IJPSR (2023), Volume 14, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 June 2022; received in revised form, 03 September 2022; accepted, 19 November 2022; published 01 February 2023

DEVELOPMENT AND EVALUATION OF LAWSONE (HENNA) NANOEMULSION FOR ANTIFUNGAL AND ANTI-BACTERIAL ACTIVITY

Nidhi Gupta, Krishna Kumar *, Neeraj Kumar and Amresh Gupta

Samarpan College of Pharmacy, Lucknow - 226028, Uttar Pradesh, India.

Keywords:

Nanoemulsion, Antimicrobial, Ternary phase diagram, viscosity

Correspondence to Author:
Krishna Kumar

Assistant Professor,
Samarpan College of Pharmacy,
Lucknow - 226028, Uttar Pradesh, India.

E-mail: krishna.pankaj101097@gmail.com

ABSTRACT: Nanoemulsions, also mentioned as submicron emulsions, ultrafine emulsions and miniemulsions, are submicron-sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions; they contain two liquids like water and oil (immiscible), stabilized by an interfacial film consisting of a suitable surfactant and co-surfactant to form a single phase. In this research, lawsone nanoemulsion was developed by using Neem oil, tween 80 and ethanol. The formulation optimization was done by designing a ternary phase diagram followed by water dilution method. We developed 6 formulations (F1-F6) with different concentrations of oil and surfactant but drug concentrations are the same in all developed formulations. Developed formulations are evaluated for different parameters like pH, solubility, drug content, viscosity, transparency, size of globules and stability study, *etc*., and found to be appropriate in all tests. Since, oil/water Nanoemulsion had greater density than water/oil systems, the stiffness values increased significantly whenever the water content increases or when the system switched to oil/water type. Antimicrobial screening of the drugs and formulations were also screened against bacterial and fungal spices and got best results. The data shows that the particle size reduces as the ratio of oil to surfactants: co-surfactants increases. As a consequence, Nanoemulsion particles settled close to the body.

INTRODUCTION: Nanoemulsions, also mentioned as submicron emulsions, ultrafine emulsions, and miniemulsions, are submicron-sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions they contain two liquids like water and oil (immiscible), stabilized by an interfacial film consisting of a suitable surfactant and co-surfactant to form a single phase.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(2).952-63

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(2).952-63

A number of surfactants with diverse characteristics (ionic or non-ionic) had been used with such Nanoemulsion. Most widely used among them were nonionic surfactants (sorbitan esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide) and zwitterions surfactants (quaternary ammonium halide).

Early nanoemulsions were oil-in-water (O/W) type emulsions with average droplet diameter ranging from 50 to 1000 nm. Nanoemulsions more recently are classified into three categories such as O/W type (oil is dispersed in aqueous phase), water-in-oil (W/O) type (water is dispersed in oil phase), and bi-continuous (microdomains of water and oil are

interdispersed within the system). Transformation among these three types can be attained by altering the components of the emulsions. Multiple emulsions are also nanoemulsions, where both O/W and W/O emulsions are present simultaneously in one system. For stabilizing these two emulsions, both hydrophilic and lipophilic surfactants are used simultaneously. Nanoemulsion offer various advantages over other dosage forms and these advantages are (1) increased rate of absorption, (2) reduced variability in absorption, (3) protection from oxidation and hydrolysis in O/W nanoemulsion, (4) delivery of lipophilic drugs after Solubilization, (5) aqueous dosage form for water-insoluble drugs, (6) enhanced bioavailability for many drugs, (7) ability to incorporate both lipophilic and hydrophilic drugs, (8) delivery systems to enhance efficacy while reducing total dose and side effects, (9) as non-toxic and nonirritant vehicles for skin and mucous membrane delivery and (10) release control by permeation of drug through liquid film, whose hydrophilicity or lipophilicity, as well as thickness, can be precisely controlled [1].

Antibiotic-resistant bacteria are becoming more common, which may reduce conventional medications' effectiveness, making patient treatments more expensive and complex. The abuse & overuse of antibiotics as well as the usage of anti-bacterial chemicals in food-producing animals to enhance animal health & increase making, has resulted in the creation of resistant microbes [2]. In 2004, it was projected that more than 70% of hazardous bacteria were resistant to at most one commercially active antibiotic [3]. *Escherichia, Klebsiella* and Pseudomonas are only a few instances of Gram-negative infections. Gram-negative bacteria are responsible for > 60% of hospital sepsis cases [4]. *Pseudomonas aeruginosa* is responsible for over 80% of these bacterial infections. Furthermore, fungi are responsible for around 40% of deaths from nosocomial infections, with *Candida* and *Aspergillus* accounting for 80% of them [5]. Antifungal drugs such as polyene antibiotics, fluoropyrimidine, Nystatin and the azole group of pharmaceuticals are presently available for the treatment of widespread mycosis. Antifungal treatment targets: Fungicides work on a variety of targets. Polyene medicines, including Itraconazole, Posaconazole, & Clotrimazole, all work on the plasma membrane. The antifungal medication also targets DNA synthesis and includes medicines like Pyrimidine analogs, such as Flucytosine. Echinocandins Caspofungin acetate is an antifungal medicine that works on the cell wall.

Herbs have been employed in traditional herbal treatment in the past. Numerous plant-derived chemicals have been shown to exhibit anti-bacterial action and a large spectrum of active ingredients has been found. Numerous studies have discovered that bioactive chemicals from medicinal herbs have anti-tumor, anti-carcinogenic, anti-bacterial, anti-bacterial, antifungal, antioxidant, antiviral, anti-inflammatory, and anti-carcinogenic activities [6]. Henna is utilized to cure a range of ailments in herbal medicine, including headaches, ulcers, rheumatoid arthritis, diarrhea, fever, diabetic [7, 8]. Furthermore, these herbs are employed to combat infection disorders. The plant's inhibitory activity against gram gram-negative, gram-positive, and fungal bacteria & fungi has been described. The existence of anthraquinones (lawsone) as key constituents of the plants accounting for roughly 1.5 percent of henna is credited with this effect [9].

Lawson is an organic dye used to color hands, fingers, nails, and hair in, Asia, Arabia, and different parts of Africa. It has a huge range of biological actions, including antimicrobial properties for hair & skin, antitumor, antimicrobial, anti-parasitic, cytotoxic properties, and anti-inflammatory properties. Many studies have shown that henna has antimicrobial properties, particularly against gram-positive bacteria, as well as antifungal action towards dermatophytes and wounds repair[10]. However, few studies have looked at its impact on nosocomial infections. The antagonistic capability of the Henna plant towards pathogenic bacteria that cause infectious diseases was also investigated.

