British Journal of Bio-Medical Research Vol.03, Issue 05, Pg.1094 - 1105, September - October 2019

Available Online at http://www.bjbmr.org

BRITISH JOURNAL OF BIO-MEDICAL RESEARCH

Cross Ref DOI: https://doi.org/10.24942/bjbmr.2019.576 Volume 03, Issue 05, Sept - October 2019

ISSN:2456-9739

Research Article

Development And Characterization Of Nanocrystal Of Mefenamic Acid With Enhanced Stability For Targeted Drug Delivery System

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ARTICLE INFO

Article History:
Received on 10th Sept 2019
Peer Reviewed on 19th Sept 2019
Revised on 16th October 2019
Published on 29th October 2019

Keywords:
Mefenamic acid, Nanocrystal, Targeted drug delivery, Rheumatoid arthritis

ABSTRACT

The objective of present work was to utilize the potential of nanocrystal to improve the stability of mefenamic acid non-steroidal anti-inflammatory drug formulated by precipitation method under bottom up technology. Nanocrystals are made up of 100% of drug and formulation was stabilized by using stabilizers. A full 2^2 factorial design was utilized to study the effect of two independent parameters namely stabilizers concentration and stirring speed. The mefenamic acid nanocrystal formulation was characterized with respect to particle size, polydispersity index (PDI), zeta-potential, encapsulation efficiency and physical morphology. The nanocrystal formulation had an average diameter of 257.6 nm, PDI of 0.335, zeta-potential of 37.48 mV and encapsulation efficiency of 82.48% respectively. The nanocrystal formulation for mefenamic acid encapsulation has been successfully developed and is suitable for targeted delivery system due to their nano-size and stability.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that affects mainly the small joints of the hands and feet. This may also lead to premature mortality, disability and compromised quality of life. In this disease there is joint inflammation, synovial proliferation and destruction of articular cartilage (Lola and 2016). Approximately 70% of patients having irreversible joint destruction are mainly affected by stiffness and devastating pain (Carvalho and Andrade 2014).

Mefenamic acid is a non-steroidal anti-inflammatory drug used in treatment pain, most commonly menstrual pain. It is typically prescribed for oral administration (Rivai *et al*., 2016). The usual oral dose of the drug is 500 mg thrice a day (Naveed and Qamar 2014).

Nanocrystal:

Nanocrystal refers to the drug in solid state, for solid particles in the nano range with minimum excipients (Gigliobiance *et al*., 2018). The nanocrystals technology used mainly for the poorly water-soluble compounds to improve the solubility and bioavailability of the drugs (Patel *et al*., 2014). Drug nanocrystals are made up of 100% drugs and can be produced by bottom-up technologies (precipitation methods) or alternatively by top-down technologies (size reduction methods) (Yarraguntla *et al*., 2016). The anti-solvent precipitation technique is more effective for the preparation of nano or micro particle of drug. In this method, the drug is dissolved in the solvent, followed by introducing into anti-solvent which results in the precipitation of drug (Rahim *et al*., 2017).

MATERIAL AND METHOD:

Material:

The following materials were used from the indicated sources, without further purification. Mefenamic acid was procured as a gift sample from Pinnacle life sciences, Baddi, Himachal Pradesh, India. Tween 80 and Dimethylformamide purchased from Thomas Baker Chemical Pvt. Ltd, Mumbai and Poloxamer 188 purchased from Sigma life sciences, India. The other chemicals were of analytical reagent grade.

Method:

Characterization of the pure active ingredient

UV visible (Ultraviolet- visible) spectrophotometer: Absorption maxima (λmax) of the drug was conducted in phosphate buffer.

Fourier-Transform Infrared spectroscopy (FTIR): FTIR range of the spectrum of active ingredient were utilizing FT-IR spectrophotometer Bruker by KBr pellet method. In the range of 4000 to 400 cm^{-1}, the spectrum was recorded.

Compatibility Studies:

FTIR will be employed to know the chemical compatibility between the drug and excipients.

FTIR analysis: FTIR analysis is used to determine the compatibility between the drug and stabilizer. The spectra were recorded for Mefenamic acid with tween-80, and poloxamer-188 in a FTIR spectrophotometer (SHIMADZU, Japan) using KBr pellets at 4000-400 cm^{-1}. To examine any changes in the shift, disappearance or appearance of the peak, the compatibility test will be investigated by measuring the IR spectra of the API and physical mixture.

NMR spectroscopy: Samples were prepared by dissolved in DMSO, with the internal standard tetramethyl silane (TMS) and irradiated with radio wave. Spectrum wave of NMR is a graph of the amount of energy absorbed against strong magnetic field.

