MIT International Journal of Pharmaceutical Sciences, Vol. 5, No. 2, August 2019, pp. 69–75
ISSN 2394-5338 (Print); 2394-5346 (Online) © MIT Publications

Original Article

Formulation and Evaluation of Nanoparticles Based Topical Gel of Indomethacin

Rupali Partani,[*a] Sourabh D Jain[b], Deepak Birla[a], Jayanti Mukherjee[a], Vimukta Sharma[b]

[a]*Shri Bherulal Pharmacy Institute, Indore Madhya Pradesh, India.*

[b]*BM College of Pharmaceutical Education and Research, Indore, Madhya Pradesh India.*

**** Corresponding Author:** E-mail: partanirupali@gmail.com*

ARTICLE INFO

Received 29 May 2019
Revised 28 August 2019
Accepted 05 September 2019

Keywords:
• Nanoparticles
• Freeze drying
• Indomethacin
• Carbopol
• Topical Gel

ABSTRACT

Objective: The objective of the present investigation is to formulate and evaluate nanoparticles (NPs) based topical gel of non-steroidal anti-inflammatory drug (NSAID) indomethacin for the treatment of arthritis, temporary relief of fever, minor aches, and pains which would attenuate the gastrointestinal related toxicities associated with oral administration.

Methods: The nanoparticles were formulated by emulsion diffusion evaporation technique using ethyl acetate, PVA (poly vinyl alcohol) and PLGA poly (lactic-co-glycolic acid) used as cross-linking agent. The freeze dried nanoparticles were evaluated using FTIR, SEM and DLS studies. The freeze dried drug loaded cross-linked PLGA nanoparticles were incorporated in carbopol 934 to disperse in small quantity of distilled water. Subsequently glycerin and triethanolamine were added with stirring till the carbopol gel gets dispersed. The in-vitro drug release of the formulated gel was evaluated using modified Franz diffusion cell containing dialysis membrane 70 (Hi-Media, Mumbai, India) having pore size 2.4 nm and phosphate buffer having pH 7.4 as the receptor medium. The permeation profiles were determined by plotting cumulative% drug release Vs time (Zero order release), log cumulative% drug remaining Vs time (first order release), cumulative % drug release Vs square root of time (Higuchi Model), log cumulative% drug release Vs log time (Korsmeyer Peppas Model) and cube root% drug remaining Vs time (Hixson Crowell Model).

Results: The Zeta value of NPs 2.41 indicated a stable formulation. It has been observed high drug entrapment efficiency. SEM data revealed the surfaces of nanoparticles were smooth and fibrous type. FTIR results indicated no interaction among the drug and the excipients. From the release kinetics data it was observed that the release of indomethacin from the PVA loaded nanoparticles exhibit anomalous (non-Fickian) diffusion for Hixson Crowell Model, first order, zero order and Higuchi Model but it closely correlated with Korsmeyer Peppas Model.

Conclusion: The research work concluded that the Indomethacin loaded NPs based topical gel formulation containing carbopol was successfully developed for topical application with good permeation potentiality as evident from its in-vitro results.

1. INTRODUCTION

Indomethacin 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid is used for the treatment of pain caused by acute and chronic arthritic condition, mild to moderate pain (including pain relief after surgery), painful menstruation, osteoarthritis, dental pain and headaches, Poor water solubility of Indomethacin in oral dosage formulations results in low bioavailability and incomplete absorption from the gastrointestinal tract. Keeping in mind to reduce systemic side effects the present study was conducted to formulate a gel which can give more specific and localized pharmacological activity. The current approaches aim to decrease indomethacin related adverse effects like gastrointestinal disorders caused on oral administration. [1] The formulation of NPs for drug delivery

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depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size. [2] In the past few years, lipid matrices became extremely popular in controlling the release of drugs. In-vitro drug release pattern of nanoparticles based gel showed fast and control release. The immediate release can be useful to improve the penetration of drug, while sustained-release supplied the drug over a prolonged period of time. [3, 4]

2. MATERIALS AND METHODS

2.1 Materials

All the chemicals like ethyl acetate, PVA (poly vinyl alcohol), PLGA poly (lactic-co-glycolic acid), Carbopol 934, glycerin and triethanolamine were purchased from Sigma - Aldrich, Switzerland. Dialysis membrane 70 (Hi-Media, Mumbai, India) MW 12,000-14,000 and the API were purchased from local vendor, Indore. All the reagents and solvents were of analytical reagent (AR) grade.

