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Research Article

Formulation of Mefenamic acid loaded polymeric nanoparticles by ionotropic gelation technique for the treatment of rheumatoid arthritis

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Drug release,
Stability

Abstract

Aim: Non steroidal anti inflammatory drugs are generally used as first line drugs for Rheumatoid arthritis. Mefenamic acid is an anthranilic acid derivative**.** The short biological half life of Mefenamic acid is 2hr. The aim of this investigation was to develop and characterize polymeric nanoparticles of Mefenamic acid by ionotropic gelation technique.

Materials and methods: For ionic gelation technique, Chitosan was used as polymer and sodium tripolyphosphate as cross linking agent. All formulations were prepared by varying the drug and polymer concentrations. The obtained nanoparticles were characterized for surface morphology, FTIR, particle size and zeta potential and evaluated for yield, drug content, entrapment efficiency, loading capacity and Invitro drug release.

Results and discussion: The mean particle size and zeta potential of the best formulation of Mefenamic acid nanoparticles for ionic gelation technique was found to be 196nm and -34 mV respectively. The drug release was found to be 96.3% till 11hrs with fickian diffusion.

Conclusions: Inotropic gelation technique was found to be the best technique for the formulation of Mefenamic acid nanoparticles as they have less particle size, greater stability and controlled drug release for 12hrs.

1. Introduction

Rheumatoid arthritis is an autoimmune disorder in which there is joint inflammation, synovial proliferation and destruction of articular cartilage and bone. Extra-articular features commonly include general malaise, fatigue, weight loss, fever and anemia. The need of treatment is to reduce pain and improve function. Drugs used in this therapy are NSAIDs, corticosteroids, TNF, IL-1 inhibitors and DMARDs i.e. disease modifying anti rheumatic drugs. NSAIDS like Mefenamic acid, Ibuprofen, Diclofenac sodium, Ketoprofen, naproxen etc. are used for the treatment. Nanoparticulation is a very useful strategy towards targeted drug delivery. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. The present conventional drug delivery systems often have side-effects and complication due to their wide distribution throughout the body fluids. The localization of drug action in injured tissue is promising way to solve this problem. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. [1,2] Chitosan is a natural polymer having excellent biodegradable properties. Methods for preparation of polymeric nanoparticles have been reviewed and they include ionic gelation, Co-acervation, solvent evaporation, spontaneous emulsification or solvent diffusion, salting out/emulsification-diffusion, supercritical fluid technology and polymerization. [4]

Ionic gelation is based on the ability of polyelectrolyte to cross link in the presence of counter ions. [5,6] Mefenamic acid is a NSAID which is an anthranilic acid derivative. [7] It is available as tablets, capsules and suspensions. The available dose for this drug is 250mg thrice a day. MA has a wide range of gastrointestinal disorders, like gastrointestinal bleeding and gastric upset.[8] MA is classified as class II on the basis of biopharmaceutical classification system, because of its poor solubility over the pH range 1.2-7.5. [9] The short biological half life of MA is 2hr. Because of short half life, frequent administration of the drug is required to maintain the desired steady state level, hence nanoparticles are formulated to achieve sustained release properties. [10] In the present study, Mefenamic acid nanoparticles were formulated using ionotropic gelation techniques.

2. Materials and Methods

2.1 Materials

Mefenamic acid was purchased from Sigma Aldrich, Bangalore and chitosan from Himedia.

2.2 Methods

2.2.1 Ionotropic Gelation Technique for Preparation of Mefenamic Acid Nanoparticles

In order to obtain nano formulations, process parameters were optimized which include concentration of cross linking agent, stirring speed, stirring time. Mefenamic acid loaded chitosan nanoparticles were prepared using ionotropic gelation technique. Chitosan solutions of 0.25%, 0.35 and 0.4% were prepared by using 0.1% acetic acid solution. The drug was dissolved in the polymer solution. It was added drop wise into 0.5% TPP solution which was kept under magnetic stirring at 700 rpm for 5hrs.The fine dispersion was collected and centrifuged using ultra centrifuge at 10000 rpm for 15min.The pellet was collected and dried. The same procedure was followed for other ratios.

