

## Experimental design and characterization of nanoemulsion based topical herbal gel developed for site-specific activity of *Glycyrrhiza glabra* extract: *In vitro* and *Ex-vivo* studies

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### ABSTRACT

*Glycyrrhiza glabra* contains the triterpenoid saponins which are hydrophilic therefore it is essential to provide lipid environment or these molecules should be micro or nanosized so easily diffuse within the aqueous regions near the outer surface of intracellular keratin filaments. The purpose of this study was to enhance the potential of *Glycyrrhiza glabra extract* (GGE) by preparing nanoemulsion formulation for transdermal application. Nanoemulsion system was developed with Tween 80 as surfactants and IPA as co-solvent and iso propyl myristate as oil for transdermal delivery of *Glycyrrhiza glabra* extract. Region of nanoemulsion was found in the pseudo-ternary phase diagrams developed at various Tween 80 and IPA ratios. The optimal nanoemulsion formulation consisted of water (14.28%), Tween 80 (38.10%), IPA (19.04%) and IPM (28.57%). *In vitro* and *Ex vivo* diffusion study shows that absorption of *Glycyrrhiza glabra extract* (MAG) was found to be fairly rapid, as compared to aqueous solution of extract. Nanoemulsion incorporated gel (NEIG) was prepared by using Carbopol 934 as a gelling agent. *In vitro* diffusion study showed that there was increase in permeation of GG extract from NEIG; also improve bioavailability of the drug. Our findings suggest that such novel w/o nanoemulsion exhibited significant antimicrobial property and NEIG useful as anti-inflammatory gel and possible alternative to traditional topical formulations with bioavailability issues.

**KEY WORDS:** NANOEMULSION, TRANSDERMAL DRUG DELIVERY, NANOEMULSION GEL, GLYCYRRHIZA GLABRA EXTRACT

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## INTRODUCTION

Over the ancient times, natural origin compounds and their formulations recognized its biological activities and health benefits. Most of the biologically active components of plants are polar like tannins, flavonoids, terpenoids etc. on the other hand, they are weakly absorbed due to their large molecular size which cannot absorb by passive diffusion and due to their pitiable lipid solubility resulting their pitiable bioavailability (Krishna and Gejjalagere 2018). Nanoemulsion has the ability to solubilize hydrophobic as well as hydrophilic drugs in their nanostructure. As nanoemulsion contain surfactants in it's composition, the application on the skin surface usually produces an increase in the membrane permeability smooth the progress of transdermal transport (Seema 2014, Chang 2013). A Nanoemulsion is a thermodynamically or kinetically stable liquid formulation. It is a dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase usually consist of small particles or droplets, with a size range of 5 nm-200 nm (Devarajan 2011). The o/w nanoemulsion is used for improving delivery of hydrophobic drugs, whereas w/o nanoemulsion preferred for incorporating hydrophilic drugs (Yen, et.al., 2018).

Nanoemulsion is of great interest as pharmaceutical, drugs, nutraceuticals, and food products & cosmetics formulation. They are used for administration through various routes like parenteral, oral, topical, ocular, pulmonary, mucosal, cosmetic, transdermal, controlled and target (Rachmawati et al., 2015 Eid et al., 2013). Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions and gel (Ghosh et al., 2013, Zhou et al., 2010, Sonnevile-Aubrun 2018).

*Glycyrrhiza glabra* contains the triterpenoid saponins which are hydrophilic so it is essential to provide lipid environment or these molecules should be micro or nanosized that can easily diffuse within the aqueous regions near the outer surface of intracellular keratin filaments. The main objective of this study is to formulate nanoemulsion of *Glycyrrhiza glabra* extract which may increase the permeation through skin and its anti-inflammatory potential.

