DESIGN AND CHARACTERIZATION OF HERBAL NANOEMULGEL FOR ECZEMA

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Abstract: *The aim of present research is to design and develop topical nanoemulgel of curcumin as effective treatment for eczema. Various oil-in-water nanoemulsions are prepared by the spontaneous emulsification method. Further, curcumin (a natural bioactive) was selected as a therapeutic agent to incorporate in to the hydrogel system to design & develop nanogel pharmaceutical products for Eczema. Curcumin possesses remarkable anti-inflammatory, anti-oxidant and anti-infective activity. Topical nanoemulsion containing 1% curcumin with different oils (oleic acid), surfactant (tween 80), co-surfactant (PEG 400) and distilled water. The nanoemulsion formulations that passed thermodynamic stability tests were characterized for appearance, pH, FTIR, Viscosity, Drug content, % Drug entrapment efficiency & In-Vitro drug diffusion study of curcumin determined by Franz diffusion cell and stability study. Nanoemulsion gel were developed by incorporation of optimized nanoemulsion in to HPMC k15 and Carbopol 934 and characterized by physical evaluation and Rheological studies.*

Keywords: *Curcumin, Thermodynamic stability study, XRD, in vitro drug diffusion.*

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INTRODUCTION

An emulsion is defined as a system containing two immiscible phases, which consists of a dispersed phase as droplets (internal phase) and a continuous phase (external phase).[1] Nanoemulsion/Sub-micron emulsion (SMEs)/Mini-emulsion are thermodynamically stable transparent or translucent dispersion of oil and water stabilized by an interfacial film of surfactant and co-surfactant molecules having a globule size of less than 100 nm.The international union of pure and applied chemistry (IUPAC) has defined nanoemulsions as dispersion made of water, oil and surfactants(s) that consists of an isotropic and thermodynamically stable system with dispersed domain diameter, with the particles formed at the nano scale by means of mechanical forces.[2]

The term "emulsion" was further explained by Alec D. Bangham and L.L. Schramm's idea of "nanoemulsion". A colloidal dispersion of liquid Crystals in a liquid is referred to as an "emulsion". Nanoemulsion are novel drug delivery systems that consists of thermodynamically stable oil& water dispersion with droplet size ranging from 5nm to 500nm.[3]

Curcumin is a natural product, isolated from the rhizomes of curcuma longa plant belongs to family Zingiberaceae and it is used orally and topically in the treatment of Eczema.Curcumin is a popular spice and colouring agent consumed worldwide. Traditionally the spice has been employed to treat numerous ailments. Turmeric, a spice that has long been recognized for its medicinal properties, has received interest from both the medicinal/scientific world and from culinary enthusiasts, as it is the major source of the polyphenol curcumin.

It aids in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidaemia may also help in the management of exercise-induced inflammation and muscle soreness, thus enhancing recovery and performance in active people. Turmeric is native to Southeast Asia and India. Turmeric is also regarded as a 'rasayana' herb, which is a branch of Ayurvedic

medicine[4]. Curcumin is the active ingredient in turmeric which has been shown to have a wide range of therapeutic effects.[5,6]

Eczema (also known as atopic eczema or atopic dermatitis) is a common and chronic, relapsing inflammatory skin disorder, characterised by intense pruritusand excoriation, with erythematous, xerotic, lichenified, fissured skin, and an increased risk of skin infection. The word "eczema" is derived from Greek word meaning "to boil over", which is a good description for the red, inflamed, itchy patches that occur during flare ups.

MATERIALS

Curcumin sample obtained from yarrow chem products. 215, Second Floor, Swastik Corporate Park, Opp. Shreya Talkies, L B S Marg, Ghatkopar (West), MUMBAI-400086. Oleic acid from Hi Media Laboratories, Tween 80 from Lobachem, Mumbai, PEG 400 from Thermo fisher scientific India pvt.ltd., Carbopol 934 from Lobachem, Mumbai, HPMC K15 from Meherchemi, Mumbai, Methyl Paraben from Ozone international, Glycerine from Vikash pharma, Triethanolamine from Molychem Thane, Mumbai.

METHODS

Preformulation study

Preformulation studies assess the physicochemical properties of a drug candidate, aiding in formulation design and molecular modifications. These properties determine how drugs interact with ingredients in dosage form development, aiming for an elegant, stable, effective, and safe product. For Curcumin, preformulation investigates its properties and interactions with other components, confirming it as the active ingredient. Early analysis guides the selection of appropriate formulation components.

Physical appearance

The physical appearance of the supplied curcumin sample was closely observed.

Determination of Melting point

The melting point of curcumin was determined by heating a small amount of the substance in a capillary tube and noting the temperature at which it melted using a thermometer.

Solubility study

Curcumin was added to various solvents (water, methanol, ethanol, chloroform, ether, castor oil, isopropyl myristate, oleic acid, tween20, 80, PEG200, PEG400) at room temperature and left for 24 hours with intermittent shaking. The supernatant was then evaluated using a UV spectrophotometer.

Estimation of curcumin by UV-Spectroscopy method

A phosphate buffer of pH 7.4 was prepared. A stock solution of curcumin was prepared by dissolving 100 mg of curcumin in a small amount of phosphate buffer to make a concentration of 1 mg/ml. Calibration curves were then constructed using different concentrations of the stock solution.

FTIR of drug and excipients

Curcumin was analyzed using FTIR spectroscopy, scanning it over the range of 4000-400 cm-1 to identify characteristic peaks that comply with reference spectra. Infrared spectra were generated with an FTIR spectrophotometer.The spectra were obtained using KBr pellet technique. The KBr pellets were prepared by 10 mg sample mixed with 200 mg potassium bromide at high compaction pressure. Thus, the prepared pellets were scanned at a resolution of 4000 cm -1 to 400 cm -1.

