European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 09, Issue 09, 2022

Original Research Article

Formulation and Evaluation of ketoprofen nanosuspension

[1]Dr. Sunil J Aher, [2]Poonam P Patil, [3]Mohsina Patwekar, [4]Dr. Faheem Patwekar

[1]Associate Professor, Department of Pharmaceutics, SRES's Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India
[2]Assistant Professor, HOD Department of Pharmaceutical Chemistry, SRES's Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India
[3]Assistant Professor, Luqman College of Pharmacy, Gulbarga, Karnataka, India
[4]Professor, Luqman College of Pharmacy, Gulbarga, Karnataka, India

Corresponding Author:
Dr. Sunil J Aher (suniljaher@gmail.com)

Abstract

The goal of the current work was to develop a nanosuspension of the drug ketoprofen in order to boost the dissolution of poorly soluble class II drugs. Ketoprofen was employed as an example because it was found to increase oral bioavailability and invitro dissolution. It was made utilizing the Quassi Emulsification Solvent Diffusion process and a variety of drug, polymer, and stabilizer. Drug entrapment and dissolution are increased as well as reduced particle size in the formulation with a ratio of 1:2 (drug: polymer). Particle size of the formulations were evaluated utilizing SEM, Zeta potential analysis and IR& DSC were used for evaluation of *in vitro* drug release studies. The formulated nanosuspensions' particle sizes were found to range between 92.20nm and 100 nm.

Keywords: Nanosuspension, ketoprofen, quassi emulsification solvent diffusion, bioavailability

1. Introduction

Poorly soluble drugs are a general problem in pharmaceutical drug formulation [1]. Typical problems associated with poorly soluble drugs are a too low bioavailability and/or erratic absorption. In case of a too low bioavailability after oral administration, parenteral administration cannot solve this problem in many cases. Due to the poor solubility, intravenous injection as a solution is not possible. Parenteral administration as a micronized product (e.g. i.m. or i.p.) does not lead necessarily to sufficiently high drug levels because the solute volume at the injection site is too low. Low saturation solubility, generally combined with a low dissolution velocity is the obstacle preventing sufficiently high blood levels. Possible exceptions are only drugs being poorly soluble but highly potent [2]. Attempts to increase the saturation solubility, and thus solving the problem, are solubility enhancement by using solubilization (e.g. mixed micelles as in Valium MMÒ for i.v. injection), non-specific or specific complexation (e.g. addition of polyethylenglycol (PEG) or use of cyclodextrins) and solvent mixtures. The limited success of these attempts is documented by the low number of products on the pharmaceutical market based on these principles. The problems to find a suitable formulation are even greater in case the drugs are poorly soluble in aqueous media