## MATERIALS AND METHODS

*Glycyrrhiza glabra* extract was received as a gift sample from Amsar Pvt. Ltd. Indore India. Tween 80, IPA, isopropyl myristate and methanol were procured from LobaChemie, Mumbai, India. All other reagents used were of analytical grade. Solubility of extract and screening of oils and surfactants for nanoemulsion: The equilibrium solubility study was performed by adding

an excess amount of *Glycyrrhiza glabra* extract in 2 mL of various oils (soybean oil, sunflower oil, coconut oil, olive oil, iso propyl myristate), surfactants (Tween 80, Tween 20, span 80, SLS, Transcutol) and cosurfactants (IPA, ethanol, n-amyl alcohol) in 5 mL capacity vials each separately vortexed using a Cyclo mixer [CM 101, REMI (INDIA)]. The solubility of *Glycyrrhiza glabra* extract in various oils and surfactants was determined using the following method. Briefly, an excess amount (approximately 200 mg) of *Glycyrrhiza glabra* was placed in a 2 mL microtube containing 1 mL of each oil. Then, the vortexed mixture was and kept for 3 days at 37° C in a shaking water bath to facilitate the solubilization. This mixture was filtered using membrane filter (Nylon, 0.45 µm, Gelman, USA). The resulting nanoemulsion were tightly sealed and stored at ambient temperature and physical stability was measured by observing periodically for the occurrence of phase separation. On basis of clarity and transparency of resultant system suitable oil, surfactant and co surfactant were used in the preparation (Rachmawati et al., 2015 Eid et al., 2013).

Construction of pseudo-ternary phase diagrams: In order to find out the concentration range of components for nanoemulsion, pseudo-ternary phase diagrams were constructed using oil titration method at ambient temperature (25 °C). On the basis of the solubility studies, IPM was selected as the oil phase. Tween 80 and TPA were selected as surfactant and co surfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and co surfactant (*Smix*) were mixed at different mass ratios such as 9:1,8:2,7:3,6:4,5:5,4:6,3:7,2:8,1:9. These ratios were chosen in decreasing concentration of surfactant with respect to co surfactant for a detailed study of the phase diagrams. For each phase diagram, oil and *Smix* at a specific ratio was mixed thoroughly at different mass ratios from 1:1 to 2:1 in different glass vials. Different combinations of oil and *Smix* were made so that maximum ratios were covered for the study to define the limits of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams of oil, *Smix* and aqueous phase were developed using the aqueous titration method. Slow titration with aqueous phase was performed for each mass ratio of oil and *Smix* and visual observations were made for transparent and easily flow able o/w nanoemulsion. For convenience, the phase diagrams were constructed by drawing "water dilution lines" representing increasing water content and decreasing surfactant-co surfactant levels. The water was titrated along dilution lines drawn from the surfactant-co surfactant to the opposite oil side of the triangle. The line was arbitrarily denoted as the value of the line intersection with the oil scale (eg, 20:80, 30:70). If turbidity appeared followed by a phase separation, the samples were considered to be biphasic. If clear and

transparent mixtures were visualized after stirring, the samples were considered monophasic. The samples were marked as points in the phase diagram. The area covered by these points was considered to be the nanoemulsion region of existence (Rachmawati *et al.*, 2015 Eid *et al.*, 2013, Ghosh *et al.*, 2013).

**Formulation development of herbal nanoemulsion:** Phase diagram demonstrate the surfactant /co surfactant in ratio 2:1 suitable for nanoemulsion formulation. The liquid nanoemulsion was prepared by dissolving extract in double distilled water (0.5-1 %) to the resultant solution added drop wise the mixture of Tween 80 (38.10%), IPA (19.04%) and then IPM (28.57%) with continuous stirring on vortex mixer. No heat is supplied during formulation. The resulting nanoemulsion was tightly sealed and stored at ambient temperature and their physical stability was measured by observing periodically for the occurrence of phase separation; nanoemulsion was also subjected for characterization. **Characterization of Nanoemulsion:** Dilution test -Small amount of nanoemulsion was placed on a clean glass slide. A drop of water added to the nanoemulsion and was mixed with the help of glass rod and their transparency was assessed visually. If the emulsion is o/w type and it's diluted with water, it will remain stable as water is the dispersion medium; but if it is diluted with oil, the emulsion will break as oil and water are not miscible with each other, (Shivhare *et al.*, 2009).