Preparation of nanoemulsion by spontaneous emulsification method [9,10]

Depending on the phase diagrams, Curcumin loaded Nanoemulsion were formulated at the different constituent's ratio by the spontaneous emulsification method. Appropriate quantities of oil (oleic acid), surfactant (tween 80) and co-surfactant (PEG 400) were weighed and mixed well. Curcumin was precisely weighed to represent 1% w/w of the total weight of the Nanoemulsions formulation, and then added to the previous mixture and stirred with a Homogenizer (3600 rpm), at room temperature (25°C+0.5) until the drug is entirely dissolved. The weighed amount of water then added drop wise to the oil phase with continuous mixing magnetic stirring for 30 min.

Table 1: Formulation of nanoemulsion

Formulations No	Sur.mix (ratio)	Oil/S.mix (ratio)	%w/w of components in Nanoemulsion formulation			Drug %w/w
			Oil	Smix	Water	
NF1	1:1	1:9	5	45	50	1
NF2	1:1	1:8	5	40	55	1
NF3	1:2	1:9	5	45	50	**1**
NF4	1:2	1:8	5	40	55	1
NF5	2:1	1:9	5	45	50	1
NF6	2:1	1:7	5	35	60	1
NF7	1:1	1:7	5	35	60	1
NF8	2:1	1:8	5	40	55	1

Figure 1: Nanoemulsion Formulation

Formulation of curcumin loaded nanoemulsion gel

Optimized nanoemulsion was testing for stability study for 3 months, stable nanoemulsions was transfer to gel from using Carbopol 934 and HPMC k15 in various concentration and analyse for different evaluation.

Nanoemulsion base gel was prepared by dispersing the weight amount of the Carbopol-934 and HPMC k15 in a sufficient quantity of distilled water. After complete dispersion, the solution was kept in dark for 24 hrs for complete swelling of Carbopol-934. The Carbopol and HPMC dispersion was mixed with selected formulations containing 1 gm of curcumin in excipients. The mixture was stirred well to get homogeneous solution so that concentration of Carbopol 934 will become 0.5% w/w. the appropriate

amount of triethanolamine was added to maintain the pH with continuous stirring to get homogeneous gel.

Table 2: Formulation of nanoemulsion gel

Formulacode	Nano Emulsion (ml)	Gelling agent %W/V		Water(ml)	Methyl Paraben (ml)	Glycerin (ml)	Triethanol Amine (ml)
		Carbopol 934	HPMC K15				
NEG1	50	1	1	50	0.1	5	Q. S
NEG2	50	1	1.5	50	0.1	5	Q. S
NEG3	50	1	2	50	0.1	5	Q. S
NEG4	50	1	2.5	50	0.1	5	Q. S

Evaluation of curcumin loaded nanoemulsions [7, 8]

Thermodynamic stability

The selected formulation is subjected to different thermodynamic stability tests.

Heating cooling cycle

The temperature of refrigerator between 4°C and 45°C of six cycles with storage at each temperature of not less than 48 hr is studied. Those formulations, which are stable at this temperature, are subjected to centrifugation.

Centrifugation

The prepared formulations that are passed for centrifugation are centrifuged at 5000 rpm for 30 min by using centrifuge. The formulations that did not show any phase separated were taken to further tests.

Measurement of pH

Ph of various nanoemulsions formulations is determining by using digital pH meter. 1 gm of nanoemulsion is dissolved in 100 ml of distilled water and pH was measured. The measurement of formulation is done in triplicate to avoid error.

Percentage drug content

1ml of nanoemulsion is mixed with 10ml of suitable solvent. Aliquots of different concentration are prepared and by using suitable dilutions after filtering the stock solution; absorbance is measured by UV spectroscopy. Drug content is calculated by using the equation obtains from linear regression analysis of calibration curve.

Viscosity determination

Viscosity of nanoemulsion is determined by using Brookfield viscometer. 20 ml of nanoemulsions filled in a 25 ml beaker and the viscosity is measured using spindle number 6 at 10 rpm.

In vitro Diffusion studies

The diffusion studies of the prepared nanoemulsions are performed by using Franz diffusion cell with the aid of cellophane membrane. Nanoemulsion sample (5ml) is taken in cellophane membrane and the diffusion studies are carried out at 37±1°C using 250 ml of (25%) ethanolic phosphate buffer (pH 7.4) as the dissolution medium. 5ml of each sample was withdrawn periodically at 1,2,3,4,5,6,7, and 8 hrs and each sample is replaced with equal volume of fresh dissolution medium in order to maintain sink conditions. Samples are analysed by UV- spectrophotometer at 425 nm for drug content.

X-Ray diffraction analysis

XRD was used to analyse drug crystallography.

Zeta potential
The formulation of nanoemulsion was tested for zeta potential using Malvern Zetasizer instrument. The analysis was carried out at 25°C.

Evaluation of nanoemulsion gel
Appearance
The formulation was observed for the presence of any particular matter. Clarity is one of the most important features of topical preparations. The appearance and clarity are determined by visual testing.

pH
pH of formulated nanoemulsion gel was ranged between 5.5-6.5. The pH of nanoemulsion gel formulation is determined by digital pH meter.
Drug content
Nanoemulsion gel, 2 gm from each formulation were taken in 100 ml volumetric flask having 10ml ethanol and stirred by magnetic stirrer for 5 minutes. The solutions were filtered using Whatmann filter paper. The absorbance of the solution was estimated spectrophotometrically (UV 1800, Shimadzu) at 425 nm using standard curve against blank.