ISSN 2515-8260 Volume 09, Issue 09, 2022

and at the same time in organic media. This excludes the use of solvent mixtures a priority. A general approach used for many years is the micronization of poorly soluble drugs by colloid mills or jet mills. The overall particle size distribution ranges from 0.1 mm to approximately 25 mm, only negligible amount being below 1 mm in the nano meter range [3]. Advances in combinatorial chemistry, biology and genetics in the recent years have led to a steady increase in the number of drug candidates under development. Due to the phospholipidic nature of cell membranes, a certain degree of lipophilicity is oftentimes a requirement for the drug compound, not only to be absorbed through the intestinal wall following oral administration but possibly also to exert its pharmacological action in the target tissue. While high lipophilicity is advantageous in terms of compound permeability, it intrinsically translates into poor aqueous solubility. Since the first step in the oral absorption process is dissolution of the drug compound in the gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on oral delivery of drug compounds [4]. Nanosizing refers to the reduction of the active pharmaceutical ingredient (API) particle size down to the sub-micron range. While reduction of particle size has been employed in pharmaceutical industry for several decades, recent advances in milling technology and our understanding of such colloidal systems have enabled the production of API particles of 100-200 nm size in are producible manner. The sub-micron particles are stabilized with surfactants or polymers in nanosuspensions which can be further processed into standard dosage forms, such as capsules or tablets, suitable for oral administration. These nano formulations offer increased dissolution rates for drug compounds and complement other technologies used to enhance bioavailability of insoluble compound (BCS Class II and IV) such as solubility enhancers (i.e., surfactants), liquid-filled capsules or solid dispersions of drugs in their amorphous state. The advantages of nano formulations in oral drug delivery have been demonstrated *in vitro* in dissolution testing and *in vivo* in both preclinical studies as well as clinical trials. Nano crystalline API has been shown to dramatically increase the rate of dissolution *in vitro* improve bioavailability, reduce variability and alleviate positive food effects for orally administered drug molecules [5]. The use of nanocrystals for dissolution enhancement is currently a popular technique in the pharmaceutical field, in which nanoparticles are produced by reducing large drug crystals down to the sub-micron range since the dissolution rate of the drug is proportional to its surface area, the major benefit of nano crystal formulations is an enhanced dissolution rate. Finally, the use of nano crystal-based systems for orally administered drugs has been found to reduce the variability of drug absorption and reduce the food effects. The solid API dissolution rate is proportional to the surface area available for dissolution as described by the Nernst-Brunner/Noyes-Whitney equation Based on this principle, API micronization has been extensively used in the pharmaceutical industry to improve oral bioavailability of drug compounds. It is evident that a further decrease of the particle size down to the sub-micron range will further increase dissolution rate due to the increase of the effective particle surface area [6, 7, 8]. For example in the case of aprepitant, the nanocrystal dispersion of 120-nm particle size exhibits a 41.5-fold increase in surface area over the standard 5 μm suspension [9]. Furthermore, as described by the Prandtl equation, the diffusion layer thickness (h) will also be decreased thus resulting in an even faster dissolution rate [10, 11].

ISSN 2515-8260 Volume 09, Issue 09, 2022

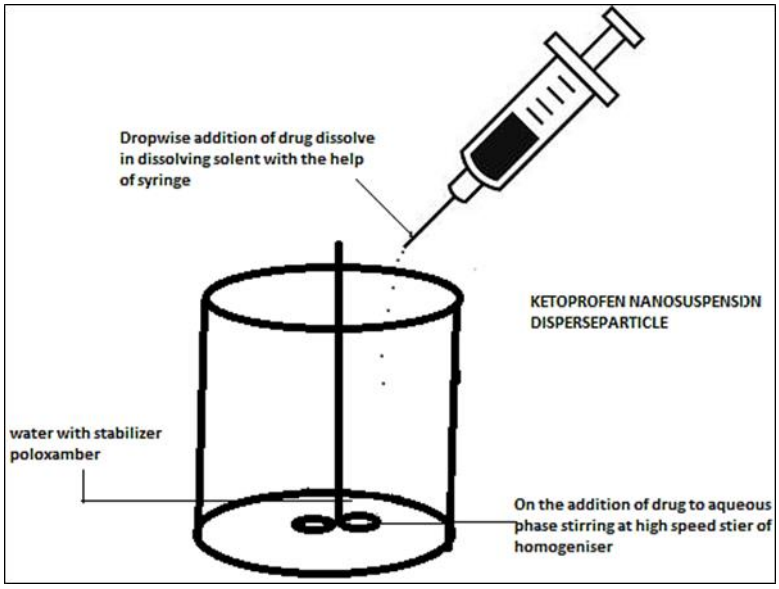


Fig 1: Schematic diagram represent the process of nanosuspension preparation

2. Materials and Methods

2.1 Materials

Ketoprofen and Poloxamber407, Eudragit RS 100, Eudragit RL 100 were purchased from Yarrow chemicals Mumbai, Maharashtra and are of AR grade.