Five formulations were prepared.F1, F2 and F3 formulations were prepared by varying the concentrations of chitosan in the range of 0.25%, 0.35% and 0.4%.F1, F4 and F5 formulations were prepared by changing the concentration of drug (1:1, 1.5:1, 2:1)

2.2.2 Characterization of the nanoparticles

Particle size measurement

It is done by Nano Partica analyzer (HORIBA SZ-100 series).Dynamic Light Scattering (DLS), also known as Photon Correlation Spectroscopy, is a common technique for measuring the size of particles in the sub micron range.

Zeta potential:

The zeta potential is a physical property which is exhibited by all particles in suspension. It is an important parameter in understanding the electric double layer repulsion and it can be measured by phase analysis light scattering.

Scanning electron microscopy

Scanning electron microscopy (SEM) is based on the incidence of a beam of accelerated electrons on the sample. These accelerated electrons interact with the sample, exciting its atoms which emit secondary electrons. According to the angle between the primary beam and the surface of the sample, it is possible to detect and analyze the surface topography.

Fourier Transforms infrared Spectroscopy (FT-IR):

The FT-IR spectra acquired were taken for the dried samples. An FT-IR (7000) spectrometer was used for the analysis in the frequency range between 4000 and 400 cms^{-1}.

2.3 Evaluation of nanoparticles

Determination of Drug content in Nanoparticle sample:

Free drug of the formulations was first determined in the supernatant by choosing a solvent in which only the free drug gets dissolved and not the other ingredients. To determine the drug content, 50mg drug equivalent to formulation was weighed accurately and transferred into three necked RBF containing 50ml of methanol. The solution was stirred at 700rpm for 3hrs using magnetic stirrer. The resultant solution was filtered and the amount of the drug in the filtrate was estimated after suitable dilution by ultraviolet (UV) spectrophotometer at 285nm.

Entrapment efficiency

Entrapment efficiency indicates the amount of drug encapsulated in the formulation. The method of choice for drug content determination is separation of free drug by ultra centrifugation, followed by quantitative analysis of the drug from the formulation. The samples were centrifuged using ultracentrifuge at 17640 rpm for 40min.

Percentage entrapment efficiency may be calculated from the following formula:

$$Entrapment\ efficiency = \frac{Amount\ of\ drug\ encapsulated\ in\ the\ formulation}{Total\ amount\ of\ drug\ in\ the\ formulation} \times 100$$

Loading Capacity

The loading capacity (L.C) refers to the polymer carrying capacity of the drug.

$$\text{Loading capacity} = \frac{Total\ \ amount\ \ of\ drug - Amount\ \ of\ unbound\ \ drug}{Nanoparticles\ \ \ weig\,ht} \times 100$$

Percentage Yield:

The yields of the prepared nanoparticles were calculated. The dried nanoparticles were weighed and the yield of nanoparticles was calculated using the formula:

$$\text{Percentage yield} = \frac{Amount\ \ of\ \ nanoparticles\ \ \ obtained}{Theore\,tical\ \ amount} \times 100$$

Drug release studies:

Drug release studies were performed by means of Orbitary shaker. Drug release from polymeric nanoparticles was determined as follows. A known amount of nanoparticles was transferred to a conical flask and 50mL of the Phosphate buffer pH 7.4 was added to the conical flask. The temperature and rotation were adjusted to 37°C and 100 rpm, respectively. 5ml aliquots were drawn at 0.5, 2, 4, 6, 8, 10, 12, and 24 hrs and it was replaced by the 5ml of fresh medium. The samples were centrifuged at 3000 rpm for 15 minutes. The samples were further analyzed using UV Spectrophotometer at 285 nm.

3. Results

3.1 Ionotropic gelation technique for Mefenamic acid (MA) loaded chitosan (CH) nanoparticles.

Mefenamic acid loaded chitosan nanoparticles were prepared by ionotropic gelation technique. In order to obtain nanoformulations, the process parameters such as concentration of cross linking agent, stirring speed, stirring time were optimized. Five formulations were prepared by varying the drug and polymer concentrations. The prepared five formulations were characterized and evaluated.

3.2 Scanning electron microscopy of Mefenamic acid (MA) loaded chitosan (CH) nanoparticles by ionotropic gelation technique.

