PREPARATION AND CHARACTERIZATION OF EUDRAGIT BASED NANOSUSPENSIONS FOR THE ORAL DRUG DELIVERY

Dr. Sunil J. Aher[1]*, Aishwarya S. Patil[2], Poonam P. Patil[3], Dr. Sangita A. Kale[4], Supriya C. Joshi[5]

Abstract

In the present research article, nanosuspension of Meloxicam (BCS Class II) was prepared using Eudragit RS100 and stabilizer Poloxamber 407 with different ratios, a total of four formulations were prepared by using Quassi emulsification solvent diffusion technique ratio of drug: polymer: stabilizer with different ratio. Formulation F2 was found with particle size 80.00-100 nm and drug entrapment of 92.93 % with a zeta potential of -15. vitro drug release shows at 10 hrs. 96.44 %. Consider being optimized formulation with an increase in the dissolution of poorly water-soluble drugs being formulated in nanocomposite in the form of nanosuspension. By applying a statistical model for dissolution Higuchi model, Kerseymere pappa's model for optimizing F2 formulation shows the First order. Optimize F2 formulation with an increase in the dissolution/saturation solubility of 23.42±0.61 (µg /mL) of poorly water-soluble meloxicam belonging to BCS Class II (reported solubility with 3.5±0.50 µg /mL). The short-term stability study results revealed that the optimized F2 formulation stored at temperature 4oC shows no change in the in vitro drug release of the formulation compared to the release study tested after the formulation 0 time which means the nanosuspension is stable at the given temperature. Were as nanosuspension stored at a temperature of 37°C ± 2°C,65 % RH ± 5 % RH comparatively there is a change in the vitro drug release. So maximum stability obtained at the temperature 4oC Polymer Eudragit RS100 can be used for the preparation of nanosuspension with the help of Poloxamer 407 as a stabilizer.

Keywords: Nanosuspension, Meloxicam, Quassi emulsification solvent diffusion, Class II drug, solubility Enhancement, bioavailability.

[1]*Department of Pharmaceutics, SRES's Sanjivani College of Pharmaceutical Education and Research, Kopargaon, MS, India-423603. Email: suniljaher@gmail.com
[2]Department of Pharmaceutics, Vilasrao Deshmukh Foundation Group of Institution, VDF School of Pharmacy, Latur, MS, India-413531
[3]Department of Pharmaceutical Chemistry, SRES's Sanjivani College of Pharmaceutical Education and Research, Kopargaon, MS, India-423603.
[4]Department of Pharmaceutics, PES Modern College of Pharmacy, Pune, MS, India-411044.
[5]Department of Pharmaceutics, Indira College of Pharmacy, Nanded, MS, India-431606.

***Corresponding Author:** Dr. Sunil J. Aher
*Associate Professor, Department of Pharmaceutics, SRES's Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Dist-Ahmednagar, MS, India-423603. Email: suniljaher@gmail.com

DOI: 10.48047/ecb/2023.12.si5a.0556

INTRODUCTION

Nanosuspensions are aqueous suspensions containing one or several submicron-sized drug substances and appropriate stabilizers. Stabilizers include excipients that enable nano grinding of the drug particles, prevent crystal growth or nano particle aggregation during storage, pH-buffering substances, preservatives, and other components that may be needed for further processing (e.g., transforming into a solid form) or administration to patients (e.g., sweeteners, colorants) [1,2]. The term nano sizing, as used in this work, describes the reduction of suspended drug particles down to the submicron size range. The main challenge in nanosuspension technology is prevention of particle agglomeration or aggregation and crystal growth. At the nanometer scale, attractive Vander Waals and dispersive forces between particles come into play. Such attractive forces increase dramatically as particles approach each other, which ultimately results in irreversible aggregation [3, 4].

Major Advantages of Nanosuspensions

It is applicable for formulating all type of drug as well its simplicity.

- ✓ Can be applied for those drugs which are poorly soluble in water.
- ✓ Administration of nanosuspension by any route.
- ✓ Reduced tissue irritation when the drug in form of nanosuspension given by subcutaneous, intramuscular route.
- ✓ By IV route of administration the rapid dissolution as well tissue targeting can be achieved.
- ✓ Orally administration of nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability.
- ✓ The absorption from absorption window of the drugs can be increased, these is due to reduction in the particle size in nanosuspension.
- ✓ In case of the inhalation & ocular there is higher bioavailability and more consistent dosing.
- ✓ The bioavailability of drugs with high log P value can be increased by converting them into nanosuspension.
- ✓ High dissolution rate increases the bioavailability of drugs.
- ✓ Ease of manufacture and little batch-to-batch variation.
- ✓ Nanosuspension shows greater physical stability.
- ✓ Nanosuspensions can be converted into other dosage forms like tablet, capsules etc.
- ✓ Crystal lattice of drugs has been changed due to increase in amorphous particles which increases solubility of drugs.
- ✓ Surface modification of nanosuspension achieves the goal of targeted drug delivery.
- ✓ Applicable to industrial scale formulations of nanosuspensions.