2.2 Methods

Preparation of zaltoprofen nanosuspensions

Ketoprofen nanosuspensions were prepared by the Quassi emulsification solvent diffusion method. The drug and polymer were co-dissolved in 5 ml of methanol. The solution was to be slowly injected with a syringe containing thin Teflon tube into water containing stabilizer poloxamber 407 and it was maintained at low temperature in ice bath protected from sun light. During injection the mixture was stirred well by a high-speed homogenizer at 5500 rpm agitation speeds. The solution immediately turned into pseudo emulsion of the drug and polymer methanol solution in the external aqueous phase. The counter diffusion of methanol and water out of and into the emulsion micro droplets respectively results into the formation of nanosuspension. Methanol was then completely removed as the stiring was continued for 5 hrs [12, 13].

2.3 Evaluation of nanosuspensions

2.3.1 Particle size analysis

Particle size analysis/evaluation was carried out with Scanning electron microscopy (SEM) Make: JEOL Model JSM-6390lv.

2.3.2 Amount of unincorporated drug

2ml of the freshly prepared nanosuspension was centrifuged at 1100rpm, 10^{o}C for 15min. Then the supernatant was analyzed at 257 nm using U.V spectrophotometer to determine the amount of unincorporated drug [14].

2.3.3 Zeta potential

Electrophoretic mobility of nanosuspension was obtained by a laser Doppler anemometer. A suitable amount of the sample (50-100µ L) was diluted with 5mL of water (HPLC grade) and

ISSN 2515-8260 Volume 09, Issue 09, 2022

placed into the electrophoretic cell of the instrument, where a potential of ±150mV was induced. The ζ-potential value was calculated by the software using smoluchowski's equation.

2.3.4 *In vitro* drug release

In vitro drug release of the nanosuspension was carried out by using USP Dissolution apparatus type 2 (paddle type). 5ml of nanosuspension was taken in a dialysis membrane consisting of a spectrap or membrane (cut-off: 1200Da). This dialysis system was tied to the paddle and the dissolution medium was Phosphate buffer p^H 7.4. Dissolution was carried in triplicate for 10hr at 37±1 ^oC temperature and 50rpm speed. At regular intervals of time 1ml of sample from the external medium was taken and replaced with fresh phosphate buffer and all the samples were analysed at 257 nm using U.V spectrophotometer.

2.3.5 Compatibility studies

Compatibility studies was performed by the DSC (make METTLER) and with Infra-red Spectroscopy.

3. Results and Discussion

In the present work nanosuspensions of Ketoprofen were formulated using different drug to polymer ratio and agitation speeds was maintained constant and prepared by quasi emulsion solvent diffusion technique. Overall, eight formulation four with Eudragit RS100 (F1, F2, F3, F4) & remaining with the Eudragit RL100 (F5, F6, F7, F8) with a different combination of drug: polymer: stabilizer. All the formulation formulate with the Ketoprofen: Eudragit RS100/Eudragit RL100: poloxamber 407 with varying ratio of 1:1:0.5 & 1:2:0.5, 1:1:1 & 1:2:1. Particle size was determined for all the formulations by scanning electron microscopy. It was found that the formulations F4 prepared at 5500 rpm speed for 5 hours with ratio of drug to polymer Eudragit RS100 (1:2) and the ratio of stabilizer 1:00 percentage had found nano size particle size prepared 92.20-100 nm with a zeta potential of -13.23. Even the *in vitro* drug release shows at 10 hrs 97.03%. The F4 formulation was found to be reduce particle size of 92.20-100 nm & consider to be optimize formulation with increase in the dissolution of poorly water soluble drug being formulated in nano composite in the form of nanosuspension. Whereas the formulations with Eudragit RL100 also found to be quite acceptable prepared at 5,500 rpm had particle size with 150 nm.