**Dye test:** Add oil soluble dye such as sudan red III in prepare a nanoemulsion with oil and surfactant. Observed a drop of nanoemulsion under microscope and under motic microscope supported with Motic images version 2. Effect of dilution was observed visually and the pH of nanoemulsion measured directly on digital pH meter. **Droplet Particle size and Polydispersity:** Droplet Particle size was measured by dynamic light scattering with zetasize ZS-90 (Malvern instruments ltd., Malvern, U.K) at Temperature 25°C, for 30 second duration. Data analysis was conducted using a software package (ELS-8000 software) provided by the manufacturer. Polydispersity index is the ratio of standard deviation to mean droplet size. Polydispersity index (PI) is a measure of particle homogeneity and it varies from zero to 1.0. **Zeta potential:** Zeta potential of nanoemulsion was determined using zetasizer ZS-90 (Malvern instrument ltd., UK). Samples were placed in clear disposable zeta cells and results were recorded. Aliquots of nanoemulsion were sampled in disposable zeta cells and zeta potentials were determined on the basis of electrophoretic mobility under an applied electrical field.

**Viscosity:** Low viscosity is required to make them good in appearance and easy to handle and packed. Also provide good spray ability. Determine by using

Brookfield Viscometer at single mode (spindle C-15) (Stoughton, A) at 100 rpm each sample evaluated in triplicate at a temperature of 25°C, and results were presented as average  $\pm$  standard deviation. **Stability Study:** The physical stability of the nanoemulsion must be determined under different storage conditions (4, 25 and 40 °C) during study cycle. Fresh preparations that have been kept under various stress conditions for extended period of time are subjected to droplet size distribution analysis and observed for any evidences of phase separation, flocculation or precipitation. Effect of surfactant and their concentration on size of droplet is also being studied. In order to estimate metastable systems, the optimized nanoemulsion formulation was diluted with purified distilled water and was centrifuged (Remi laboratories, Mumbai, India) at 3500 rpm for 30 minute at room temperature and observed for any change in homogeneity of nanoemulsion.

**Drug content of nanoemulsion:** The drug content of optimized formulation was determined by UV spectrophotometer. 10 mg equivalent of extract containing nanoemulsion was dissolved in 10 ml of methanol. The concentration of solution was found to be 100 $\mu$ g/ml. The Mono amino glycerrhizinate content was estimated at 248nm using UV-Visible spectrophotometer UV-1700. **In vitro diffusion studies:** *In vitro* diffusion of nanoemulsion and aqueous solution of extract was carried out by Franz diffusion cell having 3.0 cm diameter and 25 ml capacity. Dialysis membrane (Hi-media) having molecular weight cut off range 12000 – 14000 kDa was used as diffusion membrane. Dialysis membrane was soaked in phosphate buffer pH 5.8 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 5.8 and dialysis membrane was mounted on cell. The temperature was maintained at 32.5°C. After a pre-incubation time of 20 minutes, the nanoemulsion and aqueous solution containing extract equivalent to 10 mg were placed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for 6 hours and replaced with the same amount of fresh phosphate buffer solution, and assayed by a spectrophotometer at 248 nm.

**Development of Nanoemulsion based gel :** Gel base was prepared by dispersing the 1 g of the Carbopol 934 in a sufficient quantity of distilled water (100ml) after complete dispersion. Carbopol 934 solution was kept for 24 hours till complete swelling. Then the *Glycyrrhiza glabra* extract loaded nanoemulsion was slowly added to the above prepared gel base under magnetic stirring for the development of nanoemulsion based gel (Modi JD, Patel JK 2011)

**Physicochemical Evaluation of gel:** Gel was tested for homogeneity by visual inspection after the gel has been set in container they were tested for their appearance of

any aggregates. The pH of various gel formulations was determined by using digital pH meter. The measurement of pH of each formulation was done in triplicate and average values are calculated. Viscosity of the prepared gel was measured using Brookfield viscometer at single mode (spindle C-15) (Stoughton, A) at 100 rpm each sample evaluated in triplicate at a temperature of 25°C, and results were presented as average  $\pm$  standard deviation. One of the criteria for a gel to meet the ideal qualities is that it should possess good spread facility. It is the term expressed to denote the extent of area to which gel rarely spreads on application to skin or affected part. The therapeutic efficacy of a formulation additionally depends upon its spreading value. The spreadability of gel was tenacious utilizing the following method: Gel (0.5 g) was placed within circle of 1 cm diameter remarked on a glass plate over which a second glass plate was placed. A weight of 500g was sanctioned reposing on the upper glass plate for 5 min. The incrementation in diameter due to spreading of gel was noted (Karri et al., 2015).