2.2 Preformulation Studies

2.2.1 Determination of λmax (wavelength of maximum absorbance) for indomethacin

The solution of Indomethacin (10 µg/ml) in methanol was measured using Systronics double beam UV spectrophotometer 2203 by scanning within the range of 200 to 400 nm and the wavelength of highest absorbance was determined (Fig 1).

2.2.2 Preparation of standard curve for pure indomethacin using UV spectrophotometer

Stock solution (100µg/ml) of indomethacin was further diluted with the distilled water to achieve final concentrations of 10, 20, 30, 40, and 50 µg/ml. Each observed absorbance was plotted against concentration to prepare the standard curve of paracetamol. [5]

2.2.3 Drug Excipients interaction studies

2.2.4 Fourier Transform Infrared Spectroscopy (FTIR)

Vertex 70v FTIR Spectrophotometer (Bruker) was used to characterize any possible interaction between drug and excipients in the solid state. The spectra (Fig 2-5) were obtained in a frequency range of 670-4000cm-1. [6]

2.2.5 Differential scanning calorimetry (DSC) study

Differential scanning calorimetry (DSC) offers the possibility of detecting chemical interaction of drug and PLGA & PVA containing nanoparticles. DSC measurements were carried out on a modulated DSC Instrument (Mettler Toledo) equipped with a thermal analysis data system. [7]

3. FORMULATION OF INDOMETHACIN NANOPARTICLES

Nanoparticles were prepared by an emulsion – diffusion – evaporation technique with slight modifications. Briefly, 45 mg of Indomethacin and 50 mg of PLGA were placed in 3 ml ethyl acetate and stirred at 750 rpm for 30 minutes. Concentrations of PVA (0.5 w/v) stabilizers were placed within 6 ml of HPLC grade water heated to 140 0C and stirred at 750 rpm until fully dissolved. The organic phase was then added to aqueous phase in a drop wise manner under moderate stirring then sonicated for 5 minutes at 20 kHz using a sonic dismembrator (Fischer Scientific, Fair Lawn, NJ, USA). To facilitate diffusion, 25 ml of water was added to each emulsion under constant stirring at 750 rpm. Emulsions were stirred at 750 rpm for 4 hours to insure complete organic phase evaporation. After which, each emulsion was centrifuged (8,800 rpm or 12,000 rpm) and supernatant was collected. [8]

4. CHARACTERIZATION

4.1 Scanning Electron Microscopy (SEM)

SEM is a type of electron microscope utilizing the interaction of emitted electrons and atoms of a sample to collect information of the topography and other properties of the samples surface. SEM images have a characteristic appearance and are useful for judging the surface structure and topology of the sample. Fig 10 shows the general inner structure of an SEM.

The morphology and surface of the NP (Fig10) were observed using a scanning electron microscope (JEOL JSM-5600, Tokyo, Japan). [9]

4.2 Entrapment efficiency (EE %) of Nanoparticles

The nanoparticles suspension was centrifuged at 4500 rpm for 1hr. The supernatant solution was separated. 1 ml of supernatant was distributed in 10 ml distilled water and the absorbance was measured using UV spectrophotometer at 266 nm using water as blank. The amount of drug entrapped was determined by subtracting amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in three times and the average was calculated. The drug entrapment efficiency (EE) of nanoparticles was calculated as follows: [10, 11]

(EE %) = (Drug given − Drug loss)/Drug given × 100%

4.3 Zeta potential measurement

Zeta potential is an abbreviation for electro kinetic potential in colloidal systems. The zeta potential of the synthesized nanoparticles was determined by means of NanoPlus zeta/nano particle analyzer (Malvern Instruments, United Kingdom). The measurement of zeta potential was based on the direction and velocity of particles under the influence of known electric field. [12]

5. FORMULATION OF INDOMETHACIN LOADED NANOPARTICULATE BASED GEL

Required quantity of carbopol 934 was weighed and dispersed in small quantity of distilled water to prepare aqueous dispersion, and the dispersion was allowed to hydrate for 4 to 5 hour. Glycerol (10% w/w) was added subsequently to the aqueous dispersion equivalent to 1% of indomethacin into it.

