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DESIGN, *IN VITRO* CHARACTERIZATION& OPTIMIZATION OF MEFANIMIC ACID LOADED CARBOXY METHYL CHITOSAN NANOPARTICLE

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Key Words

ABSTRACT

In this present investigation Mefenamic acid (MA) loaded nanoparticles. Mefenamic acid loaded Carboxy methyl chitosan nanoparticles formulated by nanoprecipitation and solvent evaporation method, Mannitol is used as cryoprotectant. Drug and polymer compatibility study was carried out with FTIR and DSC study and results indicated that there were no interaction between drug and polymers. The nanoparticles were characterized in terms of nanoparticle size, surface morphology, encapsulation efficiency, in vitro drug release profilestudy. Results indicated that the particle size, drug entrapment efficiencyand drug release rate was influenced by verifying polymer ratio. The release rate of nanoparticles could be controlled by adjusting the combination of polymers in different ratios. The particle size analysis data revealed that the average particle size of the optimized formulation was 278.88 nm and the entrapment efficiency was 91.04% and drug release of about 95% at 12th hour.

INTRODUCTION

Advanced drug delivery systems have numerous advantages over conventional multi dose therapy. Much research effort in developing such drug delivery systems has been focused on controlled release and sustained release dosage forms. Now a day effort is being made to deliver the drug in such a manner so as to get optimum benefits [1,2]. There are numerous strategies in delivering therapeutic agent to the target site in a sustained release fashion. One such strategy is using nanoparticles as drug carrier [3,4]. Nanoparticles have increasing interest from every branch of medicine for the ability to deliver drugs in the optimum dosage range, often resulting in increased therapeutic efficiency of the drug and weakened side effects. Generally, nanoparticles are defined as Solid colloidal particles that include both nanospheres and nanocapsules. They can be prepared by both polymerization methods and synthesis from preformed polymers. One of the fundamental characteristics is their size, which is taken around 10nm to1000nm range [5,6]. As stated by various authors they can improve the stability of active substances and can be biodegradable with tissue and cells when synthesized from materials that are either biocompatible or biodegradable. Biocompatible nanoparticles as drug delivery vehicles provide several advantages, including high drug entrapment, protection of the encapsulated therapeutic. In this study Mefenamic acid was formulated as nanoparticle drug delivery system using carboxy methyl chitosan as polymers. Mefenamic acid is a widely prescribed NSAID

and used as first line therapy for the treatment of ailments such as Arthritis and Dysmonorrhoea. Mefenamic acid is a Non-steroidal anti-inflammatory drug (NSAID), with analgesic, and anti-pyretic properties. It is considered to be a BCS Class II drug (low soluble and high permeable). Mefenamic acid binds with prostaglandin synthetase. As these receptors have a role as a major mediator of inflammation, the symptoms of pain are temporarily reduced. Mefenamic acid has less biological half life (t1/2) of 2hrs and being an NSAID has a major side effect of gastric irritation. Formulating such drug into nanoparticles using biocompatible polymers is expected to modify andprolong the sustain release profile.

MATERIALS AND METHODS

Mefenamic acid was obtained from Sun Pharmaceutical Industries Limited Sikkim, Chitosan and methyl cellulose were procured from MSN lab Hyderabad.

FTIR Studies: Figure 3 showed the representative FT-IR spectrum of CMCS In the infrared spectra; an interesting characterization peak was in the range of 3200 – 3400 cm-1, indicating the hydrogen bonding. The hydrogen bonding in CMCS polymer was at 3312 and 3279 cm-1 respectively, and they shifted to 3323 and 3291 cm-1 after MA was encapsulated, The vibration peaks of 1500 – 1700 cm-1, corresponding to amide I and II bond, had no obvious shift in all formulations. The vibration peak at 1419 cm-1 in CMCS could be assigned to the symmetric stretching

Preparation Mefenamic acid loaded Carboxy methyl Chitosan nanoparticle (MA-CMP-NP)