The surface morphology of the nanoparticles was determined by using scanning electron microscopy. All formulations were found to be spherical in shape and were in nanorange.[**Figure 1]**

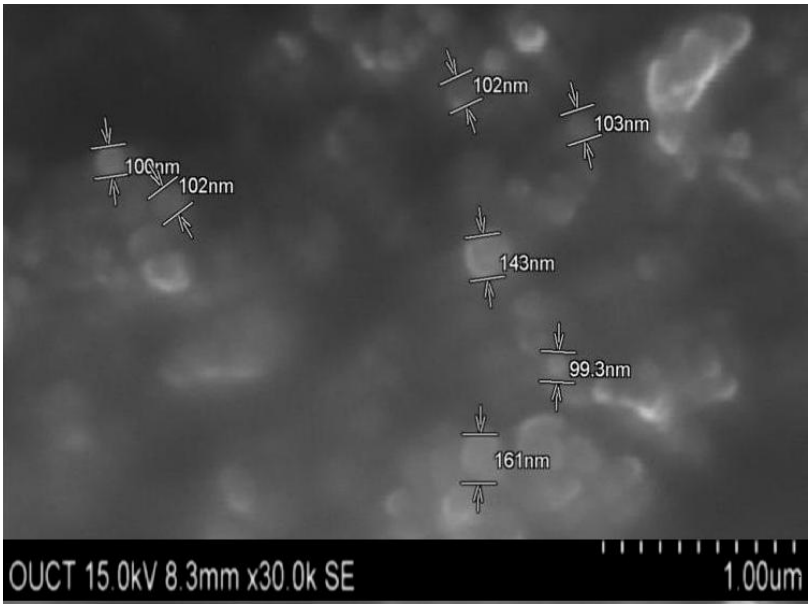

Figure 1:- SEM Images of Mefenamic acid (MA) loaded chitosan (CH) nanoparticles

Mean particle diameters

The mean particle diameters of F1, F2, F3, F4 and F5 formulations were found to be in the range of 148-210nm.Particle size distribution report of F2 formulation was depicted in **Figure 2.** Comparision of mean particle diameters of al five formulations was given in figure 3.

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Measurement Results

Date	: 28 August 2014 11:56:35
Measurement Type	: Particle Size
Sample Name	: F2-CH
Scattering Angle	: 90
Temperature of the holder	: 25.0 deg. C
T% before meas.	: 2776
Viscosity of the dispersion medium	: 3.094 mPa.s
Form Of Distribution	: |Standard|
Representation of result	: Scattering Light Intensity
Count rate	: 1992 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	194.6 nm	47.4 nm	182.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	194.6 nm	47.4 nm	182.0 nm

Cumulant Operations

Z-Average : 151.3 nm

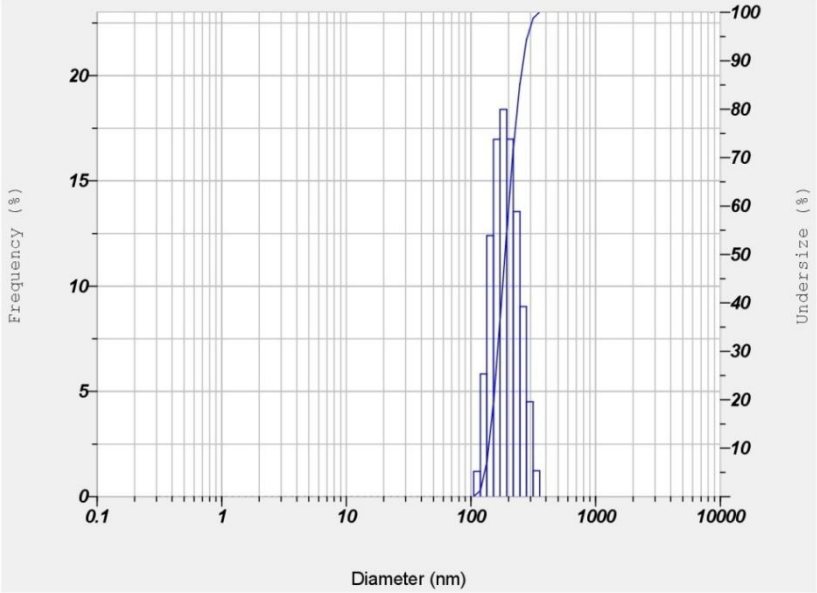

Figure 2: Particle size distribution report of F2 formulation of CH MANPs.

