Creation and Assessment of an Aspirin-Loaded Nano-emulsion for Improved Topical Administration

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Abstract- **The present study investigated the anti-inflammatory and analgesic activities of novel aspirin oil-inwater (o/w) nanoemulsion and water in oil in water (w/o/w) nano multiple emulsion formulations generated using ultrasound cavitation techniques. The objective of this work was to increase the bioavailability of aspirin. Aspirin is a non-steroidal anti-inflammatory drug. Aspirin is classified in the non-selective Cox inhibitors. The results show that oral administration of Nanoemulsion and nano multiple emulsion containing aspirin(60mg/mg) significantly reduced pain. The aspirin is used to relieve mild to moderate pain arthritis and inflammation and aspirin also prevent the blood clots and reduce the risk of heart attacks and strokes. Both nano formulations number of abdominal constriction in acetic acid induce writing model. One of the most efficient disperse nanosystems is nano emulsion having droplet size ranging to sub Micron size nano emulsions are thermodynamically stable,clear,isotropic liquid mixtures of oil,water surfactant and Co surfactant. The droplet size of nanoemulsions falls typically in the range 20-200 nanometer.The stability of nanoemulsion formulations can be maintained by a surfactant and co-surfactant. These experimental studies suggest that nano emulsion and nano multiple emulsion produce a pronounced anti-inflammatory & analgesic effects in rats and may be candidates as new nanocarriers for pharmacological NSAIDS in the treatment of inflammatory disorders and alleviating pains. The present study is important for the evaluation of anti-inflammatory and analgesic properties of both the Nanoemulsion and nanomultiple emulsion generated using ultra sound cavitation technique.**

Index Terms- **Anti-inflammatory, Stability, Viscosity, Nanoemulsion, Zeta potential**

I. INTRODUCTION

A mixture of two or more liquids that are typically immiscible or unmixable, with one liquid dispersed in the water, is called an emulsion. Water-in-oil emulsion and oil-in-water emulsion are the two varieties. The interfacial layer of an appropriate surfactant and co-surfactant stabilizes nanoemulsions, which are transparent or translucent, thermodynamically stable, heterogeneous dispersions of two immiscible phases, aqueous and oil.Because of its anti-inflammatory, antipyretic, analgesic, and platelet-anti-aggregation qualities, aspirin (acetyl salicylic acid) is a well-known non-steroidal anti-inflammatory medication that is used extensively.

It is always available over-the- counter in the form of tablets and capsules. After oral administration of aspirin absorbed aspirin is rapidly de- acetylated to salicylate, And it was reported that the vast majority of circulating salicylate is bound to plasma proteins. Similar to other NSAIDs, aspirin inhibits prostaglandin synthesis by inhibiting enzyme cyclo-oxygenase early in the synthetic pathway. In fact “inhibition of prostaglandin synthesis as the main mechanism of therapeutic action of aspirin like drugs.” Although adequate inflammation and pain relief is achieved with the currently available aspirin dosage forms like tablets or capsules. Some of their serious side effects in the gastrointestinal tract, kidney and platelets are major limitation to their routine use in therapy.

Define:- Nanoemulsion can be defined as oil-in-water (o/w) emulsion with mean droplet diameters ranging from 50 to 1000nm,usually the droplet size is between 100 to 500nm.

1.1.Advantages of nanoemulsion:-

1. Increase the rate absorption.
2. Eliminates variability in absorption.
3. Helps solublize lipophilic drug.
4. Provides aqueous dosage form for water insoluble drugs.
5. Increase bioavailability.
6. Rapid and efficient penetration of the drug moiety.
7. Liquid dosage form increases patient compliance.
8. Less amount of energy requirement.
9. Nanoemulsion are thermodynamically stable system and the stability allows self-emulsion of the system.

1.2.Disadvantages of nanoemulsion:-

1. use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
2. Limited solubilizing capacity for high-melting substance.
3. The surfactant must be non-toxic for using pharmaceutical applications.
4. Nano emulsion stability is influenced by environmental parameters such as temperature, pH.

1.3.Limitation of nanoemulsion:-

1. The formulation of nano emulsion is an expensive process due to size reduction of droplets is very difficult as it requires special kind of instruments and process methods for example homogenizer arrangement is an expensive process.
2. oswald ripening could damage the nano emulsion.
3. Changing of pH may cause stability problems.