Key to drug nanoparticle technology is the successful compensation of the extra free energy of freshly exposed surfaces [5]. The tendency of the smaller particles in a suspension to dissolve and re-crystallize on the larger particles represents a mode of instability, termed Ostwald ripening. Ostwald ripening becomes important with particles smaller than 0.5 μm. In general, the speed of Ostwald ripening is governed by molecular diffusion or surface reaction. Diffusion-controlled growth predominates if the particle size distribution in the suspension is large (i.e in presence of an important fraction of smaller particles) and the solubility is high. In this situation, Ostwald ripening can be lowered by narrowing the particle size distribution. The alternative mechanism of surface reaction predominates under very low super saturation, i.e., when the solubility of the smaller and larger particles is similar. Ostwald ripening via reaction-controlled mechanism can be prevented by the addition of polymeric stabilizers to the suspension. Irrespective of the mechanism, particle growth can be prevented or at least minimized by steric hindrance and/or electrostatic repulsion. Steric hindrance is primarily achieved by adsorbing polymers onto particles, while for electrostatic repulsion, ionic surfactants or polymers are used. Use of surfactants or surface-active polymers additionally promotes wetting and dispersion of the drug particles, which are usually very hydrophobic. Commonly used polymeric stabilizers for nanosuspensions include cellulose ethers, such as hydroxypropyl cellulose (HPC) and hydroxypropyl methylcellulose (HPMC), povidone, and poloxamers (types 188, 407 and 338). Commonly used surfactant stabilizers are either non-ionic, such as the polysorbate types, or anionic, such as sodium dodecyl sulfate (SDS) and docusate sodium (SD). For effective nanosuspension stabilization, the drug substance: stabilizer ratio may vary between 20:1 to 2:1, (w/w). While insufficient amounts of stabilizers remain ineffective for preventing particle agglomeration, excessive quantities may promote crystal growth by Ostwald ripening. Naturally, only excipients with established safety profiles should be used for the stabilization of

nanosuspensions. Particle size reduction can be achieved mainly by bottom-up and top-down processes [6,7] Particle formation through micro-precipitation, chemical synthesis, or complexation; particle comminution (Nano sizing) through high energy homogenization [8,9] Processes can also be combined to achieve synergistic effects as adopted in the NANOEDGE ® technology platform.

A widely used process in nanosuspension technology is wet media milling (Nanocrystals® technology) in high-shear energy mills. In this process, drug substance powder is suspended in an appropriate medium (mostly an aqueous or aqueous-organic solution containing appropriate stabilizers). To the drug dispersion, milling medium is added under continuous stirring; which is made up of different materials and can be obtained in different sizes (typically between 0.1 and 1 mm). The slurry of drug suspension and milling beads is then introduced into the milling chamber of an appropriate mill. Shear forces generated by the movement of the milling medium lead to particle size reduction. Hardness of particles governs the milling time , viscosity of the drug suspension, temperature, size and density of the milling medium, and total energy input during milling [10] . Milling time can be vary from minutes to hours. [11]

APPLICATION OF NANOSUSPENSIONS

Bioavailability enhancement

Many drug they are classified in BCS from that the class-II, IV in this class the drug belong to poor water solubility or low gastrointestinal permeability these will result in the poor bioavailability of the drug. Nanosuspension formulation solve the problem of poor water solubility& gastrointestinal permeability and they're by increase in the bioavailability of the drug. It has been observed that the dissolution rate was improved in diclofenac when it was formulated in the nanosuspension. Diclofenac nanosuspension was prepared and the rate of dissolution in the stimulated gastric fluid for sixty minute was conducted at the same time coarser suspension dissolution rate was determined result show 10 % release from the coarse suspension and the 50% from the nanosuspension. Celecoxib have poor bioavailability was enhance by preparing the celecoxib nanosuspension, the prepared nanosuspension show enhance dissolution compared to the micron sized particle. Spironolactone and budesonide are poorly soluble drugs. By using the different proportion of the surface-active agent, the prepared nanosuspension exhibit the enhance flux across coca-2 cell monolayer than the saturated solution. As enhance or higher bioavailability is the result of enhance flux of the preparation. Fenofibrate having the low water solubility as well as the low oral bioavailability and was increase with the nanosuspension compare to the suspension with micro-size [12]

MATERIALS AND METHODS

Materials

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

Methods

Quassi emulsification solvent diffusion technique the Meloxicam nanosuspension were prepared. [12] The Meloxicam (15mg) was kept constant and Eudragit RS100 were co-dissolved in 6 to 7 ml of methanol it was stirred continuous for 3 Hrs. The solution was to be slowly injected with a syringe containing thin Teflon tube into 20 ml 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 6000-6500 rpm speed for 5 Hrs. 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. Formulation was prepared with varying polymer & stabilizer ratio overall 4 formulation of drug meloxicam were prepared with polymer Eudragit RS100 & stabilizer such as poloxamber 407(Pluronic F127) and the formulation were code as F1, F2, F3, F4 (13,14).