**Extrudability study:** The formulations were filled in the collapsible tubes after the gels were set in the container. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm. ribbon of gel in 10 second. **In vitro drug release:** *In-vitro* diffusion study of nanoemulsion incorporated gel (NEIG) and extract in gel base was carried out by Franz diffusion cell having 3.0 cm diameter and 25 ml capacity. Dialysis membrane (Hi-media) having molecular weight cut off range 12000 – 14000 kDa was used as diffusion membrane. Dialysis membrane was soaked in phosphate buffer (PB) pH 5.8 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 5.8 and dialysis membrane was mounted on cell. The temperature was maintained at 37°C. After a pre-incubation time of 20 minutes, the NEIG and extract gel containing extract equivalent to 10 mg were placed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for 4-6 hours and replaced with the same amount of fresh phosphate buffer solution, and assayed by a spectrophotometer at 248 nm.

**Ex Vivo Skin Permeation Studies:** The hairs of rat abdominal region were clipped with animal clippers. Full thickness dorsal skin was carefully excised and subcutaneous fat was removed with a dull scalpel then soaked in phosphate buffer pH 5.8. Diffusion cell was filled with phosphate buffer pH 5.8. The skin samples were mounted diffusion cell with stratum corneum side up. Diffusion cell was filled with phosphate buffer pH 5.8 and dialysis membrane was mounted on cell. The temperature was maintained at 32.5°C. After a pre-incubation time of 20 minutes, the NEIG and extract in gel base equivalent to 10 mg were placed in the donor

chamber. Samples were periodically withdrawn from the receptor compartment for 4-6 hours and replaced with the same amount of fresh phosphate buffer solution, and assayed by a spectrophotometer at 248 nm.

**Skin Irritation studies:** The skin irritation study for nanoemulsion based gel was carried out on Swiss albino mice weighing 25-35 g. The experiments were carried as protocol approved from Institutional Animal ethical committee. The animals were kept under standard laboratory conditions and housed in polypropylene cages. The animals were divided into three groups. Group I was taken as negative control received normal saline and Group II served as positive control received formalin solution and Group III applied with nanoemulsion based gel formulations. A single dose of the NEIG was applied to right ear of the mice keeping left as control in Group III. In group II formalin solution was applied; development of erythema or skin irritation was visualized after a total period of 21 days (Sonia K et al., 2011)

#### **In-Vivo Pharmacodynamics Study Using Carrageenan-Induced Rat Paw Edema**

**Anti-inflammatory activity:** It was evaluated on the basis of the inhibition of the carrageenan-induced hind paw edema. The rats of either sex (180-200) were divided into four groups each group containing six animals. The rats were fasted for 12 hrs prior to induction of edema however water was provided. To ensure uniform hydration, the rats receives 5ml of water by stomach tube. Thirty minutes later acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of carrageenan in normal saline in left hind paw of the rats. The NEIG formulation (0.25g) or base was applied topically with gentle rubbing to the paw of each rat of test group one hour before and one hour after the carrageenan challenge. Rats of the control groups received only the gel base and standard marketed formulation diclofenac gel BP applied in the same way as a reference standard. The paw volume was measured at 0, 1, 2, 3, 4, 5 and 6 hour using the digital Plethysmometer (Ugo Basile, Italy). The percentage inhibition of paw volume in test groups was compared with the control group (Prakash PR et al., 2010., Meshram GG et al., 2016)

$$\text{Percentage inhibition of edema} = (1 - V_t/V_c) \times 100$$

Where,  $V_t$  is the inflammatory increase in paw volume in test groups and  $V_c$  is the inflammatory increase in paw volume in normal control group of rats. Percentage inhibition of edema is proportional to anti-inflammatory activity.