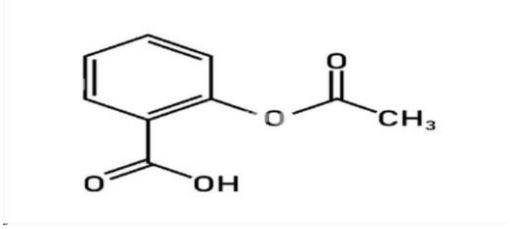
1.4.Componets of Emulsion:

1. Oil:- Ex. Coconut oil, Olive oil.
2. Surfactant:- Ex. Tween 20, Tween 80, Lecithin.
3. Co-surfactant:- Ex. Propylene glycol, 1-Butanol.
4. Aqueous phase:- Ex. Buffers, Electrolytes. Types of nanoemulsion:-

1. Oil in water,
2. Water in oil,
3. Bi-continuous nano emulsion.

II. DRUG PROFILE

1.Proper name:-Aspirin (salicylic acid)
Chemical name:-2-Acetoxybenzoic acid
Molecular formula:$C_9H_8O_4$
Molecular mass:-180.158g/mol
Structural formula:-



PHYSICOCHEMICAL PROPERTIES:-
Physical state:Crystalline powder Color:-White
Odour:-vinegar-like smell
Taste:-bitter taste. Boiling point:-140^{0}c
Melting point:-136^{0}c
P^H :- 3.5
Nature:-Aromatic and Acidic
Solubility:-soluble in water and Organic solvent

III.MECHANISM OF ACTION

a Cyclooxygenase-1 (cox-1) inhibitor it is a modifier of the enzymatic activity of cyclooxygenase-2 (cox- 2).
Other NSAIDs (Ibuprofen),which bind reversibly to this enzyme. aspirin binding is irreversible.
It also blocks thromboxane A2 on platelets in an irreversible fashion preventing platelet aggregation.
Administration:-

- aspirin can be administered by orally rectal and intravenous route.
- it is available in different doses, the lowest dose being 81mg, also called a baby aspirin.

PHARMACOKINETIC:-
3.1.ABSORPTION:- Aspirin absorption from the gastrointestinal tract (GIT) deends on the formulation state. When consume as a liquid preparation, it is rapidly absorbed as opposite to tablets.
The pKa of aspirin is 3.5 most aspirin is mostly absorbed in the stomach, aspirin absorption is P^H sensitive at the level of the small intestine.
3.2.DISTRIBUTION:- This drug is distributed to body tissues shortly after administration. it is called cross the placenta, the plasma contains high levels of salicylate, as well as tissues such as spinal, peritoneal, and synovial fluids, saliva and milk. the kidney, liver, heart and lungs are also found to be rich in salicylate concentration after dosing. Low concentration of salicylate are usually low and minimal concentration are found in feces, bile and sweat.
3.3.PROTEIN BINDING:- 50% 90% Of a normal therapeutic concentration salicylate (a main metabolite of acetylsalicylic acid) binds plasma proteins, particularly albumin, while acetyl salicylic acid itself binds negligibly acetyl salicylic acid it's the ability to bind to and acetylate many proteins, hormones, DNA, platelets and hemoglobin.
3.4.METABOLISM:- Acetylsalicylic acid is hydrolyzed in the plasma to salicylic acid. plasma concentration of aspirin following after administration of the extended-release form are mostly undetectable 4 to 8 hours after ingestion of a single dose. salicylic acid or the major at 24 hours. following a single dose of extended-release acetyl salicylic acid.
3.5.EXCRETION:- Excretion of salicylates occurs mainly through the kidney, by the processes of glomerular filtration and tubular excretion, in the form of

free salicylic acid, salicyluric acid and additionally phenolic and acyl glucuronide.

PHARMACODYNAMICS:-

Almost 90% of COX Inhibition can be achieved with the administration of 160 to 325 mg of aspirin. These effects last for about 7 to 10 days which usually corresponds with the lifespan of a platelet. Prostacyclin inhibition can be achieved with the use of higher doses.

This inhibition occurs in the endothelial cells of blood vessels.