NCL Formulation:

Selection of Solvent:

Solvent for crystal preparation was selected by checking the solubility of the drug in different solvents by means of visible observation with the naked eyes under normal light. Solvents used for this study were Ethanol, Methanol, Distilled Water, Chloroform and N-N Dimethyl formamide. Weighed quantity of drug (50 mg) separately with various Solvent (5 mL each) was solubilized to the solvents at room temperature in 10 mL glass vials. After

dissolution, the solubility of drug Mefenamic Acid) in each solvent was observed visually the under normal light.

Selection of Stabilizers:

Stabilizers are selected on the basis of drug polymer compatibility test.

Method of preparation of nanocrystal:

Nanocrystals were prepared by precipitation method under bottom up technique.

Step- I: Drug is dissolved in N-N dimethyl formamide. Heat the solution at until boil after boiling cool the solution in the ice bath for 1 h. Crystals were form, filter the crystal and collect them.

Step- II: Prepare the solution of stabilizers (tween 80 & poloxamer 188). To this solution add Mefenamic acid crystal. Agitate the solution overnight. Keep the solution for precipitation. Filter the nanocrystals, dry them.

Table 1: Formulation of Various Batches of Nanocrystals

S. No.	Formulation code	Stabilizer ratio (Tween 80: Poloxamer 188)	Rotation (rpm)
1.	NCL-1	20% : 10%	1500
2.	NCL-2	20% : 10%	2000
3.	NCL-3	15% : 5%	1500
4.	NCL-4	15% : 5%	2000

Table 2: Variables and levels used in 2^2 factorial design for mefenamic acid loaded nanocrystal

Factors	Levels	
	-1	**+1**
X_1	1500	2000
X_2	15%:5%	20%:10%

Where, X1= Stirring speed, X2= Concentration of stabilizers (%)

Table 3: The central composite experimental design for Mefenamic acid loaded nanocrystal

S. No.	Formulation code	X_1	X_2
1	NCL-1	-1	+1
2	NCL-2	+1	+1
3	NCL-3	-1	-1
4	NCL-4	+1	-1

Characterization and Evaluation studies of Mefenamic acid loaded Nanocrystal:

Particle size and polydispersity index

The particle size analysis and Polydispersity index of the nanocrystal were determined using a Zeta sizer (Nano plus at BBAU, Lucknow). To determine the particle size of obtained nanocrystal was diluted with distilled water (up to 2mL) in a cuvette.

The Polydispersity index was studied to determine the narrowness of particle size distribution. PDI will have the range between 0.1 to 0.7 and sample having broad size distribution have the PDI range > 0.7.

Zeta potential

The Zeta potential, reflecting the electric charge on the particle surface and indicating the physical stability of colloidal systems, measured using a zeta sizer after suitable dilution with distilled water.

Determination of drug loading and entrapment efficiency (Ali *et al*., 2015) (Uner *et al*., 2013) (Sethuraman *et al*., 2018)

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 amount of drug entrapped in nanocrystal formulation was investigated by centrifugation of 5 mL of formulation at 19000 rpm for 1 h at 4ºC. Then dilution of supernatant was prepared and absorbance was taken by UV-spectrophotometer at 284nm. The percent of entrapment efficiency (%EE) and drug loading (%DL) was calculated according to following formula:

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

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

Scanning Electron microscope (SEM):

Scanning electron microscope was employed to investigate the surface morphology of the prepared optimized formulation of Nanocrystal. It was observed by SEM (scanning electron microscopy) at central instrumentation facility, IIT (BHU), Varnasi.

***In vitro* drug release study**

The dissolution medium used was freshly prepared 6.8 phosphate buffer. Dialysis membrane, previously soaked overnight, was tied to one end of a specially designed glass cylinder (open at both ends). 5 mL of formulation was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 100 mL of dissolution medium maintained at 37 ± 5ºC so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at low speed using magnetic stirrer. An aliquot of 1ml of the sample was withdrawn from the receiver compartment at pre-determined time intervals and replenished with fresh medium. Samples were analyzed by UV-Visible spectrophotometer at a wavelength of 284 nm. Data obtained from *in vitro* release studies were fitted to various kinetic equations to find out the mechanism of Mefenamic acid release from nanocrystal.

Stability study

The NCL dispersion was stored at room temperature (approximately 25ºC) and refrigerator temperature (approximately 4-10ºC) for one month. The initial formulation of these studies analyzed for particle size, Polydispersity index and entrapment efficiency.

RESULT AND DISCUSSION:

Characterization of the pure active ingredient

By UV-visible (Ultraviolet-visible) spectrophotometer: Absorption maxima (λmax) of the mefenamic acid was conducted in methnaol is depicted in Table 4 & Figure 1.

