CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.6599194

Available online at: http://www.iajps.com *Research Article*

TO DESIGN AND DEVELOP SOLID LIPID NANOPARTICLES BASED NANOGEL FOR DERMAL DELIVERY OF MELOXICAM

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Article Received: April 2022 **Accepted:** April 2022 **Published:** May 2022

Abstract:

Topical drug delivery can be defined as application of medication containing formulation to the skin to directly treat the cutaneous or subcutaneous disorders and diseases like acne or fungal infections by providing the drug to the surface of the skin or within the skin. In spite of many advantages of transdermal and dermal drug delivery over other drug delivery system, relatively few topical drug formulations are commercially available in market. The main challenging step in the topical delivery is the crossing of most impermeable epithelia of human body that is stratum corneum. Stratum corneum becomes a barrier for the exogenetic substances. Hence this fact is to be considered at the time of formulating a new formulation for the topical administration of drug so that maximum penetration of the drug into the skin without irreversible disturbing the skin barrier function can be achieved.

KEYWORDS- Nanoparticles, Nanogel, Meloxicam

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Please cite this article in press Shivani Sandip Patil et al, ***To Design And Develop Solid Lipid Nanoparticles Based Nanogel For Dermal Delivery Of Meloxicam.,*** *Indo Am. J. P. Sci, 2022; 09(5).*

1. INTRODUCTION:

1.1 Anatomy and Physiology of Skin and barrier properties

Skin is one in every of the biggest organ, separates the foremost stable internal environment from the foremost unstable external environment. Skin composes of epidermis, dermis and subcutis, each plays a fundamental role of maintaining analytical balance and protection of skin from microorganisms, dust and varied weather conditions. Refer Figure 1 for illustration of Skin.

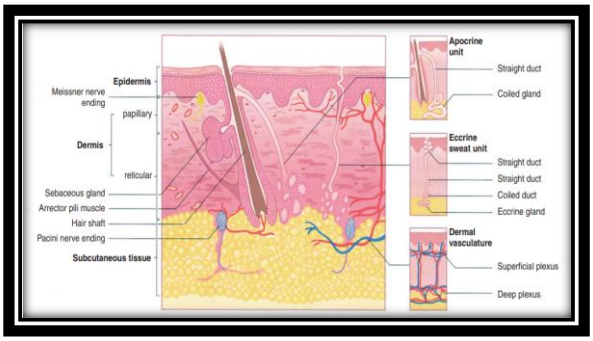


Figure 1: Cross-Section of Human Skin

It performs many vital functions, including protection against external physical, chemical, and biologic assailants, likewise as prevention of excess water loss from the body and a task in thermoregulation. The skin is continuous, through the mucous membranes lining the body's surface. The system is made by the skin and its derivative structures. The skin consists of three layers: the epidermis, the dermis, and subcutaneous tissue. The outmost level, the epidermis, contains of a selected constellation of cells referred to as keratinocytes, which function to synthesize keratin, a long, threadlike protein with a defending role. The center layer, the dermis, is fundamentally made from the fibrillar structural protein called collagen. The dermis lies on the subcutaneous tissue, or panniculus, which contains small lobes of fat cells called lipocytes. The thickness of those films varies considerably, observing on the geographic location on the anatomy of the body. The eyelid, for instance, has the thinnest layer of the epidermis, measuring but 0.1 mm, whereas the palms and soles of the feet have the thickest epidermal layer, measuring approximately 1.5 mm. The dermis is thickest on the rear, where it's 30–40 times as thick because the overlying epidermis Epidermis forms the outermost layer of skin. The cells of epidermis travel upward and become dead flat cell called horny layer. stratum composed of corrneocytes and intercellular lipids which forms the compact impermeable layer. Dermis forms the elastic layer below the epidermis. Subcutaneous layer encompasses sheet of fat rich connective tissue attaching the dermis to the underlying structure of skin.

2. NEED

Nanogels composed of nanosize particles formed by physically or chemically cross-linked polymer networks that swells in a good solvent. The nanogel systems have proven their potential to carry drugs in controlled, continuous and targetable mode. With the promising field of polymer sciences, it has now become predestinated to make smart nano-system which can found effectual for treatment, detecting as well as clinical trials progress.

Nanogels is been proving as a promising drug delivery system and offers variety of characteristics like on site drug delivery system, sustained release formulation, high drug entrapment properties, water solubility, biodegradability, low toxicity etc. Due to these multi functionality properties and features nanogel utilized extensively in many drug deliver fields. Composite with polymers, metals and other active molecules nanogel turned out as excellent drug delivery system.

Topical administration of the MLX drug is apparently an attractive choice since it would reduce the chances of drug associated gastrointestinal and systemic side effects, and would allow an increased level of drug locally. Although, topical application of MLX offers the advantage of delivering a drug directly to the disease site in order to maximize local effects without concurrent systemic activity yet, no formulation of MLX is available in the market for topical use. The most difficult aspect of the topical drug delivery system is the formidable barrier properties of the stratum corneum (SC), the outermost layer of the skin that prevents percutaneous absorption of drugs.

3. AIM

To Design and Develop Solid Lipid Nanoparticles Based Nanogel for Dermal Delivery of Meloxicam

4. OBJECTIVES

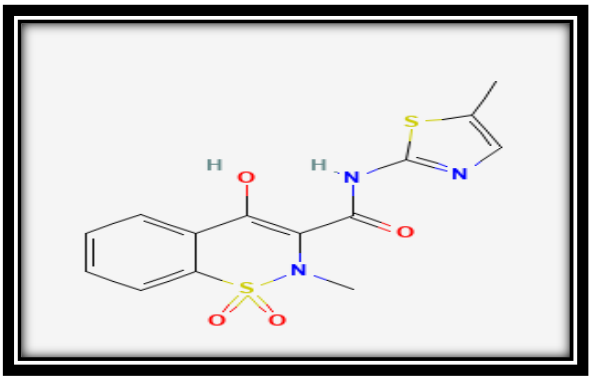
1. To select the suitable excipients for formulation of Solid Lipid Nanoparticles based Nanogel through preliminary trials to achieve desired Entrapment Efficiency, Ex-Vivo Permeation Studies and In-Vitro drug release.
2. To undertake drug excipient compatibility study using DSC and FTIR
3. Using GELRITE as polymer in varying concentrations for Solid Lipid Nanoparticles based Nanogel formulation
4. Formulation of Nanogel of MLX