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Triethanolamine was added in small quantity and in regular interval to the above dispersion using a magnetic stirrer with a controlled speed of 1200 rpm. Stirring was continued till the carbopol get dispersed. The gel was allowed to stand overnight to remove entrapped air [13-16].

6. EVALUATION OF INDOMETHACIN LOADED GEL

6.1 Physical appearance & Homogeneity

Physical appearance and homogenecity of gel was observed visually. [17]

6.2 Spreadability study of NP gel

The spreadability of the gel formulations was determined using an apparatus made with two glass plates. [18] Excess amount of gel was placed between glass slides (10 x 10 cm^2). A weight of gel 100g was placed on the upper glass plate for 5 min to compress the formulation. The time required to separate the slides in seconds was taken as the measure of spreadability. The spreadability was calculated by using the following formula.

$$S = M \times L/t$$

Where S is spreadability, M is weight tied on upper slide. L is the length of glass slide, t is time taken. [19]

6.3 *In vitro* Release Study of Indomethacin NP Gel

The i*n vitro* release studies were performed using modified Franz diffusion cell (Fig 1) to evaluate the amount of Indomethacin released from the formulation. This cell consists of donor compartment, acceptor compartment, Dialysis membrane 70 (Hi-Media, Mumbai, India, MW cut-off between (12000-14000), magnetic stirrer, thermostatic water bath and sampling device. The surface area of the release membrane was 3.14 cm2. [20] The receptor medium was approximately 15 ml and composed of phosphate buffer saline (PBS), pH 7.4, and stirred by magnetic bar at 700 rpm to avoid different concentrations within the acceptor medium and to minimize stagnant layers. [21-22] Nano particulate indomethacin based gel (equivalent to 1 mg of drug) [23-25] was placed in the donor compartment. During the experiments, the solution in receptor side was maintained at 37 °C ± 0.5 °C. After certain time interval, 3 ml of the sample medium was withdrawn from receiver compartment through side tube and same volume of freshly prepared receptor medium were added. The samples were analyzed by UV-Vis spectrophotometer at 266 nm. For the prepared formulation, the release studies were performed in triplicate. [26]

In vitro studies were performed to find out the release rate of the drug from the indomethacin gel formulation using carbopol, The cumulative percentage release of Indomethacin from prepared Indomethacin gel were investigated for a period of 8 hours. Each sample was analyzed in triplicate. [27]

Fig. 1 In vitro drug release study using Franz diffusion cell

7. RESULTS & DISCUSSION

7.1 Preparation of standard curve for pure Indomethacin using UV method

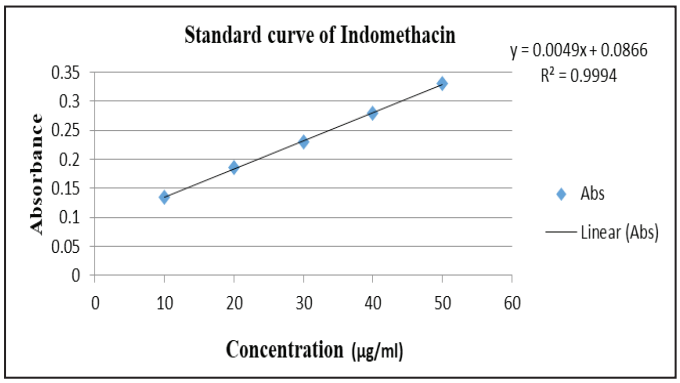

Fig. 2 Standard curve of Indomethacin

7.2 Fourier Transform Infrared Spectroscopy (FTIR)

It was revealed from the results of FTIR studies that, there were no physical or chemical incompatibilities between drug and excipients used to prepare final formulation of nanoparticles**.** Based on these results, it is confirmed that no major shifting and no loss of fundamental peaks between the spectra of indomethacin and PLGA loaded indomethacin nanoparticles took place. In addition all other peaks normally present in the cross-linked starch nanoparticles were seen in the nanoparticles. Therefore, it can be concluded that there is no strong chemical interaction between Indomethacin and PLGA, PVA and nanoparticles.