Determination of viscosity
Viscosity of nanoemulsion gel was determined by using Brookfield viscometer. 20 gm of nanoemulsion gel was filled in a 25 ml beaker and the viscosity was measured by using spindle number S6.

Spreadability
It is determined by apparatus which consists of a wooden block, which is provided by a pulley at one end. The spreadability is measured on the basis of 'slip' and 'Drag' characteristics of nanoemulsion gel. A ground glass slide is fixed on this block. An excess of nanoemulsion gel (about 2g) under study is placed on this ground slide. The nanoemulsion gel is then sandwiched between this slide and another glass slide. A 1 kg weight is placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the nanoemulsion gel between the slides. By putting weight of 1kg, the time (in seconds) required by the top slide to cover a distance of 7.5 cm with the help of stirring attached to the hook is noted.
A shorter interval indicates better spreadability, which is calculated by the formulae:
S=M.L/T

In vitro drug diffusion study
The diffusion studies of the prepared nanoemulsion gel were carried out in Franz diffusion cell through a cellophane membrane. Gel sample (0.5g) was taken in cellophane membrane and the diffusion studies were carried out at 37±1°C using 250 ml of (25%) ethanolic phosphate buffer (pH 7.4) as the dissolution medium.5ml of each sample was withdrawn periodically at 1,2,3,4,5,6,7, and 8 hrs and each sample was replaced with equal volume of fresh dissolution medium in order to maintain sink condition. Samples were analysed by UV-visible spectrophotometer at 425 nm for drug content.

Stability study
Formulation of nanoemulsion gel is placed in collapsible tubes with proper sealing and for a short term accelerated stability study at 40±2°C, 75±5%RH as per ICH Guidelines. The formulation was withdrawn after particular period of interval; the physical stability was evaluated by visual inspection for physical changes (such as phase separation and drug precipitation) and evaluated for drug content and in vitro drug diffusion.

RESULTS AND DISCUSSION

Organoleptic properties

Organoleptic properties of Curcumin are found to be as per USF NF monograph.

Table 3: Organoleptic Properties

Properties	Specification as per IP 1996	Result
Colour	Yellow	Yellow
Odour	Characteristics	Characteristics
Nature	Amorphous/ Crystalline	Amorphous

Melting point analysis

The melting point of Curcumin was observed to be 172°C-180°C Using a glass capillary method.

Solubility

The solubility study of curcumin in different solvent was found to be, curcumin was soluble in ethanol and methanol, soluble in oleic acid, tween 80 and PEG 400, slightly soluble in Benzene, Acetone, Insoluble in Ether. Therefore, oleic acid was used as oil phase, tween 80 as surfactant and PEG 400 as co-surfactant in pseudo-ternary phase diagram for preparation of nanoemulsion.

Table 4: Solubility study

Sr.no.	Solvent system	Specification as per reference standard	Result
1.	Ethanol	Soluble	Soluble
2.	Oleic acid	Soluble	Soluble
3.	Tween 80	Soluble	Soluble
4.	PEG 400	Soluble	Soluble
5.	Methanol	Soluble	Soluble
6.	Water	Insoluble	Insoluble
7.	Chloroform	Soluble	Soluble
8.	Ether	Insoluble	Insoluble

Identification of the drug by UV spectroscopy

The UV spectrum of Curcumin in buffer solution pH 7.4 in the range of 200-600nm. The spectrum indicates that the absorption ʎ max of curcumin was 425 nm which is match with ʎ max given in Indian Pharmacopoeia.

Table 5: Standard calibration curve of Curcumin

Conc.(µg/ml)	Absorbance (ʎmax)
2	0.205
4	0.461
6	0.695
8	0.985
10	1.191

UV shows that Curcumin gives maximum absorption at 425 nm and figured in linear standard calibration curve shown in fig no.4

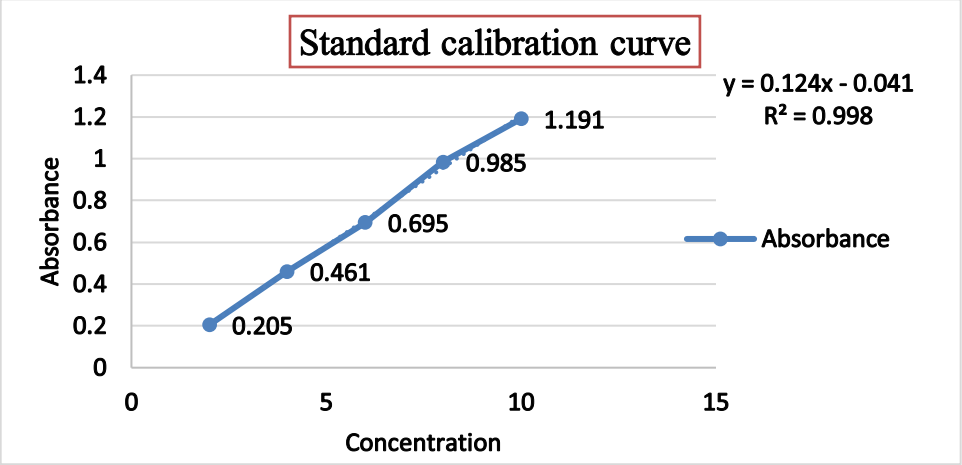

Figure 2: Standard calibration curve of curcumin

Identification of drug by FTIR spectroscopy

The IR spectrum of the pure curcumin sample recorded by FTIR spectrophotometer is shown in fig no. 5. The frequencies of the functional group of curcumin in the reported range which indicates that the obtained sample was of curcumin and was pure.

