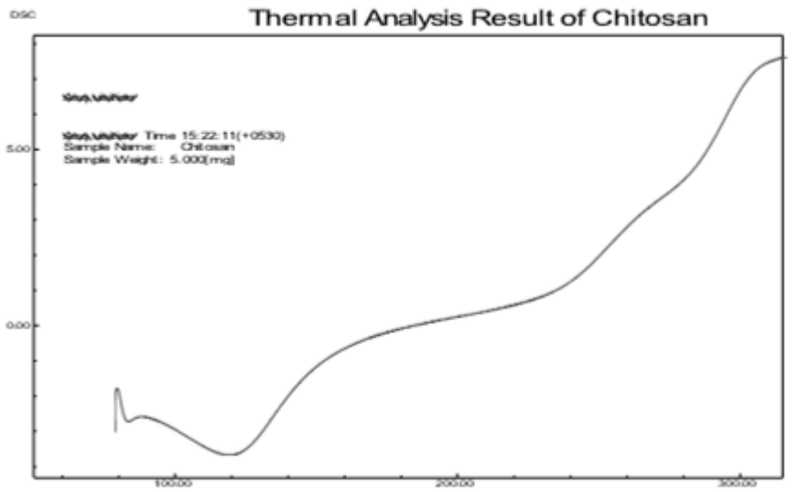


Fig 5: DSC Curve of Chitosan

Fig 6 displayed DSC thermograms obtained for TPP, CS, IM, and IM loaded nanoparticles The IM, CS, TPP showed an endotherm at 160, 108, and 40 oC respectively corresponding to their melting temperatures. However, the endothermic peak characteristic of IM was not detected in the thermograms obtained for IM-loaded nano-particles. These results suggest that IM was dispersed molecularly in the CS polymer network in both carrier systems**.**

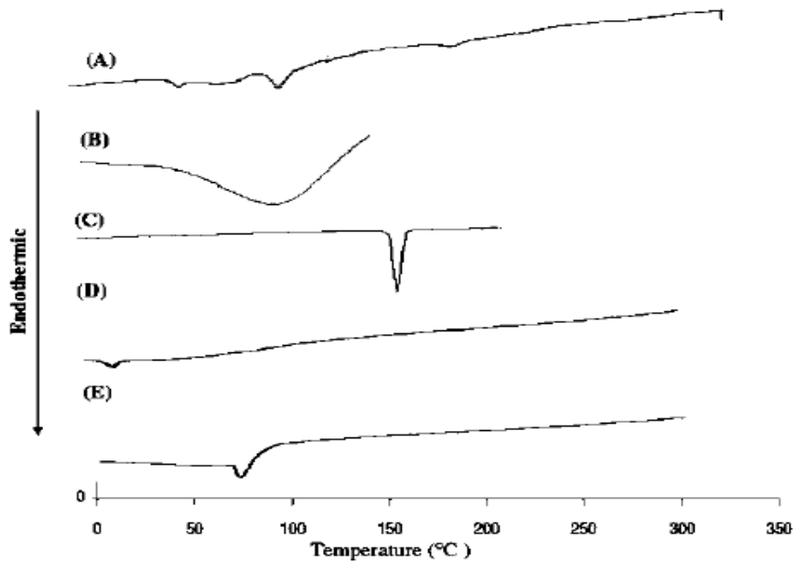
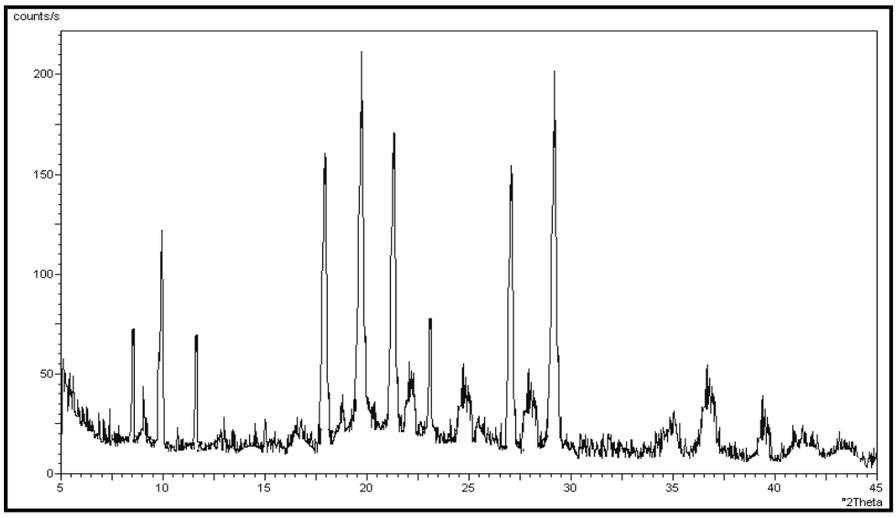