Table 1: Details about formulation contents of polymeric nanosuspension batches

Batch	Drug (mg)	Polymer (mg)	Surfactant Poloxamer 407 (%)	Distilled water (mL)
		Eudragit RS100		
F1	15	15	0.5	20
F2	15	30	0.5	20
F3	15	15	1	20
F4	15	30	1	20

Evaluation of Nanosuspensions

Compatibility Studies:

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

Particle Size Analysis

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

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 362 nm using U.V spectrophotometer to determine the amount of unincorporated drug [14]

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 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. [15]

***In Vitro* Drug Release:**

In vitro drug release of the nanosuspension was carried out by using USP Dissolution apparatus type2 (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^{0}C 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 analyzed at 362 nm using U.V spectrophotometer. Statistical dissolution model like Higuchi model, Korsemeyer pappas model was applied for optimize F2 formulation to proves release kinetic.

Short term stability

Short term stability studies were performed on optimize formulation for three months stored at two set of condition first set at 4 ^{o}C in the refrigerator and second at 37 ^{o}C ±2 ^{o}C ,65 % RH ± 5 %RH (Humidity cabinet make REMI Instrument ltd). The sample were removed at the interval of 0,1,2,3 month and evaluate for the in vitro drug analysis. As well as any change in physical appearance is observed. [16]

RESULTS AND DISCUSSION:

Compatibility Studies

A) SEM Scan: The SEM of the optimize F2 formulation show particle size 80-100 nm. Were as the formulation F1 to F4 show particle size in range of 80 nm-200nm

B) DSC Analysis

The Meloxicam physical state raw as well as in the combination for compatibility studies was determined and no interaction between the Meloxicam and polymer as well as stabilizer observed.

C) IR Spectra: Raw Meloxicam and polymer Eudragit RS100, 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.