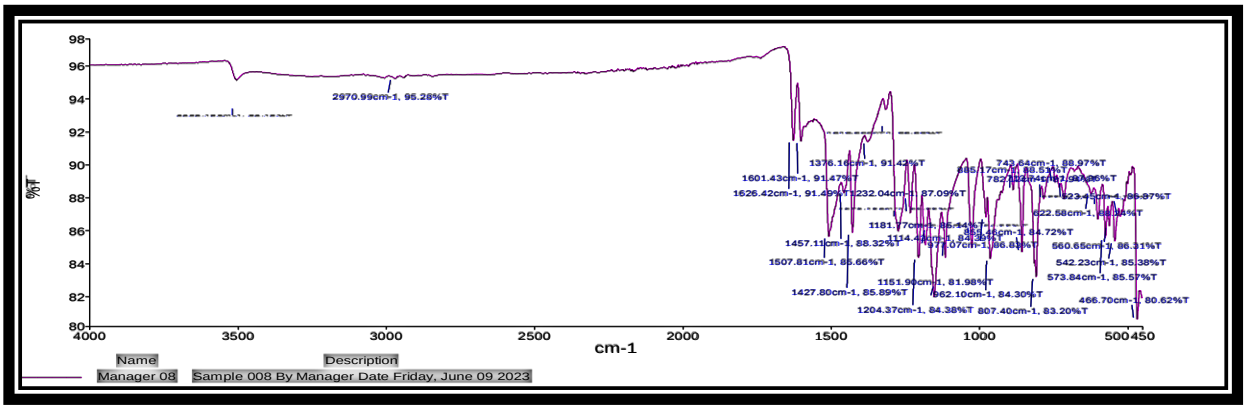

Figure 3: FTIR of Curcumin

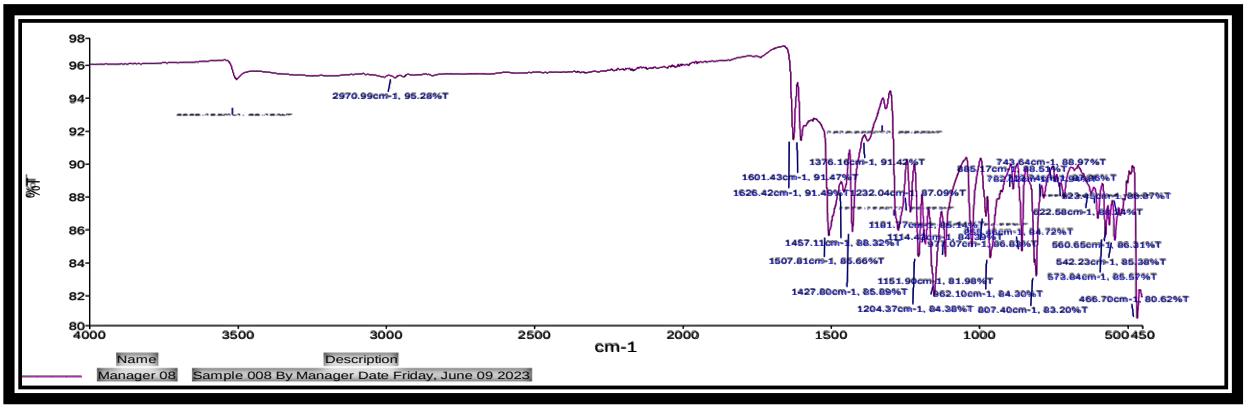

Figure 4: FTIR of drug+ excipients

Form the ftir study drug compatabile wirth all the excipients.

Nanoemulsion Characterization
Thermodynamic stability

Table 6: Thermodynamic stability study

Formulation Code	Heating cooling cycle	Centrifugation
F1	Stable	No phase separation
F2	Stable	No phase separation
F3	Stable	No phase separation
F4	Stable	No phase separation
F5	Stable	No phase separation
F6	Stable	No phase separation
F7	Stable	No phase separation
F8	Stable	No phase separation

pH, Viscosity and % Drug content

Table 7: Appearance pH and drug content nanoemulsion formulation

Formula code	pH	Viscosity (cp)	Drug content (%)
F1	5.2±0.3	4221	78.55
F2	5.7±0.3	4830	83.87
F3	6.3±0.2	5040	90.12
F4	5.2±0.3	4325	82.48
F5	5.5±0.3	4332	76.37
F6	5.4±0.2	4556	81.12
F7	5.3±0.2	4470	80.06
F8	5.0±0.3	4585	79.56