Figure 1: UV spectrum of Mefenamic acid

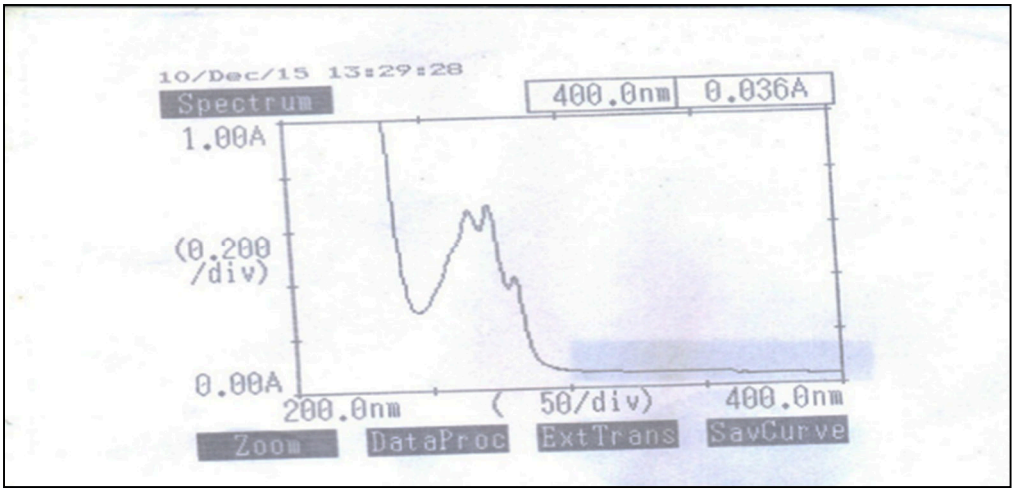


Table 4: Absorption maxima (λmax) of Mefenamic acid

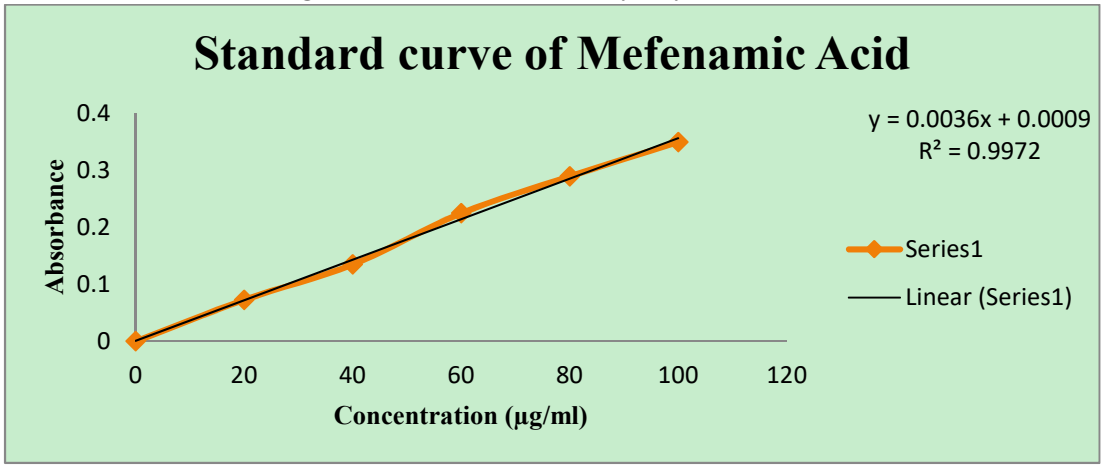
Drug	Solvent	λ_{max} (nm)
Mefenamic acid	Methanol	284

The calibration curve graph of mefenamic acid was made in methanol, which is depicted in Table 5 & Figure 2. Coefficient regression is 0.9972 along a value of slope 0.0036 & value of Y-interception 0.0009. It indicates a straight-line relationship between absorbance and concentration.

Table 5: absorbance maxima at different concentration of Mefenamic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	0	0
2.	20	0.073
3.	40	0.135
4.	60	0.225
5.	80	0.29
6.	100	0.35

Figure 2: Calibration curve of Mefenamic acid



FTIR Studies: This study indicates the pure drug which is showing the respective IR peaks along with the presence of functional groups of mefenamic acid. (Table 6 & Figure 3)