ADVERSE EFFECT:-

1. Hypersensitivity 2.Reye syndrome 3. Intracerebral hemoglobin

INDICATIONS OF ASPIRIN

a) Angina pectoris b) Fever c) Ischemic stroke d) Pain e) Rheumatoid arthritis f) Myocardial infarction g) Cardiovascular risk reduction

IV. LITERATURE SURVEY

1. Manmit Jaiswal et al.3 Biotech 2015. These are the thermodyanamically stable isotonic system in which two immiscible liquids are mix to form a single phase by means of an emulsifying agent. The main difference between emulsion and nanoemulsion lies in the size and shape of particles disperse in the continuous phase. In this review, the attention is focused to give a basic idea about its formulations, methods of preparation, characterization techniques, evaluation parameters and various applications of nanoemulsion.

2. Kumar S. et al.2014. Role of nanoemulsion in pharmaceutical sciences, focusing on their ability to improve drug delivery. Nanoemulsion are thermodyanamically stable systems were two immiscible liquids are mixed,using surfactants and co surfactants to form a single phase. These systems offers advantages for drug delivery dye to their ability to encapsulate and protect drugs, enhance bioavailability, and target specific areas.

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4. Tim J Wooster et al. Langmuir.2008. Nanoemulsion made with high viscosity oils,such as long chain triglycerides were considerably larger than Nanoemulsion prepare with low viscosity oils such as hexadecan. The physical properties of the oil phase and nature of surfactant layer were found to have a considerable impact on nanoemulsion formation and stabilization.

5. Chavan SR. et al. 2014. Nanoemulsion are submicron size emulsion that is under extensive investigation as a drug carriers for improving the delivery of therapeutic agents. These are the thermodyanamically stable isotropic systems in which two immiscible liquids are mixed to form a single phase by means of an appropriate surfactant or it mixes with a droplets diameter approximately in the range of 0.5-100um. Nanoemulsion show great promise for the future of cosmetics, diagnostics, drug therapies and Biotechnologies.

6. Hari Katepalli et al.2014. Surfactant and colloidal particles are often used to stabilize emulsion, surfactant are amphiphilic molecules; they minimize the energy required for the emulsion formation by reducing oil water interfacial tension. The combination of optical, confocal and cryogenic scanning electron microscopic were used to determine the final stability and structure of the emulsion.

V. MATERIAL

Ingredient use in aspirin-loaded nanoemulsion for enhance topical delivery:-

1. Sodium lauryl sulfate
2. Citric acid
3. Castor oil
4. Ethanol
5. Vanilline
6. Brilliant green

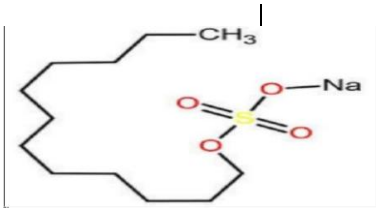
5.1.SODIUM LAURYL SULFATE:

Proper name:- Sodium lauryl sulfate

Chemical name:-Sodium dodecyl sulfate

Molecular formula:-$C_{12}H_{25}NaO_4S$

Molecular mass:-288.38g/mol



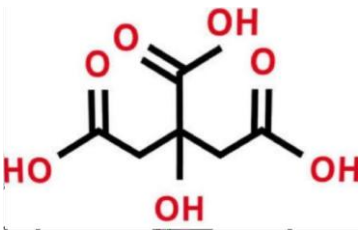
5.1 Sodium lauryl sulfate

Physicochemical properties**:-**
Physical state:- Powder or flake or crystal
Color:- White or slightly yellowish
Odour:- Characteristic
Solubility:- Soluble in water
Uses:- SLS use in surfactant and various application like cosmetic, pharmaceuticals, and cleaning products

5.2.CITRIC ACID :

Proper name :- Citric acid
Chemical name:- 2-hydroxy-pane-1,2,3-tricarboxylic acid Molecular formula:- $C_6H_8O_7$
Molecular mass:- 192.124g/mol