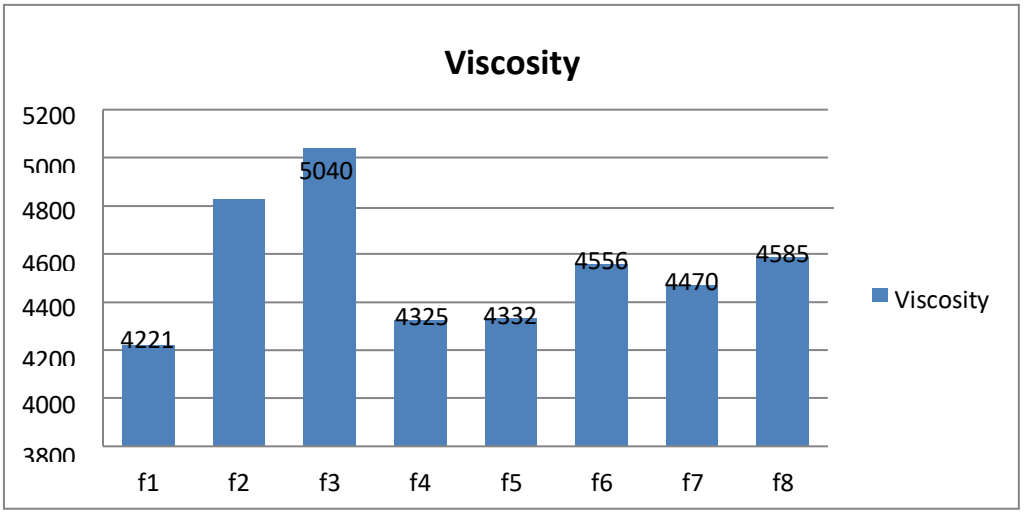

Figure 5: Viscosity of NE

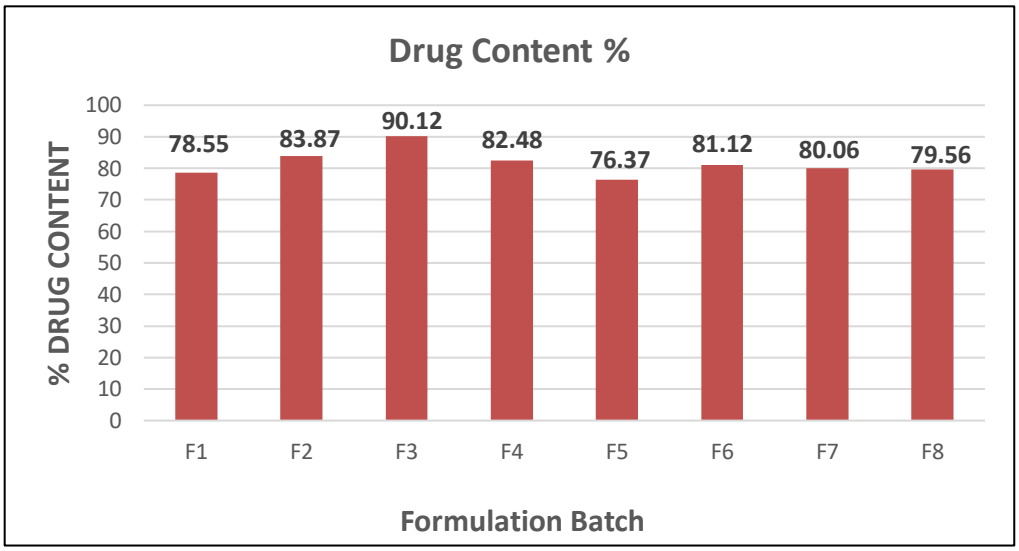

Figure 6: %Drug content of NE

SEM

A SEM and TEM images of microscopic evaluation of optimized formulation F3 was found to be spherical shape of droplets as shown below figure 8 &10. Nanoemulsion prepared by using tween 80 and PEG 400 with the ratio of 1:2, the size of nanoemulsion was found to be 20-200nm shown in figure no. 8.