Figure 3: FTIR Spectra of Mefenamic acid

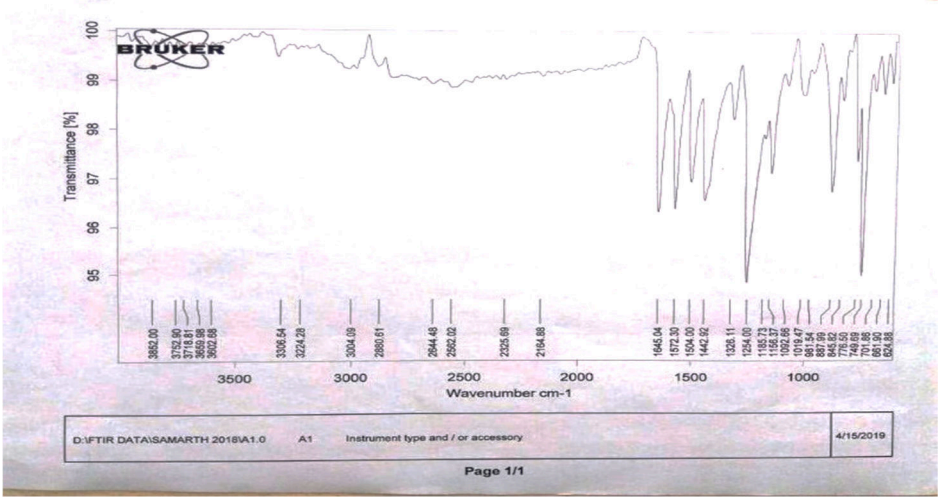


Table 6: IR characterization of Mefenamic acid

Reported Peak	Observed Peak	Assignments
3500-3100	3306.54	-NH(stretch)
3300-3200	3224.28	-OH(stretch)
3000-2850	2880.61	-CH(stretch)
1750	1645.04	C=O(stretch)
1500-1400	1442.92	C=C(stretch in aromatic ring)

Compatibility studies

FTIR analysis: The FTIR of mefenamic acid with Stabilizers was determined. The functional groups of pure drug and stabilizers were found to be correlative. The FTIR of the stabilizers and mefenamic acid shown in Figure 4, Figure 5 & Table 7, Table 8. From the obtained result, there were no interaction between mefenamic acid and stabilizers. Hence Mefenamic acid and selected stabilizers were compatible with each others.

Figure 4: FTIR spectrum of Mefenamic acid with poloxamer 188

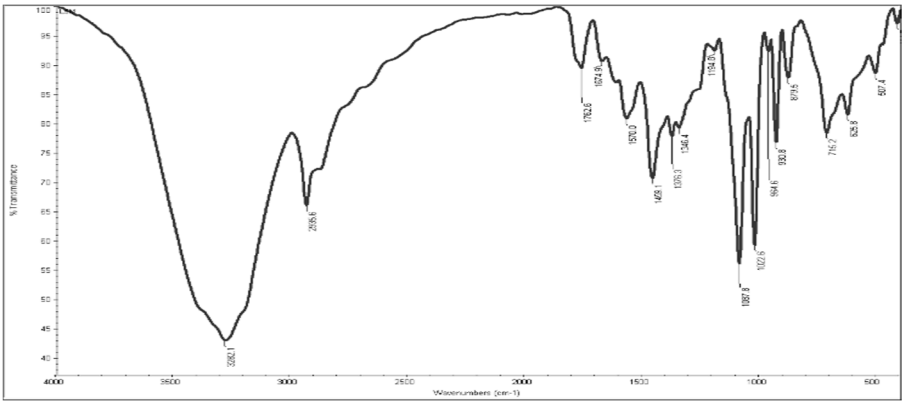


Figure 5: FTIR spectrum of Mefenamic acid with tween 80

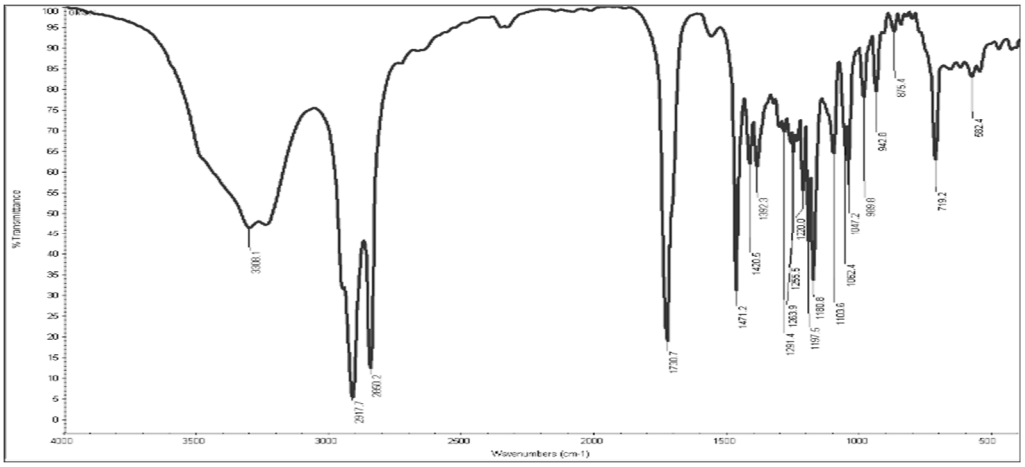


Table 7: IR characterization of drug and poloxamer 188

Reported Peak	Observed Peak	Assignments
3500-3100	3282.1	-OH(stretch in hydrogen bond)
2970-2850	2935.6	-CH(stretch)
1750-1700	1762.6	C=O(stretch)
1680-1620	1674.9	C=C(stretch)
1500-1400	1570	C=C(stretch in aromatic ring)
1500-1430	1459.1	-CH(def)
1400-1300	1376.3	-CH(def in –CH3)
1350	1346.4	C-O(stretch)
1020-950	964.6	-CH(def in out of plane)
700-600	625.8	-NH (def out of plane)