5.2 Citric acid

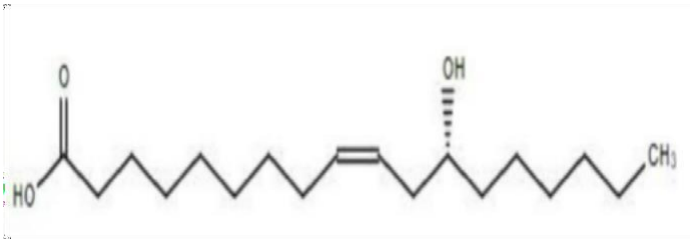


Physicochemical properties:-

Physical state:- White crystalline powder and granules
Odour:- Odorless
Taste:- Strongly taste
Solubility:- Very soluble in water P^H :- 3-6
Density:- 1.66g/cm^3
Uses:- Antioxidant, Lighten skin tone, P^H Balancer, Preservative.

5.3.CASTOR OIL:

Proper name:- Castor oil
Chemical name:- Glycerol triricinoleate Molecular formula :- $C_{18}H_{34}O_3$ Molecular mass:- 933g/mol

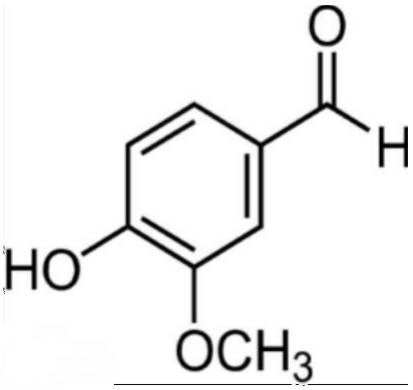


5.3Castor oil

Physicochemical properties:-
Physical state:- Viscous in liquid
Viscosity:- 889.3
centistokes Density:- 0.959g/ml
Refractive index:- 1.480
P^H:- 6.2-7.0
Color:- Yellow or White
Odour:- A faint characteristic
Uses:- Relieving constipation, moisturizing skin and hair, and reducing Inflammation.

5.4.VANILLINE:-

Proper name:- Vanilline
Chemical name:- Phenolic aldehyde Molecular formula:- $C_8H_8O_3$ Molecular mass:- 152.15g/mol



5.4Vanilline

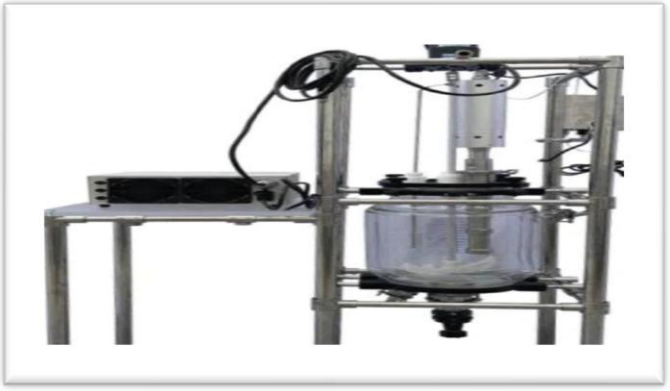
Physicochemical properties:

Physical state:- crystalline powder
Color:- White
Solubility:- Slightly soluble in water and readily soluble in ethanol
Density:- 1.056g/cm^3
$P^{H:-}$ 4.3
pKa:- 7.38
Uses:- As a flavouring agent, food beverages industry, product in like Ice cream and bake products.

VI. METHODOLOGY

Methods of preparation:

Ultrasonic emulsification- To break the big droplets into nano size, thus forming nanoemulsion. In this Technique premixed emulsion is exposed to agitation at ultrasonic frequency of 20 kHz reducing the droplets to nano droplets size. The emulsion is passed through high shear region to form droplets with uniform size distribution. The Water jacket Is regulate the temperature. Sonotrodes also known as sonicator probe consisted of piezoelectric quartz crystals as the energy provides during ultrasonic emulsification. On application of alternating electric voltage, these sonotrode contract and expand. Formulated emodin- loaded nanoemulsion by using ultrasonic emulsification method at a frequency of 20 kHz achieve mean diameter of emodine-loaded nanoemulsion was found to be in the range of 10 to 30 nanometer