5. To evaluate the prepared Nanogel for Mean Particle size, Zeta potential, Production yield, Entrapment Efficiency and Drug loading, Surface morphology, XRD, DSC, Swelling Studies, and In-Vitro Drug release
6. Designing, development, Optimize and evaluation of Solid Lipid Nanoparticles based Nanogel containing MLX using Design Expert 12.0 Software
7. To study the effect of formulation variables selected on Entrapment Efficiency, Ex-Vivo

5. DRUG PROFILES

5.1 Meloxicam

Table 1: MLX Drug Profile

Name	Meloxicam
Chemical Structure	 **Figure 2:** MLX Chemical Structure
IUPAC Name	4-hydroxy-2-methyl-N-(5-methyl-1,3-thiazol-2-yl)-1,1-dioxo-1λ6,2-benzothiazine-3-carboxamide
CAS No.	71125-38-7
Molecular Weight	351.4 g/mol
Molecular Formula	C14H13N3O4S2
Melting point (°C)	254 °C

6. MATERIALS AND METHODS:

6.1 Materials

Table 2: Materials used

S. No	Name
1	Meloxicam
2	Polysorbate 20
3	Polysorbate 80
4	GELRITE (Gellan Gum)
5	Methanol
6	DMSO
7	Triethanolamine
8	Propylene Glycol
9	Ethanol
10	Sodium Hydroxide
11	Glycerin
12	Sodium Chloride
13	Potassium phosphate monobasic
14	Sodium phosphate dibasic

6.2 Preparation of Stock & Buffer Solutions

1. Hydrochloric acid buffer pH1.2: 50ml of 0.2M potassium chloride and 85ml of 0.2 M HCl were taken in a 200 ml volumetric flask and made up to the volume with water.
2. Phosphate buffer pH 6.8: Dissolve 60.5 g of disodium hydrogen phosphate and 46 g of potassium dihydrogen phosphate in water add 100 ml of 0.02 M disodium edetate and 20 mg of mercuric chloride and dilute with water to produce 1000ml.
3. Phosphate buffer pH 7.4: 50 ml of 0.2 M potassium dihydrogen phosphate and 39.1 ml of 0.2 M NaOH were taken in a 200 ml volumetric flask and made up to the volume with water.
4. Sodium hydroxide solution (0.2 M): Accurately weighed 8.0 gm of sodium hydroxide was dissolved in 1000 ml of distilled water.
5. Potassium dihydrogen phosphate (0.2 M): Accurately weighed 27.218 gm of potassium dihydrogen orthophosphate was dissolved in 1000 ml of distilled water.
6. Potassium chloride (0.2 M): Accurately weighed 14.91 gm of potassium chloride was dissolved in 1000 ml of distilled water.

6.3 Solid State Characterization of Drug

6.3.1 Fourier Transfer Infrared Spectroscopy

Drug was mixed with Potassium Bromide in a ratio of 9:1 which was triturated and blended evenly. The mixture was further compressed into pellets on a motorized pellet press at pressure of 15 ton. The prepared pellets were then scanned over range of 4000 – 400 cm^{-1} to get the IR spectra. Functional group determines was studied visually by interpreting the peaks observed.

6.3.2 Differential Scanning Calorimetry

Drug was hermitically sealed in perforated aluminum pan using crimper and heated at constant rate of 10°C/min over the temperature ranges of 30-300°C at 20mL/min nitrogen purging on a Mettler Toledo DSC apparatus, Switzerland

6.3.3 Melting Point Determination

Capillary Method was employed for Melting Point Determination. Drug was filled in a one end sealed capillary tube and was placed in a Liquid Paraffin bath in a Thiele's Tube. Upon visual inspection, temperature on which the solid starts turning into a liquid was noted down.

6.3.4 Solubility Analysis

The preparation of any dosage form, it required to know the solubility of drug. The solid dosage form need particular solvent to dissolve, and produces pharmacological effect to body. Additionally, the bioavailability of drug present in solid dosage form depends upon solubility of drug. If drug is sparingly soluble in solvent, then it produces minimum therapeutic response due to less availability of drug to receptors. Hence solubility of drug play important role in therapeutic effects of drug.

Solubility of drug was studied in Methanol, Ethanol, DMSO, 0.1N HCl, pH 1.2 HCl buffer, pH 6.8 phosphate buffer to study the behavior of the drugs.

Saturated solutions of both drugs with each solvent were made in 10ml glass vials and were set aside in an Orbital Shaking Incubator (Remi Instruments, Mumbai, India) at 32ºC for 24 hours at 50rpm. The solutions were filtered using a 0.45 µm filter and diluted as required for further analysis. The solutions were analyzed by UV-2450 UV-Vis Spectrophotometer (Shimadzu, Japan) at wavelength of λmax against blank (blank contained same solvent in which drug was suspended).

6.4 Drug-Excipients Incompatibility Studies

6.4.1 Fourier Transfer Infrared Spectroscopy

The Fourier Transform – Infrared (FT-IR) spectroscopy has numerous applications in pharmaceutical field. It is widely used in determination of identification of known and unknown compound. Apart from this it can also be used in evaluating the drug interaction. During formulation the active ingredient are used mixed with various excipients to give proper shape and appearance. Sometimes it happens after mixing the active ingredients with excipient, it produces incompatibility due to drug excipient interaction. The incompatibility of drug can alter the potency of formulation. It can also produce adverse effects to the body. Hence for pharmaceutical industries it is prime work to check the drug and excipient incompatibility.
Drug was mixed with all excipients in equal proportion forming a physical mixture were all compressed as a KBr pellet respectively for each sample at a ratio of 9:1. The prepared pellets were then scanned over range of 4000 – 400 cm^{-1} to get the IR spectra. Functional group determination was studied visually by interpreting the peaks observed and any changes in parent peaks were observed.

6.4.2 Differential Scanning Calorimetry

Physical Mixture of drug and excipients was prepared for both drugs and sealed in a pre-washed ampoule. It was set aside in a Programmable Environmental Test Chamber, Remi Instruments Ltd. Mumbai for 28 days. Following that the sample was hermitically sealed in perforated aluminum pan and heated at

constant rate of 10°C/min over the temperature ranges of 30-300°C at 20mL/min nitrogen purging on a Mettler Toledo DSC apparatus, Switzerland.