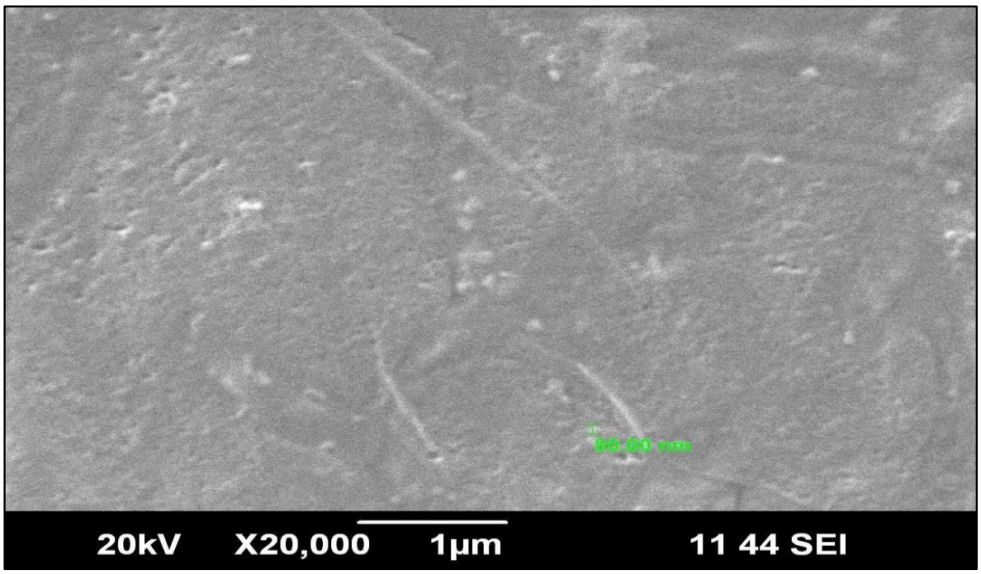

Fig.1: SEM of F2 formulation

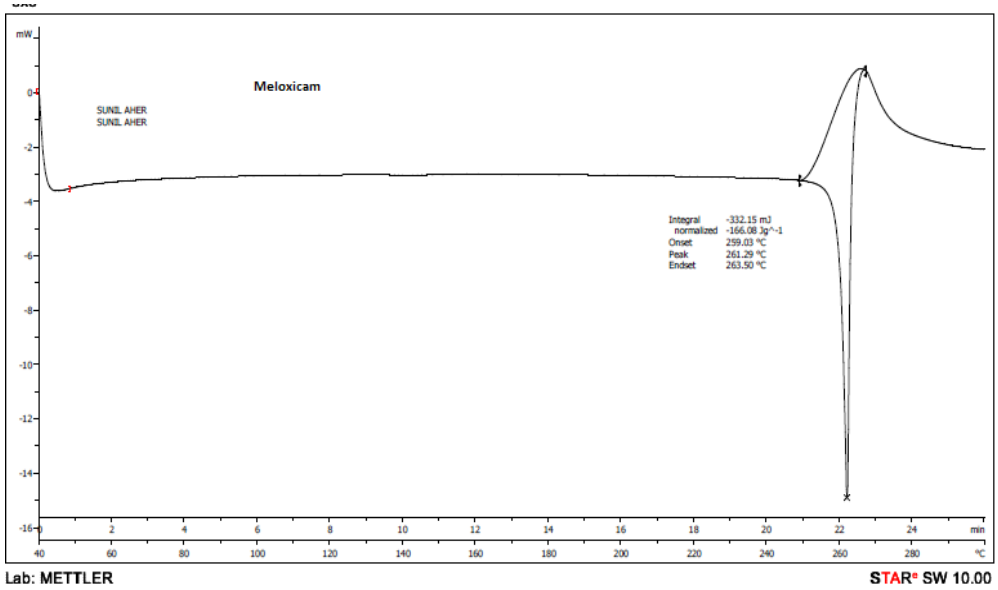

Fig.2: DSC of Meloxicam

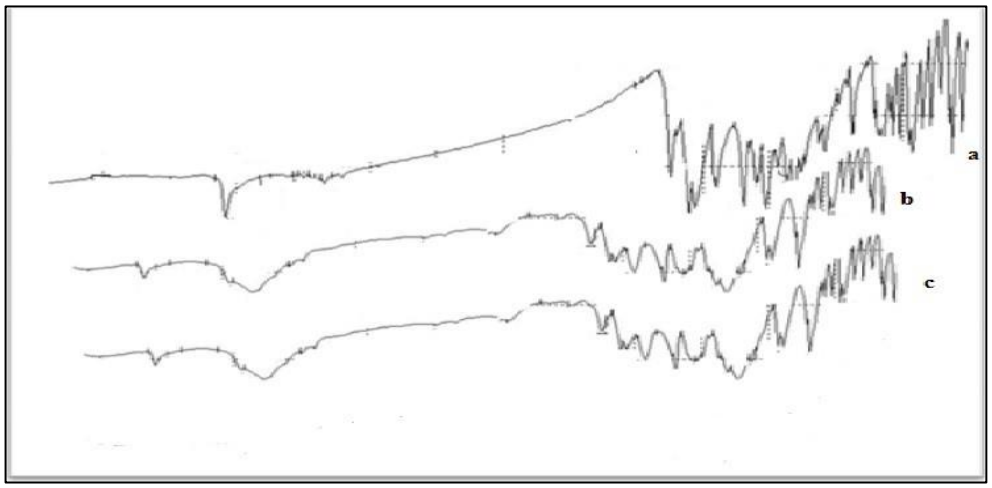

Fig.3: IR spectra for compatibility study: Meloxicam (a); Eudragit RS100 (b), Poloxamer 407 (c)

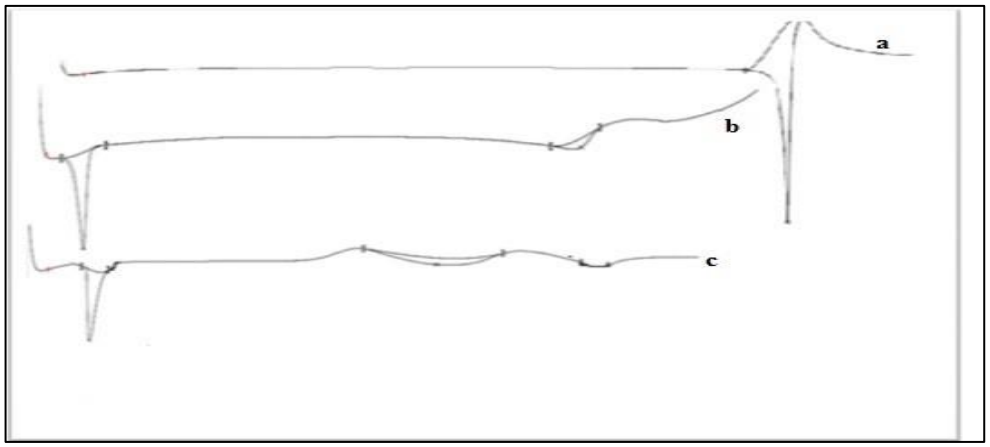

Fig.4: DSC thermographs for compatibility study: Meloxicam (a); Eudragit RS100 (b); Poloxamer 407 (c)

In the present work nanosuspensions of Meloxicam were formulated using different drug to polymer ratio and agitation speeds was maintained constant and prepared by quasi emulsion solvent diffusion technique. Overall, four formulation four with Eudragit RS100 (F1, F2, F3, F4) with a different combination of drug: polymer: stabilizer. All the formulation formulates with the Meloxicam: Eudragit RS100: 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 F2 prepared at 6000 rpm speed for 5 hours with ratio of drug to polymer EudragitRS100 (1:2)and the ratio of stabilizer 0.5 percentage had found nano size particle size prepared 80.00-100 nm with a zeta potential of -15 .The F2 formulation was found to be reduce particle size of 80-100 nm & consider to be optimize formulation with increase in the dissolution/saturation solubility of 23.42±0.61 (µg /mL) of poorly water soluble meloxicam belong to BCS Class II(reported solubility with 3.5±0.50 µg /mL) and in vitro drug release 96.44% at 10 hrs. being formulated in nano composite in the form of nanosuspension .