Table 8: IR characterization of drug and tween 80

Reported Peak	Observed Peak	Assignments
3500-3100	3308.1	-OH(stretch)
2970-2850	2917.7	-CH(stretch)
1550-1450	1471.2	-CH(bend)
1450-1000	1420.5	C=CH(stretch)
1300-1250	1392.3	C=C(stretch)
1350-1150	1197.5	C-O(stretch)
1000-950	989.8	-CH(def)

NMR studies: This study indicates the pure drug which is showing the amount of energy absorbed against strong magnetic field. (Table 9, Table 10 & Figure 6, Figure 7)

Figure 6: C-13 NMR Spectrum of Mefenamic acid

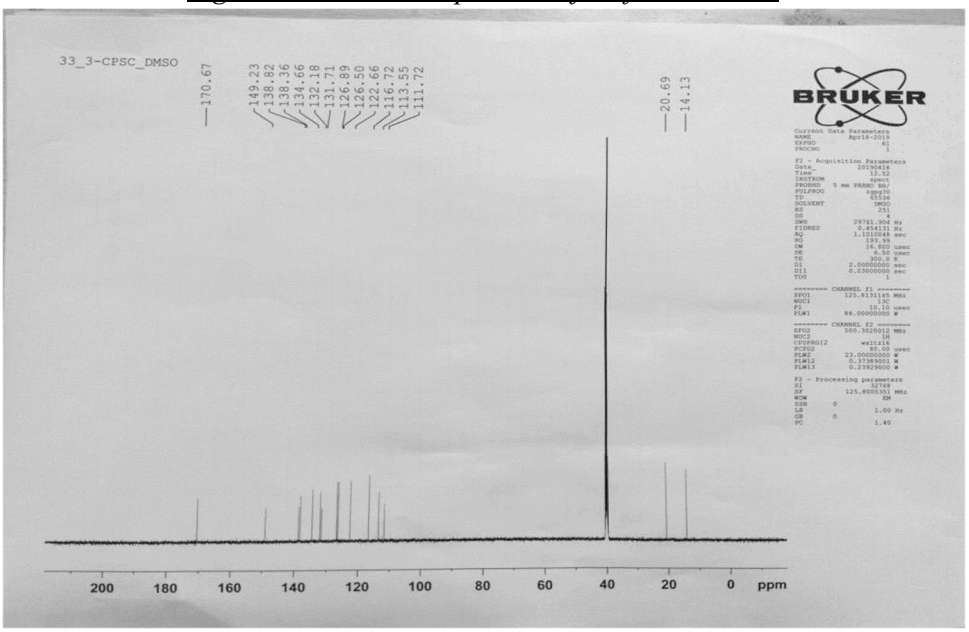


Figure 7: H-1 NMR Spectrum of Mefenamic Acid

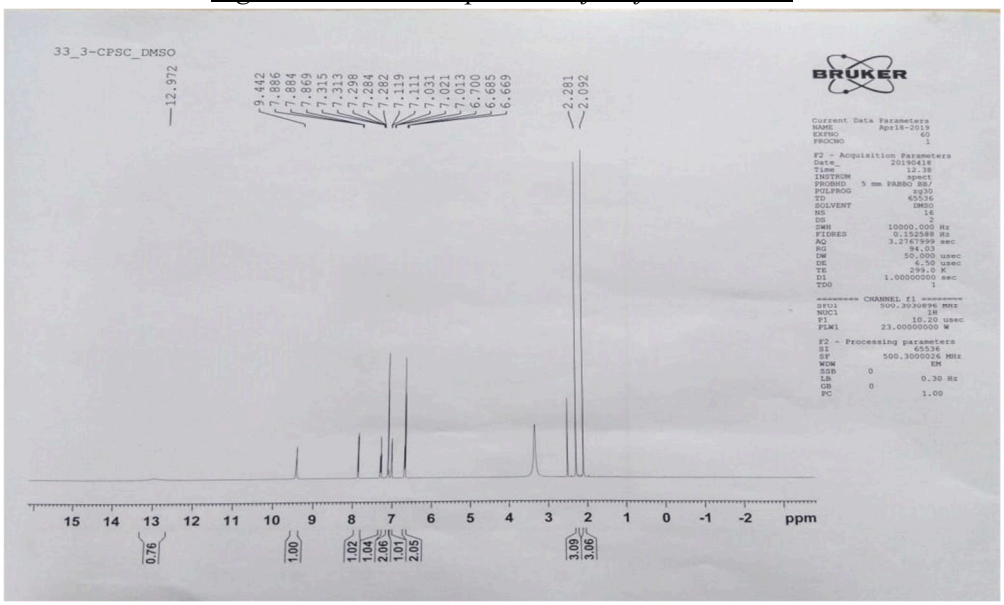


Table 9: C-13 NMR Characterisation of Mefenamic Acid

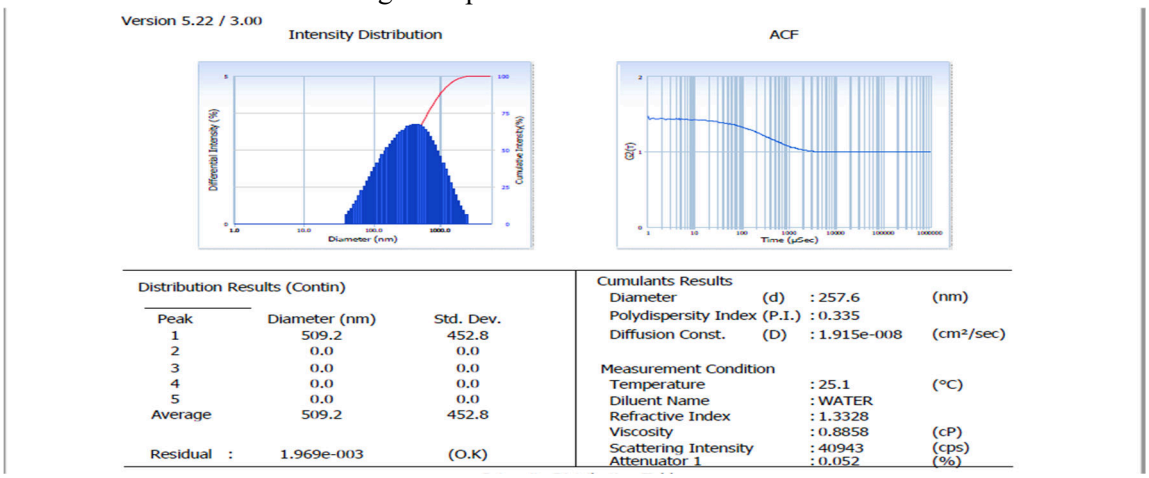
Chemical Shift (ppm)	Integration	Multiplicity	Proton from groups
170.67	1	Singlet	C=O