3.1 Particle size distribution

The particle size distribution of formulation F1, F2, F3, F4 these formulate with Eudragit RS100 with varying proportion of Ketoprofen: Eudragit RS100: poloxamber407 was found to be in between 92-100 nm. Whereas the formulation F5, F6, F7, F8 these formulated with Eudragit RL100 with the varying ratio of Ketoprofen: Eudragit RL100: poloxamber 407 particle size was found to be in between 150-250 nm.

3.2 Amount of unincorporated drug

The amount unincorporated was found to 9.64% with a optimize formulation F4 (Formulated with the Eudragit RS100). And F5 show unincorporated 13.30%.

3.3 Zeta potential

It was found that the formulations F4 prepared at 5500rpm speed for 5 hours with ratio of drug to polymer EudragitRS100 (1:2) and the ratio of stabilizer 1:00 percentage had found

ISSN 2515-8260 Volume 09, Issue 09, 2022

nano size particle size prepared 92.20-100 nm with a zeta potential of -13.23

3.4 *In vitro* drug release

All the formulation *in vitro* drug release study was determined among that F4 & F5 found to be optimize. And *in vitro* drug release shows at 10 hrs 97.03% with the F4 formulation was found to be reduce particle size of 92.20-100 nm & consider to be optimize formulation with increase in the dissolution of poorly water-soluble drug being formulated in nano composite in the form of nanosuspension. The formulation F5 show the *in vitro* drug release at the end of ten hour of 95.90%.

3.5 Compatibility studies

a) **IR Spectra:** Raw Ketoprofen and polymer Eudragit RS100, Eudragit RL100 Stabilizer Poloxamber 407. IR Spectra was determined which demonstrate the chemical structure with functional group of the drug is not changed as per as compatibility is concerned.

b) **DSC Analysis**: The Ketoprofen physical state raw as well as in the combination for compatibility studies was determined and no interaction between the Ketoprofen and polymer as well as stabilizer observed.

c) **SEM Scan:** The above SEM are of the optimize formulation F4 and F5. On a scale we can clearly see the F4 formulation of Ketoprofen: Eudragit RS100 Particle size 92.20 to 100 nm were as the formulation F5 Formulation of Ketoprofen: Eudragit RL100 show particle size of 150 nm-200nm.

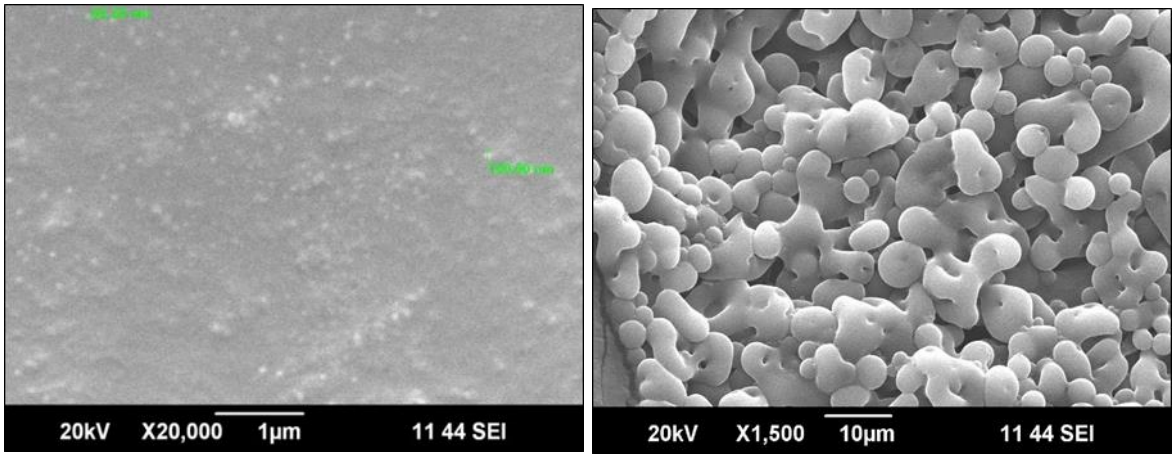
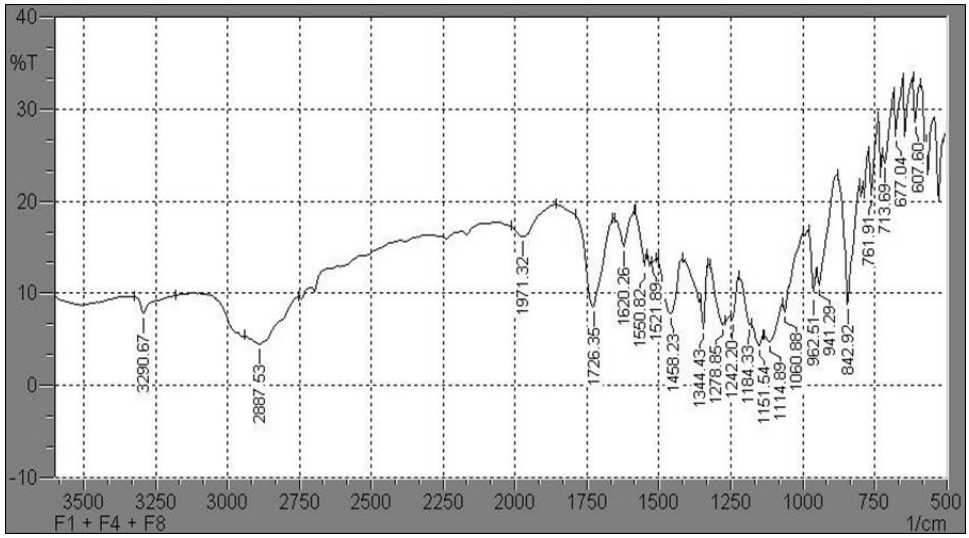