Pathways for Delivery of Drugs through Human Skin: Drug particles in interaction with the surface of the skin can enter through three different routes: hair follicles, sweat ducts & sebaceous glands (together known as the shunted or appendageal route), or immediately through the *stratum corneum* **Fig. 1**. Scientists have argued the relative relevance of the shunted or appendageal route against transportation over the stratum corneum for

years and the lack of an appropriate experimental model to separate the three paths has made things even more confusing. The usage of hydrated skin or epidermis membranes in vivo investigations is common because the swell accompanied by hydration closes appendages. The pre-steady-State penetration of polar compounds and flux of large polar molecules or ions that have difficulties diffusing over the entire stratum corneum, according to scheuplein and colleagues, is caused by a follicular shunt pathway. The corneum of the stratum is composed of 10-20 levels of corneocytes from 10-15m while dry to 40m when wet. It consists of keratin-rich keratinocytes (bricks) imbedded in a fibrous tissue (mortar) mostly composed, phospholipids, lipids, sterol, triglycerides sulfate, and sterol/wax esters. Corneocytes are elongate, polygonal, and flat (0.2-1.5 m thick, 34-46 m in diameter) instead of brick-shaped, which is important to remember. Waters is an important constituent of the stratum corneum, acting as a plasticizer to prevent cracking and assisting in producing a natural moisturizing factor (NMF) that helps keep the skin supple. Hydrophilic substances are assumed to dissipate via the lipid matrix between intracellular keratin filaments, while lipophilic compounds are thought to dissipate through the soluble areas near the external surface of intracellular keratin filaments [11] (intracellular or transcellular route) (intracellular route) **Fig. 2**

Structure of Nanoemulsion: Based on the quantities of the ingredients, a combination of oil, water, and surfactants can produce a wide range of morphologies and stages. In this aspect, the mobility of the surfactant film is critical. The presence of numerous diverse structures, such as droplet-like forms, aggregates, and bicontinuous constructions, will be enabled by a flexible surfactant layer, which will widen the range of Nanoemulsion presence. The presence of a bicontinuous structure will be hampered by an extremely stiff surfactant coating, which will limit the spectrum of existence. Depending on the component ratio, structural studies can indicate the presence of normal emulsifiers, anisotropic crystalline hexagon or cubic phases, lamellar structures, and Nanoemulsion. The interior architecture of a Nanoemulsion carrier is critical for phase conductivity and, as a result, drug diffusion in the corresponding phases.

The intricate phase behavior and varied microstructures seen in macroemulsion systems have piqued the interest of scientists [12].

MATERIAL AND METHOD: The Lawsone (Drug) was obtained from Himedia Pvt. Ltd., Mumbai, India. Neem oil was used as an oil phase purchased from Devinez Pvt. Ltd. and fulfilled by Amazon. All other chemical used in this study was appropriate grades supplied by justified vendors. The equipment used in this study during work was electronic balance, UV/Vis spectrophotometer, Micropipette, autoclave, water bath, magnetic stirrer, incubator, homogenizer, Brookfield viscometer, digital pH meter, Vortex shaker, FTIR spectrophotometer, melting point apparatus, and thermometers, etc. The required apparatus such as beaker, volumetric flask, measuring cylinder, glass rod RD bottles, etc. were used for the experiment.

Preformulation Studies: Preformulation is a design phase in which researchers characterize the chemical, physical, and mechanical characteristics of the drug material to create a dosage form that is effective, durable, and secure. As a result, Preformulation experiments are required to describe the medicine so that the delivery of drugs can be properly designed. The following are some of the pre-formulation investigations that were carried out as part of this project:

- Melting point
- Solubility
- Uv-spectroscopy
- FTIR
- PH

Melting Point: Volumetric flask of one end closed is filled with enough dry powder to produce a 0.25cm long to 0.35cm column at the end of the hole that is tightly compacted as tightly as feasible by gentle hammering on a solid surface. The device is operated following industry best practices. The block is warmed until it reaches a temperature approximately 30° below the projected melting point. The capillary is put into the hot plate, and the temperature is raised at a rate of around 1 to 2°C per minute until the melting is finished. The start of

melt is classified according to the temperature at which the sensor signal initially leaves its initial value, and the end of melt, or the melting point, is described as the temperature at which the sensor signal achieves its final value. Both of these temperatures are inside the melting range [13].

Solubility Studies: Solubility is the interaction of components of a mixture to establish homogeneous biochemical dispersion. At room temperature, 10 mg of sample was placed independently in 10 ml of several solvents in firmly capped tubes & shaken. The table shows the solubility characteristics of two medicines in several solvents **Table 1** [14].

TABLE 1: SOLUBILITY PROFILE

Descriptive term	Solvent is required for 1 part of the solute
Easily dissolving	≤1
dissolves without restriction	1-10
Soluble	10-30
Dissolve in Moderate Amounts	30-100
Dissolve in a Minor Amount	100–1000
Only a little bit dissolve	1000-10,000
Insoluble to the point of being almost insoluble	≥10,000

pH: A pH meter with a glass electrode is used to determine pH. The power of H+ concentration in a solution is represented by pH. The equation that follows defines it. The natural log of the mutuality of H+ in dilute fluids well matches this amount.

The pH of Nanoemulsion compositions was determined utilizing an ELICO LI120 pH meter that had been adjusted before being used with a buffer solution at pH 4 & pH 9.2.

A specific quantity of the formulation was measured out, dissolved with measured filtered water, & thoroughly mixed. The electrodes of the pH meter were submerged in the pH formulation that had been created.

A specific amount of the mixture was measured, diluted with measured filtered water, and thoroughly mixed. For pH measurement, the electrodes of the pH meter were submerged in the produced formulation determination. A pH meter was used to determine the pH of around 2gm of formulation distributed in 20ml of purified water [15].

FTIR Analysis: Responsive units in a molecule are identified using FTIR. The drug was examined at 4mm/s with a precision of 2cm over a wave number range of 400 to 4000cm-1 on a KBr disc. The typical peaks were captured on film [16].

UV-spectroscopy: *Lawsonia innermis* was subjected to a UV spectroscopic test to determine its authenticity. Using the dilution procedure, the *Lawsonia innermis* was dissolved in ethanol in a 10 ml volumetric flask and diluted up to 10 times.