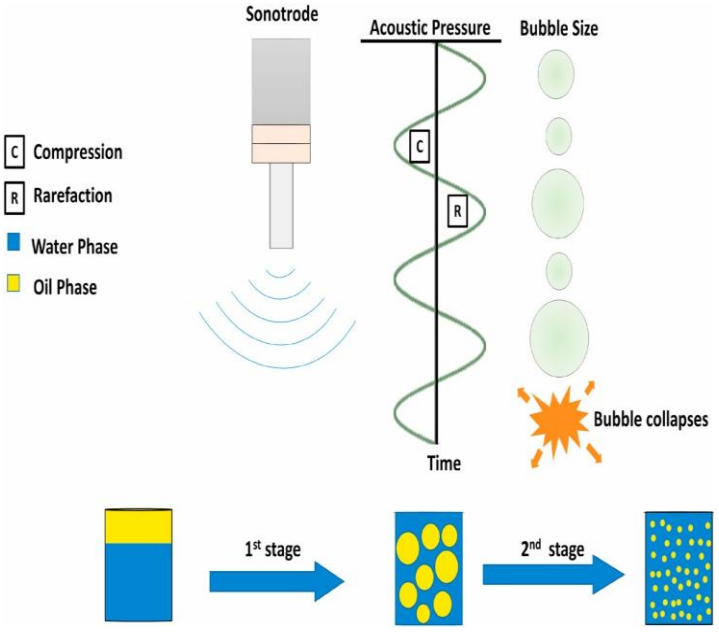


FIGURE:-6.1. ULTRASONIC EMULSIFICATION

VII. EVALUATION TEST

1.Characterization and Evaluation of Nanoemulsion:
Different characterization parameters for Nanoemulsion include thermodynamic stability studies, Scanning electron microscopy, Droplet size analysis, viscosity, refractive index, *in-vitro* skin permeation studies, skin irritation test .

2.Thermodynamic Stability Studies[10]:
To overcome the problem regarding the thermodynamic stability, stability study were performed, which are as follows:

3.Heating Cooling Cycle:
Heating and cooling cycle was done in refrigerator ranging the temperature between 4°C and 45°C for 48 hours. The formulations which were stable at these temperatures were subjected to centrifugation test

4.Centrifugation:
Centrifugation study for the selected formulations was done at 3500 rpm for 30 min. Formulations that did not show any phase separation were taken for the freeze thaw stress test

5.Freeze Thaw Cycle:
Three freeze thaw cycles were carried out between a temperature - 21°C and +25°C where the formulation was stored for not less than 48 hours at each temperature. Those formulations, which passed these thermodynamic stress tests, were selected for further study

6.Nanoemulsion Droplet Size Analysis:
Droplet size distribution of the Nanoemulsion was determined by photon correlation spectroscopy, which analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer 1000 HS (Malvern Instruments, UK). Light scattering was monitored at 25 °C at a 90° angle.Droplet size distribution studies were performed at a fixed refractive index of the respective formulation [11].

7.Polydispersity Index:
The average diameters and polydispersity index of samples were measured by photon correlation spectroscopy. The measurements were performed at 25^0 C using He-Ne laser.

8.Viscosity:
Brookfield DVE viscometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA) was used for the determination of viscosity of the formulations. About 0.5 g of sample was taken for analysis without dilution the

sample by using spindle no. 63 at different rpm at 25±0.5°C[12].

9.Refractive Index:
The refractive index of placebo formulation and drug loaded formulations was determined using an Abbe-type refractometer (Macro Scientific Works, Delhi, India)[13].
10.PH:
The apparent pH of the formulation was measured by using digital pH meter which is standardized previously[14].
11.Scanning Electron Microscopy (SEM):
Morphology and structure of the Nanoemulsion were studied using Scanning electron microscopy. It was used to reveal the form and size of Nanoemulsion droplets. Observations was performed as, a drop of the Nanoemulsion was directly deposited on the holey film grid and observed after drying.

12.Zeta Potential:
Zeta potential for microemulsion was determined using Zetasizer HSA 3000 (Malvern Instrument Ltd., UK). Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment[11].