Fig 2: SEM of Optimized F4 and F5 formulations



ISSN 2515-8260 Volume 09, Issue 09, 2022

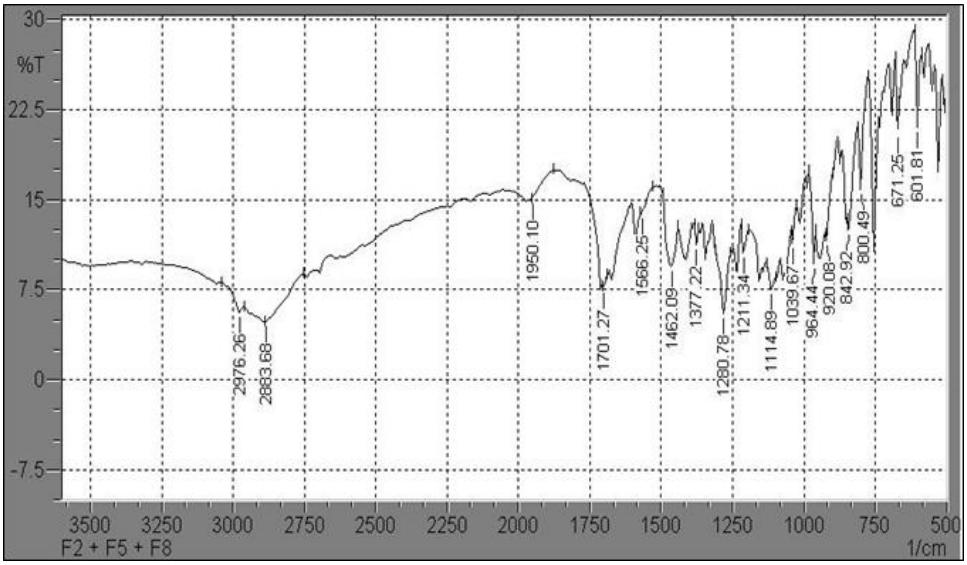
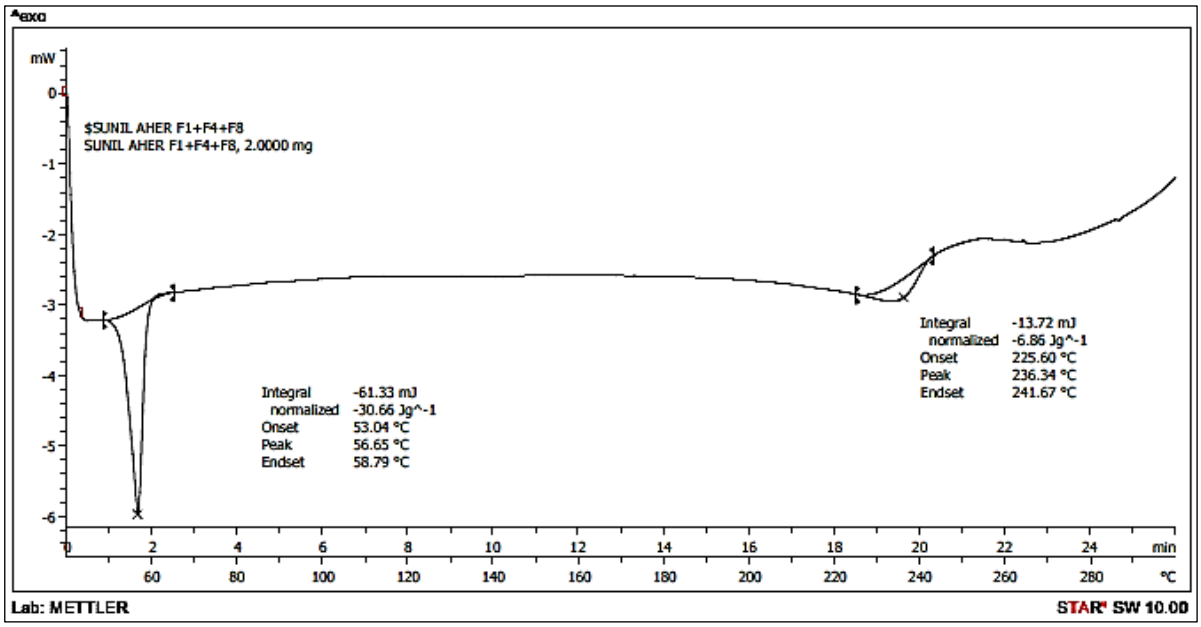