UV spectroscopy was used to establish the ethanol's maximum concentration of Lawsonia innermis. *Lawsonia innermis* standard curve in ethanol was also displayed.

Calibration Curve: The calibration curve was created by immersing one gram of *Lawsonia innermis* in 100 mL of ethanol as a stock solution. Using the serial dilution procedure, we create solutions with varying concentrations of *Lawsonia innermis* from a stock solution. As a result, the OD was measured at 426nm using a UV spectrophotometer [17].

Development of Nanoemulsion:

Optimization of Co-surfactants, Oils and Surfactants: Optimization of the concentration of surfactants, oils and co-surfactants was carried out using various ratios.

The ratio of surfactant and co-surfactant mixture (Smix) varies from 1:1, 1:2, 1:3 & 1:4. The ratio of the Smix and oil concentration is 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9.

Aqua distilled water is added by performing the titration and then stirred by using a magnetic stirrer till it converts to translucent and no phase separation [18].

Optimization of Formulation: Researchers have used numerous formulation & optimization strategies such as the ternary phase transition, Box-Behnken design, & D-optimization designs, according to diverse literature.

We used a ternary phase diagram **Fig. 1** and a design of experiment technique to optimize the formulations in this investigation [19].

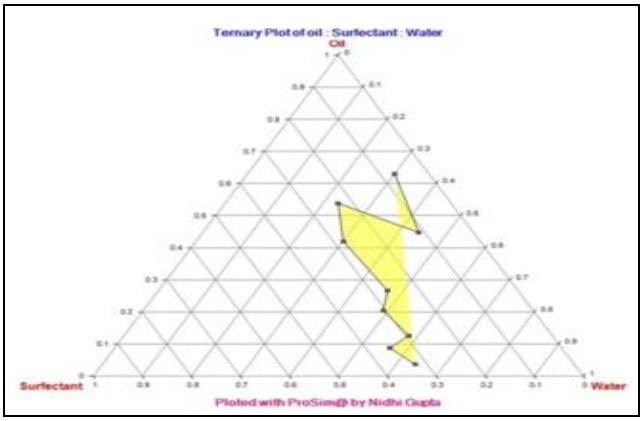

FIG. 1: TERNARY PHASE DIAGRAM

Nanoemulsion Formulation: Formulation of the Nanoemulsion was carried out by using the method known as the spontaneous emulsification method. Total 6 formulations were carried out, known as F1, F2, F3, F4, F5 and F6 **Table 2**. The Lawsonia was added to the oil phase consisting of standardized butylated hydroxytoluene; further, the Smix solution was added to it, which is a mixture of co-surfactant and surfactant, then allowed for stirred with the magnetic stirrer until a homogeneous mixture was prepared. Then the water is added by using the mode of titration and then left for stirring condition until the Nanoemulsion will be formed which is noticeable by the development of a translucent solution [20].

Protocol:

- **Oil Phase:** Neem oil was taken as an oil phase for developing Nanoemulsion.
- **Aqueous Phase:** Take distilled water and add tween 80 (surfactant).
- Lawsonia is dissolved in ethanol (co-surfactant) and mixed with an aqueous phase. Both the phases (aqueous and oil phase) were heated in a water bath up to 7°C.
- The aqueous phase was placed on a magnetic stirrer, and the oil phase was added dropwise into the aqueous phase to develop the coarse emulsion. Allowed the Coarse emulsion to stir on a magnetic stirrer for 20 min to get the Nanoemulsion.

TABLE 2: FORMULATION DEVELOPMENT

Sr. no.	Ingredients	F1	F2	F3	F4	F5	F6
				Lawsone			
1	Oil	17.5	16.1	13.1	14.2	10.5	19.1
2	Tween 80	10.6	8.25	10	11.5	10	6.9
3	Ethanol	1.25	1.25	1.25	1.25	1.25	1.25
4	Distilled water	50 qs.	50 qs.	50 qs.	50 qs.	50 qs.	50 qs.

Evaluation of Developed Nanoemulsion: The following criteria were used to evaluate the Nanoemulsion after it had been formed:

Optical Transparency: Inspecting the specimen in a clean & visible vessel in the vicinity of good lighting versus reflecting into the eye and viewing against a dark and white lighted background was used to evaluate the formulation's optical transparency.

Viscosity: Using a Brookfield Viscometer, this process evaluates the fluid velocity. Viscosity is a measurement of fluid friction that can be thought of as the internal friction that occurs when one stratum of fluid moves about another. The ratio of combing force to the shear rate is measured by viscosity. Verify also that viscometer has been properly optimized If not, use software to calibrate. Similar to the Calibration Standards, the specimen container and quantity should be similar. Equilibrate the sample's temperatures with the temperature specified in the requirement. (±1°C). Utilizing the bubble level on the rear of the viscometer, double-check that it is level. The device with the spindles connected as well as the speed set as specified in the design specifications for the Brookfield LV-II. After ten min, the primary screen will flash 00.0.

Immersion of the appropriate spindle in the specimen to the notch on the spindles shaft as specified in the design specifications. Allowing air bubbles to form is not a good idea. Connect the viscometer to the spindle. The spindle must not contact the container's bottoms or edges and should be centered. Make sure the viscometer is appropriate. The spindle number 64 was revolved at 60 revolutions per minute. Nanoemulsion

specimens were permitted to stay at room temperature for 30 minutes before readings were conducted [21].

Droplet size Test: Zetasizer was used to evaluate the best Lawsonia Nanoemulsion formulations. The specimen was inserted into this chamber and served as a delivery and control chamber. The chamber was then filled with a suitable solvent. A laser beam was now expected to slide on the sample cell. They were sent towards the sensor after completing the necessary amount of runs. The formula's particle diameter range and the mean particle size can be determined using this information. A particles analyzer can be used to estimate the mean particle size of Nanoemulsion formulations [22].

Phase Separation Test: The Nanoemulsion stability was assessed using the centrifugal test. About 4-8 g of the finished Nanoemulsion composition was placed in a test tube and agitated at 5000 rpm for around 10 minutes at 250°C in this test. As a result, the Nanoemulsion gel was visually examined for any signs of phase separating [23].

Anti-bacterial and Antifungal Analysis:

Requirements: Micropipettes, microbiological growth, extract operating fluids, conical flask, tips, marker, Petri plates, nutrient agar medium, potato dextrose agar media, nutrient agar media, sterile nutrient agar media, nutritional agar media, and nutrient broth media.