6.5 Analytical Method Development

6.5.1 Determination of λ_{max} for MLX

10 mg drug was suspended in 100 ml methanol to prepare a stock solution and 10ppm sample was taken out and studied for its UV Spectra photometrically on a UV- 2450 UV-Vis Spectrophotometer

6.5.2 Preparation of Stock Solution:

Accurately weighed 10 mg of MLX was transferred to a 100 ml volumetric flask, dissolved in 10 ml Methanol by shaking manually for 10 min. The volume was adjusted with the same up to the mark to give the final strength, i.e.100 µg/ml.

6.5.3 Preparation of Calibration Curve of APZ

Different aliquots of MLX in the range 0.2-1 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with distilled water to get concentrations 2, 4, 6, 8 and 10 µg/ml, respectively. The solutions remained scanned on a spectrophotometer in the UV range 200–400 nm. The absorbance was noted at 354 nm.

6.6 Formulation Of Mlx Solid Lipid Nanoparticles

SLN were prepared by film hydration technique. The mixture of vesicle-forming ingredients namely lecithin and cholesterol was dissolved in a volatile organic solvent (dichloromethane and methanol) in a round-bottom flask. The rotating evaporator was rotated at 60°C for 45 min. Then the organic solvent was removed with gentle agitation and the organic solvent evaporated at 60°C, leaving a thin film of lipid on the wall of the rotary flash evaporator. The aqueous phase containing Meloxicam drug was added slowly with intermittent shaking of the flask at room temperature and sonicated for 30 min. The obtained nanolipid solution remained cool by insertion in the freezer. The composition of the nanolipid is presented in Table 3.

Table 3: Formulation design for MLX SLN

Ingredients (%)	F1	F2	F3	F4	F5
MLX	5	5	5	5	5
Lecithin	5	2.5	7.5	4	6
Cholesterol	5	7.5	2.5	6	4
Dichlormethane: Methanol (1:1)	25	25	25	25	25
Water	60	60	60	60	60

Formulation of Nanogel was equipped on the basis of drug entrapment efficacy of prepared SLNs. The batch of SLN that gave maximum entrapment was selected for preparation of Nanogel

6.7 Formulation of MlX Nanogel

After conducting trial batches, the excipients and their concentration ranges were selected and thus formulation chart was designed by using Design Expert 12.0 software Design Expert 12.0 software was used to create formulation design for the purpose of optimization of SLN for MLX. Design of experiments is a method by which purposeful changes to input factors of process in order to observe the effects on the output can be made. DOE's can and have been performed in virtually every industry on the planet— agriculture, chemical, pharmaceutical, electronics, automotive, hard goods manufacturing, etc. Service industries have also benefited by obtaining data from their process and analyzing it appropriately. Traditionally, experimentation has been done in a haphazard one-factor-at-a time (OFAT) manner. This technique is inefficient and very frequently yields misleading results. On the other hand, factorial plans are a very simple type of DOE, need only a minimal numeral of runs, yet they allow you to identify interactions in the process. This information leads to breakthroughs in process understanding, thus improving quality, reducing costs and increasing profits.

Various factors were studied under hit and trial method and out of them, Two independent factors suited the experiment's need viz. The concentration of GELRITE (%) and Ratio of Tween 20:80 (%). Dependent factors were Entrapment Efficiency (%) and In-Vitro drug release (%) as these three parameters address the essence of oral films. Central composite randomized design was selected as it was best suited for the experiment to screen via Response Surface Methodology in the following study.

Table 4: Independent Factors for Formulation Design

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	GELRITE	%	Numeric	7.93	22.07	-1 ↔ 10.00	+1 ↔ 20.00	15.00	4.08
B	20:80	%	Numeric	7.93	22.07	-1 ↔ 10.00	+1 ↔ 20.00	15.00	4.08

Responses were selected as follows

Table 5: Responses for Formulation Design

Response	Name	Units
R1	Entrapment Efficiency	%
R2	In-Vitro drug release	%

A total of 13 runs were obtained out of which 5 were replicates. Hence a total of 9 definite experiments were obtained to be conducted practically

Table 6: Formulation Table for MLX Nanogel composed by using Central Composite Design

Formulation Code	MLX (%)	GELRITE (%)	Tween 20:80 (%)	Diluent to make 100%
F1	5	10	10	Q.S
F2	5	20	10	Q.S
F3	5	10	20	Q.S
F4	5	20	20	Q.S
F5	5	7.92893	15	Q.S
F6	5	22.0711	15	Q.S
F7	5	15	7.92893	Q.S
F8	5	15	22.0711	Q.S
F9	5	15	15	Q.S

The Meloxicam nanogel was synthesized with the aid of Gelrite as a polymer. Accurately weighed 5 mg of MLX SLNs and variable concentration of GELRITE was dissolved in 1% v/v methanol followed by the drop wise addition of Tween 20:80 (1:1) at the rate of 2 ml/min with constant stirring for 3 h by using magnetic stirrer at 1000 rpm. pH was adjusted by gel Triethanalamine(0.05%). The mixture was allowed to achieve room temperature which resulted in gel formation.

6.7.1 Isolation of Nanogel

Centrifugation of Nanogel dispersion was done for the sepration of nanoparticles by using Optima "MAX-XP" ultracentrifuge at 45,000 rpm for 35 minutes. Deposited particulate was redispersed in minimum quantity of water with appropriate concentration of mannitol.

6.7.2 Stabilization of Nanogel by Lyophilisation

The nanogel was successfully freeze dried using the bench top freeze dryer. Lyophilisation was carried out for optimised batch at -75°C with 5% mannitol as a cryoprotectant. The role of cryoprotectant is to prevent nanoparticle aggregation and facilitate solvent removal by sublimation during the process of freeze-drying. The obtained lyophilised powder was found to be dry, porous and friable after 72h. The vacuum was maintained at 76 mTorr.

6.8 Characterization of Mlx SLN and Nanogel

6.8.1 Mean Particle size

The MPS were determined by PCS with a Malvern Zetasizer (Nano ZS 90, Malvern ltd., UK). The measurement using PCS is predicated on the sunshine scattering phenomena during which the statistical intensity fluctuations of the scattered light from the particles within the measuring cell are measured. Before the measurements, all samples were diluted with double H2O to supply an appropriate scattering intensity.