Fig 6: DSC of (A) TPP, (B) chitosan, (C) indomethacin, (D) IM-loaded nanoparticle Formulation -1, and (E) IMNP- 4

2.3.5. XRD Studies:

Fig 7: XRD Studies of Indomethacin



2.4.1 Measurement of particle size of Nanoparticles

The results of formulations and characterizations indicate that NPs prepared with chitosan Concentration of 0.3% (F3) was found the best formulations among the five optimized formulations (F1-F5) with an average particle size of 401 nm. All the optimized formulations has shown monomodal size distribution. With increasing chitosan concentration an increase in the average particle size were found in the range of 301 to 750 nm. The results of Particle size were shown in table 6.

2.4.2. Zeta potential and Surface morphology

The polydispersity index (PDI) of the all the formulations (F1-F5) was found low, indicating that the developed chitosan-nanoparticles arehomogeneously dispersed in the dispersion medium. An increase in the values (0.115 to 0.215) of PDI was observed with increasing concentration of chitosan in the five optimized formulations (Table 6).

All the chitosan nanoparticles were found to have positive zeta potential values (Table 6). The positive values of zeta potential are due to the presence of cationic chitosan on the surface of nanoparticles. A high magnitude of absolute zeta potentials are indicative that the developed chitosan-nanoparticles are having good dispersion stability.

Formulations	Z-Average(nm)	PDI	Zeta-potential(mV)
F1	301.52 ± 15.75	0.115 ± 0.075	+36.05 ± 2.18
F2	389.85 ± 18.45	0.145 ± 0.029	+28.14 ± 3.23
F3	401.54 ± 14.25	0.198 ± 0.054	+31.25 ± 3.45
F4	430.95 ± 10.56	0.200 ± 0.018	+27.14 ± 4.26
F5	559.12±5.21	0.210 ± 0.018	+26.19 ± 2.48
F6	758.25 ± 12.65	0.295 ± 0.036	+19.19 ± 2.48

Surface Morphology:

Transmission electron microscopy (TEM) observation has shown that the nanoparticles were discrete and isolated in their distribution and having spherical morphology with solid dense structure. Transmission electron microphotographs of chitosan-NP-INDO (F3) which were shown in figure 8.

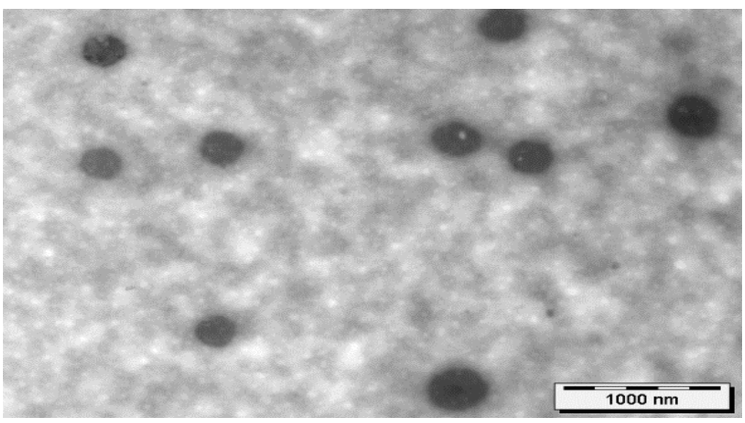


Figure8: Transmission electron microphotographs of chitosan-NP-INDO (F3) presenting solid dense spherical structure in the nano-range.