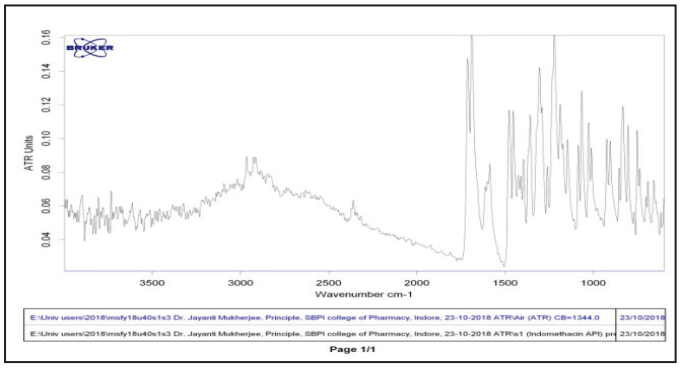

Fig. 3 FTIR data of indomethacin

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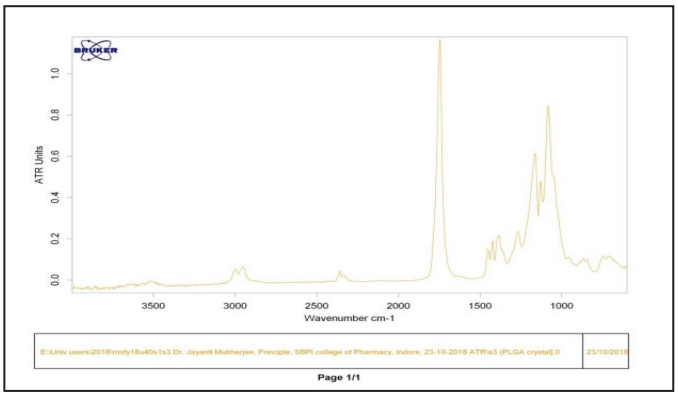

Fig. 4 FTIR data of PLGA crystal

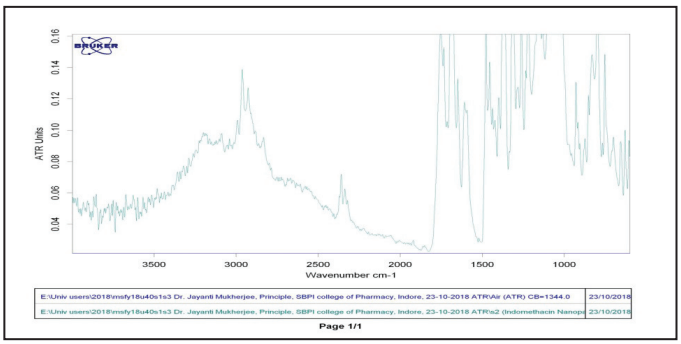

Fig. 5 FTIR data of indomethacin nanoparticles

7.3 Differential Scanning Calorimetery (DSC)

It was revealed from the spectrum of DSC studies that, there were no physical or chemical incompatibilities between drug and excipients used to prepare final formulation of nanoparticles.