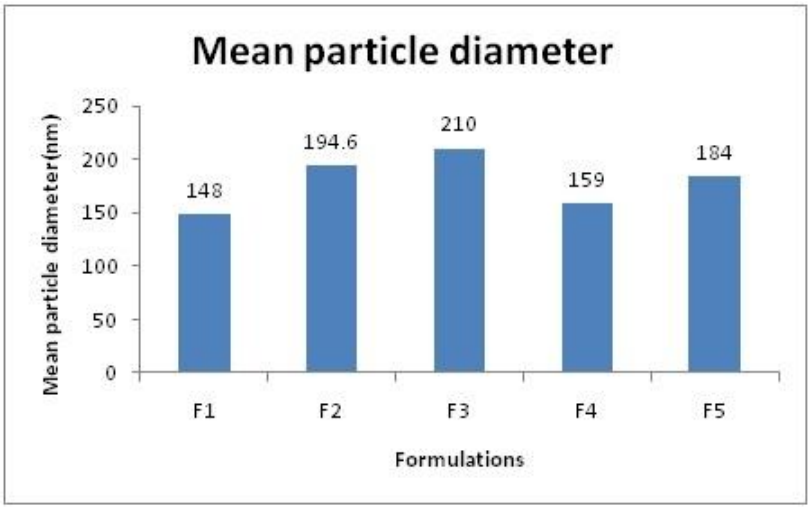

Figure 3:- Comparison of mean particle diameters of mefenamic acid loaded chitosan F2 formulation

Zeta potential values

The zeta potential values of F1, F2, F3, F4, and F5 formulations were found to be in the range of 30-48mV

[**Figure 4**]. The arbitrary zeta potential value is ± 30 mV. So, all formulations were found to be stable.

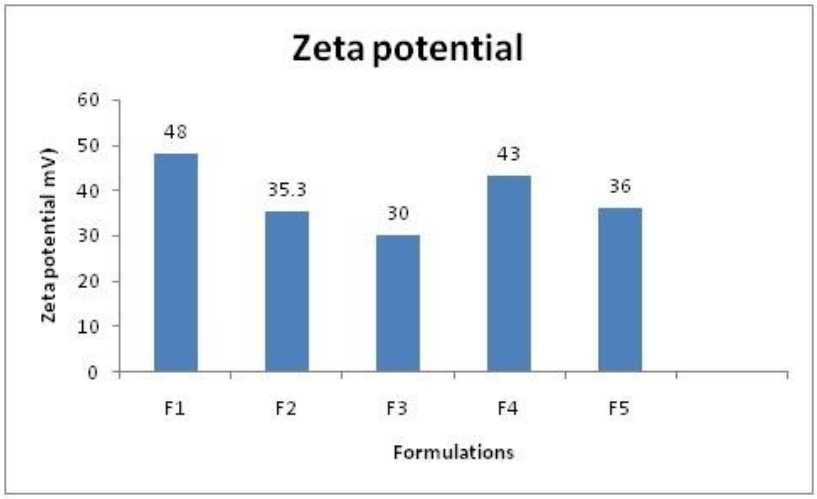

Figure 4:- Comparison of zeta potential values of mefenamic acid loaded chitosan formulations

Comparison of Product yields

The product yields of all five formulations were found to be 93.5%, 94.4%, 90.3%, 92.3% and 94%. It is depicted in [**Figure 5].** Among all the formulations, F2 was considered to have higher yield.

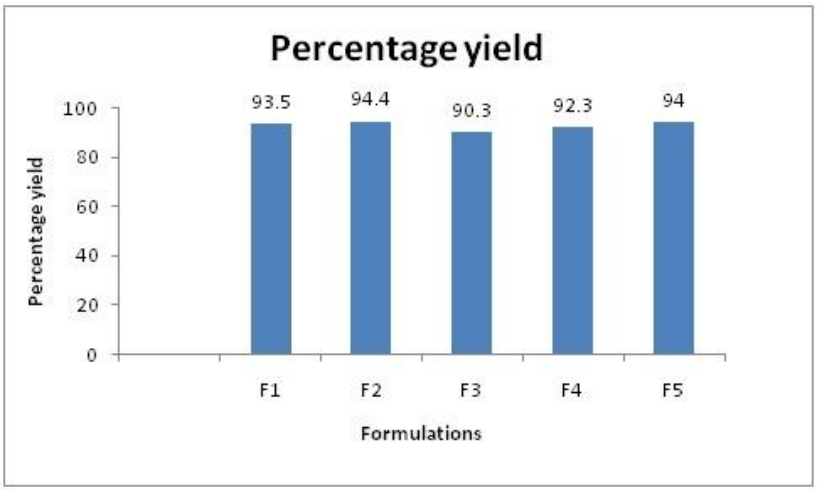

Figure 5: Comparison of product yields of F1, F2, F3, F4, F5 formulations of CH MANPs.