Preparation of MA-CMC-NP: MA-CMC-NPs containing mefanamic acid-NPs was prepared using combined technique of solvent evaporation and nanoprecipitation technique with slight modification**.** This is atwo-step process, in the first step, emulsification of the polymer solution into aqueous phase containing a surfactant is done. Then in the second step evaporation of polymeric solvent is carried out, inducing polymer precipitation of the nanoparticles (**Reis et al., 2006)**. The calculated quantities of Carboxy methyl chitosan and tween 80 and PVA were varied according to the experimental design approaches depicted in table no 1**.**Carboxy methylChitosan was dissolved in an organic solvent acetone (10 mL) and separately tween 80 dissolved in double distilled water. The organic solvent was added slowly to the aqueous phase containing tween 80 with a constant stirring on magnetic stirrer at room temperature. The evaporation of the organic solvent was performed at a temperature range of 65-80^{0}C which involves precipitation process lead to formation of nanoparticles. The obtained nanoparticle was ultrasonicated for different time interval (2-8 min.) at 60-80 KHz amplitude) for 1 cycle and allowed to cool at room temperature. The developed MA-CNPs were lyophilized using the freeze dryer at a chamber pressure (20pa) and cold trap temperature (-120ºC) in the entire process. The study was performed for 24 h for freezing, 4 h for primary drying at 0ºC, followed by 10ºC for 2 h and 15ºC for 1.5 h and secondary drying at 25ºC for 3 h. Mannitol (3%) was added as a cryoprotectant to avoid lysis of NPs

FTIR study: Compatibility studies were carried out to know the possible interactions between MA and polymers used in the formulation. Physical mixtures of drug and polymers were prepared to study the compatibility. Drug polymer compatibility studies were carried out using FTIR spectroscopy (ATR technique). IR spectra of drug andalong with excipients were seen in between 600-4000 cm^{-1}

Determination of % Entrapment efficiency and % yield

The amount (10 ml) of MA loaded nanoparticles suspension was carefully transferred to centrifugation tube. The nanoparticles in the form of sediment was separated from the solution by ultracentrifugation at 15,000 rpm at 4℃ for 40 mins. The supernatant wascarefully decanted and analyzed by UV spectrophotometer (Lab India 3200) for MA at 287 nm. The % entrapment efficiency and % drug loading were calculated using equation as given below

$$\text{Entrapment Efficiency \%} = \frac{Total\ drug - Free\ drug}{Total\ drug} \times 100\ \%$$

$$\text{Drug Loading \%} = \frac{Total\ drug - Free\ drug}{Nanoparticle\ Weight} \times 100$$

Particle size measurement: Particle size measurements and polydispersity of MA nanoparticles was determined by Brookhaven Instrument Corporation, NY. Lyophilized MA nanoparticles were dispersed in double distilled water and analyzed in three readings per nanoparticles sample. The poly dispersity was also calculated based on the volumetric distribution of particles

Drug release study: The *in vitro* drug release profile of MA-loaded nanoparticles formulation has been studied using a dialysis bag. Approximately 100 mg of MA nanoparticle suspension were taken into a dialysis bag (molecular weight cut-off, 12 KDa, Himedia, India) and placed in a beaker containing 150 ml of tris buffer solution. Then, the beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at 37 ± 0.5℃ throughout the study. Samples (5 ml) were withdrawn at definite time intervals (1, 2, 3, 4, 6, 8, 10 and 12 h) and replaced with equal amounts of fresh buffer. The samples were analyzed for drug concentration by UV-Vis spectrophotometer at 287 nm. Moreover, MA-CMC-NPs, prepared in the optimal condition were observed using SEM (Fig. 4). All nanoparticles were spherical or ellipsoidal in shape with a smooth surface and well dispersed without aggregation. Spherical particles with uniform particle size in the nanoscale formed, ranging from 154 to 201 nm. The aggregates, usually having a rod shape, as observed in the SEM photos were probably formed during the drying process. The particle size of nanoparticles obtained after cast drying was in good agreement with that measured in an acidic aqueous system presented in the next section. The effects of CMC concentration and sonication time on particle size and polydispersity index (PDI) of MA-loaded CMC loaded nanoparticles were summarized in (**Table no 2)**. The particle size increased linearly from 154 to 201 nm with the increase of CMC concentration (**Table no 2)**. These trends were in accordance with previously reported results (Hu et al., 2008; Gan, Wang, Cochrane, & McCarron, 2005). The above results showed the interaction effect of Carboxy methyl chitosan concentration and Sonication time on the EE% and DL% of MA loaded CSNPs.. These result revealed that EE% varies from 59.12±3.30% to86.65±1.16, F8 having high Carboxy methyl chitosan concencentration and longest sonication time showed 86.12% Entrapment Efficiency due to cross linking of chitosan with drug. DL% varied .from 10.51% to 19.31 %. **(Table no 3).** This could be attributed to the binding of hydroxyl groups of MA to positively charged amino groups on CS molecules by electrostatic interaction These results might be attributed to an increase number of interacting units at higher polymer concentrations and to cross-linker levels that lead to the observed increase in particle size and decrease entrapment efficiency. The formulation of F1 to F9 showed wide range of drug release as shown in Fig. 10. In case of formulations (F2, F4and F8) showed more than 98% drug release from in 24 h respectively. F6 showed early release of drug Results of drug release showed that as the concentration of polymer increased, percentage drug release increased because of increase in the entrapment efficiency. But after some level percentage drug release decreased because of decrease in entrapment efficiency. Thus the formulation F8 showed highest percentage 99.11% cumulative drug release. In 24hr it is due to enhanced concentration of chitosan methyl cellulose which modifies the drug release