Principle: The antifungal testing of medicinal plants against test pathogens was done using the agar well diffusion method.

Procedure:

- Nutrient agar medium was made and placed into sterile plates after being autoclaved (sterile).
- After that, the media was allowed to set.
- In each Petri plate labeled Escherichia coli and *Staphylococcus aureus* (Bacterial species), 20 l of the pathogen was disseminated (Fungal species).
- Using sterile tips, 4 wells of 8mm diameter were bored after 5-6 minutes of spreading.
- Then, using a micropipette, 50 l of the extract was put into each well.
- The plates were then incubated for 24 hours at 370°C [24].

***In-vitro* Release Study:** A customized Franz diffusion (FD) cell was used for the in vitro release of drug testing. Between the donor and receptor compartments of the FD cell, a dialysis layer was positioned, and approx. 2 g of the optimized Lawsone formulation was applied on the dialysis membrane. Phosphate buffer (PH 5.4) has been used as a dissolution medium. The cell's temperature was kept constant at 37°C using flowing water jackets and a mechanism. The intended apparatus was mounted on a magnet stirrer, and magnetic beads were used to continuously mix the dissolving media.

After that, the samples (5ml) were taken from the receptors chamber at appropriate intervals and replaced with an equivalent volume of a new medium. The cumulative percent release of drugs was estimated using a twin-beam spectrophotometer and spectrophotometric examination of samples at 426 nm [25].

Stability Test: The capacity of a certain formulation in a particular vessel /closure system to stay inside its chemical, physical, microbiological, pharmacological, and toxic parameters during its shelf life is known as the stabilization of a pharmaceutical formulation [26]. The storage durability of Nanoemulsion compositions is a serious issue as it is a big problem in the creation of commercially available products. Chosen Nanoemulsion compositions (ME-3) were considered as a high polyethylene bottle and held in plastic cages at a temperature of 40°C and relative humidity of 75 percent. The stability tests were conducted out over a three months period the proportion of medication concentration in the Nanoemulsion composition was tested and verified at frequent intervals [27].

RESULT& DISCUSSION:

Melting Point: The result was founded to be 181°C. The average value was taken from triplicate readings. The reference melting point is about 250 – 251°C.

Solubility: Solubility of curcumin in various solvents was performed by the given method in the methodology section. The obtained data are as follows **Table 3.**

TABLE 3: SOLUBILITY PROFILE OF LAWSONIA

S. no.	Solvent	Solubility
1	D.W.	Partially
2	C_2H_5OH	high
3	$CHCl_3$	Partially
4	0.1N sodium hydroxide	Slightly

Standard Curve for Ethanol: Absorbance of different concentrations of curcumin solution was measured at λ max 429 nm. Table 4 shows a calibration curve in **Fig. 2.**

TABLE 4: STANDARD CURVE DATA

Sr. no.	Absorbance	Concentration (µg / ml)
1	0.212	2
2	0.495	4
3	0.712	6
4	0.982	8
5	1.212	10

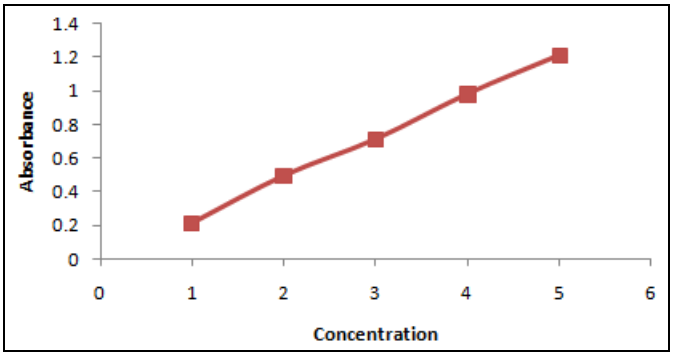

FIG. 2: STANDARD GRAPH FOR ETHANOL

FTIR Analysis: 1249 cm^{-1} owing largely to the overlapped stretch vibration of O=S and carbonyl (C=O) characteristics. Stretch vibrations at 3419cm^{-1} owing to O-H groups and high-intensity band 1740 cm^{-1} apportioned to blended vibration such as stretch carbonyl bond vibration (C=O), in-plane bending vibrations all over aliphatic CC-C, CC=O, and in bending vibration around aromatic NH stretch of keto and enol setups. All obtained IR data and their graph is given in **Table 4** and **Fig. 3.**

TABLE 4: IR DATA OF LAWSONE

Sr. no.	Functional groups	Wave number in (cm-1)
1	H – O stretch	3419.97
2	H- N- stretch	2924.23
3	H- C- (Aromatic) stretch	2858.58
4	Carbonyl O=C stretch	1740.99
5	NH(Amide) stretch	1194.64
6	O=S stretch	1249.63
7	H –C bending	829.27

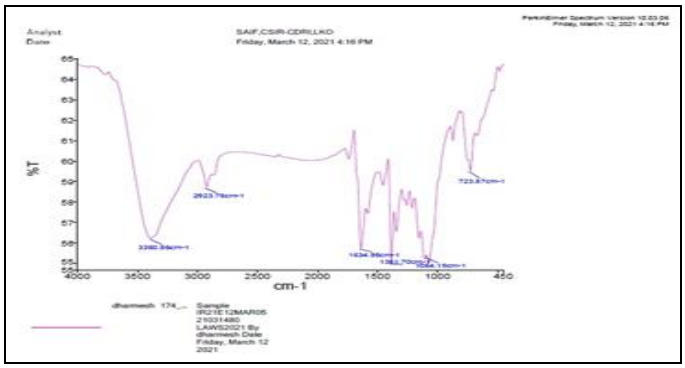

FIG. 3: IR SPECTRA OF LAWSONE

FIG. 4: FORMULATED NANOEMULSION

Characterization of Nanoemulsion:

TABLE 5: APPEARANCE OF FORMULATION

Formulation	Appearance
F1	Milky
F2	Opalescent
F3	Clear
F4	Reddish
F5	Reddish
F6	Reddish

Determination of pH: The obtained PH data of all batches are given in **Table 6.**

TABLE 6: PH DETERMINATION OF FORMULATED GELS

Formulation	pH
F1	6
F2	5.9
F3	6.12
F4	6.3
F5	6.9
F6	6

Viscosity: The viscosity of all batches was checked by Brookfield viscometer at different rpm; obtained viscosity data is given in **Table 7.**

TABLE 7: VISCOSITY ANALYSIS OF FORMULATIONS

Formulation	Viscosity (centipoise)			
RPM	10	20	50	100
F1	600	500	440	400
F2	200	200	200	220
F3	280	200	280	260
F4	320	380	168	320
F5	480	420	360	284
F6	560	640	352	248

Size of Particle Test: On the basis of the evaluation of all of the five formulated Nanoemulsion gel, two formula F1 and F4 was selected as best **Fig. 5, 6**.