7.4.4 Drug entrapment efficiency:

The mean encapsulation efficiencies (%EE) and the mean drug loading capacities (%DL) of the optimized (F1-F6) formulations are presented in **Table 7**. Lower value of %EE and %DL in case of F2 might be due to the lower concentration of chitosan. F3 is having good %EE and %DL capacity as formulated with optimum concentration of chitosan and having satisfactory drug release in the 12 h in vitro release study, so it was considered as the best formulation amongst the six optimized chitosan-nanoparticles. F4 is also having good encapsulation and drug loading, but here the particle sizes are very high as compared to F3.

Table 7: Encapsulation efficiency and Drug Loading capacity of Prepared Nanoparticle Formulations.

Formulations	Encapsulation(%EE)	Drug Loading(%DL)
F1	62.65 ± 3.74	12.85 ± 1.78
F2	59.35 ± 2.64	10.23 ± 1.26
F3	81.25 ± 4.75	21.51 ± 1.46
F4	62.47 ± 3.12	14.25 ± 1.08
F5	68.25 ± 4.43	16.72 ± 1.35
F6	34.12± 6.43	11.72 1.35

2.5. *In vitro* drug release study

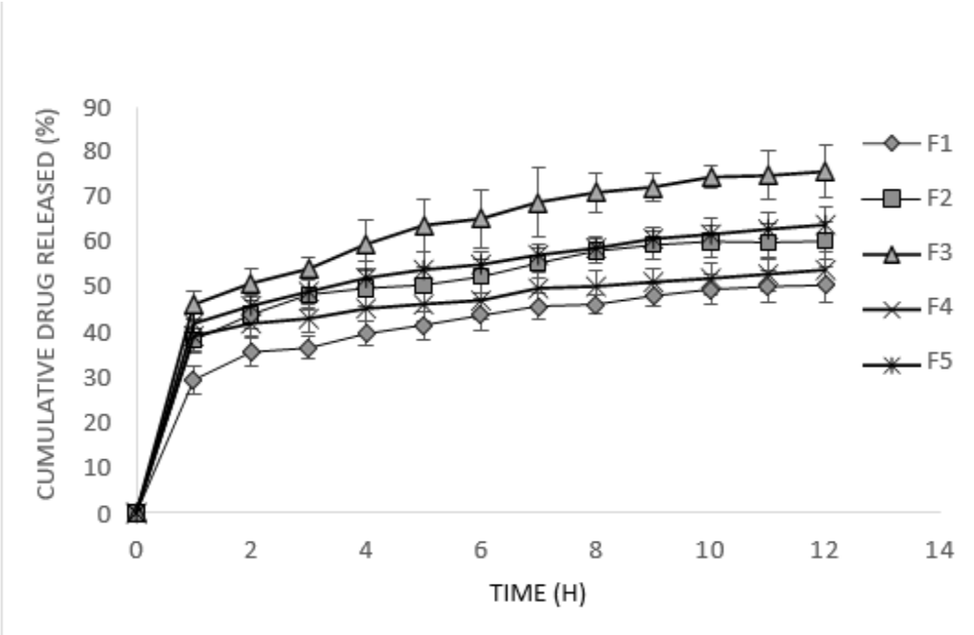
In vitro release profile of indomethacin from chitosan-nanoparticles in PBS (pH 7.4) are presented in **Fig 9.** The release of drug from chitosan-nanoparticles should be sustained and it is important, as it would allow for a prolonged retention of the drug at the target surface, would increase drug bioavailability and prolong therapeutic effect. The release of indomethacin from the chitosan-nanoparticles follow a biphasic pattern with an initial burst and rapid release followed by a slower release period. Up to 60 minutes the rapid release phase was lasted in all the five optimized formulations and in this duration about 30-50% of the indomethacin was found to be released from the chitosan-nanoparticles, which is an agreement with the study of drug-loaded chitosan nanoparticles .