Particle Size Distribution

The particle size distribution of formulation F1, F2, F3, F4 these formulate with Eudragit RS100 with varying proportion of Meloxicam: Eudragit RS100: poloxamber407 was found to be in between 80.0-200 nm. And the optimize formulation F2 the particle size was 80.00 – 100 nm.

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

Drug	Particle size(nm)
F1	125-150
F2	80.00-100
F3	120-150
F4	150-200

Amount of Unincorporated Drug:

The amount unincorporated was found to 7.07 % with a optimize formulation F2 (Formulated with the Eudragit RS100).

Table 3: Percentage drug unincorporated and entrapped for nanosuspension

Drug Formulation	% Drug Unincorporated	% Drug Entrapped
F1	20.82	79.18
F2	7.07	92.93
F3	14.23	85.77
F4	19.60	80.40

Zeta Potential:

It was found that the formulations F2 prepared at 5500rpm speed for 5 hours with ratio of drug to polymer EudragitRS100 (1:2)and the ratio of stabilizer 0.5 percentage had found nano size particle size prepared 80.00-100 nm with a zeta potential of -15.

Table 4: Zeta potential of nanosuspension

Sr. no.	Formulation	Zeta Potential (mV)
1	F1	16
2	F2	-15
3	F3	11
4	F4	16

***In-vitro* drug Release:**

All the formulation in-vitro drug release study was determined among that F2 found to be optimize. And in vitro drug release shows at 10 hrs 96.44 % .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. By applying statistical model for dissolution Higuchi model, Korsemeyer pappas model for optimize F2 formulation it shows First order.

Table 5: Percentage cumulative drug release of nanosuspension

TIME	% Cumulative drug release			
	MRS-F1	MRS-F2	MRS-F3	MRS-F4
0	0	0	0	0
1	17.18	20.14	16.92	15.92
2	23.18	30.44	26.14	22.16
3	31.22	38.76	35.13	30.19
4	40.16	48.11	40.2	39.11
5	48.91	56.75	53.18	45.48
6	51.21	68.45	62.11	51.64
7	59.99	75.11	71.25	57.51
8	67.81	83.88	79.13	65.27
9	76.16	90.13	87.35	74.98
10	87.2	96.44	94	85.33

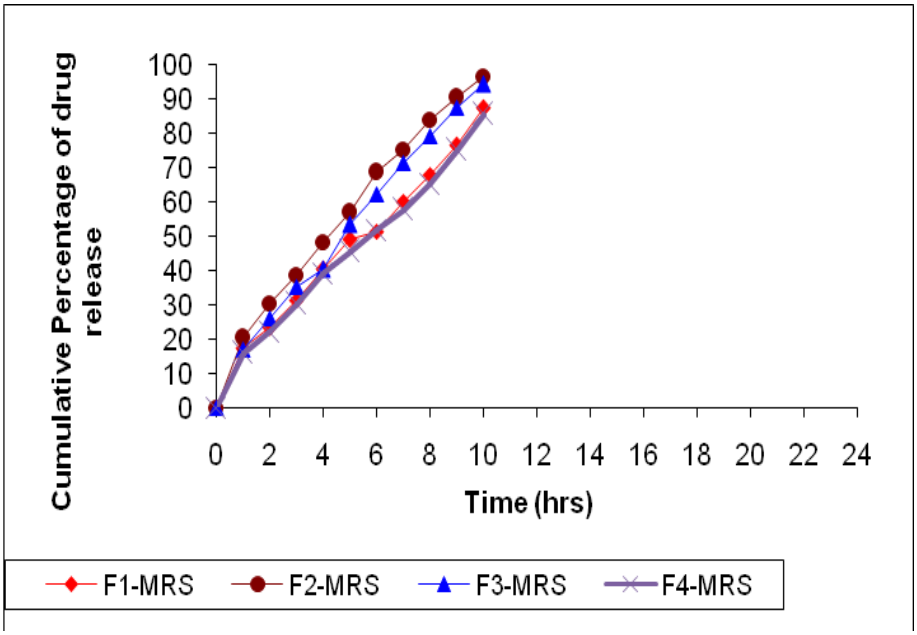

Fig 5: Graph for the Percentage cumulative drug release of Meloxicam nanosuspension with Eudragit RS100

Table 6: Data of Higuchi plot for F2 Formulation

Sr. no: F2	Time	Square root of time	cumulative %drug release
1	0	0.00	0
2	1	1.00	20.14
3	2	1.41	30.44
4	3	1.73	38.76
5	4	2.00	48.11
6	5	2.24	56.75
7	6	2.45	68.45
8	7	2.65	75.11
9	8	2.83	83.88
10	9	3.00	90.13
11	10	3.16	96.44