**Antimicrobial Study:** Broth macro dilution Assay: Broth dilution is a technique in which a suspension of bacterium of a appropriate concentration is tested against varying concentrations of an antimicrobial

agent. As nanoemulsion o/w it will break if directly mixed with broth media which was prepared in water so the 1% and 2% nanoemulsion was dissolved in DMSO so that final concentration was 2.5mg/ml and 5mg/ml the and *Glycyrrhiza glabra* extract 5mg/ml and 10 mg/ml was prepared in DMSO. A suspension of different bacterium and fungi was poured in above test tubes. The tubes with bacterial suspension were incubated at 37 °C for 24 hours and with fungal culture were incubated at 30°C for 48 hours. After incubation observed for growth or turbidity (Das K et al., 2010).

#### MIC by Micro titer plate method

Different concentrations of nanoemulsion and extract were prepared in DMSO. An equal volume i.e 1µl of bacterial suspension/fungal suspension and 1µl each concentration was added to the wells initial absorbance was measured at 620nm and plates were incubated at 37°C for 24 h after incubation, plates are examined for changes in turbidity as an indicator of growth. Initial absorbance and absorbance after incubation were compared. The lowest concentration of drug that reduces, by more than 50% or Concentration with sharp decline in absorbance value is the MIC (Hasan et al., 2010, Selvamohan et al., 2012, Dasari et al., 2014).

## RESULTS AND DISCUSSION

Development of nanoemulsion formulation depends on physicochemical properties of drugs. The solubility of the drug is most important factor as the ability of nanoemulsion to maintain the drug in solubilised form. Solubility of the lipophilic drugs in the oil phase and hydrophilic drugs in aqueous phase is an important criterion for the selection of oils and water respectively. GGE and MAG are hydrophilic in nature. The solubility of MAG in different oils was determined since solubility of MAG was higher in aqueous phase as compared to oil phase, w/o nanoemulsion was developed for transdermal delivery of MAG. In order to select suitable oils and surfactants for good solubilizing of herbal extract of *Glycyrrhiza glabra* in various oil and surfactant were determined.

Ingredients	Formulations composition (% w/v)				
	FA1	FB1	FC1	FD1	FE1
Distilled Water	5.5	5.5	25.0	14.28	5.0
Tween 80	25.00	25.00	38.89	38.10	30.0
IPA	25.00	25.00	19.44	19.04	15.0
IPM	44.44	44.44	16.66	28.57	50
Extract	0.0	0.5	0.5	0.5	0.5

Table 2. Formulation composition of Nanoemulsion Batch 2

Ingredients	Formulations composition (% w/v)				
	FA2	FB2	FC2	FD2	FE2
Distilled Water	5.0	5.0	5.0	14.28	14.28
Tween 80	30.0	30.0	30.0	38.10	38.10
IPA	15.0	15.0	15.0	19.04	19.04
IPM	50	50	50	28.57	28.57
Extract	0.25	0.50	0.75	1.0	0.50

Medium and long chain triglyceride oils with different degree of saturation have been tried in the development of nanoemulsion. Oils tried were soybean oil, sunflower oil, coconut oil, olive oil, iso propyl myristate (IPM) etc. Surfactants employed were Tween 80, Tween 20, span 80, SLS, Transcutol and co-surfactant was IPA, ethanol, n-amyl alcohol.

The solubility of *Glycyrrhiza glabra* was determined indifferent oils. It was evident that *Glycyrrhiza glabra* exhibited highest solubility in isopropyl myristate (195.71±0.91 mg 2 mL<sup>-1</sup>) as polarity of the poorly soluble drugs favors their solubilization in small/medium molar volume oils. Edible oils cannot depict large micro emulsion region due to their rancid nature. IPM was selected for the preparation of nanoemulsion due to its well-known permeation enhancing property and biocompatibility. The most critical problem related in the development of nanoemulsion based drug delivery systems is the toxicity of the surfactants. Large amounts of surfactants may cause skin irritation when administered transdermally. It is therefore important to determine the surfactant concentration properly and use the minimum concentration in the development of nanoemulsion formulation (Alam et al., 2010).