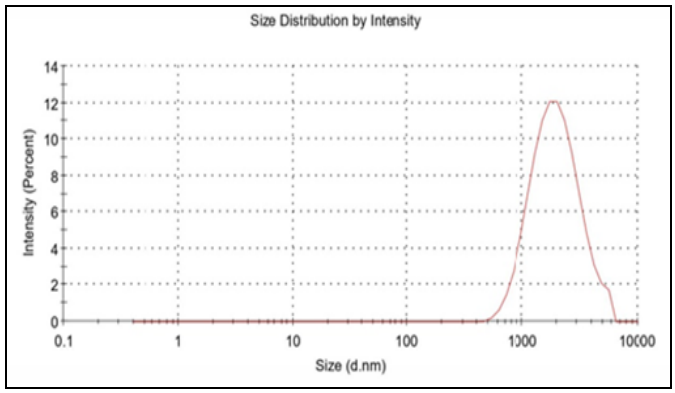

FIG. 5: PARTICLE SIZE DATA OF F1 BATCH

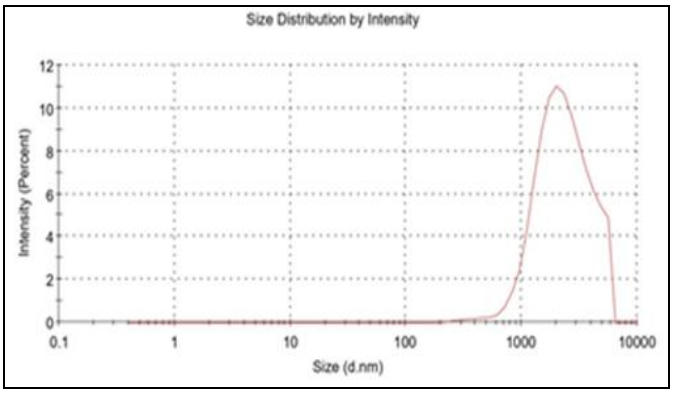

FIG. 6: PARTICLE SIZE DATA OF F2 BATCH

Drug Content: Drug content was estimated by the standard curve of Lawsone **Table 8.** Phase separation test **Table 9.** *In-vitro* release **Table 10.**

TABLE 8: PERCENT DRUG CONTENT OF NANOEMULSION FORMULATION

Sr. no.	Formulation	% Drug content
1	F1	86.12
2	F2	86.12
3	F3	83.75
4	F4	86.14
5	F5	86.45
6	F6	89.75

TABLE 9: PHASE SEPARATION TEST OF ALL SIX FORMULATIONS

Formulation code	observation
F1	No phase separation
F2	No phase separation
F3	No phase separation
F4	No phase separation
F5	No phase separation
F6	No phase separation

TABLE 10: *IN-VITRO* % DRUG RELEASE OF FORMULATIONS

Sr. No.	Time (min)	F1 (In %)	F2 (In %)	F3 (In %)	F4 (In %)	F5 (In %)	F6 (In %)
1	0	0	0	0	0	0	0
2	30	4.54	5.82	3.66	2.93	7.35	6.87
3	60	7.59	11.69	6.47	8.07	8.88	8.55
4	90	19.07	20.28	17.39	14.34	13.37	17.39
5	120	23.41	32.16	22.77	21.88	18.11	18.99

6	150	32.08	47.90	32.08	32.99	23.09	27.67
7	180	49.03	51.39	39.23	41.8	30.8	34.89
8	210	52.16	62.52	49.11	53.6	41.16	41
9	240	67.17	73.84	54.89	62.11	57.54	53.2
10	270	75.36	74.80	62.76	72.07	60.19	66.13
11	300	84.36	75.93	71.51	81.63	71.51	72.63

Antimicrobial analysis **Table 11 Fig. 7.**

TABLE 11: ANTIMICROBIAL ANALYSIS OF THE DRUG AND FORMULATIONS

Pathogens	Zone of inhibition (mm)						
	Drug	F1	F2	F3	F4	F5	F6
S. aureus	16	14	26	19	24	19	20
E. coli	30	38	44	36	46	38	36
A. niger	12	22	18	28	17	25	24

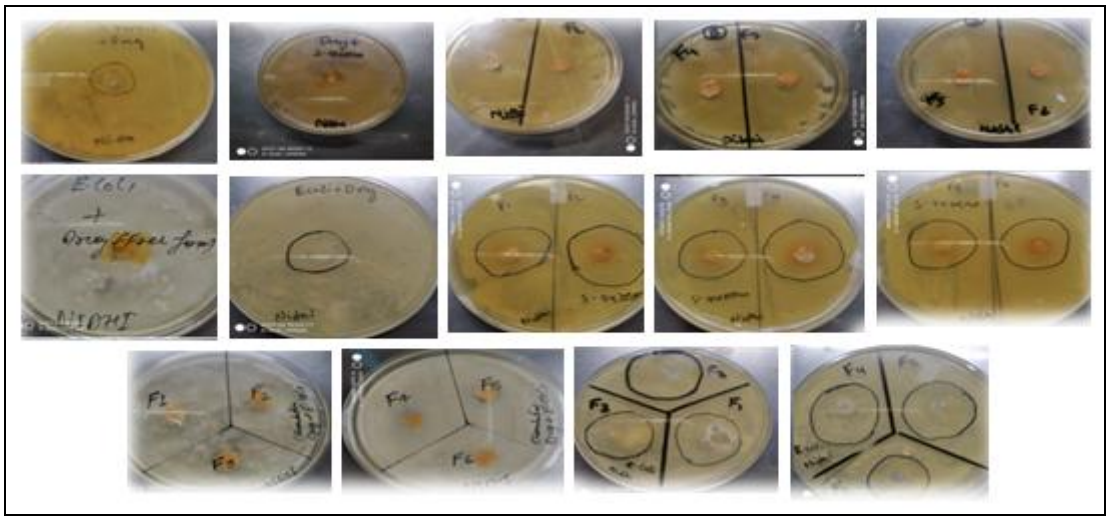

FIG. 7: ANTIMICROBIAL SCREENING OF SAMPLES

Stability Data of Developed Formulation: After the 3 month of storage the some parameter was checked and found unchanged during storage **Table 12.**