13.In-vitro skin permeation studies
In-vitro skin permeation studies were performed on a Franz diffusion cell with an effective diffusion area of 3.14 cm^2 and 25 mL of receiver chamber capacity, using rat abdominal skin. The full thickness of rat skin was excised from the abdominal region and hairs were removed with an electric clipper. The subcutaneous tissue was removed surgically and the dermis side was wiped with Ethanol to remove adhering fat. The cleaned skin was washed with distilled water and stored at –21 °C until further use. The skin was brought to room temperature and mounted between the donor and receiver compartments of the Franz diffusion cell where the stratum corneum side was facing the donor compartment and the dermal side was facing the receiver compartment. Initially, the donor compartment was empty and the receiver chamber was filled with phosphate buffer saline (PBS) pH
7.4. The receiver fluid was stirred with a magnetic rotor at a speed of 100 rpm. After complete stabilization of the skin, 5 mL nanoemulsion formulation (20 mg mL–1 Asprin or 1 g of Asprin (20 mg g^{-1}) was placed into the donor compartment and sealed with paraffin film to provide occlusive conditions. Samples were withdrawn at regular intervals (0.5,1, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 24 h) filtered through 0.45mm membrane filter and analyzed for drug content by using UV- Visible spectroscopy at 245nm[15].

14.Permeation Data Analysis:
The permeation profiles were constructed by plotting the cumulative amount of Asprin permeated per unit dialysis membrane area (g/cm^2) versus time. Linear regression analysis was used to calculate the steady state flux (Jss, g/cm^2/hr) (20) Asprin by using the slope of the plot. The following equation was used to determine the permeability co-efficient (Kp) of the drug through the stratum corneum[16].
Kp = Jss/c
Where,
C is the initial concentration of the drug in the donor compartment.

The penetration enhancing effect was calculated in terms of enhancement ratio (ER) by using the following equation[12].
Er = Jss of formulation/Jss of control
15.Skin irritancy test:
Skin irritancy test was done on male Swiss albino mice, weighing 25–30 g. The animals were kept under standard laboratory conditions, temperature (25±1°C) and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, six per cage, with free access to standard laboratory diet (Lipton Feed, India) and water ad libitum. A single dose of 10 µL of the Nanoemulsion was applied to the left ear of the mice, with the right ear as a control. The development of erythema was monitored for 6 days[17].

VIII.RESULTS AND DISCUSSION

8.1.Solubility of Aspirin.:

The maximum solubility of Aspirin was found in water (1.22±0.145mg/ml) as compared to other oils. High drug solubility was found in SLS (5.49 ± 0.120 mg/ml) and Ethanol was selected as co- surfactant as it forms stable Nanoemulsion, also acts as permeation enhancer. Therefore, SLS and Ethanol were selected as surfactant and cosurfactant, respectively, for the phase study.

Table 01: Solubility of Aspirin **in oils.**

Oil	Concentration of drug (mg/ml)
Castor Oil	0.521 ± 0.010
Citic acid	0.358 ± 0.014

Mean ± SD, n=3

Table 02 :Solubility of Aspirin **in surfactants**.

Surfactant	Concentration of drug (mg/ml)
SLS	0.521 ± 0.010

Table 03: Composition of selected Nanoemulsion formulations

A total of 11 formulations were selected based on their ability to form oil in water Nanoemulsions which are selected from pesudoternary phase diagram of each Smix as shown in the table no.3.

			Component %(w/w)		
Code	**Oil/Smix ratio**	**Oil/Smix**	**Oil**	**Smix**	**Water**
NE1	1:1	1:4	10	40	50
NE2	1:1	1:5	10	50	40
NE3	1:2	1:2	15	35	50
NE4	1:2	1:3	15	45	40
NE5	2:1	1:4	10	40	50

8.2.Dispersion stability studies:

Nanoemulsions are thermodynamically and physically stable systems and are formed at a particular concentration of oil, surfactant and water, making them stable to phase separation, creaming or cracking. It is the thermo stability that differentiates Nanoemulsion from emulsions with kinetic stability and eventually phase separation. Thus, the formulations were tested for their physical (dispersion) stability by using centrifugation, heating-cooling cycle and freeze-thaw cycle. Only those formulations which survived dispersion stability tests were selected for further study. The compositions of selected formulations are given in table 4. Except NE2 and NE4remaining all the formulation were passed the stability tests.

Table 04: Stability studies of formulations.