Amongst various surfactants screened, Tween 80 exhibited (96.8±1.47 mg 2 mL<sup>-1</sup>) highest solubilization capacity of GGE. Whereas various co-surfactants were screened for solubility as well miscibility with surfactant, Isopropyl alcohol IPA was revealed greater solubilization capacity (101.98±1.02 mg 2 mL<sup>-1</sup>) as well as it forms transparent system (99.87 %T). The screening of surfactant and co-surfactants on the basis of solubility is difficult because all surfactant and co surfactant cannot solubilise all type of oil phase. Surfactant chosen must be able to lower the interfacial tension to a very small value to aid the dispersion process during the preparation of the nanoemulsion, provide a flexible film that can readily deform around droplets. aqueous phase, the second representing oil and the third representing a mixture of surfactant and co surfactant at a fixed mass ratio.

Figures 1 represent the pseudo ternary phase diagrams for nanoemulsion systems along with the ratios of sur-

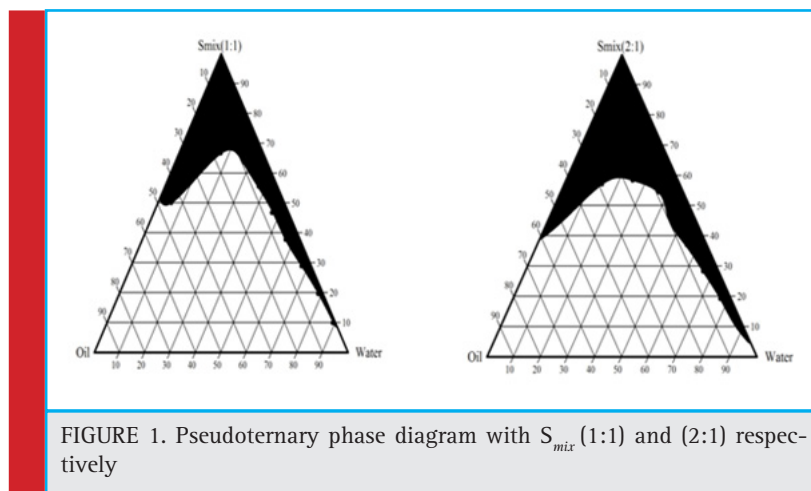


FIGURE 1. Pseudoternary phase diagram with  $S_{mix}$  (1:1) and (2:1) respectively

factant and co surfactant, as 1:1 and 2:1. The change in the area of nanoemulsion region can be very well observed in the ternary phase diagram as the ratio of surfactant to co surfactant was changed from 1:1 to 2:1. When ratio of surfactant to co surfactant was 2:1, there was increased in nanoemulsion region, because of high concentration of surfactant. Nanoemulsions are considered to be thermodynamically stable systems that are formed at a particular concentration of oil, surfactant, and water, with no phase separation, creaming, or cracking. Selected formulations from phase diagram were subjected to different stress stability testing like heating cooling cycle, centrifugation, and freeze-thaw cycle. During physical stability testing, some formulations became turbid and in some phase separation occurred. One reason of this instability in nanoemulsions may be due to the Ostwald ripening, in which molecules move as a monomer and coalescence of small droplets takes place, resulting in the formation of large droplets by diffusion processes driven by the gain in surface free energy. The other reason may be that when temperature quench occurs during stress stability study, instability of nanoemulsion occurs due to separation of oil phase and droplet distribution of smaller size is favoured by the change in curvature free energy. Only those formulations, which showed no phase separation, creaming, cracking, coalescence, and phase inversion during stress stability tests, were selected for further studies (Osanloo et al., 2018).

Novel nanoemulsion of *Glycyrrhiza glabra* extract was prepared by the spontaneous emulsification method (oil phase titration method), Isopropyl myristate was used as oil phase components. Optimized nanoemulsion consists of water (14.28%), Tween 80 (38.10%), Isopropyl alcohol (19.04%) and Isopropyl myristate (28.57%). Nanoemulsion developed was clear and transparent. From dilution and dye test it can be concluded that the system was w/o type with pH  $5.59 \pm 0.01$ ,  $5.78 \pm 0.005$