TABLE 12: STABILITY DATA OF SOME PARAMETERS

Sr. no.	Formulation	% Drug Content	pH	Visual Appearance	
				Color	Phase Separation
1.	F1	86.12	5.9	Brown	No
2.	F2	86.12	6.0	Brown	No
3.	F3	52.12	6.0	Brown	No
4.	F4	84.14	6.5	Brown	No
5.	F5	86.12	7.0	Brown	No
6.	F6	89.14	6.5	Brown	No

DISCUSSION: Transdermal delivery devices make therapeutic amounts of medication ingredients easier to pass through to the epidermis and into the bloodstream for systemic effects. Topical application of medications having systemic effects has various advantages over other methods, including avoiding The drug's first-pass metabolism in the liver and its associated toxicity, regulating the rate of delivery, and altering drug trafficking in the circulatory system. Two factors are considered while constructing a transdermal delivery system: obtaining enough flux over the skin and minimizing skin permeation lag time. As it promotes epidermal permeability *via* 2 mechanistic situations: (a) lipids fluidization & (b) lipid stage segregation, Oleic -acid is an epidermal permeability booster candidate. Oleic acid promotes skin permeation by altering the permeability of the stratum corneum. According to the conclusions of the solubility investigation, the

drug's thermodynamics activity in the formulations is a primary driving force. Because non–ionic surfactants are often less harmful and cause less skin irritation, they were chosen. The HLB value of a microemulsion of the O/W type. Tween-20 HLB value (16.7), necessary HLB for oleic acid in O/W type emulsion, and HLB value of span-20 (8.6). To increase the porosity profiles, Tween-20, a hydrophilic non-ionic detergent, was utilized to construct these microemulsion complexes. In substitution of propylene glycol, other co-surfactant such as ethanol, butanol and isopropyl alcohol have been used, but propylene glycol was found to have the best clarity and cumulative percent release.

The co-surfactant in microemulsion lowers the surfactant film's interfacial tension leading to a more flexible & kinetic layers structure. The thermodynamically drive power for discharge reflects the drug's comparative activity in different phases. Since medications may be transferred with active ingredients for aqueous dispersion & the extraction solvent to the surface of the epidermis, the comparative activity can be employed to measure the epidermis permeate flux. Furthermore, because surfactants & co-surfactant may be present in each phase, lawsone can be partially dissolved in the exterior phase. The discharge of lawsone form of the active ingredient can replenish the depleted of lawsone from the exterior phase due to penetration into the skin. Oily combinations of tween-20, oleic acid, and propylene glycol, on the other hand, boosted medication solubility. After comprehensive screening for physical properties and appearance, the final surfactant-cosurfactant ratios were determined.

F-1 through F6 pH values (6, 5.9, 6.12, 6.3, 6.9, 6) units only.pH revealed the smallest variations in all cases. Due to the pH of the epidermis range from 5-7.0, the pH of the optimized lawsone emulsions formula. It is appropriate for topical and transdermal applications. The produced formulas have steadily grown in thickness (450, 200, 250, 300, 430, 410 cps), as evidenced by the stiffness of the microemulsion system (F1-F6). As anticipated from microemulsion, all specimens displayed Newtonian flow characteristics. Since oil/water microemulsion had greater density than water/oil systems, the stiffness values increased significantly whenever the water content increased or when the system switched to oil/water type. Antimicrobial screening of the drugs and formulations was also screened against bacterial and fungal spices. The optimized microemulsion formula (has particle sizes ranging from 1000-1100 nm with Pdl 0.249-0.290. The data shows that the particle size reduces as the ratio of oil to surfactants: co-surfactants increases. Consequently, microemulsion particles settled close to the body, resulting in a large concentration gradient and improved lawsone absorption.

ACKNOWLEDGEMENT: This research did not receive any specific grant from funding agencies in public, commercial, or non-profit sectors. The author acknowledges the support of Parents and Krishna Kumar in this manuscript.

CONFLICTS OF INTEREST: The author declares no conflict of interest, financial or otherwise.

REFERENCES:

1. Payyal SP, Rompicherla NC, Sathyanarayana SD, Shriram, RG and Vadakkepushpakath AN: Microemulsion Based Gel of Sulconazole Nitrate for Topical Application. Turkish Journal of Pharmaceutical Sciences 2020; 17(3): 259–264.
2. Scomoroscenco C, Teodorescu M, Raducan A, Stan M, Voicu SN, Trica B, Ninciuleanu CM, Nisto CL, Mihaescu, CI, Petcu C & Cinteza LO: Novel Gel Microemulsion as Topical Drug Delivery System for Curcumin in Dermatocosmetics. Pharmaceutics 2021; 13(4): 505.
3. Atipairin A, Chunhachaichana C, Nakpheng T, Changsan N, Srichana T & Sawatdee S: Development of a Sildenafil Citrate Microemulsion-Loaded Hydrogel as a Potential System for Drug Delivery to the Penis and Its Cellular Metabolic Mechanism. Pharmaceutics 2020; 12(11): 1055.
4. Hashem FM, Shaker DS, Ghorab MK, Nasr M & Ismail A: Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. AAPS Pharm Sci Tech 2011; 12(3): 879–886.
5. Chien YW: Transdermal Drug Delivery and Delivery Systems, in: Y. W. Chien, Novel Drug Delivery Systems, 2nded, Chapter 7, Marcel Dekker New York 1992; 301-80.
6. Vyas SP and Khar RK: Targetted and Controlled Drug Delivery Novel Carrier System 1sted, CBS Publishers and Distributors. New Delhi 2002; 411-447.
7. Hadgraft J and Guy R: In; Transdermal Drug Delivery, Marcel Dekker, Inc., NewYork and Basel 35: 296.
8. Prausnitz MR, Mitragotri S and Langer R: Current Status and Futyre Potential of Transdermal Drug Delivery. Nature Reviews, Drug Discovery 2004; (3): 115-124.
9. Elias PM, Brown BE, Fritsch P, Goerke J, Gray GM and White RJJ: Invest Dermatol 1979; 73: 339-48.
10. Govil SK: In; Tyle, P., Eds., Drug Delivery: Fundamentals and Application, Marcel Dekker, Inc., New York 1998; 385-406