149.23 -111.72	12	multiplet	C in aromatic ring
20.69	1	singlet	CH_3
14.13	1	singlet	CH_3

Table 10: H-1 NMR Characterisation of Mefenamic Acid

Chemical Shift (ppm)	Integration	Multiplicity	Proton from groups
12.972	1	Singlet	COOH
9.442	1	singlet	NH
7.284 - 7.886	7	multiplet	H in aromatic ring
2.281	1	singlet	CH_3
2.092	1	singlet	CH_3

Evaluation of Mefenamic acid nanocrystal

Particle size and polydispersity index (PDI) analysis: In the evaluation of nanocrystal particle size plays a very crucial factor because it will affect the amount of drug absorption & drug release. If the particle size small, large will be the interfacial area for drug diffusion. The particle size and PDI was found to be 257.6 nm and 0.335. (Table 11 & Figure 8)



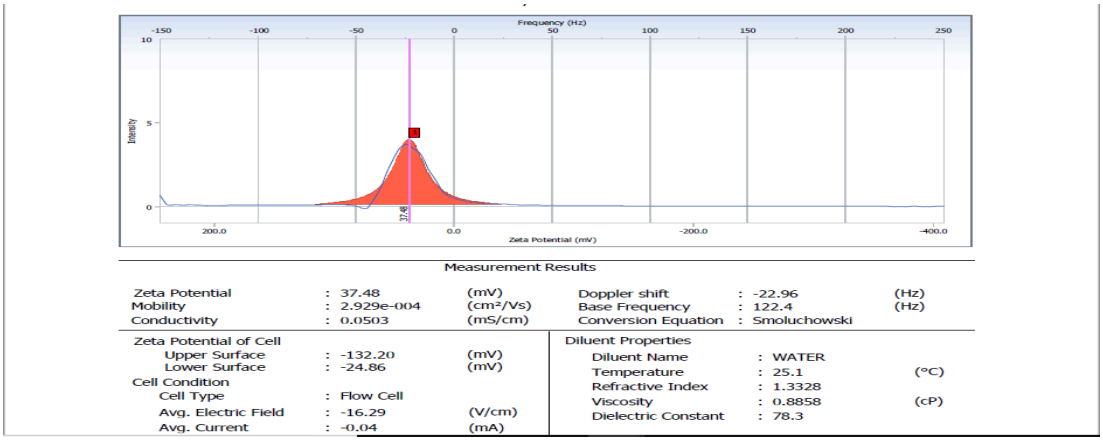
(Figure 8: Optimized formulation NCL-4 polydispersity index)