6.8.2 Zeta potential

The zeta potential (ZP), reflecting the electric charge on the particle surface and indicating the physical stability of colloidal systems, was measured by determining the electrophoretic mobility using the Malvern Zetasizer (Nano ZS 90, Malvern ltd., UK).

The measurements were performed with diluting in double-distilled water. It was measured using Dip cell with applying field strength 20 V/cm and the average of the zeta potential was given from 30 runs.

6.8.3 Production yield:
The production yield of nanogel formulation were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of nanogel.
Production Yield = Practical/ Theoretical Yield * 100

6.8.4 Entrapment Efficiency and Drug loading
Percent Entrapment efficiency (EE) is defined as the percentage of drug incorporated into the polymeric nanogel relative to the total drug added. It specifies how much percent of drug is included in the particles and how much percent of free drug are still present in the dispersion medium. For this both, MLX SLNs and NG were centrifuge at 45,000 rpm for 35 min ; 1.0 mL of the supernatant collected after centrifugation was diluted with 3.0 mL of DMSO and methanol and then make up volume up to 10 ml in 10ml volumetric flask and measured spectrophotometrically at 354 nm using UV-Visible spectrophotometer (UV 1700,Shimadzu, Japan). The entrapment efficiency and standard deviation was calculated.

Drug loading (DL) refers to the percentage of drug incorporated into the polymeric nanogel relative to the total weight of the nanogel (i.e. polymer + drug). For this, CRM from Lyophilized flakes was extracted by triturating 10mg powder with DMSO and methanol in mortar pestle and diluted up to 10 ml in volumetric flask . MLX content in methanolic extract was analyzed spectrophotometrically (UV 1700, Shimadzu, Japan) at 354 nm, against the standard methanolic solution of MLX. (Paradkar et al 2007, Patil et al 2005).

The %EE and% DL were calculated using the following Eq. (2) and (3), respectively.

Entrapment efficiency (%)= (Total amount of drug-un-entrapped drug)/(Total amount of drug) x 100
Drug loading (%)= (Actual amount drug in nanogel)/(Total amount of drug) x 100

6.8.5 Surface morphology:
The morphology of nanogel were examined by scanning electron® microscopy (JSM 6390, Japan). Samples of nanogel were dusted onto double-sided tape on an aluminum stub and coated with gold using a cold sputter coater to a thickness of 400°A, and then imaged using a 20 kv electron beam.

6.8.6 X-ray Diffraction Studies
X-ray diffraction patterns optimized MLX NG formulation was obtained using X-ray diffractometer (BruckerAxs, D8 Advance; Germany) in which Cu-Kα line used as a source of radiation by operating at the voltage 40 kV and the current applied was 30 mA.

6.8.7 Differential Scanning Calorimetry
Thermal analysis was performed using a differential scanning calorimetry (DSC) (Mettler-Toledo, Zurich, Switzerland) for optimized formulation. The samples, weighing 2 mg, were analyzed in sealed and pin-holed standard 40 µl aluminium pan, with a heating rate of 10°C/min from 30°C to 300°C and during the measurement the sample cell were continuously purged with nitrogen at a flow rate of 40 ml/min.

6.8.8 Swelling Studies:
The degree of swelling was calculated by finding out weight of swollen nanogels . The swelling behavior of the nanogels was studied at two different pH 6.8. The swelling ratio was calculated using the following formula after determining the dry as well as wet weight of the lyophilized, nanogel after sufficient exposure to the corresponding pH solution.
Swelling ratio= Final- Initial Weight * 100

6.8.9 *In vitro* Drug Release
In vitro diffusion study of Nanogels was carried out by Franz diffusion cell having 2.0 cm diameter and 25 ml capacity. Dialysis membrane(Himedia) having molecular weight cut off range 12000 – 14000 kDa was used as Diffusion membrane. Pieces of dialysis membrane were soaked in Phosphate buffer solution (PBS) pH 6.8 with 1% nanogel for 24 h prior to experiment. Diffusion cell was filled with PBS pH 6.8 and dialysis membrane was mounted on cell. The temperature was maintained at 37oC. After a pre-incubation time of 20 minutes, the lyophilized powder equivalent to 50mg of MLX was dispersed in 3ml of PBS and was placed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for 11hours and replaced with the same amount of fresh PBS, and assayed by a UV spectrophotometer at 354 nm. In this type of nanogel drug release through stimuli responsive If alteration in pH then drug release start.

6.8.10 Accelerated stability study
The stability study was done for the optimized film formulation of MLX NG. The prepared NG was kept in glass vial, then placed in desiccators for period of 90 days at room temperature, ambient humidity and

then evaluated for analyzing MPS, ZP and % EE with a time interval of 30 days.

7. RESULTS:

7.1 Solid State Characterization of Drug

7.1.1 Fourier Transfer Infrared Spectroscopy

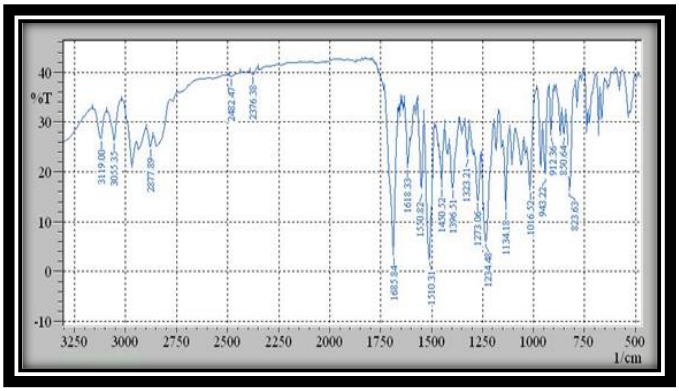


Figure 3: FTIR spectra of MLX

Table 7: Principal peak and chemical group present in IR spectra of MLX

Observed peaks	Reported peaks	Interpretation of chemical groups
3510.56	3500-3450	C=O stretch carbonyl
3014.84	3100-3000	=C-H stretching alkene
2943.47	3000-2850	C-H stretching alkane
1602.90	1650-1580	C=O, carbonyl group
1585.54	1685-1550	C=C stretch aromatics
1429.30	1450-1400	C-H bend alkenes
1381.08	1320-1000	C-O stretch alcohols, esters, carboxylic acid