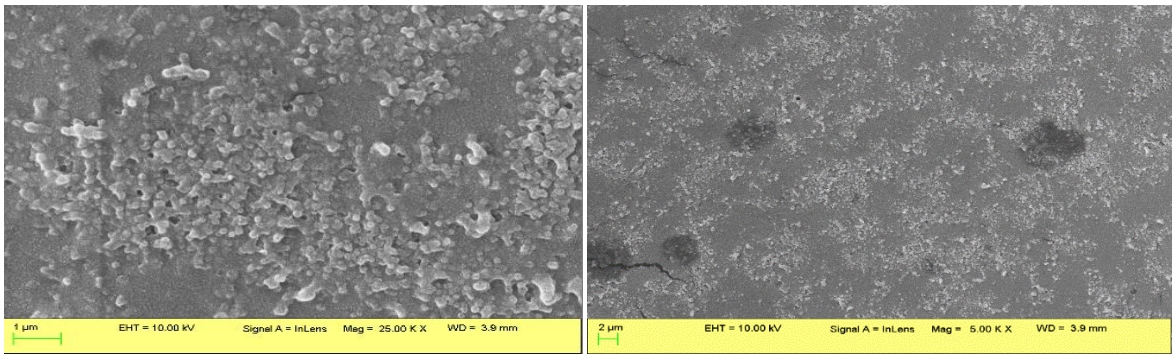

Figure 7: SEM images of optimized nanoemulsion formulation

Microscopic Study

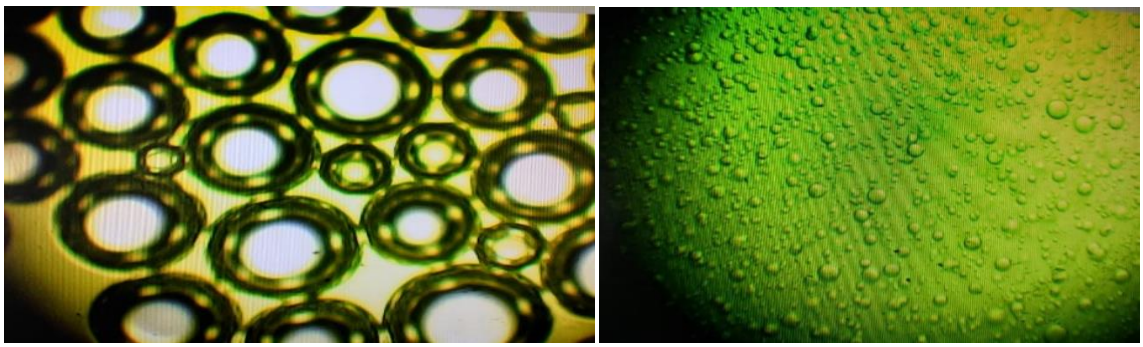

Figure 8: Microscopic images of nanoemulsion formulation

Tem

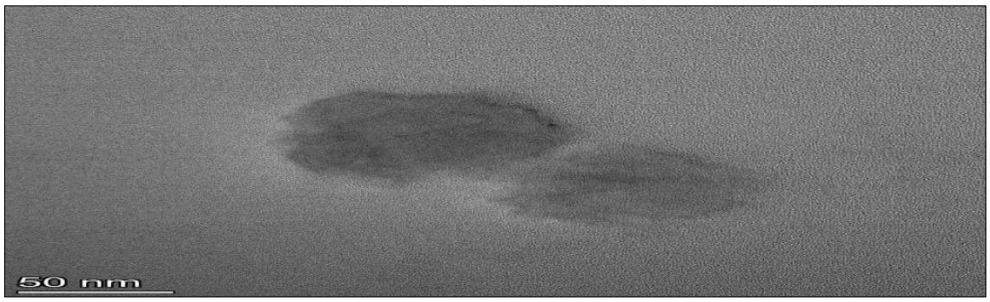

Figure 9: TEM image of optimized nanoemulsion formulation

XRD

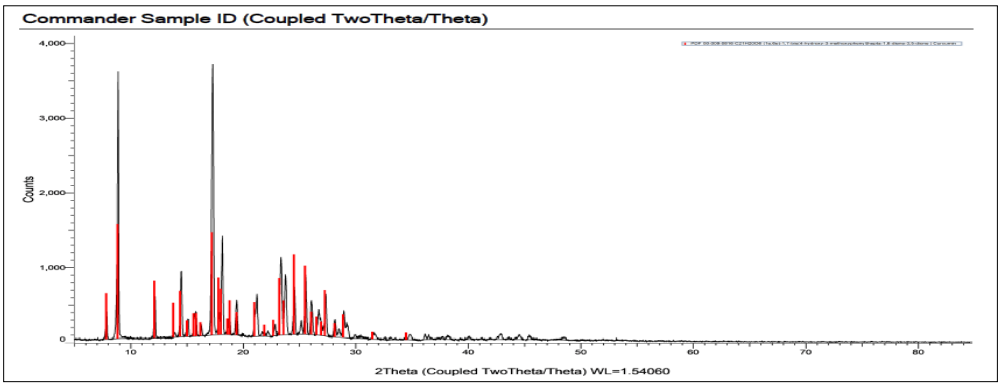

Figure 10: XRD image of optimized nanoemulsion formulation

Form the XRD it was observed that curcumin was found crysatalline in nature

Zeta Potential

Zeta potential of nanoemulsion was shown in fig. no. 2 zeta potential value of F3 was found to be –0.5mv. The surface charge on nanoparticles was determined by Zeta potential. Values in the range of -30mv to +30mv of either charge characterize stable formulations.

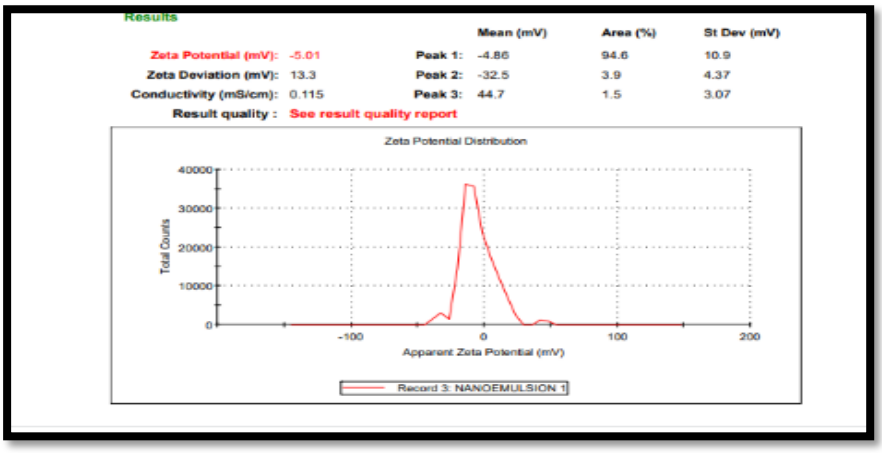

Figure 11: Zeta Potential

Evaluation of nanoemulgel

Table 8: Evaluation parameter of nanoemulgel

Sr. no.	Formulation Code	Appearance& Colour	Viscosity (cps)	pH	Homogeneity	% Drug content	Spreadability (gms.cm/sec)
1	NEG1	Transparent Yellow	43400	6.5	Homogeneous	78.88	57.4
2	NEG2	Transparent Yellow	46567	6.4	Homogeneous	87.90	40.84
3	NEG3	Transparent Yellow	52500	6.5	Homogeneous	89.23	68.5
4	NEG4	Transparent Yellow	65300	6.3	Homogeneous	80.45	59.68