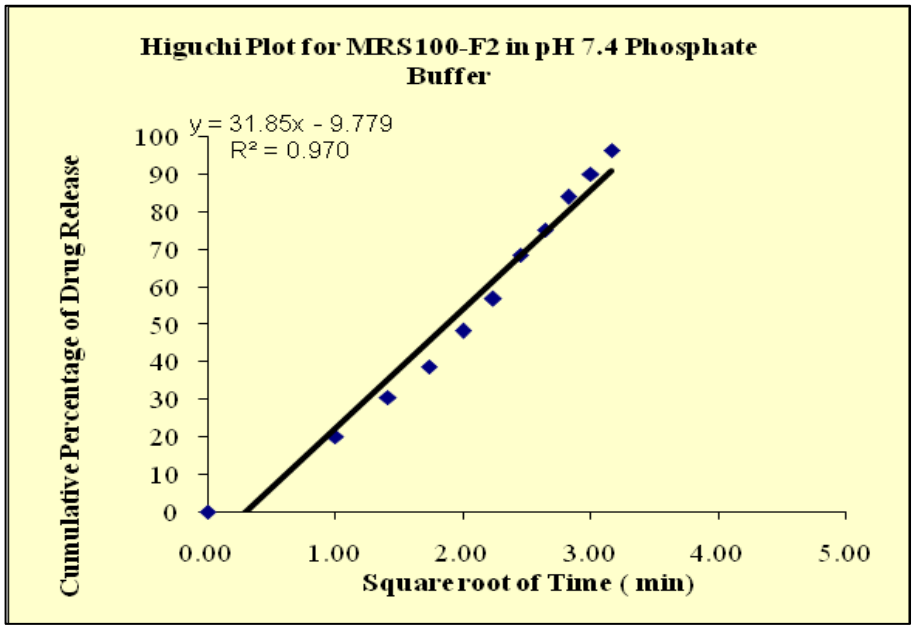

Fig 6: Higuchi plot for F2 Formulation

Table 7: Data of Korseymeres plot for F2 Formulation

Sr. No	Time	log time	log cumulative % drug release
1	0	0.00	0.00
2	1	0.00	1.30
3	2	0.30	1.47
4	3	0.48	1.58
5	4	0.60	1.68
6	5	0.70	1.75
7	6	0.78	1.83
8	7	0.85	1.87
9	8	0.90	1.92
10	9	0.95	1.95
11	10	1.00	1.98

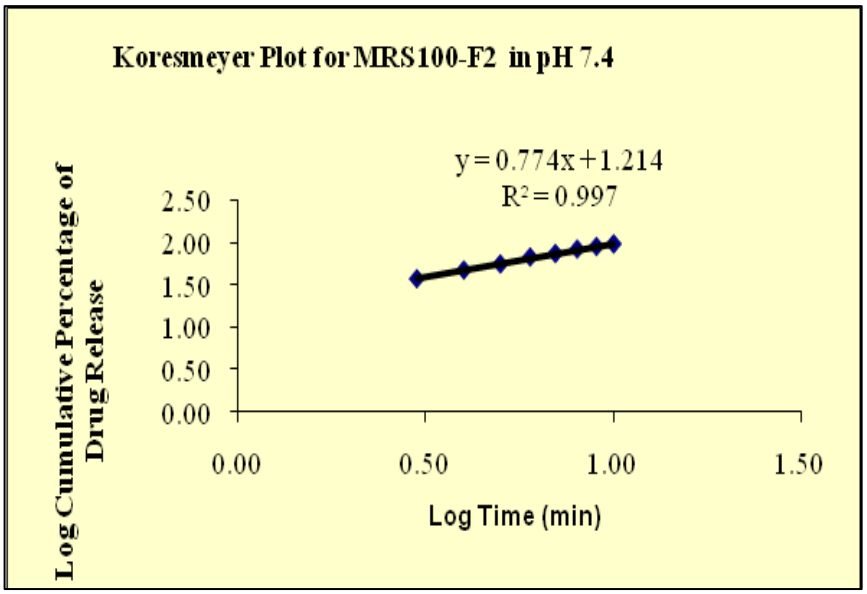

Fig 7: Korseymer peppas plot for F2 Formulation

Short term stability : Short term stability Studies was performed for three month for the optimize F2 formulation of the Meloxicam nanosuspension, result revealed that the formulation store at temperature 4 ºC shows no change in the invitro drug release of the formulation compare to the release study tested after the formulation 0 time that means the nanosuspension are stable at the given temperature .Moreover the physical appearance of the all the nanosuspension was found to be up to the mark that confirmed that the nanosuspension are stable and no growth or crystal formation observed. On the other hand, the nanosuspension store at a temperature 37ºC ± 2 ºC ,65 % RH ± 5 % RH comparatively there is change in the vitro drug release. So maximum stability obtained at the temperature 4°C