7.1.2 Differential Scanning Calorimetry

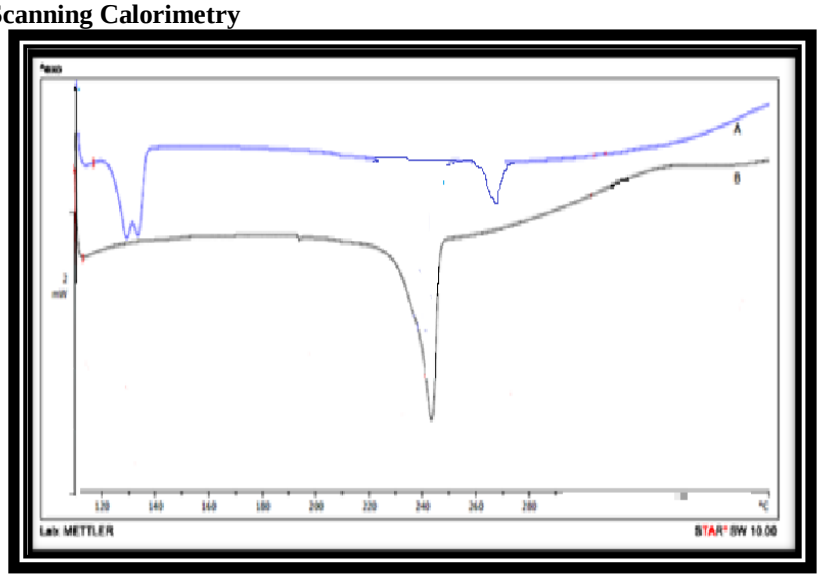


Figure 4: Overlay of DSC Thermogram of MLX, Physical mixture

7.1.3 Melting Point Determination

Melting point of MLX was found by glass capillary method to be 245- 256 ^{0}C. The observed melting point of MLX was confirmed with the standard melting point of MLX.

7.1.4 Solubility Analysis

Table 8: Solubility of MLX in different solvent system

S/N	Solvent	Observed solubility mg/ml
1	Methanol	16±0.000
2	DMSO	15±0.180
3	Methanol :DMSO (98:2)	16±0.90
4	Methanol	29±0.90
5	Phosphate buffer 6.8	9.88±0.05

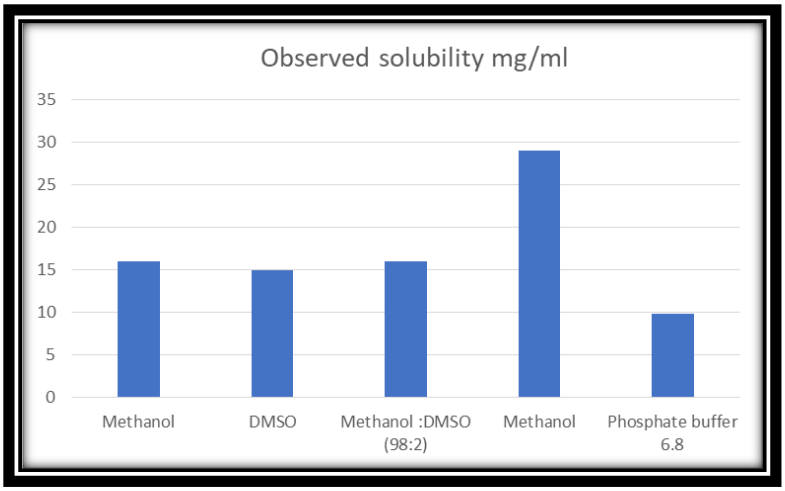


Figure 5: Solubility of Mlx In Different Solvent Systems

7.2 Drug-Excipients Incompatibility Studies

7.2.1 Fourier Transfer Infrared Spectroscopy

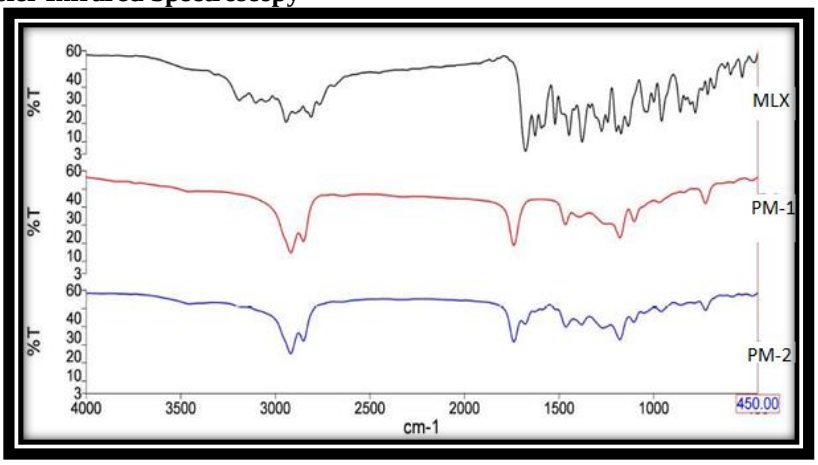


Figure 6: FTIR Spectra of MLX and their Physical Mixture

7.3 Analytical Method Development

7.3.1 Determination of λ_{max} for MLX

The solution of MLX in methanol was found to exhibit maximum absorption at 354 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature.