Table 11: Data of particle Size and PDI

S. No.	Formulation	Average	Polydispersity Index
1	NCL-4	257.6	0.335

Zeta potential analysis: The ZP represents the electrical charge to the Nanocrystal surface. The greater the ZP value, the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. It is currently admitted that higher ZP values, either positively or negatively charged, mean that dispersion will have greater long-term stability. The ZP value was found to be 37.48mV (Figure 9).

(Figure 9: Zeta potential of nanocrystal of optimized batch)



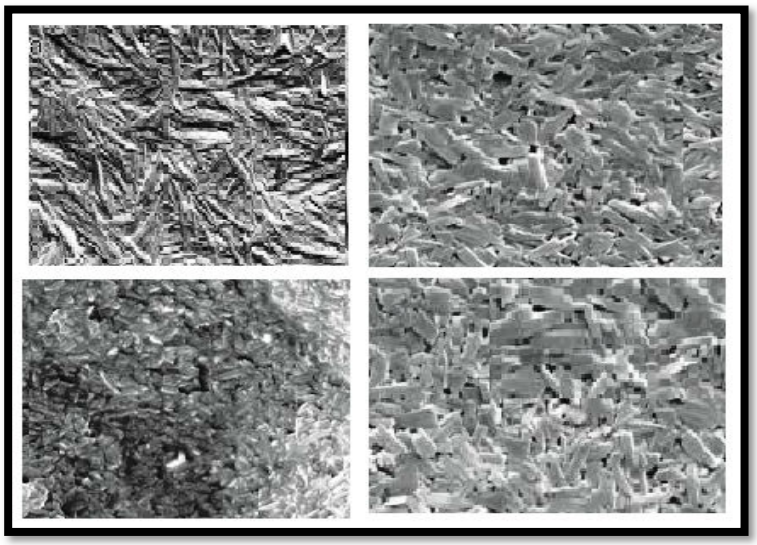
Determination of drug loading and entrapment efficiency: Entrapment efficacy studies are performed to check the amount of mefenamic acid entrapped in nanocrystal. The entrapment efficiencies of nanocrystal made from different concentrations of stabilizers. Due to the presence of a sufficient amount concentration of stabilizers, there was an increase in entrapment efficacy and helps in solubilize the drug and stabilizing the entrapped drug molecule. The entrapment efficiency was found be in the range of 64.6% to 82.48%. The maximum entrapment efficiency was found to be 82.48%. (Table 12)

Table 12: %Entrapment efficiency and % drug loading of NCL

S. No.	Formulation code	% Entrapment efficiency	% Drug loading
1.	NCL-1	64.6	2.16
2.	NCL-2	71.22	12.82
3.	NCL-3	76.52	13.65
4.	NCL-4	82.48	14.56

Scanning Electron Microscope: From the SEM photographs of Mefenamic acid nanocrystal sample, which is depicted in figure 10. The Mefenamic acid nanocrystal having their regular particles, the size around 220-400 nm which is very near to the size necessary for targeted delivery i.e. 200nm. The result was also supported by the non-significant difference with the measured particle size of optimized batch i.e. 257.6 nm.

(Figure 10: SEM image of different batches)



In Vitro drug release study: In present study, the drug was entrapped in lipid matrix and thus lipid enhances the absorption & bioavailability of drug. Thus, Nanocrystal broadens the absorption window and bioavailability of mefenamic acid. Drug release was observed for 8 hours in buffer solutions. The % CDR obtained in phosphate buffer pH 6.8 was 90.56%. (Table 13 & Figure 11, Figure 12).