Table 8: Data of Invitro drug release of optimizing formulation F2 during the stability study at temperature 4 ºC and different interval

Time (Hrs)	F2-1 Month at 4 º C	F2-2 Month at 4 º C	F2-3 Month at 4 º C
0	0	0	0
1	19.23	17.85	16.01
2	29.17	25.47	20.14
3	37.54	34.87	29.06
4	47.21	39.04	35.95
5	55.67	52.65	44.32
6	68.13	61.89	54.06
7	74.08	70.54	64.84
8	82.43	78.42	74.52
9	89.11	86.82	85.76
10	96.26	95.18	96.78

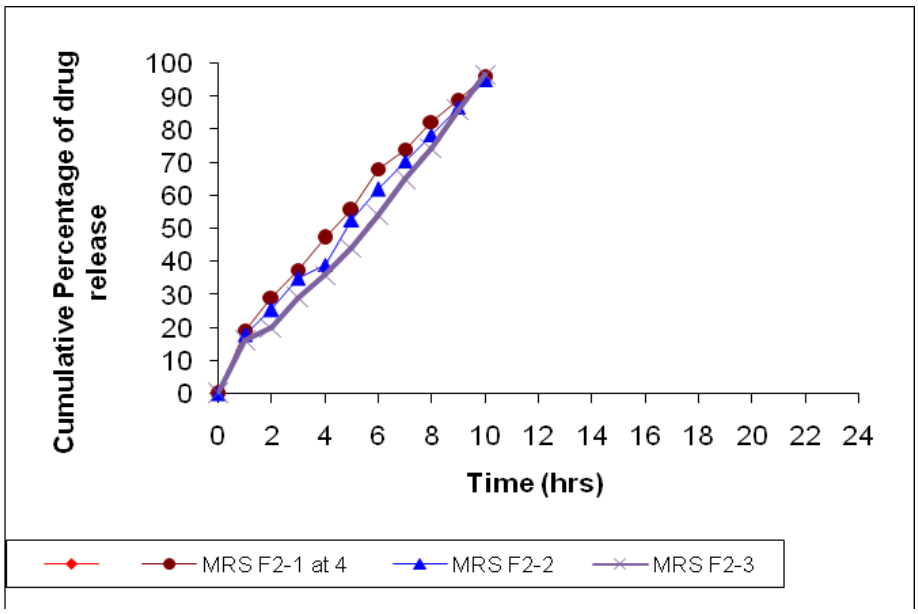

Fig 8*: In vitro* drug release of optimize formulation F2(Meloxicam) during the stability study at temperature maintained at 4 ºC for different interval (1,2,3 Month)

Table 9: Data of Invitro drug release of optimizing formulation F2 during the stability study at temperature 37 ºC and different interval

Time (Hrs)	MRS F2-1Month at 37 º C	MS F2-2 Month at 37 º C	MRS F2-3 Month at 37 º C
0	0	0	0
1	14.21	14.77	12.83
2	26.37	22.11	21.14
3	34.17	29.23	28.12
4	36.37	38.32	37.76
5	50.43	47.9	44.91
6	59.85	52.02	51.86
7	68.49	59.43	58.06
8	78.12	68.93	66.32
9	85.01	75.32	73.85
10	95.21	81.77	78.4

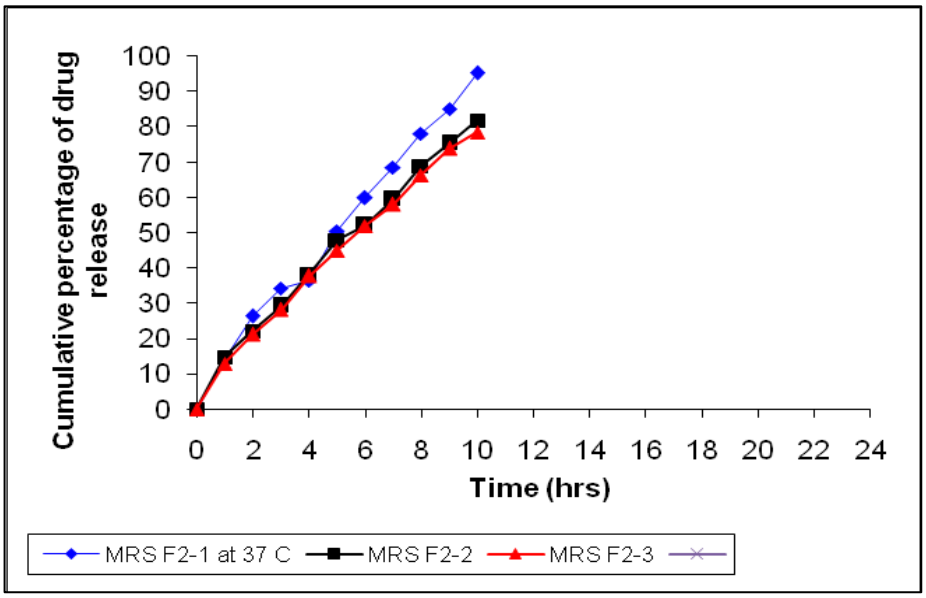

Fig 9: *In vitro* drug release of optimize formulation MRS-F2 (Meloxicam) during the stability study at temperature maintained at 37ºC for different interval