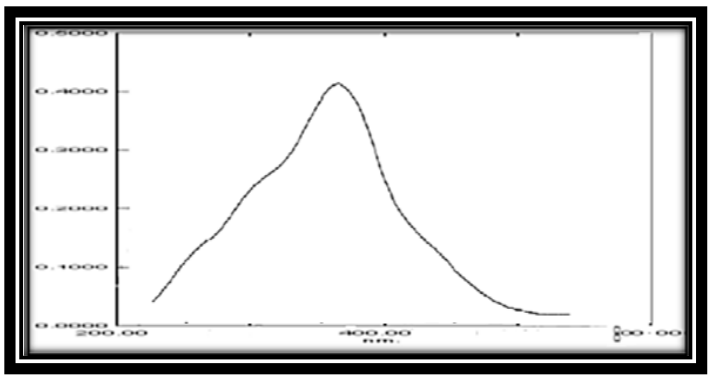


Figure 7: UV spectrum of MLX in Methanol

7.3.2 Preparation of Calibration Curve of MLX

Table 9: Standard calibration curve of MLX in Methanol

Sr. No.	Concentration (µg/ml)	Absorbance
1.	2	0.136
2.	4	0.316
3.	6	0.481
4.	8	0.631
5.	10	0.795
6.	12	0.939

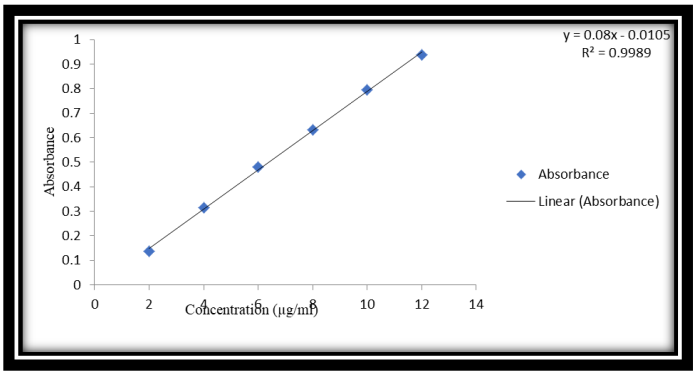


Figure 8: Standard calibration curve of MLX in Methanol

7.4 Characterization Of MlX SLN And Nanogel

7.4.1 Selection of suitable MLX SLN formula for further preparation of Nanogel

Formula F1 was selected as the most optimized formula for preparation of Nanogel. This decision was based upon the Entrapment Efficiency results (99.98 %) for F1 SLNs.

7.4.2 Mean Particle size

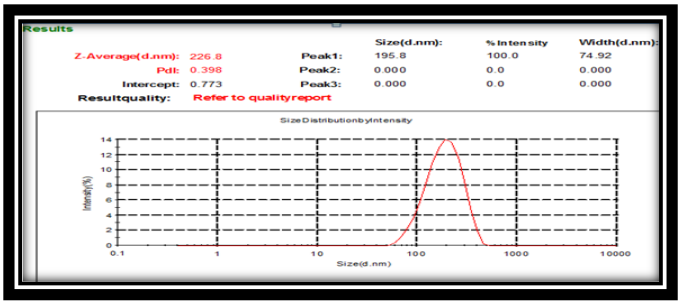


Figure 9: Particle size analysis of Optimized Formulation of MLX NG

7.4.3 Zeta potential

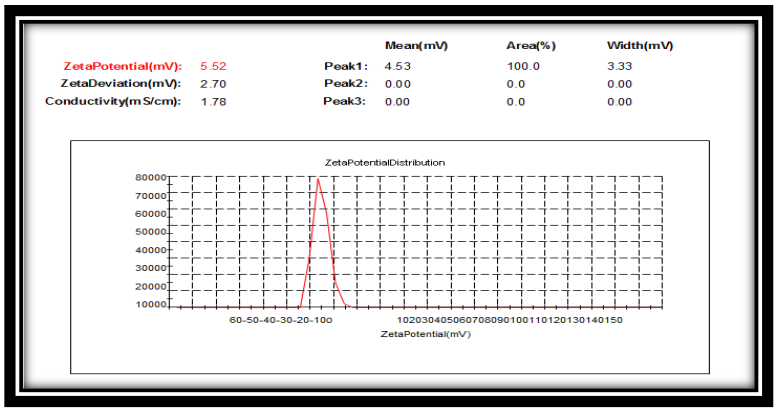


Figure 10: Zeta potential distribution of Optimized Formulation of MLX NG

7.4.4 Production yield, Entrapment Efficiency and Drug loading

Table 10: Results

Formulation Code	Production yield (%)	Entrapment Efficiency (%)	Drug loading (%)
F1	77.2	98.7	7.3
F2	69.8	99.8	6.5
F3	74.6	89.0	7.7
F4	79.0	95.4	5.8
F5	72.1	99.9	7.4
F6	74.2	100.8	8.2
F7	78.0	94.3	9.5
F8	73.2	89.1	8.3
F9	70.0	91.1	7.1

7.4.5 Surface morphology:

Morphological study of optimized NG was done by taking SEM pictures of prepared MLX NG. It was revealed that they were irregular in shape and porous in nature with smooth surface.

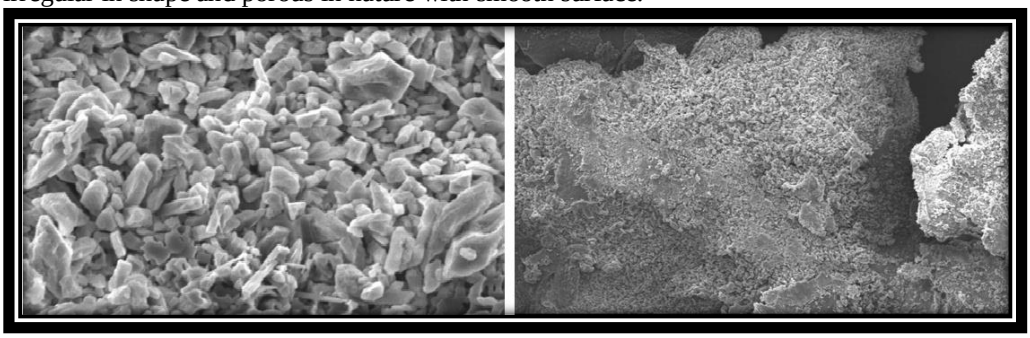


Figure 11: SEM Images of MLX -loaded Optimized Formulation of NG

7.4.6 X-ray Diffraction Studies

XRD for optimized MLX NG shows amorphous property thus also confirming resemblance with SEM images and proving to be smooth on surface