Comparison of Drug contents

The drug contents of all five formulations were found to be 84.5%, 94.2%, 93.5%, 90% and 91.1%. It is depicted in **Figure 6.** Among the, F2 showed higher drug content.

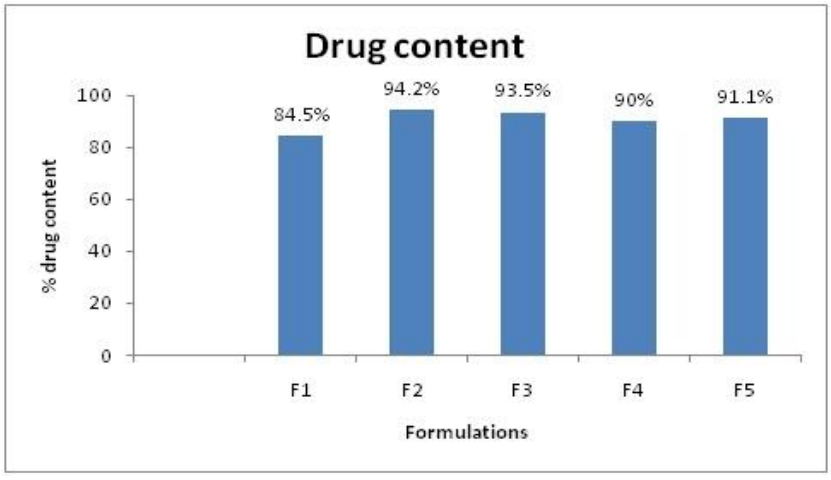

Figure 6: Comparison of drug contents of F1, F2, F3, F4, and F5 formulations of CH MANPs.

Comparison of Entrapment efficiencies

The entrapment efficiencies of F1, F2, F3, F4 and F5 formulations were found to be 61%, 84%, 80%, 81% and 82.3%. F2 has greater entrapment efficiency than the other formulations (shown in Figure 7.

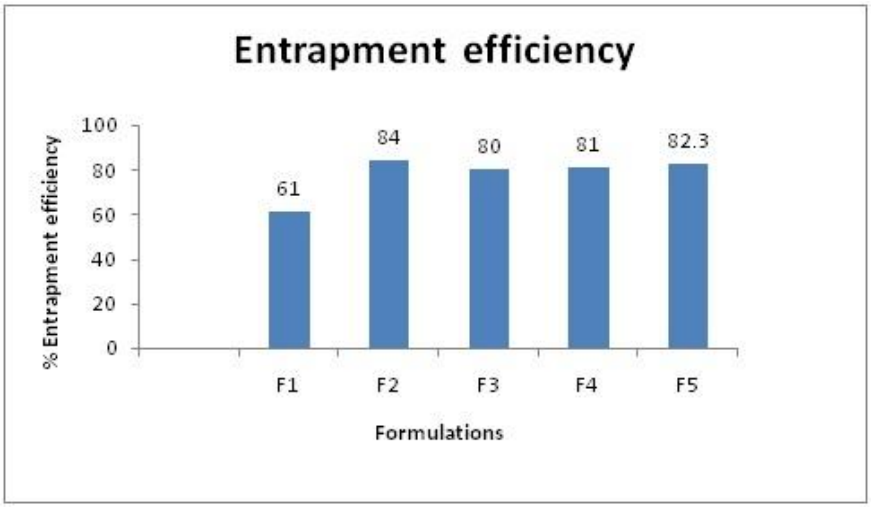

Figure 7: Comparison of entrapment efficiencies of F1, F2, F3, F4, F5 formulations of CH MANPs.