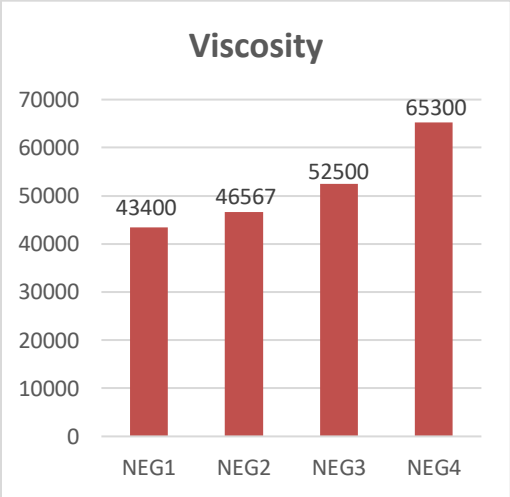

Figure 12: Viscosity of NEG

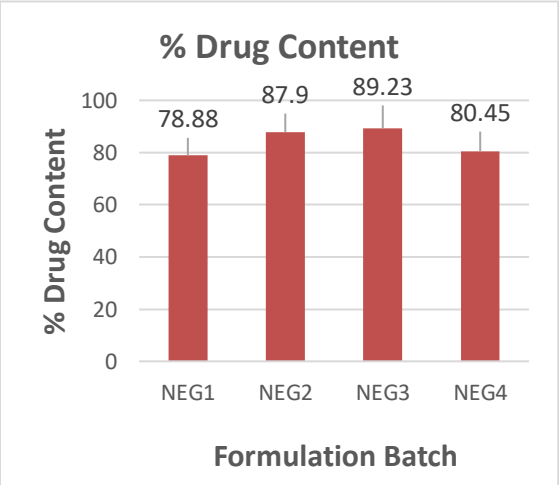

Figure 13: % Drug content of NEG

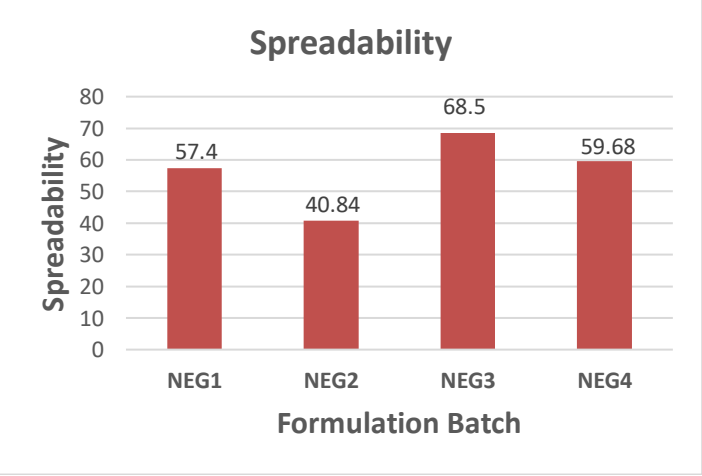

Figure 14: Spreadability of NEG

In vitro diffusion studies

Table 9: In vitro drug diffusion study

% Cumulative Drug diffusion Study					
Time in (hrs)	**NEG1**	**NEG2**	**NEG3**	**NEG4**	**Conventional gel**
0	0	0	0	0	0
1	6.54±0.3	10.70±0.3	11.32±0.2	9.79±0.4	5.85±0.2
2	15.68±0.2	14.50±0.1	16.54±0.5	14.77±0.3	10.86±0.4
3	20.89±0.3	19.86±0.2	23.89±0.4	21.66±0.2	15.56±0.5
4	39.75±0.5	40.69±0.3	42.62±0.2	38.43±0.4	22.37±0.2
5	50.51±0.2	53.39±0.5	57.53±0.3	54.12±0.2	35.34±0.3
6	61.72±0.1	60.55±0.3	65.23±0.4	60.05±0.4	42.21±0.2
7	79.25±03	71.15±0.2	86.57±0.1	75.17±0.3	49.20±0.3
8	81.37±0.2	83.93±0.1	91.93±0.3	85.63±0.1	54.23±0.2