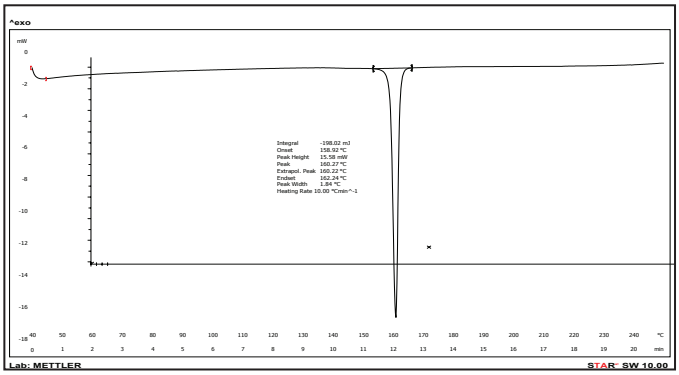

Fig. 6 DSC data of Indomethacin

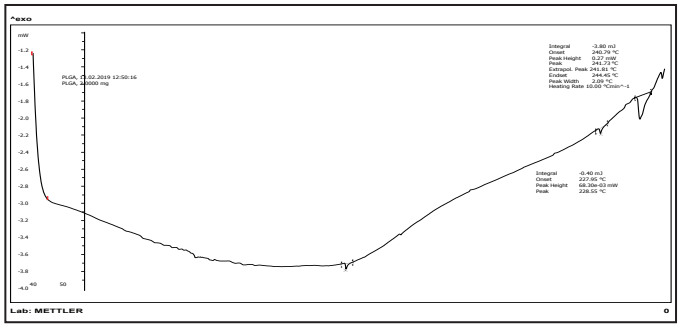

Fig. 7 DSC data of PLGA

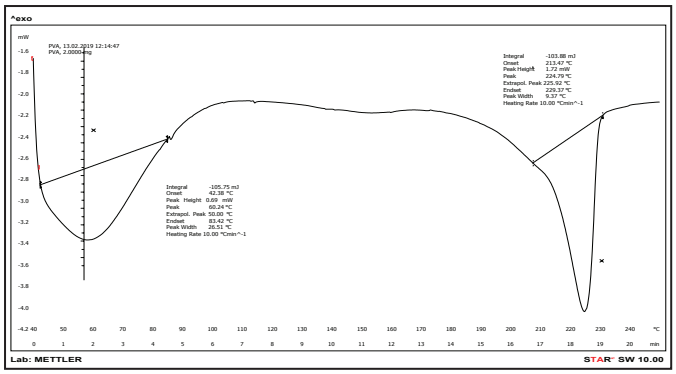

Fig. 8 DSC data of PVA

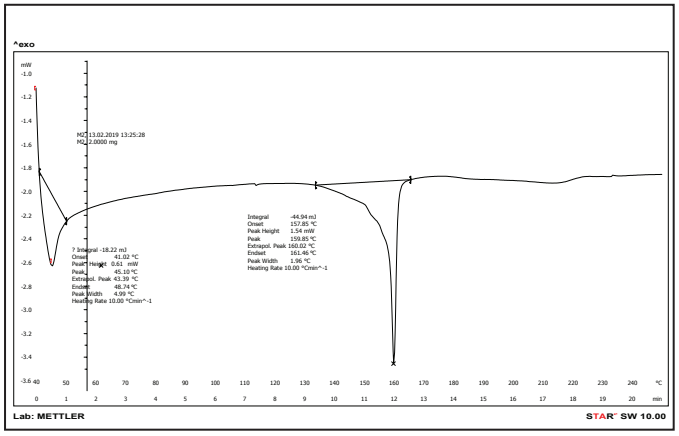

Fig. 9 DSC data of Indomethacin nanoparticle

7.4 Scanning electron microscopy (SEM) study

Imaging of nanoparticles by SEM is expected to provide information on nanoparticle morphology and size. Examination of SEM photographs of the nanoparticles revealed that the surfaces were smooth and flaky in structure as seen in Figure 10.

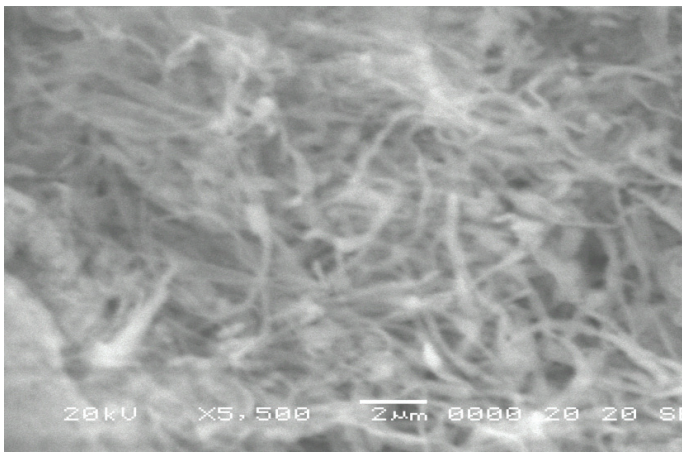

Fig. 10 SEM of Indomethacin nanoparticles

7.5 Particle size and zeta potential measurement

Particle size and particle size distribution are important factors in the therapeutic performance of NPs. From DLS measurements, individual particle size diameter and polydispersity index are 1842.3 nm and 2.41 respectively.