Comparison of loading capacities

The loading capacities of F1, F2, F3, F4 and F5 formulations were found to be 39.6%, 36.4%, 27.9%, 52% and 54.2%. Loading capacity of F5 formulation was more when compared with other formulations. **[Figure 8]**

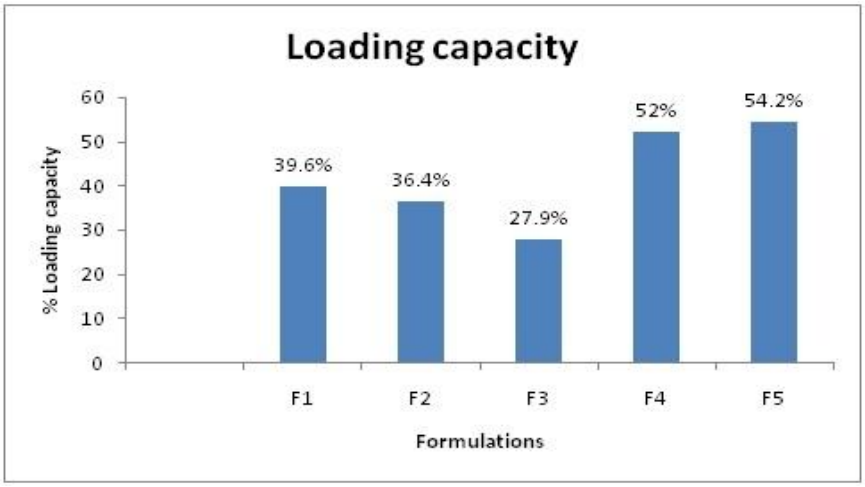

Figure 8:- Comparison of loading capacities of F1, F2, F3, F4, F5 formulations of CH MANPs.

In vitro drug release studies

The drug release studies were performed for 24hrs.The percentage of drug release from F4 and F5 was found to be 94.6% and 96.8% respectively with in a time period of 6hrs. From F2 formulation the percentage of drug release was found to be 96.3% in 12 hr and F3 formulation was able to control up to 10hrs with 97.6% of drug release. Among all the formulations, F2 was found to be the best formulation as it controlled drug release up to 12hrs with 96.3% drug release. In F1, F3, F4and F5 formulations, the polymer concentration was not sufficient to retard the drug release. Comparative drug release profile was given in figure 9.

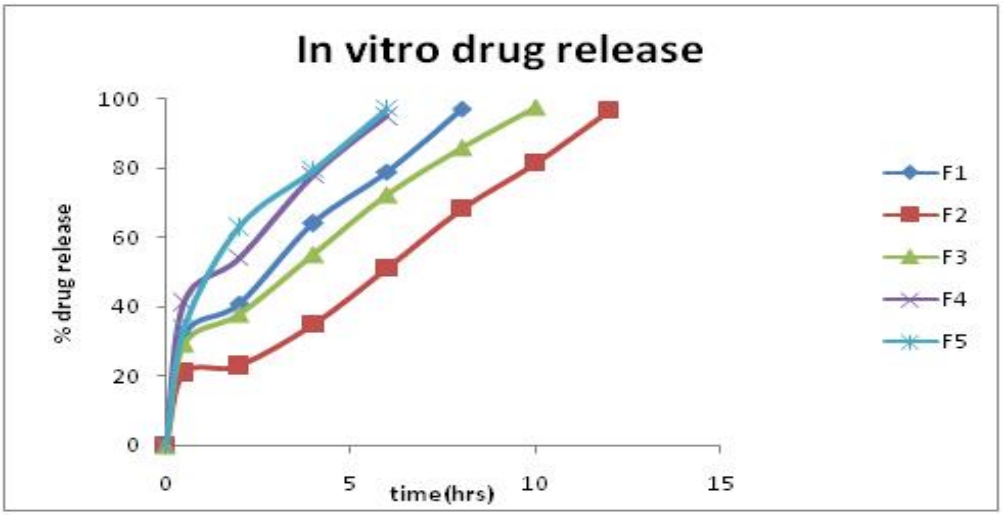

Figure 9:-Comparative drug release studies of CH formulations

The drug release data of F2 formulation was fitted into various kinetic plots in order to determine the mechanism and order of release.