The initial fast release of indomethacin from chitosan-NP at pH 7.4 was thought due to the fast dissolution of the drug molecules adsorbed on the surface of nanoparticles which diffuse out easily in the initial phase of incubation, moreover large specific surface area of nanoparticles could adsorb the drug, hence initial burst release was assumed due the desorption of indomethacin from the surface of chitosan-nanoparticles.

After the initial burst release, the release rate slow down that might be due to the changed mechanism of drug i.e. diffusion of indomethacin through the matrix of chitosan. The overall ‘‘rate’’ of drug release (i.e. fraction of drug content of the nanoparticles released at a particular time point) increased as the drug encapsulation in the nanoparticles increased (81.25% encapsulation in F3, shown highest drug release). It may be assumed that the differences in the release of indomethacin among the chitosan-nanoparticles at pH 7.4 (SIF) was because of the differences in encapsulation of drug in chitosan-nanoparticles (F1-F5). So, encapsulation of drug efficiently control the rate of drug release. From the *in vitro* results it can be assumed that the chitosan nanoparticles would reveal a similar sustained release *in vivo*, which is an indication that that chitosan-nanoparticles could be considered as a controlled drug delivery system for indomethacin.

Table 9: Percentage drug release

Formulation code	Percentage drug release					
	2 h	4h	6h	8h	10h	12h
F1	11.2 ± 0.6	19.5 ± 0.8	25.0 ± 1.4	27.9 ± 1.8	31.6 ± 0.2	48.9 ± 0.2
F2	19.2± 1.2	22.4± 1.4	29.6 ± 0.4	35.4 ± 0.2	59.4± 1.8	71.1 ± 1.4
F3	20.4 ± 0.6	27.6 ± 1.8	35.3± 1.4	47.9± 1.2	61.2± 0.2	79.9± 1.2
F4	19.6 ± 1.2	27.4 ± 0.4	33.8 ± 1.4	35.0 ± 1.2	44.1± 0.8	52.74±1.5
F5	16.4 ± 0.8	29.7 ± 1.4	30.2 ± 1.0	37.6 ± 0.2	55.1± 0.8	58.12±1.9
F6	40.2± 0.3	52.9±0.5	75.9±6.0	92.3±1.8	-	-



When the indomethacin loaded chitosan nanoparticles exposed with the release medium, the aqueous phase imbibes into the nanoparticles, causes the hydrolytic degradation of the chitosan molecules and thus release of drug occurs. Various mathematical models have been reported to quantify the drug release from different delivery carriers where the drug is homogeneously distributed throughout the carrier system with an initial drug concentration higher than their solubility in the carrier material i.e. monolithic dispersions.[48-51]

The drug release behavior from all the five optimized indomethacin loaded chitosan nanoparticles were subjected to fit in to various drug release kinetic models and it was found that overall curve fitting (Table 10) represented the drug release from chitosan-nanoparticles followed Highuchi-matrix kind of release mechanism as evidenced by their higher values of coefficients of determination (R2) of the straight line equations (Table 10). From the slopes of straight lines of the fitting equations, the 'n-values' were calculated that were found in the range of 0.0259 to 0.0823, which suggested a Fickian diffusion release mechanism, that described the sustained release of Indomethacin from the chitosan-NPs up to 12 h. Since, normally the diffusion-controlled release of drug illustrates a significantly higher release rate initially which is might be due to the small diffusion pathways at the starting time-points[52]. Therefore, the bursting phenomenon may be attributed to a normal diffusion-controlled drug release.

Table 10: Drug release model study by fitting *in vitro* release study

Release Models	Formulation F1	Formulation F2	Formulation F3	Formulation F4	Formulation F5
Zero order	0.9366	0.9183	0.9472	0.9728	0.9625
First order	0.9577	0.9432	0.9757	0.9821	0.9834
Higuchi Model	0.9912	0.9858	0.9853	0.9930	0.9970
Korsemeyer Peppas	0.9909	0.9845	0.9847	0.9722	0.9917
Hixkon crosswell	0.9512	0.9358	0.9660	0.9793	0.9774