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Particle Size

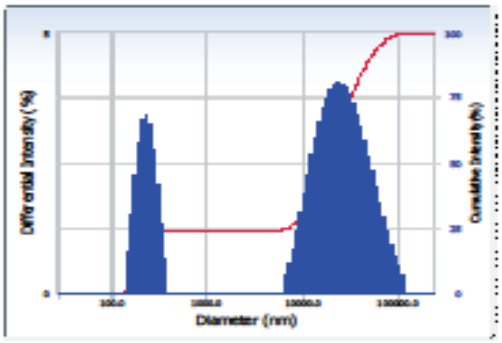
NanoPlus
Common

Intensity Distribution S/N : 407714

User	: Common	Group	: SBPI	Repetition	: 1/3
Date	: 12/24/2018	File Name	: INDNP r		
Time	: 13:33:53	Sample Information	: INDNP SIZE		
SOP Name	: Monika			Security	: No Security

Version 5.10 / 3.00

Intensity Distribution



Ln(G2(τ)-1) vs τ

Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	246.1	55.8
2	32,506.6	21,004.5
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	24,668.0	22,922.1

Residual : 2.647e-003 (O.K)

Cumulants Results

Diameter	(d)	: 4842.3	(nm)
Polydispersity Index	(P.I.)	: 1.882	
Diffusion Const.	(D)	: 1.642e-009	(cm²/sec)

Measurement Condition

Temperature	: 24.8	(°C)
Diluent Name	: Methanol	
Refractive Index	: 1.3312	
Viscosity	: 0.5490	(cP)
Scattering Intensity	: 21246	(cps)
Attenuator 1	: 19.57	(%)

Intensity Distribution Table

d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)	d (nm)	f(%)	f(cum.%)
30.0	0.0	0.0	290.0	2.7	20.2	2803.2	0.0	24.3	27096.2	4.0	65.2
32.8	0.0	0.0	317.5	2.1	22.3	3069.4	0.0	24.3	29670.1	3.9	69.1
36.0	0.0	0.0	347.7	1.3	23.6	3361.0	0.0	24.3	32488.5	3.8	72.9
39.4	0.0	0.0	380.7	0.7	24.3	3680.3	0.0	24.3	35574.7	3.6	76.6
43.1	0.0	0.0	416.9	0.0	24.3	4029.8	0.0	24.3	38953.9	3.4	80.0
47.2	0.0	0.0	456.5	0.0	24.3	4412.6	0.0	24.3	42654.2	3.2	83.2
51.7	0.0	0.0	499.9	0.0	24.3	4831.8	0.0	24.3	46705.9	2.9	86.1
56.6	0.0	0.0	547.3	0.0	24.3	5290.8	0.0	24.3	51142.6	2.6	88.7
62.0	0.0	0.0	599.3	0.0	24.3	5793.4	0.0	24.3	56000.7	2.3	91.1
67.9	0.0	0.0	656.3	0.0	24.3	6343.7	0.0	24.3	61320.2	2.0	93.1
74.3	0.0	0.0	718.6	0.0	24.3	6946.3	0.3	24.6	67145.1	1.7	94.9
81.4	0.0	0.0	786.9	0.0	24.3	7606.1	0.6	25.2	73523.2	1.5	96.3
89.1	0.0	0.0	861.6	0.0	24.3	8328.6	0.9	26.1	80507.3	1.2	97.5
97.6	0.0	0.0	943.5	0.0	24.3	9119.8	1.2	27.3	88154.7	0.9	98.4
106.9	0.0	0.0	1033.1	0.0	24.3	9986.0	1.5	28.8	96528.6	0.7	99.1
117.0	0.0	0.0	1131.2	0.0	24.3	10934.6	1.9	30.7	105698.0	0.5	99.6
128.1	0.0	0.0	1238.7	0.0	24.3	11973.3	2.3	33.0	115738.3	0.4	100.0
140.3	0.0	0.0	1356.3	0.0	24.3	13110.7	2.7	35.7	126732.4	0.0	100.0
153.6	0.8	0.8	1485.2	0.0	24.3	14356.1	3.0	38.7	138770.8	0.0	100.0
168.2	1.5	2.3	1626.2	0.0	24.3	15719.8	3.3	41.9	151952.8	0.0	100.0
184.2	2.2	4.6	1780.7	0.0	24.3	17213.0	3.5	45.5	166386.9	0.0	100.0
201.7	2.9	7.5	1949.9	0.0	24.3	18848.1	3.7	49.2	182192.2	0.0	100.0
220.9	3.3	10.8	2135.1	0.0	24.3	20638.5	3.9	53.1	199498.8	0.0	100.0
241.9	3.4	14.2	2337.9	0.0	24.3	22598.9	4.0	57.1	218449.3	0.0	100.0