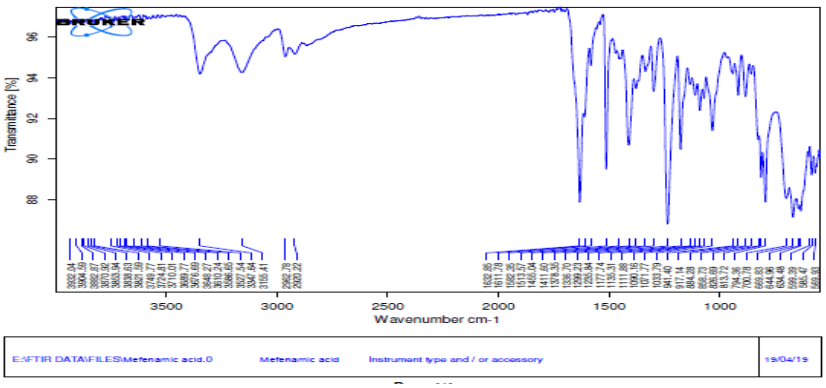

Figure: 1- FTIR of Mefanimic acid

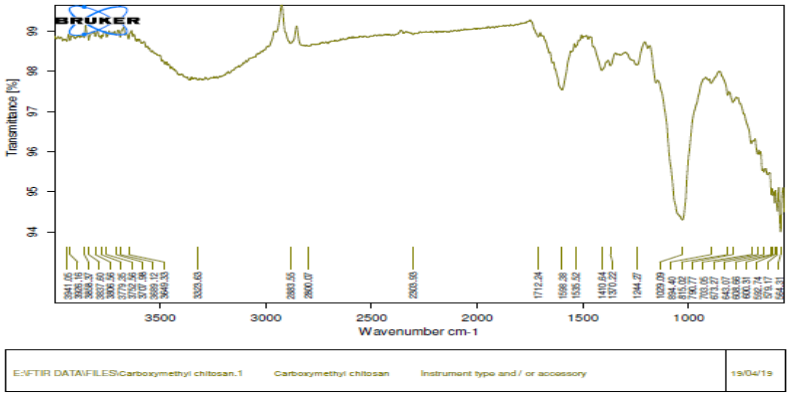

Figure: 2- FTIR (CMC)

Factor	Level Used, Actual Coded		
Independent variables	Low (-1)	Medium (0)	High (+1)
A = CMC (%)	2	4	6
B = Tween 80 (%)	3	5	7
C = Sonication time (minute)	2	5	8