CONCLUSION

It is concluded that the polymer Eudragit RS100 with a ratio of drug 1: 2 with a help of stabilizer poloxamer is effective for formulating the stable nanosuspension for a sustained release of Meloxicam drug with increased in dissolution rate meloxicam nanosuspension can be prepared using Quassi emulsification solvent diffusion method using poloxamber 407 as a stabilizer. Poloxamber 407 is essential to achieve a particle size close 80-100 nm for optimize formulation were as other formulation the particle size was observed in between 80 to 200 nm. Invitro drug release shows at 10 hrs. 96.44 % for optimize formulation. Statistical dissolution model like Higuchi model, Korsemeyer pappas model was applied for optimize F2 formulation it proves first order release kinetic. Moreover, the short-term stability studies were performed on optimize F2 formulation Moreover the physical appearance of nanosuspension was up to the mark that confirmed that the nanosuspension are stable and no growth or crystal formation observed at 4 ^{o}C and nanosuspension store at a temperature 37^{o}C ± 2 ^{o}C ,65 % RH ± 5 % RH comparatively there is no change in the vitro drug release. The results show the suitability of method for the preparation of stable nanosuspension for water insoluble drugs. Polymer Eudragit RS100 can be used for the preparation of nanosuspension with a help of Poloxamer 407 as stabilizer.

REFERENCES:

1. Merisko-Liversidge, E.M., Liversidge, G.G., Drug nanoparticles: formulating poorly water-soluble compounds. Toxicol. Pathol. 2008; 36, 43–48
2. Nakach, M., Authelin, J.R., Chamayou, A., Dodds, J., Comparison of various milling technologies for grinding pharmaceutical powders. Inter. J. Mineral. Process. 2004; 74, S173–S181
3. Pouton, C.W., Lipid formulations for oral administration of drugs: nonemulsifying, self-emulsifying and ‘‘self-microemulsifying’’ drug delivery systems. Eur. J. Pharm. Sci. 2000; 11, 93–98
4. Kesisoglou, F., Panmai, S., Wu, Y., Application of nanoparticles in oral delivery of immediate release formulations. Curr. Nanosci. 2007; 3, 183–190
5. Carrier, R.L., Miller, L.A., Ahmed, I., 2007. The utility of cyclodextrins for enhancing oral bioavailability. J. Control. Rel. 123, 78–99
6. Muller, R.H., Benita, S., B ohm, H.L., Emulsions and Nanosuspensions for the formulation of poorly soluble drugs. Med Pharm GmbH Scientific Publishers, Germany, 1998; pp. 54–102
7. Muller, R.H., Jacobs, C., Kayser, O., Nanosuspensions as particulate drug formulations in therapy: rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 2001; 47, 3–19
8. Nakarani, M., Patel, P., Patel, J., Murthy, R.S.R., Vaghani, S.S., Cyclosporine a nanosuspension: formulation, characterization and in vivo comparison with a marketed formulation. Sci. Pharm. 2010; 78, 345–361
9. Leevers, S.J., Vanhaesebroeck, B., Waterfield, M.D., Signalling through phosphoinositide 3-kinases: the lipids take centre stage. Curr. Opin. Cell Biol. 1999; 11, 219–225
10. Sigfridsson, K., Lundqvist, A.-J., Strimfors, M., Particle size reduction for improvement of oral absorption of the poorly soluble drug UG558 in rats during early development. Drug Dev. Ind. Pharm. 2009; 35, 1479–1486
11. Stengera, F., Mendeb, S., Schwedesc, J., Peukertd, W., Media milling in stirred media mills. Chem. Eng. Sci. 2005; 60, 4557–4565
12. Bhargavi A, Technical Review of Nanosuspensions, International Journal of Pharmacy & Technology, 3(3), 2011, 1503-1511.
13. Dr Sunil J Aher, Poonam P Patil, Moshina Patwekar, Dr Faheem Patwekar .,European Journal of Molecular & Clinical Medicine., 2022,Volume09, Issue09.
14. M.S. Muthu & S. Singh., Current Drug Delivery., 2009, **6**, 62.
15. P. Rosario, R. Nadia, B. Claudio, M. Francesco, M. Adriana & P. Giovanni., AAPS Pharma sci tech., 2006, **7**, 27.
16. P. Dandagi., S Kerur., A Gadad ., Polymeric ocular nanosuspension for controlled release of acyclovir: in vitro release and ocular distribution, Iranian Journal of Pharmaceutical Reseach2009,volume 8,78-86.