2.7 Stability study Results

The physicochemical stability studies for a period of 6 months on the optimized chitosan-nanoparticles were performed. After 3 of storage at 25°C, an increase in the particle sizes ranged 8.73-14.71 nm (F1), 13.40-19.41 nm (F2), 7.31-12.61 nm (F3), 12.37-17.31 nm (F4), 9.64-14.30 nm (F5) and decrease in the zeta-potentials ranged 2.79-3.90 (F1), 0.991.58 (F2), 1.10-1.66 (F3), 0.79-1.09 (F4), 0.54-1.18 (F5) were observed. The percent encapsulation of indomethacin was lowered by 2.13-4.39 (F1), 2.11-3.14 (F2), 0.81-2.02 (F3), 1.32-2.92 (F4), 2.13-5.67 (F5), reduction in the percent drug contents were found in the range of 0.85-1.66 (F1), 1.03-1.99 (F2), 0.35-0.86 (F3), 0.69-1.01 (F4), 0.88-1.11 (F5) and decrease in percent drug released was found in the range of 1.24-2.98 (F1), 2.16-3.74 (F2), 1.03-2.36 (F3), 2.28-3.44 (F4), 1.92-2.61 (F5) The particle size analysis carried out on the storage formulations after 3 months, a small increase in the diameter were found, which might be due to swelling and water adsorption behavior of chitosan-NPs. This swelling nature and degradation of chitosan in the aqueous phase might be the reason for drug expulsion from NPs and hence there were decreased encapsulation efficiency and drug release from chitosan-nanoparticles after long term storage.

Table 11: Results of Stability Studies

Formulations	Z-Average (nm)	Zeta-potential (mV)	Encapsulation (%EE) ± SD	Drug content (%) ± SD	Cumulative drug release (%) ± SD
At initial time					
F1	449.52 ± 15.75	+36.05 ± 2.18	62.65 ± 3.74	100.00	51.25 ± 1.78
F2	301.85 ± 18.45	+28.14 ± 3.23	59.35 ± 2.64	100.00	62.28 ± 1.26
F3	401.54 ± 14.25	+31.25 ± 3.45	81.25 ± 4.75	100.00	75.75 ± 1.46
F4	658.25 ± 12.65	+27.14 ± 4.26	62.47 ± 3.12	100.00	53.56 ± 1.08
F5	430.95 ± 10.56	+26.19 ± 2.48	68.25 ± 4.43	100.00	62.15 ± 1.35
At 3rd month					
F1	459.25 ± 14.15	+33.26 ± 2.13	60.52 ± 2.43	99.15 ± 0.56	50.01 ± 1.08
F2	315.25 ± 15.78	+27.15 ± 3.12	57.24 ± 3.17	98.97 ± 1.87	60.12 ± 2.01
F3	408.85 ± 15.45	+30.15 ± 2.35	80.44 ± 4.75	99.65 ± 1.01	74.72 ± 1.25
F4	670.62 ± 18.57	+26.35 ± 2.56	61.15 ± 3.54	99.31 ± 1.05	51.28 ± 1.07
F5	440.59 ± 16.25	+25.65 ± 3.08	66.12 ± 3.28	99.12 ± 1.02	60.23 ± 1.23

CONCLUSION:

The present Research relates to the Preparation of Indomethacin Chitosan loaded Nanoparticles.The Indomethacin Nanoparticles were prepared by using Ionotropic Gelation Method, wherein the polymers used in the preparation were chitosan.This study confirms that Inotropic gelation method is suitable for the preparation of Indomethacin nanoparticles, in view of the fact that high encapsulation efficiency was obtained. Indomethacin nanoparticles showed a higher dissolution rate of the drug which suggests that lower doses of this molecule.Out of all the prepared formulations F3 with the Indomethacin concentration of 40mg showed the highest entrapment efficiency of 81.25 % when compared to that of other formulations and the highest extent of release (79.9%).The prepared Indomethacin chitosan loaded nanoparticles would be capable of reducing the frequency of administration and the dose-dependent side effects associated with the repeated administration of conventional Indomethacin tablets.Using ionotropic gelation method, good average sized positively charged chitosan-nanoparticles with high encapsulation efficiency; satisfactory drug release and good storage stability properties could be produced.

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