Table: 1 - Formulation variable with coded value

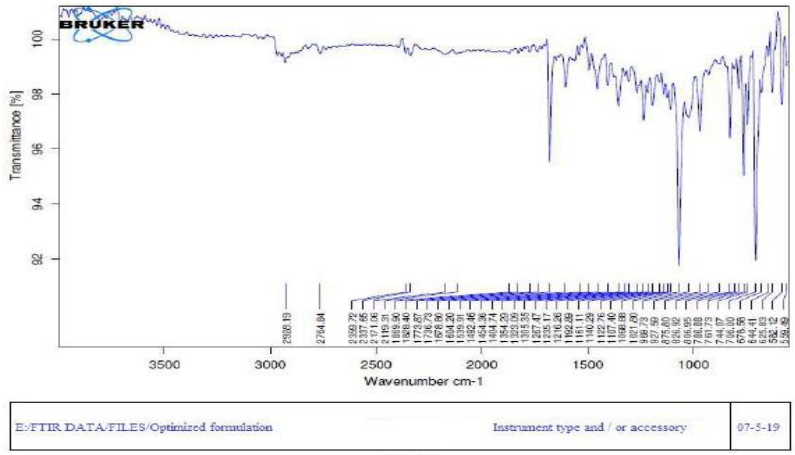

Figure: 3 - FTIR Optimized formula

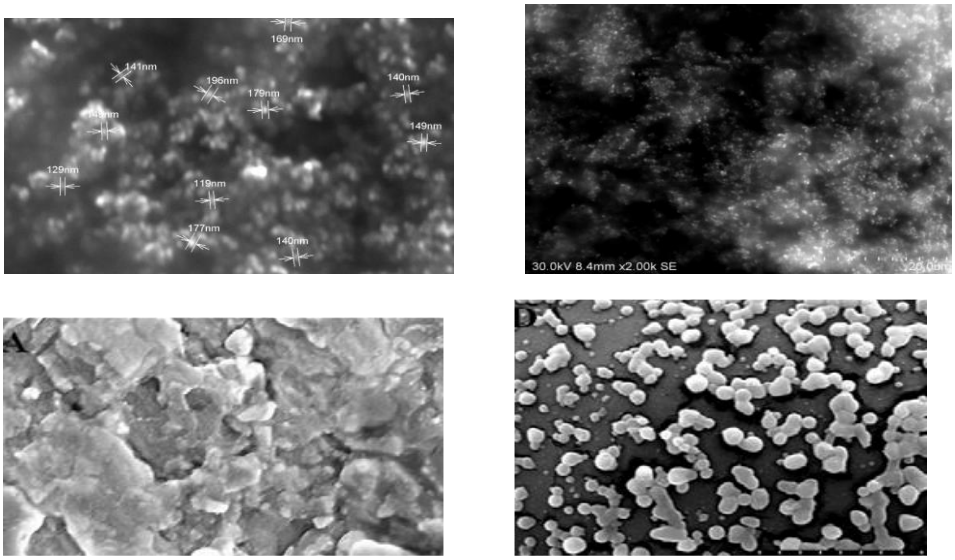

Figure: 4 - Scanning electron microscopy images of MA-CMC-NP

Particle Size of Mefenamic acid loaded Carboxy methyl Chitosan

Formulation Code	CMC	Sonication time	Particle size	PDI
F1	+1	+1	158.7±13.4	0.234±0.0235
F2	0	+1	170.3±11.2	0.264±0.0245
F3	-1	+1	168.7±12.4	0.234±0.0335
F4	+1	0	160.3±11.2	0.264±0.0205
F5	0	0	154.7±13.4	0.214±0.0335
F6	-1	0	177.3±11.2	0.134±0.0435
F7	+1	-1	201.7±13.4	0.278±0.0425
F8	0	-1	171.3±10.2	0.134±0.0515
F9	-1	-1	159.2±12.4	0.264±0.0235

Table: 2Particle Size of Mefenamic acid loaded Carboxy methyl Chitosan **MA-CMC-NP**
N=3 (Mean± SD)