As drug loading increased 0.5, 1% the particle size of optimized nanoemulsion varies, 104.2 nm to 185.3 nm but almost it is in range. It has been reported that the smaller particle size of the emulsion droplets may lead to more rapid absorption and improve the bioavailability. Polydispersity is the ratio of standard deviation to mean droplet size, so it indicates the uniformity of dispersity. Polydispersity index (PDI) is a measure of particle homogeneity and it varies from 0.0 to 1.0. PDI 0.186 - 0.451 found it closer to zero so the more homogeneous are the particles. Polydispersity signifies the uniformity of droplet size within the formulation. The polydispersity value of the formulations was very low (<0.4) which indicated uniformity of droplet size within the formulation. The more negative zeta potential, greater the net charge of droplets and more stable the emulsion. Zeta potential values lower than -30 mV generally indicate a high degree of physical stability the zeta potential found -9.5 to -13.8. It shows little change with increase in con-

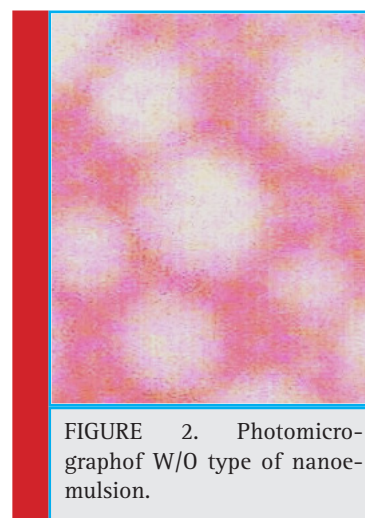


FIGURE 2. Photomicrograph of W/O type of nanoemulsion.

Table 3. The influence of mixture components on characterization of prepared nanoemulsion Batch -1

Parameters	FA1		FB1		FC1		FD1		FE1	
	Initial	After 1 month	Initial	After 1 month	Initial	After 1 month	Initial	After 1 month	Initial	After 1 month
Zeta size	353.4	Unstable	247.1	Unstable	203.2	Unstable	245.4	206.3	236.5	183
PDI	0.568		0.339		0.26		0.249	0.384	0.249	0.204
Zeta potential	-44.7		-37.5		-44.3		-34	-16	-34.4	-27.4

Table 4. The influence of mixture components on characterization of prepared nanoemulsion batch-2

Parameters	FA2	FB2	FC2	FD2	FE2
Zeta size	208	175.1	183.5	185.1	104.2
PDI	0.202	0.198	0.234	0.451	0.186
Zeta potential	-35.3	-33.6	-19.2	-13.8	-9.5

centration of extract but all the values are in range and the nanoemulsion was found to be stable on thermodynamic stability study. The viscosity of nanoemulsion at room temperature was  $67.43 \pm 0.15$ CP. Generally, it was observed that the viscosity of the nanoemulsion formulations was very low. Lower viscosity is one of the characteristics of nanoemulsion formulations (Gurpreet & Singh 2018.) The drug content was found to be 97.89% (Table 3, 4)

Evaluation of prepared nanoemulsion was carried out for its physicochemical parameters. It was found that w/o nanoemulsion was thermodynamically stable and no or little effect of drug concentration on it. The formulation is safe for topical application.

In vitro diffusion through the dialysis membrane of formulation shown in Table 5, Figure 3.

After 3.5 hours drug release from the nanoemulsion- was 95.30% while drug release from aqueous solution of extract was 33.17% which is much less as compared to NE. It was observed that rate of diffusion was improved for nanoemulsion attributed to their nano range size. The significant difference in permeation between nanoe-

mulsion formulations and aqueous solution could be due to the mean size of droplets.

Ex vivo permeation through rat skin of formulation shown as

Ex vivo permeation study results shows that drug permeation through skin in 210 min (3.5 hr) is 92.26% and through aqueous solution of extract it was only 33.61%. This proved that the nanoemulsion enhance the ability of drug to permeation through the skin.

Nano emulsion incorporated gel found to be homogeneous with pH $5.65 \pm 0.02$  viscosity  $118.66 \pm 0.30$  and spreadability  $5.36 \pm 0.11$  with good extrudability property. Physical parameters of gel evaluation are given in Table 7.

Skin irritation studies: It was observed that nanoe-mulsion incorporated gel (NEIG) application on Swiss albino mice no signs of erythema and skin irritation even on application for 21 days. Thus, the developed formulation is non-sensitizing and safe for use. In vitro diffusion through the dialysis membrane of formulation seen in Table 8, Figure 5.