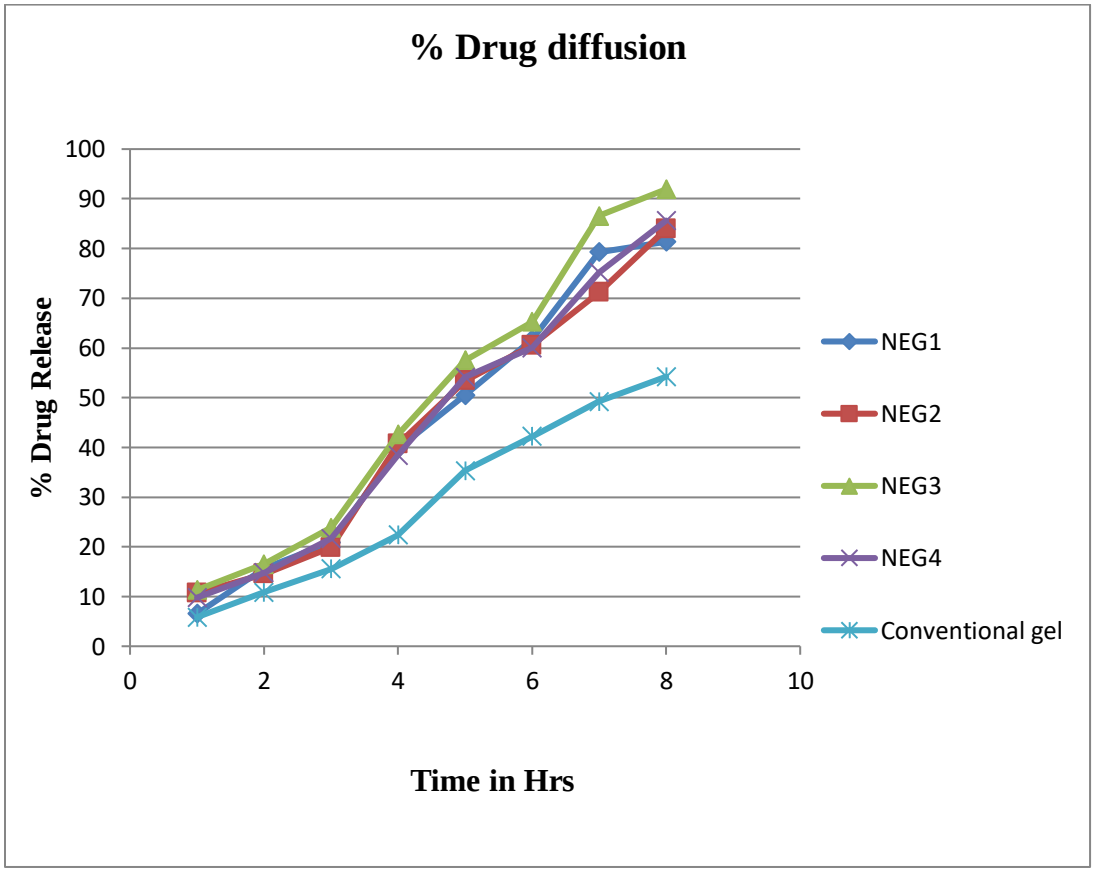

Figure 15: In Vitro Drug Diffusion

Stability studies

The accelerated stability study of nanoemulsion gel batch 3 was carried out; there is no significant change in pH, drug content and % drug diffusion in NEG3 formulation at 40°C temperature and 75% RH. The

result is shown in table no.10

Table 10: Stability Study of NE gel

Parameters	**Initial**	**1 Month**	**2 Month**	**3 Month**
Appearance	Transparent yellow	NC	NC	NC
Viscosity	52500	NC	NC	NC
pH	6.5	6.3	6.2	6.1
Drug content	89.23	88.55	87.02	86.32

NC= No Change

Table 11: In vitro drug diffusion study after stability

Times(hrs.)	**Month 1**	**Month 2**	**Month 3**
0	0	0	0
1	8.23±0.2	8.19±0.3	8.18±0.4
2	20.54±0.4	19.33±0.4	18.33±0.2
3	31.64±0.3	30.01±0.5	29.34±0.3
4	46.6±0.2	45.32±0.3	44.54±0.2
5	63.35±0.5	62.12±0.2	61.24±0.4
6	76.23±0.1	75.23±0.4	74.18±0.3
7	86.57±0.5	85.01±0.1	84.43±0.2
8	92.93±0.2	90.57±0.3	89.87±0.1

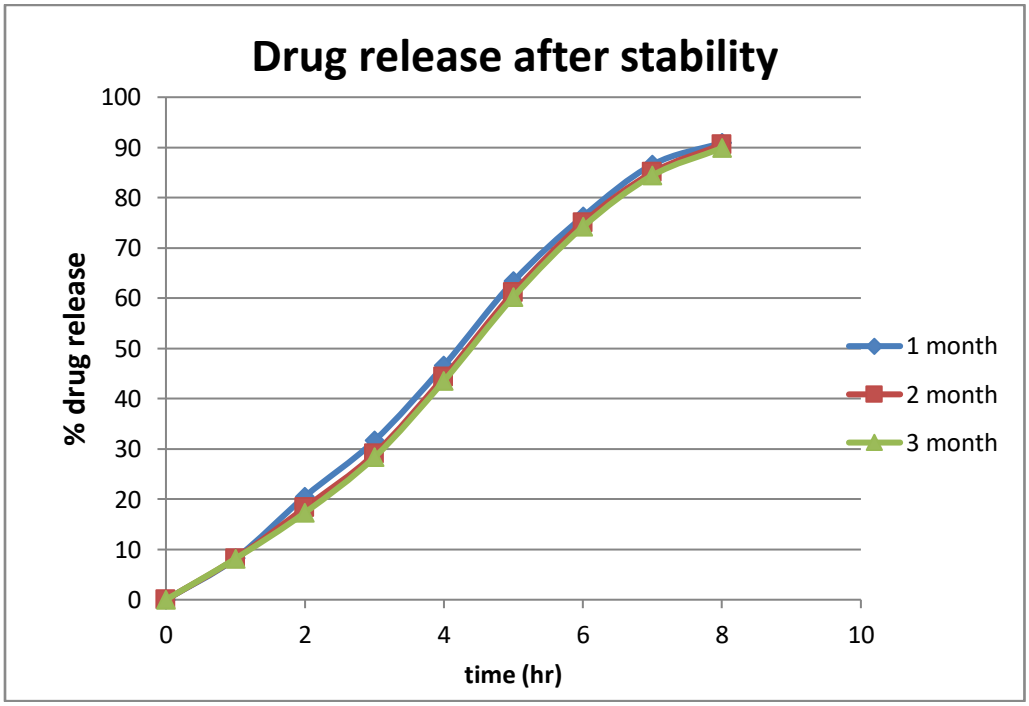

Figure No. 16: In vitro drug diffusion study after stability

CONCLUSION

Curcumin loaded nanoemulsion was prepared by the spontaneous emulsification technique. FTIR spectrum revealed that drug compatibles with the excipients. The formulated nanoemulsion was evaluated and optimized nanoemulsion was chosen for the preparation of nanoemulsion gel. All the formulations were evaluated for pH, drug content, viscosity, spreadability, drug diffusion study and accelerated stability study and results were within the limits. Among all the formulations of nanoemulsion F3 was the best formulation, and it was further converted to gel form. The optimized formulation of nanoemulsion gel i.e., NEG3 showed better drug content, In-vitro drug diffusion (as compared to conventional gel) and good anti-inflammatory, anti-oxidant, anti-bacterial activity. The stability studies carried out for three months and the formulation was found to be stable. It can be concluded that Curcumin nanoemulgel can be better alternative for conventional topical gel and effectively used for the treatment of Eczema.

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