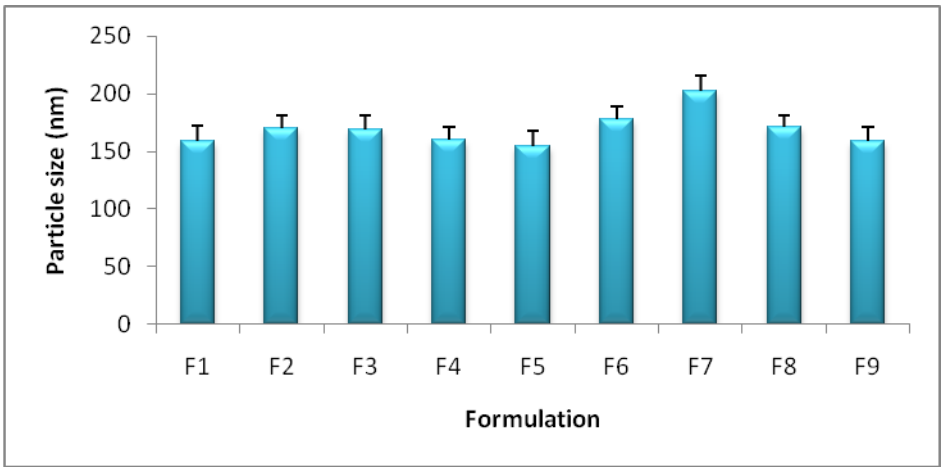

Figure: 5 Particle size MA-CMC-NP

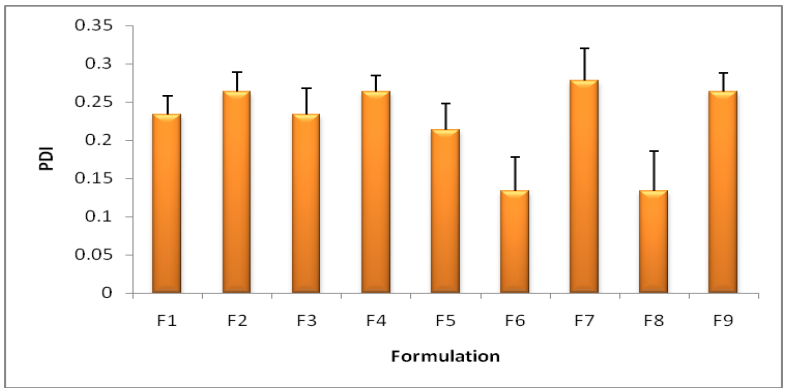

Figure: 6- Polydispersity Index*Mefenamic acid loaded Carboxy methyl Chitosan* MA-CMC-NP

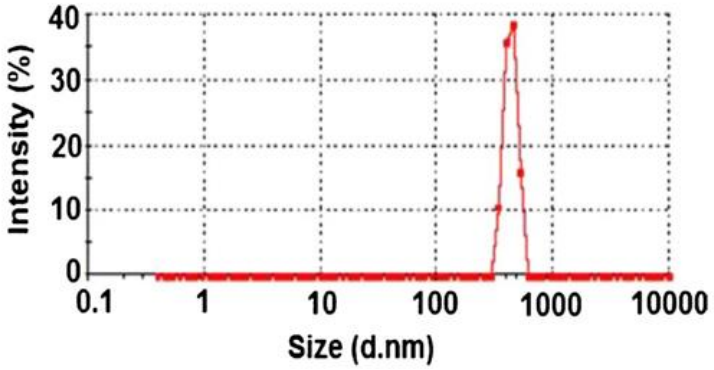

Figure: 7 Size distribution of MA-CMC-NP

Encapsulation Efficiency & Drug Loading % of Mefenamic acid loaded Carboxy methyl Chitosan MA-CMC-NP

Formulation Code	CMC	Sonication time	EE%	DL%
F1	+1	+1	59.19±1.38	12.31±1.24
F2	0	+1	79.18±2.19	11.21±1.04
F3	-1	+1	81.52±1.27	10.51±1.03
F4	+1	0	79.52±3.39	11.01±1.64
F5	0	0	82.19±1.69	15.31±0.24
F6	-1	0	80.16±1.44	12.31±1.94
F7	+1	-1	78.02±1.30	18.31±1.29
F8	0	-1	86.12±1.09	19.31±1.38
F9	-1	-1	79.02±1.29	11.31±1.44

Table 3: 3EE% and DL% of MA-CMC-NPN=3 (Mean± SD)