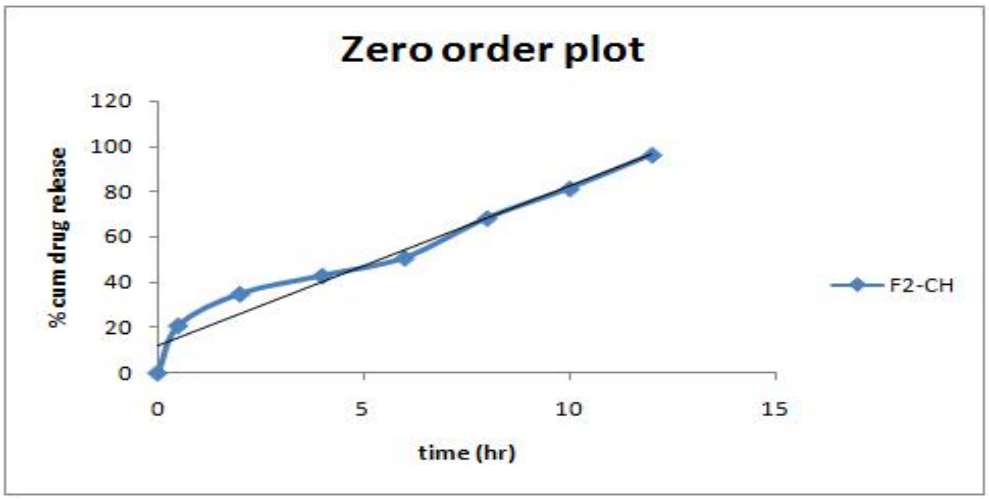

Figure 10:- Zero order plot of F2 formulation of CH MANPs

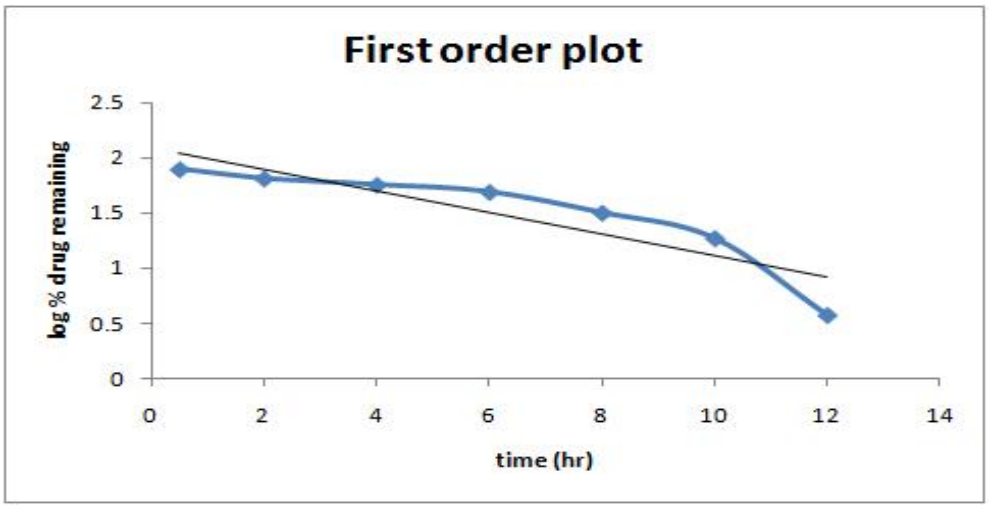

Figure 11:- First order plot of F2 formulation of CH MANPs

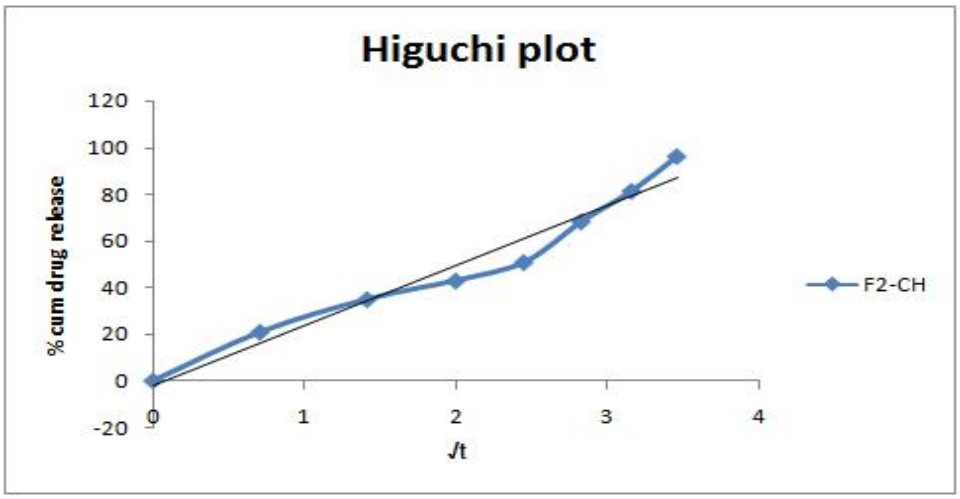

Figure 12:- Higuchi plot of F2 formulation of CH MANPs

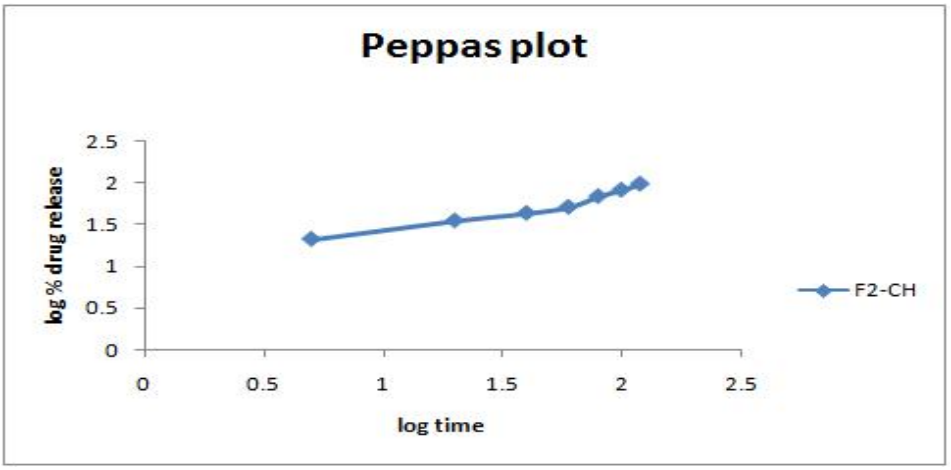

Figure 13: Peppas plot of F2 formulation of CH MANPs

The curve fitting data revealed that the release followed First order kinetics and Higuchi and Peppas plots, stated Fickian diffusion controlled in all the formulations.

4. Discussion

Mefenamic acid polymeric nanoparticles (MANPs) were formulated by solvent evaporation and ionotropic gelation techniques using Eudragit S-100, Ethyl cellulose and Chitosan polymers.

For ionotropic gelation technique, a natural polymer such as chitosan was selected for the study. Chitosan is a biocompatible, biodegradable and natural polymer. Here the process parameters including stirring speed, concentration of cross linking agent and stirring time were optimized. Five formulations were prepared by varying the drug and polymer concentrations. Nanoparticles prepared by ionic gelation method using chitosan as polymer and sodium tripolyphosphate as cross linking agent produced particles of good stability. [17,18] It seems that the relatively lower viscosity of chitosan with a concentration as low as 0.35% and an appropriate concentration of TPP (0.5 %w/v) promoted the formation of nanoparticles.[19] All formulations were in nano range and found to be spherical with optimum stability. The mean particle diameter of F1 was found to be less as the drug and polymer proportions are equal. The zeta potential of the F1 formulation was found to be more because of the minimum mean diameter. The polymer appeared to have good compatibility with the entrapped Mefenamic acid as there was no clear evidence of interaction between the two compounds. Among five formulations, In vitro drug release data of F2 formulation showed 96.3% of drug release sustained up to 11hrs. Entrapment of the drug within the gel network of the chitosan matrix may have made the formulation more stable. [20] Though F1 formulation has got minimum diameter and higher stability, F2 was considered to be the best based on the entrapment efficiency and sustained release properties.

5. Conclusions

MANPs were formulated by solvent evaporation and ionotropic gelation techniques. Comparative study was done between the polymers of solvent evaporation technique and also between the techniques.F3 of ED was found to be the best formulation for the preparation of MANPs with particle diameter of 103.6nm, greater stability(59.5mV) and higher entrapment efficiency(85.7%) and 98.2% of drug release in 12hrs.

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