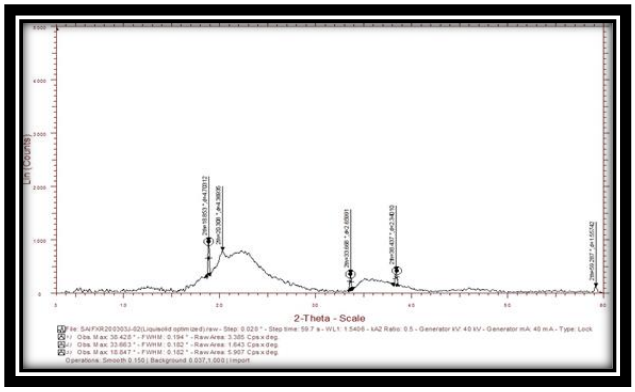


Figure 12: XRD of MLX -loaded Optimized Formulation of NG

7.4.7 Differential Scanning Calorimetry

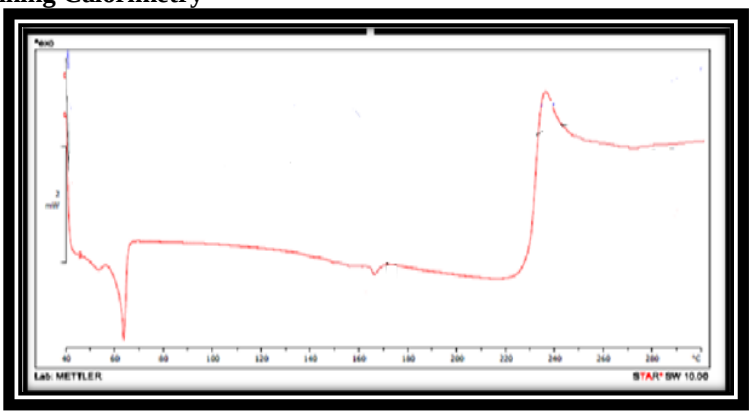


Figure 13: DSC thermogram of Optimized formulation of MLX –NG

7.4.8 Swelling Studies:

The swelling ratio was found to be 0.5 at pH 5.4 and 0.2 at pH 7 for optimized formulation

7.4.9 In vitro Drug Release

Table 11: - In-Vitro drug release studies for MLX NG formulations

Formulation Code	Drug release at 30 minutes (%)
F1	93.2
F2	96.4
F3	94.5
F4	97.8
F5	98.1
F6	99.5
F7	100.8
F8	94.3
F9	92.9

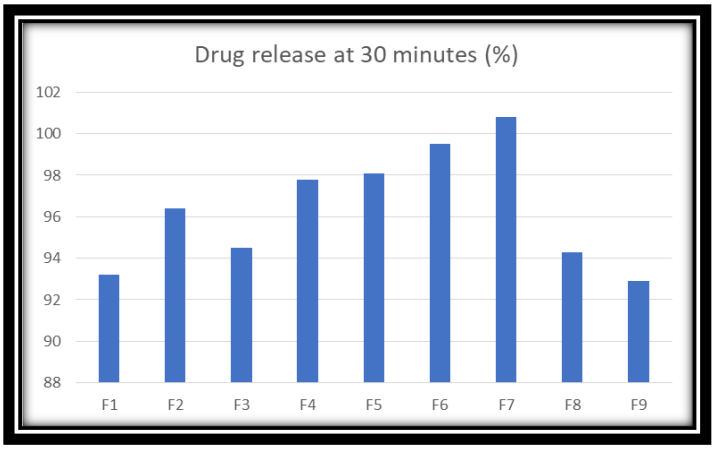


Figure 14: - In-Vitro drug release studies for MLX NG formulations

Formulation F7 showed maximum drug release at 100.8 % in 30 minutes. Hence, F7 was considered as optimized formulation.

Table 12: Accelerated stability study

Stability parameter	Test period			
	0 Days	**30 Days**	**60 Days**	**90 Days**
MPS (nm)	226.2 ± 0.027	227.2 ± 1.80	229.9 ± 0.03	230.1 ±0.013
PDI	0.3 ± 0.19	0.3 ± 0.53	0.3 ± 0.57	0.3 ± 0.96
% EE	96.66 ± 1.18	94.02 ± 0.02	90.98 ± 1.05	87.01 ± 1.35

8. CONCLUSION:

Hence, this study aims to enhance the permeation and administration of MLX by formulation of Meloxicam into a in-situ Nanogel by using GELRITE (Gellan Gum) as a polymer.

Suitable excipients were selected for formulation of Solid Lipid Nanoparticles based Nanogel through preliminary trials to achieve desired Entrapment Efficiency, Ex-Vivo Permeation Studies and In-Vitro drug release. Drug excipient compatibility study using DSC and FTIR was performed Using GELRITE as polymer in varying concentrations for Solid Lipid Nanoparticles based Nanogel formulation followed by Formulation of Nanogel of MLX. The prepared Nanogel was evaluated for Mean Particle size, Zeta potential, Production yield, Entrapment Efficiency and Drug loading, Surface morphology, XRD, DSC, Swelling Studies, and In-Vitro Drug release. At last, conducted the ageing studies of the optimized formulation.

Fourier transformed infrared spectra of MLX was taken by using the KBr disk method. The scanning range was 450 to 4000 cm^{-1} and the resolution was 1cm-1. The obtained IR spectra of drug sample given in Fig 13. Observed peaks of the drug are shown in Table 13 which are similar to the standard IR spectra of drug reported in the literature. To verify the existence in the physical interaction between drug and excipients, sample was analyzed by differential scanning calorimetry (DSC). The DSC results presented in (Fig.14) demonstrated an endothermic peak for MLX at 250 ^{0}C corresponding to the melting point. The physical mixture Thermogram was nearly identical to that of pure MLX and showed an endothermic peak at 270^{0}C. Melting point of MLX was found by glass capillary method to be 245- 256 ^{0}C. The observed melting point of MLX was confirmed with the standard melting point of MLX. The solubility of MLX was assessed in different solvent system viz., Methanol and DMSO mixture and Phosphate buffer saline pH 6.8 at 37 ± 0.5°C. FTIR study of MLX, its physical mixture in ratios of 1:9 and 1:1 shows no significant drug-drug interactions. The solution of MLX in methanol was found to exhibit maximum absorption at 354 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature. Thus the procured drug sample of MLX complies with the reference spectra. Graph of absorbance vs. concentration was plotted and found to be linear over the range of 2 to 12µg/mL indicating its compliance with Beer's and Lambert's law. Formula F1 was selected as the most optimized formula for preparation of Nanogel. This decision was based upon the Entrapment Efficiency results (99.98 %) for F1 SLNs.