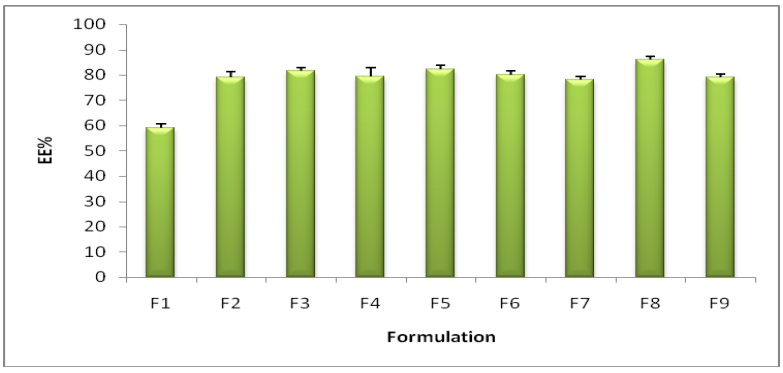

Figure: 8 - EE% Size MA-CMC-NP

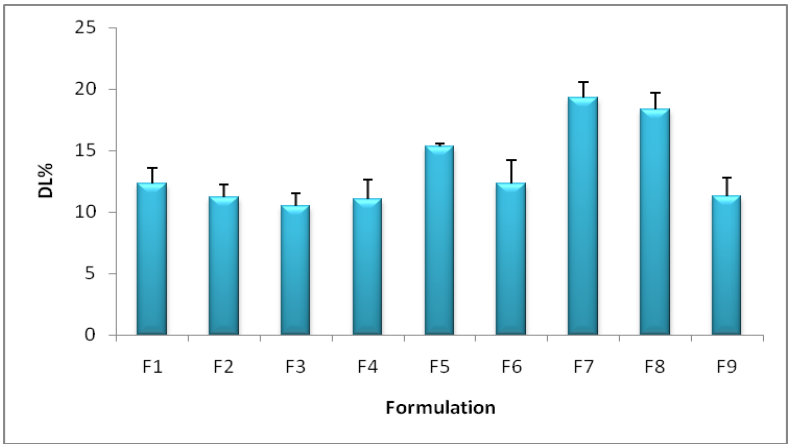

Figure: 9 DL% size MA-CMC-NP

Time(hr)	*In-vitro* Cumulative Drug Release %									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	Control
0	0	0	0	0	0	0	0	0	0	0
8	53	42	49	44	39	46	28	33	28	72.33
10	61.37	59	68.03	59.2	49.1	65	40	59	40	96.32
12	65.04	61.02	71	69.43	59	79	57	78	57	100.1
14	71.34	65.03	73.09	82.33	65	83	62	83	62	100.5
16	73.13	82.04	75.01	93.12	78	91	75	89	69	100.9
20	88.05	85.11	81.06	98.08	81	100	79	96	73	100.9
24	93.02	98.33	83.33	100	89	100	88	99.11	88	100.9
26	100	100	85.45	100	98	100	99	100	91	100.9

Table: 4- *In-Vitro* Release Profile of MA-CMC-NP

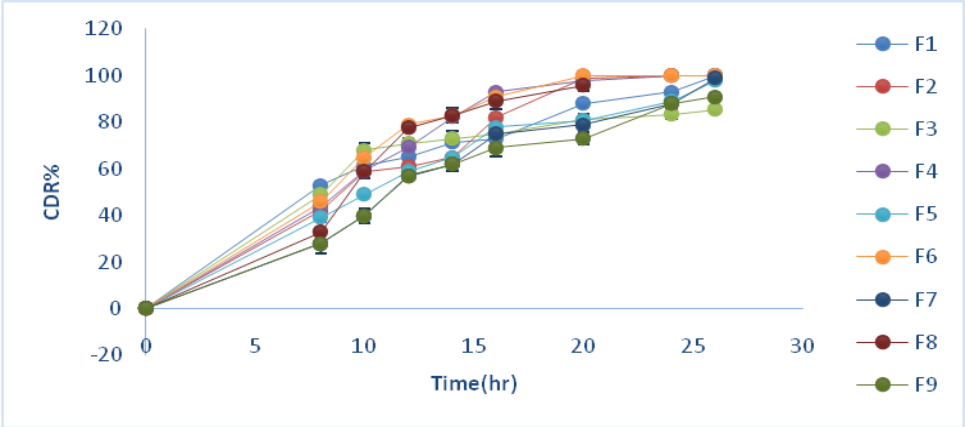

Figure: 10- *In-Vitro* Release Profile of MA-CMC-NP

CONCLUSION

Mefenamic acid loaded Chitosan methyl cellulose nanoparticles were prepared by Nano precipitation technique. The obtained nanoparticles were characterized by Scanning electron microscopy. The images clearly reveal that the particles were in nano range. The entrapment efficiency was found to be between 59.19±1.38 to 86.12±1.09 The and loading capacity was found to be between 10.51±1.03 to 19.31±1.44.The in vitro cumulative release was found to be 99.11% in 24hr in F8 formulation Therefore, the present investigation showed promising result of nanoparticles of Mefenamic acid loaded in chitosan methyl cellulose in F8 batch was proved as best formulation

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