Code	**Heating and cooling**	**Centrifugation**	**Freeze-thaw cycle**	**Inferences**
NE1	Pass	Pass	Pass	Pass
NE2	Pass	Pass	Pass	Pass
NE3	Pass	Pass	Pass	Pass
NE4	Pass	Pass	Pass	Pass
NE5	Pass	Pass	Pass	Pass

8.3.Droplet size measurements:

The mean droplet size and polydispersity index were calculated from intensity, volume and bimodal distribution assuming spherical particles. All the Nanoemulsion had small average droplet diameter between 10 to 100 nm. A small droplet sizes are very much prerequisite for drug delivery as the oil droplets tend to fuse with the skin thus providing a channel for drug delivery. Polydispersity index (PI) is a measure of particle homogenicity and it varies from 0.0 to 1.0. The closer to zero the polydispersity value the more homogenous are the particles. Formulations showed their PI in between 0.314 to 0.91 that indicates acceptable homogenicity. Zeta potential of all Nanoemulsion formulation was found between -9.22 to -0.044 mV in the 100 times diluted (Table 06). Nanoemulsion formulation consists of non-ionic components which show relatively neutral charge, it means it will not affected by body membrane charge during absorption.

Table 05: Droplet size analysis and zeta potential

Code	Mean Particle size (nm)	Polydispersity index	Zeta potential
NE1	42.91 ± 7.16	0.366	-6.8
NE2	28.6 ± 9.0	0.314	-9.22
NE3	50.67 ± 6.03	0.44	-4.71
NE4	65.83± 11.25	0.621	-4.31
NE5	80.61 ± 13.45	0.81	-5.21

8.4.Viscosity:

Formulation NE2 had the least viscosity (27.33 ± 1.15cps) compared to other formulation. This may be due to the lower oil content. The difference in viscosity between formulations NE1 and NE2 was not significant. Viscosity of all Nanoemulsion formulations was very low as expected..

8.5.PH of Nanoemulsion formulations:

The pH value of all developed Nanoemulsion formulations was in the range of 6.7-7.21, which is well within the limits of skin pH i.e. 5.6-7.5. Hence, it was concluded that all the formulations could not produce any local irritation to the skin. The results are shown in table 06.

Table 06: viscosity and pH of Nanoemulsion formulation

SAMPLE CODE	VISCOSITY (cP)	pH
NE1	31.66 ± 1.52	7.16 ± 0.11
NE2	27.33 ± 1.15	6.93 ± 0.05
NE3	40 ± 2	6.86 ± 0.05
NE4	50.66 ± 3.05	6.7 ± 0.10
NE5	34 ± 2	7.06 ± 0.11

Mean ± SD, n=3

8.6.Refractive index:

Refractive index of placebo formulations and drug loaded formulations was determined using an Abbes refractometer. The values of the refractive index of drug loaded formulations and placebo formulations are given Table 7. Therefore it can be concluded that the Nanoemulsion formulations were not only thermodynamically stable but also chemically stable and remained isotropic. Thus there were no interactions between Nanoemulsion excipients and drug.

Table 07: Refractive index of Nanoemulsion formulations

SAMPLE CODE	Refractive Index of Formulation	Refractive Index of Placebo formulation
NE1	1.324 ± 0.021	1.326 ± 0.001
NE2	1.313 ± 0.001	1.314 ± 0.001
NE3	1.328 ± 0.02	1.329 ± 0.01
NE4	1.333 ± 0.002	1.335 ± 0.001
NE5	1.371 ± 0.001	1.372 ± 0.002

Mean ± SD, n=3

IX. CONCLUSION

The optimized formulation contained 10 % of oil phase (citric acid), 50 % of surfactant mixture (SLS as surfactant and Ethanol as co-surfactant) and 40 % of distilled water. From the studies, it is observed that the formulated Nanoemulsions and Nanoemulsion gel released up to 87.63 and 76 % of the drug, respectively. The formulation was nonsensitizing and safe for use prepared with non-irritating, pharmaceutically acceptable ingredients. No additional permeation enhancers were needed to be added since the excipients themselves acted as permeation enhancers. A high percent inhibition of edema was observed with the N (72 %) as compared with the normal Aspirin (38 %). Thus, it can be concluded that the developed Nanoemulsion-based gel have a greater potential for topical drug delivery as compared to conventional formulations.

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