11. Jain NK: Contolled and Novel Drug Delivery, Cbs Publisher and Distributor 1st Ed 100
12. Jayaswal SB and Sood R: The Eastern Pharmacist 1987; 30(357): 47-50.
13. Hoar TP and Schulman JH: Transparent Water-In-Oil Dispersions. The Oleopathic Hydro-Micelle. Nature 1943; 152: 102-103.
14. Schulman JH, Stoeckenius and W Prince LM: Mechanism of Formation and Structure of Microemulsions by Electron Microscopy. J Phys Chem 1959; 63: 1677-1680.
15. Vyas SP and Khar RK: Submicron Emulsions in Targeted and Controlled Drug Delivery Novel Carrier System; Cbs Publishers and Distributors, New Delhi 2002; 282-302.
16. Jadhav KR: applications of microemulsion based drug delivery system. Curr Drug Deliv 2006; 3(3): 267-73.
17. Cordero J, Alarcon L, Escribano E, Obach R and Domenech J: A comparative study of the transdermal penetration of a series of nonsteroidal anti-inflammatory drugs. J Pharm Sci 1997; 86: 503–508.
18. Prince LM: Microemulsions. In: Lissant KJ (Ed), Emulsions And Emulsion Technology, New York: Marcel Dekker 1974; 125-178.
19. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, Lopez QM, Marty JP and Guy RH: Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur J Pharm Biopharm 1997; 43: 37-42.
20. Celebi N, Kıslal O and Tarımcı N: The effect of β-cyclodextrin and penetration additives on the release of naproxen from ointment bases. Pharmazie 1993; 48: 914-9179,
21. Gasco MR, Gallarate M, Trotta M, Bauchiero L, Gremmo E, Chiappero O. J Pharm Biomed 1989; 7: 433.
22. Holmberg K: Handbook of Applied Surface and Colloid Chemistry. Chichester, New York Wiley 2002.
23. Giustini MS and Murgia G: Palazzo. does the schulman's titration of microemulsions really provide meaningful parameters. Langmuir 2004; 20(18): 7381-4.
24. Giustini M: Microstructure and dynamics of the water-in-oil ctab/npentanol/n-hexane/ water microemulsion: a spectroscopic and conductivity study. Journal of Physical Chemistry 1996; 100(8): 3190-98.
25. Lawrence MJ and Rees GD: Microemulsion-based media as novel drug delivery systems. Adv Drug Deliv Rev 2000; 45(1): 89-121.
26. Googman and Gilman's The Pharmacological Basis of Therapeutics.11th Edition 2005; 1415.
27. Rang and Dale's pharmacology 6 the dition, Anti-inflammatory activity of NSAIDsp 215; 1304-1309.
28. Kalra R, Mulik RSA, Badgujar L and Paradkar AR: Development and characterization of microemulsion formulations for transdermal delivery of aceclofenac: a research. International Journal of Drug Formulation & Research 2010; 1(1): 359-386.
29. Anjali CH and Madhusmita Dash N: Chandrasekaran, amitava mukherjeeanti bacterial activity of sunflower oil microemulsion, International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2: 123-128.
30. Mrunali RP, Rashmin BP, Jolly RP and Kashyap KB: Investigating the effect of vehicle oninvitro skin permeation of ketoconazole applied in o/w microemulsions, Acta Pharma Sciencia 2010; 52: 65-77.
31. Fathy I, Abd-Allah, Hamdy MD and Ahmed MS: Preparation, characterization, and stability studies of piroxicam loaded microemulsions in topical formulations, original article. Drug Discoveries & Therapeutics 2010; 4(4): 267-275.
32. Tian QP, Li P and Xie KC: Investigation of microemulsion system for transdermal drug delivery of amphotericin B, Chem Res Chinese Universities 2009; 25(1): 86-94.
33. Rohit RS, Chandrakant M and Kiran AW: Fluconazole Topical Microemulsion: Preparation and Evaluation Research. J Pharm and Tech 2009; 2(2): 352-358.
34. Zhai, Guo C, Yu A, Gao Y and Cao F: Microemulsion-based hydrogel formulation of penciclovir for topical delivery. Int J Pharm 2009; 378(1-2): 152-8.
35. Arun K, Dhamankar, Jagdish V Manwar and Dipak D Kumbhar: The Novel Formulation Design of O/W Microemulsion of Ketoprofen for Improving Transdermal Absorption. International Journal of Pharmtech Research 2009; 1(4): 1449-57.
36. Mostafa DM: Investigation of a Transdermal Microemulsion Delivery System for Fluoxetine Hydrochloride. Asian Journal of Pharmaceutical Sciences 2008; 3(2): 68-79.
37. Gamal M: Self-Microemulsifying and Microemulsion Systems for Transdermal Delivery of Indomethacin: Effect Of Phase Transition. Colloids Surf B Biointerfaces 2010; 75(2): 595-600.
38. Ambade KW, Jadhav SL, Gambhire MN, Kurmi SD, Kadam VJ and Jadhav KR: Formulation And Evaluation Of Flurbiprofen Microemulsion. Current Drug Delivery 2008; 5(1): 32-41.
39. Gamal M Maghra: by GM: Transdermal Delivery of Hydrocortisone from Eucalyptus Oil Microemulsion: Effects of Cosurfactants. IJP 2008; 355(1-2): 285-92.
40. Ke-Shu Y, Ting-Xu Y, Hong G and Ji-Zhong L: Evaluation of Transdermal Permeability of Pentoxifylline Gel: *in-vitro* skin Permeationand *in-vivo* Microdialysis Using Wistar Rats. Orignal Article Drug Discov Ther 2007; 1(1): 78-83
41. Anna K, Nissim G: Microemulsion as transdermal drug delivery vehicles. Advance In Colloidal and Interface Science 2006; 123-6: 369-85.
42. Huabing C, Xueling C, Danrong D, Jin L, Huibi X and Xiangliang Y: Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int J Pharm 2006; 315: 52-58.
43. Gupta S, Moulik SP, Lala S, Basu MK, Sanyal SK and Datta S: Designing and testing of an effective oil in-water microemulsion drug delivery system for *in-vitro* application. Drug Delivery 2005; 12(5): 267-273.
44. Haubing C, Xueling C, Weng T, Xiaozhi Z, Zhonghong G, Yajing Y and Huibi X: A syudy of microemulsion systems for transdermal delivery of triptolide. J Control Release 2004; 98: 427-436.
45. Fang, Yann LL, Chia CC, Chia H and Hung T: Lipid nano/submicron emulsions as vehicles for topical flubriprofen delivery. Drug Delivery 2004; 11: 97-105.
46. Baroli B, López-Quintela MA, Delgado-Charro MB, Fadda AM and Blanco-Mendez J: Microemulsions for Topical Delivery of 8-Methoxsale. J Control Release 2000; 69(1): 209-18.
47. Mohammed C and Manoj V: Aerosol-otmicroemulsions as transdermal carriers of tetracaine hydrochloride drug development and industrial pharmacy. Research Paper 2000; 26(5): 507–512.
48. Shaul M, Muhamad A, Joseph F P and Simon B: *Ex-vivo* permeation study of indomethacin from a submicron emulsion through albino *Rabbit cornea*. J Control Release 1997; 44: 55-64.
49. Kavitha K, Mangesh R: Design and evaluation of transdermal films of lornoxicam. International Journal of Pharma and Bio Sciences 2011; 2(2): 54-62.