Figure 11: In Vitro drug release of various batches

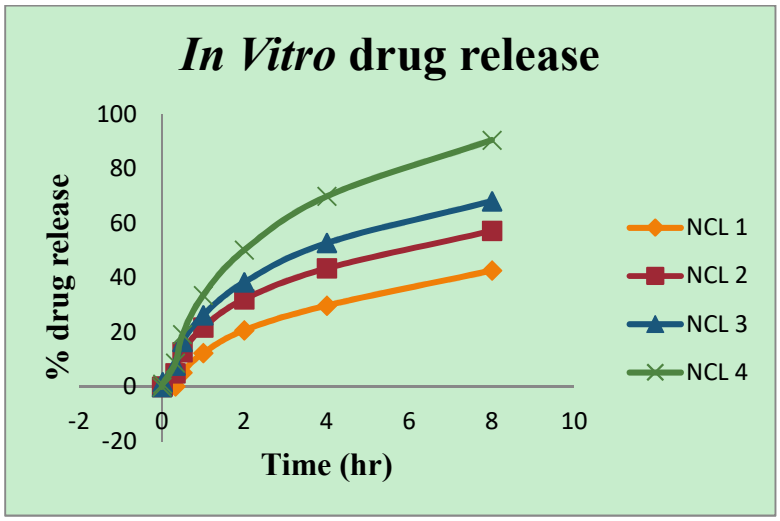


Figure 12: In-vitro drug release of optimized batch NCL- 4

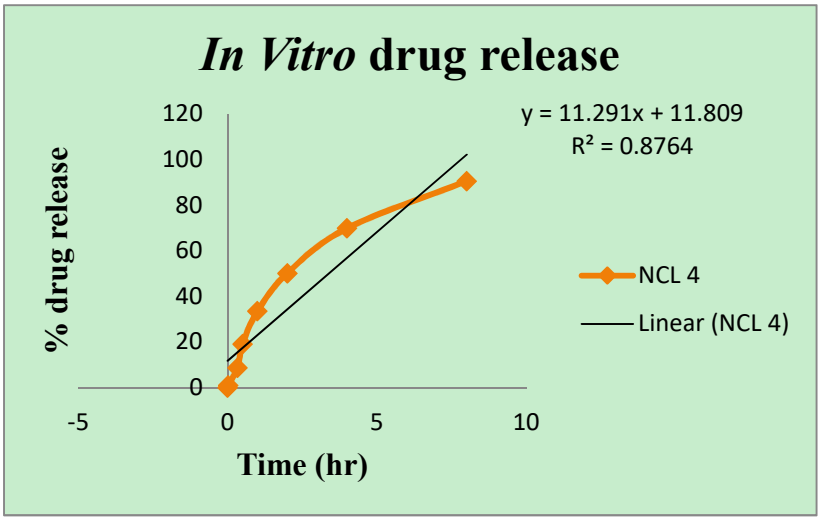


Table 13: Percentage of released Mefenamic acid after 8 h

S. No.	Formulation code	% Drug release
1.	NCL-1	42.69%
2.	NCL-2	57.23%
3.	NCL-3	68.12%
4.	NCL-4	90.56%

Stability study: The NCL dispersion was stored at room temperature (approximately 25ºC) and refrigerator temperature (approximately 4-10 ºC) for one month. During the period of investigation, no changes found in the formulation.

CONCLUSION

In this study, MA nanocrystals were prepared by using 2^2 factorial designs with different stabilizers ratio and stirring speed, and was evaluated by particle sizes, SEM, % entrapment efficiency, % drug loading, *in vitro* drug release were studied. The goal of the present study was to develop and evaluates the Mefenamic acid nanocrystals to enhance bioavailability and stability of the formulation.

The reason for this is that they were developed by considering the industrial needs e.g. scale up, qualification and validation, simple technology, low cost etc. The mefenamic acid nanocrystals were successfully prepared by precipitation method under bottom up technology. The mefenamic acid nanocrystal showed improvement in rate of in-vitro drug release study. The improvement can be attributed to change in ratio of stabilizers and stirring speed, reduced particle

ACKNOWLEDGEMENT:

All the authors are highly thankful to Pinnacle life sciences, Baddi, Himachal Pradesh, India for providing mefenamic acid as a gift sample.

CONFLICT OF INTEREST:

All the authors hereby declare that there were no conflicts of interest/Competing Interests in the publication of the manuscript or an institution or product that is mentioned in the manuscript and/or is important to the outcome of the study presented. Rajat Srivastava, Manoj Kumar Mishra, Amit Kumar Patel and Krishna Kushwaha have no individual or combined conflict of interest during the publication.

FUNDING SOURCES:

All the authors hereby declare that there were no sources of funding received for the research submitted to the journal.

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How to cite this article:
Rajat Srivastava, Manoj Kumar Mishra, Amit Kumar Patel, Krishna Kushwaha. ***Development And Characterization Of Nanocrystal Of Mefenamic Acid With Enhanced Stability For Targeted Drug Delivery System.*** **Br J Bio Med Res , Vol.03, Issue 05, Pg.1094 - 1105, September - October 2019. ISSN:2456-9739 Cross Ref DOI : https://doi.org/10.24942/bjbmr.2019.576**

Source of Support: Nil **Conflict of Interest:** None declared.