The particle size and PDI the drug free NG was found to be 201nm and 0.3 respectively. After then drug loading partical size of MLX loaded NG was increase 226 nm there was no significant change in PDI. Partical size of NG a crucial factor because it determines the rate and extent of drug release as well as drug absorption. The slighter droplet size provides a greater interfacial surface area for drug absorption. The particles having average diameter up to 300 nm could be easily transported Parental route. In addition, it was advised that the slighter droplet size permit a faster release rate. Also, it has been reported that the smaller particle size may lead to more rapid absorption and improve the bioavailability. If PDI is lower than 0.1, it might be associated with a high homogeneity in the particle population, whereas high PDI values suggest a broad size distribution. The obtained zeta potential of MLX-loaded NG was found to be 5.52. The Zeta potential represents the electrical charge to the NG surface. The greater the ZP value, 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, indicates that the dispersion having long term stability. Morphological study of optimized NG was done by taking SEM pictures of prepared MLX NG. It was revealed that they were irregular in shape and porous in nature with smooth surface. XRD for optimized MLX NG shows amorphous property thus also confirming resemblance with SEM images and proving to be smooth on surface DSC was a basic method to investigate the crystallization or amorphous state of drug in the compounds and NG by determining the variation of temperature and energy at phase transition. (Fig. 23) shows DSC curve of MLX NG. The disappearance of the endothermic peak of MLX in the MLX–NG powder suggests that the drug is completely encapsulated in the polymer crosslinking matrix and converted to amorphous state from crystalline state.

Formulation F7 showed maximum drug release at 100.8 % in 30 minutes. Hence, F7 was considered as optimized formulation. From stability studies, it was observed that particle size was slightly increased from 226.2 ± 0.027 nm to 227 ± 1.80 nm and % EE was decreased to 87.01 ± 1.35 % during storage. Additionally, there was not much change in PDI means, initially it was 0.189 ± 0.89 and changed to 0.262 ± 1.045. Minimum loss of % EE indicates that the drug was retained within the matrix carriers during the stability period and minimum loss of drug was occurred. The obtained results revealed that there was no significant change in the MPS, PDI and % EE indicating that they were found to be stable at 25 ± 2°C, 60 ± 5% RH for a total period of 3 months.

9. REFERENCES:

1. Pandya JB, Parmar RD, Soniwala MM, Chavda JR. Solid lipid nanoparticles: overview on excipients. Asian Journal of Pharmaceutical Technology & Innovation. 2013;1(3):01-9.
2. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian journal of pharmaceutical sciences. 2009 Jul;71(4):349.
3. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. European journal of pharmaceutics and biopharmaceutics. 2000 Jul 3;50(1):161-77.
4. Kaur IP, Bhandari R, Bhandari S, Kakkar V. Potential of solid lipid nanoparticles in brain targeting. Journal of Controlled release. 2008 Apr 21;127(2):97-109.
5. Almeida AJ, Souto E. Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Advanced drug delivery reviews. 2007 Jul 10;59(6):478-90.
6. Vyas SP, Khar RK. Controlled drug delivery concepts and advances. vallabh prakashan. 2002;1:411-7.
7. Vyas SP, Khar RK. Controlled drug delivery concepts and advances. vallabh prakashan. 2002;1:411-7.
8. Lee CH, Chien YW. Drug delivery: Vaginal route. InEncyclopedia of Pharmaceutical Science and Technology, Fourth Edition 2013 Jul 1 (pp. 1236-1259). CRC Press.
9. Üner M, Yener G. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. International journal of nanomedicine. 2007 Sep;2(3):289.
10. Kaur IP, Bhandari R, Bhandari S, Kakkar V. Potential of solid lipid nanoparticles in brain targeting. Journal of Controlled release. 2008 Apr 21;127(2):97-109.
11. zur Mühlen A, Schwarz C, Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery–drug release and release mechanism. European journal of pharmaceutics and biopharmaceutics. 1998 Mar 1;45(2):149-55.
12. Kuo YC, Chen HH. Entrapment and release of saquinavir using novel cationic solid lipid nanoparticles. International journal of pharmaceutics. 2009 Jan 5;365(1-2):206-13.
13. Paliwal R, Rai S, Vaidya B, Khatri K, Goyal AK, Mishra N, Mehta A, Vyas SP. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic

delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2009 Jun 1;5(2):184-91.
14. Suresh G, Manjunath K, Venkateswarlu V, Satyanarayana V. Preparation, characterization, and in vitro and in vivo evaluation of lovastatin solid lipid nanoparticles. Aaps Pharmscitech. 2007 Mar;8(1):E162-70.
15. Teja VC, Chowdary VH, Raju YP, Surendra N, Vardhan RV, Reddy BK. A glimpse on solid lipid nanoparticles as drug delivery systems. J Glob Trends Pharm Sci. 2014;5(2):1649-57.
16. Ekambaram P. *Formulation and Evaluation of PH Triggered In Situ Gelling System of Levofloxacin* (Doctoral dissertation, Madurai Medical College, Madurai).
17. Abdelbary G, Fahmy RH. Novel Drug Delivery. AAPS Pharm. Sci. Tech. 2009;10(1):1.
18. Harivardhan Reddy L, Murthy RS. Etoposide-loaded nanoparticles made from glyceride lipids: formulation, characterization, in vitro drug release, and stability evaluation. AAPs PharmSciTech. 2005 Jun;6(2):E158-66.
19. Sandhu P, Bilandi A, Kumar S, Rathore D, Bhardwaj S. Additives in topical dosage forms. IJPCBS. 2012;2(1):78-96.
20. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharmaceutical research. 1995 Mar;12(3):413-20.
21. Dao Thanh T. Desarrollo galénico de nuevas formulaciones inyectables de meloxicam y amoxicilina sódica para uso veterinario.
22. Burke A, Smyth E, FitzGerald GA. Analgesic-antipyretic agents; pharmacotherapy of gout. The pharmacological basis of therapeutics. 2006;1:706.
23. Oliveira IM, Fernandes DC, Cengiz IF, Reis RL, Oliveira JM. Hydrogels in the treatment of rheumatoid arthritis: Drug delivery systems and artificial matrices for dynamic in vitro models. Journal of Materials Science: Materials in Medicine. 2021 Jul;32(7):1-3.
24. PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 54677470, Meloxicam; [cited 2022 Apr. 30]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Meloxicam