50. Parikh BA, Najmuddin M, Kulkarni U and Hariprasanna RC: Preparation and evaluation of sustained release matrix tablets of lornoxicam using tamarind seed polysaccharide. International Research Journal of Pharmacy 2011; 2(4): 130-133.
51. Phani KGK, Gangarao B and Lova RNS, Kotha: Preparation and evaluation of sustained release matrix tablets of lornoxicam using tamarind seed polysaccharide. International Journal of Pharma Research and Developmentn 2011; 2(12): 89-98.
52. Syed NU, Anup KR, Martand K and Vinod KSM: Formulation and Evaluation of sustained release matrix tablets of lornoxicam. International Journal of Drug Development & Research 2011; 3(1): 31-65.
53. Ganesh NS: Deecaraman chronomodulated drug delivery system of lornoxicam using natural polymers. Journal Of Pharmacy Research 2011; 4(3): 825-828.
54. Sandeep KD, Shashidhar RK, Amit KT and Suddhasattya Dey: Design and evaluation of buccal patches of lornoxicam. Int J of Pharma and Bio Sciences 2010; 1(4).
55. Bhavsar KC, Gaikwad PD, Bankar VH and Pawar SP: development and validation of uv-spectrophotometric method for simultaneous estimation of paracetamol and lornoxicam in bulk and tablet dosage form. International Journal of Pharmacy & Technology 2010; 2(2): 429-439.
56. Lakshmi S, Lakshmi KS and Tintu T: Simultaneous spectrophotometric estimation of paracetamol and lornoxicam in tablet dosage form. International J of Pharmacy and Pharma Sciences 2010; 2(4): 166-168.
57. Rajesh SJ, Swadesh N, Sabita A and Vikas D: Taste masking of lornoxicam by polymer carrier system and formulation of oral disintegrating tablets. International Journal of Drug Delivery 2009; 27-31.
58. Berry H, Bird H A and Black C: A double blind, multicone, placebo controlled trial of lornoxicam in patients with osteoarthritis of the hip and knee. The Eular Journal 1992; 51(2): 238-242.
59. Fathy IA, Hamdy M, Dawaba and Ahmed M: Evaluation of the anti-inflammatory and analgesic effects of piroxicamloaded microemulsion in topical formulations. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(2): 66-70.
60. Nahla Barakat, Ehab Fouad and Azza Elmedany: Enhancement of skin permeation and anti-inflammatory effect of indo methacinusing microemulsion. Research Article 2011; 5(3): 141-149.
61. Ashutosh M, Ravichandran V, Prateek KJ, Vinod KD and Ram KA: Synthesis, haracterization and Pharmacological Evaluation of Amide Prodrug of Flurbiprofen. J Braz Chem Soc 2008; 19(1): 89–100.
62. Buritova J and Besson JM: Dose related anti-inflammatory and analgesic effects of lornoxicam: a spinal c- fos protein study in the Rat. Inflammation Research 1998; 47: 18-25.
63. Ravic M, Johnston A and Turner P: Clinical pharmacological studies of some possible interactions of lornoxicam with other. Drugs Postgrad Med J 1990; 66(4): 30-34.
64. Hitzenberger G, Welte SR, Takacs F and Rosenow D: Pharmacokinetics of Lornoxicam in Man. Postgrad Med J 1990; 66(4): 22-6.
65. Julia A, Balfour AF, Barradell LB and Lornoxicam: A Review of its pharmacology and therapeutic potential in the management of painful and inflammatory conditions. Drugs 1996; 51(4): 639-57.
66. Berg J, Christoph T and Fellier H: The Analgesic NSAID Lornoxicam Inhibits Cox-1/ Cox-2. The Formation of II-6 *in-vitro*. Naunyn-Schmied Arch Pharmacol 1998; 358(2): 716.
67. Welte SR The Formation of II-6 *in-vitro* Rabbeseda X: Lornoxicam, ANew Potent Nsaid With Improved Tolerability Profile. Drugs of Today 2000; 36(1):55-76.
68. Smolinske SC: Hand book Of Food, Drug, And Cosmetic Excipients. Boca Raton, Fl: CRC Press 1992; 295-301.
69. Francoeur MI, Golden GM The Formation of II-6 *in-vitro* Potts RO: Oleic Acid: its effects on stratum corneum in relationto (trans) dermal drug delivery. Pharm Res 1990; 7: 621-627.
70. Murakami T, Yoshioka M The Formation of II-6 *in-vitro* Yumoto R: Topical delivery of keloid therapeutic drug tranilast, by combined use of oleicacid and propyleneglycol as a penetration enhancer: evaluation by skin microdialysis in rats. J Pharm Pharmacol 1998; 49–54.
71. Khossravi M, Kao YH Mrsny RJ: The Formation of II-6 *in-vitro* the Formation of II-6 *in-vitro* Sweeney TD: Analysis methods of polysorbate 20: a new method to as sessthest ability of polysorbate 20 and established methods that may over look degraded polysorbate 20.
72. Arulanantham K and Genel M: Central nervous system toxicity associated with ingestion of propyleneglycol. J Pediatr 1978; 93: 515–516.

How to cite this article:
Gupta N, Kumar K, Kumar N and Gupta A: Development and evaluation of laws one (henna) nanoemulsion for antifungal and antibacterial activity. Int J Pharm Sci & Res 2023; 14(2): 952-63. doi: 10.13040/IJPSR.0975-8232.14(2).952-63.