Fig 3: FT-IR of Optimized F4 and F5



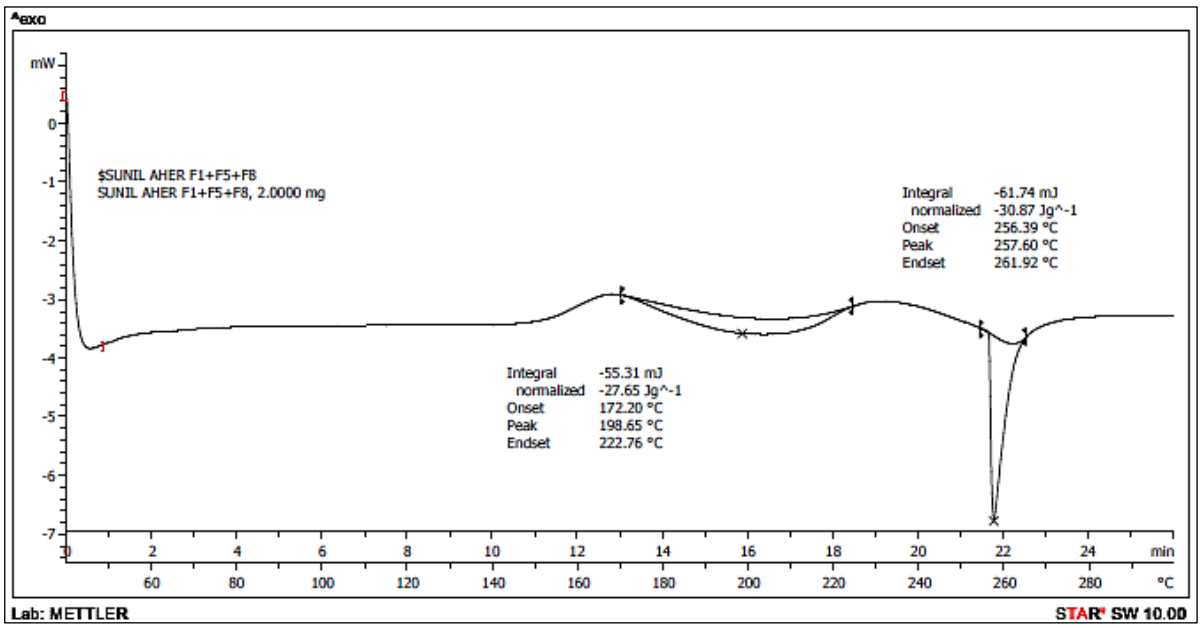


Fig 4: DSC of F4 and F5

ISSN 2515-8260 Volume 09, Issue 09, 2022

Table 1: Details about formulation contents of polymeric nanosuspension batches (Ketoprofen)

Batch	Drug (mg)	Polymer (mg)		Surfactant Poloxamer 407 (%)	Distilled water (mL)
		Eudragit RS100	Eudragit RL100		
F1	75	75	-	0.5	Qs
F2	75	150	-	0.5	Qs
F3	75	75	-	1	Qs
F4	75	150		1	Qs
F5	75		75	0.5	Qs
F6	75	-	150	O.5	Qs
F7	75		75	1	Qs
F8	75		150	1	Qs

Table 2: Showing the drug and the particle size in nanometer (nm)

Drug	Particle size(nm)
F1	158-189
F2	165-178
F3	110-127
F4	92.20-100
F5	150-200
F6	234-247
F7	289-306
F8	302-320

Percentage drug unincorporated and entrapped for nanosuspension

Table 3: Percentage drug unincorporated and entrapped for nanosuspension

Drug Formulation	% Drug Unincorporated	% Drug Entrapped
F1	21.34	78.66
F2	18.65	81.35
F3	17.61	82.39
F4	9.64	90.36
F5	9.34	90.66
F6	17.89	82.11
F7	34.65	65.35
F8	21.08	78.92

Zeta potential (mV)

Table 4: Zeta potential of nanosuspension

Sr. No.	Formulation	Zeta Potential (mV)
1.	F1	-56.78
2.	F2	-54.09
3.	F3	-59.87
4.	F4	-13.23
5.	F5	-23.78
6.	F6	50.87
7.	F7	53.76
8.	F8	54.23

ISSN 2515-8260 Volume 09, Issue 09, 2022

Cumulative drug release %

Table 5: Percentage cumulative drug release of nanosuspension

Time (Hrs.)	% Cumulative drug release							
	F1	F2	F3	F4	F5	F6	F7	F8
0.	0	0	0	0	0	0	0	0
1.	12.69	14.90	15.11	18.59	10.11	11.09	12.36	13.4
2.	23.78	24.35	2242	27.64	21.79	19.28	20;.34	22.65
3.	35.70	34.75	32.86	39.87	31.77	29.40	28.60	30.59
4.	42.88	47	45.02	47.12	42.70	38.61	36.88	39.37
5.	52.57	55.35	53.94	55.97	54.10	48.25	49.75	48.58
6.	63	61.45	62.90	64.56	64.40	52.78	55.32	59.10
7.	71.55	74.88	76.55	77.34	74.55	67.34	65.89	69.55
8.	82.97	84.15	82.49	85.99	84.00	72.22	75.23	83.77
9.	87.55	89.73	90.34	91.64	90.15	81.11	82.30	90.16
10.	94.63	95.20	94.55	97.03	96.89	90..67	91.12	95.09