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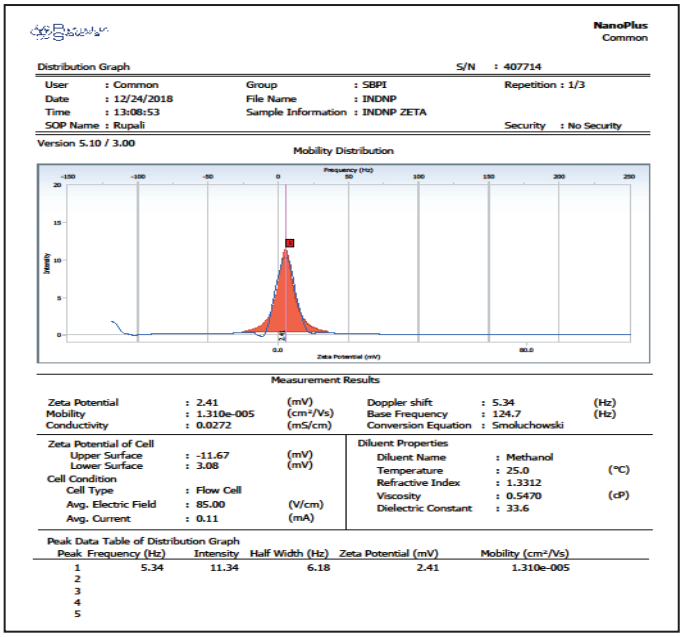

Fig. 11 Particle size measurement and zeta potential

7.6 Entrapment efficiency

The entrapment efficiency of Paracetamol in the prepared formulation was determined and recorded in Table 1. It is seen that the entrapment efficiency ranges 9.18±5.80

Table 1 Percentage entrapment efficiency data

Formulation	Percentage Entrapment Effieciency
Paracetamol Nanoparticles	9.18±5.80

7.7 Release order kinetics study

Data obtained from in vitro release studies were fitted to various kinetics equations to find out the mechanism of drug release from the gel formulation. The kinetic models used were zero order, first order, Higuchi model, Korsmeyer Peppas model and Hixson Crowell model. From the release kinetics data it was observed that the release of indomethacin from the PLGA loaded nanoparticles exhibit anomalous (non-Fickian) diffusion for Hixson Crowell Model, first order and Higuchi Model whereas it closely follows zero order release and also highly correlated with Korsmeyer Peppas Model.

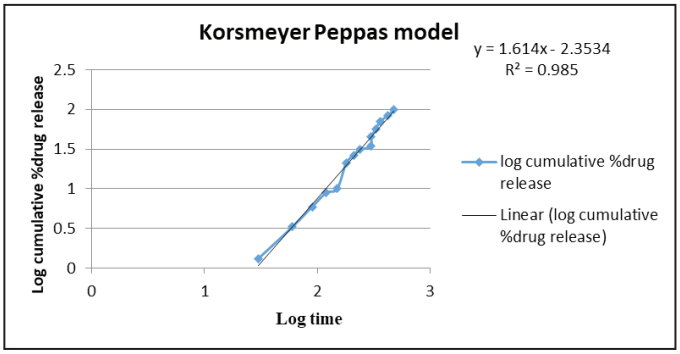

Fig. 12 Korsmeyer Peppas model

8. CONCLUSION

The solid indomethacin nanoparticles were successfully developed by emulsion diffusion evaporation technique. Ingredients used in this study were economic and safe. Physicochemical characterization including particle size, particle size distribution, zeta potential, scanning electron microscopy and *in-vitro* release profile were carried out. *In-vitro* drug release pattern of immediate release topical gel showed fast and control release. Immediate release topical application can be useful to improve the penetration of drug & maintain the loading dose. Characterization of indomethacin nanoparticles reveals a good kind of product which could be reproduced for commercial purpose. Entrapment efficiency and drug release were good and up to the acceptable range.

Acknowledgement

The authors are thankful to Dr V. Ganeshan, Dr Uday Deshpande, and Dr GN Okram of UGC-DAE Consortium, DAVV, Indore and Dr Kinny Pandey of IITI, for providing sophisticated instrumental facilities. The authors gratefully acknowledge Madhya Pradesh Council of Science and Technology (MPCST), Bhopal, for providing the fellowship to pursue the research project.

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