Conclusion

It is concluded that the polymer Eudragit RS100 with a ratio of drug 1: 2 with a help of stabilizer poloxamber is effective for formulating the stable nanosuspension for a sustained release of Ketoprofen drug with increased in dissolution rate Ketoprofen nanosuspension can be prepared using Quassi emulsification solvent diffusion method using poloxamber 407 as a stabilizer (F4 Formulation). Poloxamber 407 is essential to achieve a particle size close 92.20-100 nm. The formulations along with Eudragit RS 100 polymer shows significant drug release shown in dissolution profile of the formulations. Nanosuspension of ketoprofen shows significant drug release determined using phosphate buffer having pH 7.4. The results show the suitability of method for the preparation of stable nanosuspension of water insoluble drugs. Both the polymer Eudragit RS100 & Eudragit RL 100 can be used for the preparation of nanosuspension with a help of stabilizer.

Acknowledgement

Authors wish to express their sincere thanks to Hon. Mr. Amit Dada Kolhe saheb Trustee, Sanjivani rural education society, Kopargaon for his constant encouragement and support.

Conflict of interest

Authors do not have any conflict of interest.

References

1. Muller RH, Benita S, Bohm B. (Eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs, Medpharm Scientific, Stuttgart; c1998.
2. Taifa S, Muhee A, Bhat RA, Nabi SU, Roy A, Rather GA, *et al*. Evaluation of Therapeutic Efficacy of Copper Nanoparticles in Staphylococcus aureus-Induced Rat Mastitis Model. Journal of Nanomaterials; c2022.
3. Filippos Kesisoglou, Santipharp Panmai, Yunhui Wu. Nanosizing-Oral formulation development and biopharmaceutical evaluation, Advanced Drug Delivery Reviews. 2007;59:631-644.
4. Bhakay A, Merwade M, Bilgili E, Dave RN. Novel aspects of wet milling for the production of micro-suspensions and nanosuspensions of poorly water soluble drugs. Drug Dev. Ind. Pharm. 2011;37:963-976.

ISSN 2515-8260 Volume 09, Issue 09, 2022

5. Shinde MU, Patwekar M, Patwekar F, Bajaber MA, Medikeri A, Mohammad FS, *et al*. Nanomaterials: A potential hope for life sciences from bench to bedside. Journal of Nanomaterials; c2022. p. 1-13.
6. Kesisoglou F, Santipharp P, Wu Y. Nanosizing-oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 2007;59:631-644.
7. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions: a promising drug delivery strategy, J Pharm. Pharmacol. 2004;56(7):827-840.
8. Patwekar M, Patwekar F, Alghamdi S, Kamal M, Allahyani M, Almehmadi M, *et al*. Vancomycin as an Antibacterial Agent Capped with Silver Nanoparticles: An Experimental Potential Analysis. Bio Med Research International; c2022.
9. Mosharraf M, Nystrom C. The effect of particle size and shape on the surface specific dissolution rate of micronized practically insoluble drugs, Int. J Pharm. 1995;122:35-47.
10. Mothilal Mohan, Manasa Veena, Damodharan Narayanasamy, Manimaran Vasanthan, Shaik Nelofar. Development & Evaluation of Aceclofenac Nanosuspension Using Eudragit Rs100, Asian Journal of Biochemical and Pharmaceutical Research Issue. 2012;2(2):1-9.
11. Friedrich RB, Fontana MC, Beck RCR. Quim nova. 2008;31:11-31.
12. Shah R, Magdum C, Patil SK, Dhanya CK, Nilofar N. Research J Pharm. and Tech. 2008;1(4):43.
13. Muthu MS, Singh S. Current Drug Delivery. 2009;6:62.
14. Rosario P, Nadia R, Claudio B, Francesco M, Adriana M, Giovanni P. AAPS Pharma Sci. tech. 2006;7:27.