After 3.5 hours drug release from the NEIG 98.19% while drug release from extract in gel base is 36.54% which is much less as compared to NEIG. It was observed that there was increase permeation of GG extract from NEIG. It was observed that the when nanoemulsion incorporated in Carbopol gel it will enhance its permeation than nanoemulsion as such.

The nanoemulsion shows the positive susceptibility test for all the six bacterial and two fungal cultures so

Table 5. In Vitro Drug Diffusion study of Nanoemulsion and Extract

Time (Minutes)	% Cumulative Drug Release (Mean $\pm$ SD) of Nanoemulsion	% Cumulative Drug Release (Mean $\pm$ SD) of Extract
15	17.30 $\pm$ 0.41	8.083 $\pm$ 0.60
30	21.57 $\pm$ 0.62	10.85 $\pm$ 0.98
60	26.22 $\pm$ 1.42	13.35 $\pm$ 0.61
90	38.64 $\pm$ 1.20	16.75 $\pm$ 0.18
120	54.66 $\pm$ 0.72	20.00 $\pm$ 0.62
150	68.80 $\pm$ 1.09	24.18 $\pm$ 0.23
180	82.58 $\pm$ 0.60	28.56 $\pm$ 2.28
210	95.30 $\pm$ 0.97	33.17 $\pm$ 1.25

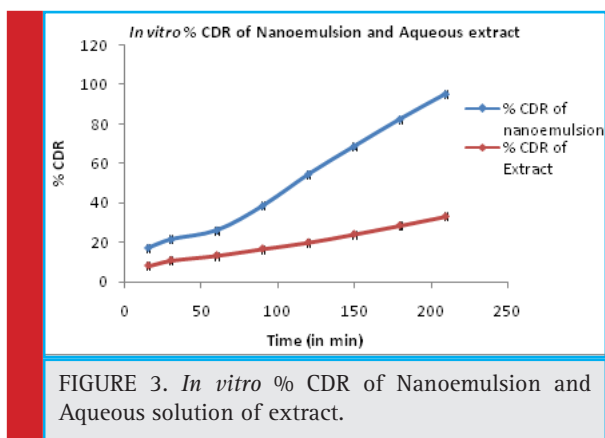


FIGURE 3. In vitro % CDR of Nanoemulsion and Aqueous solution of extract.

subjected for MIC determination. MIC of nanoemulsion and extract given in Table 9.

As the MIC of Nanoemulsion was less than Extract for all the bacterial and fungal cultures we can say that nanosized droplets have more energy and surfactant to destabilize the targeted microbes. The nanoemulsion particles are thermodynamically driven to fuse with lipid-containing organisms. When sufficient nanoparticles fuse with the pathogens, they release part of the energy

trapped within the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death.

The results of anti-inflammatory activity i.e. increase in paw volume at different time interval and % inhibition after topical administration of Diclofenac gel, NEIG, Extract in gel are given in Table 10 and Figure 6

All the values expressed mean±S.E.M. (percent inhibition) n=6. Data was analyzed by one way ANOVA followed by Dunnet test \*\*  $P < 0.05$  , \*\*\*  $P < 0.001$

Statistical analysis showed that the topical preparation has significant inhibition of carrageenan induced rat paw edema when compared with control group. It was also observed that the % inhibition of edema by NEIG was greater than standard diclofenac gel and Extract in gel base. Glycyrrhizaglabra extract has anti-inflammatory activity so it reduces the paw edema but when we use NEIG activity increases two fold which could be due to enhanced permeation of MAG through skin. So it was concluded that the nanosize of extract in NEIG responsible for better absorption so as to increase its bioavailability and therapeutic effect (Mahboobian 2017, Rai et al., 2018).

Table 6. Ex-vivo Drug permeation study data

Time (Minutes)	% Cumulative Drug Release (Mean ± SD) of Nanoemulsion	% Cumulative Drug Release (Mean ± SD) of Extract
15	16.77±0.12	9.25±0.72
30	20.12±0.29	11.11±0.53
60	23.85±0.25	13.31±0.27
90	36.60±0.98	14.96±0.23
120	48.23±0.40	21.37±0.54
150	65.36±0.95	25.23±0.19
180	83.68±0.19	30.17±0.78
210	92.26±0.37	33.61±0.71

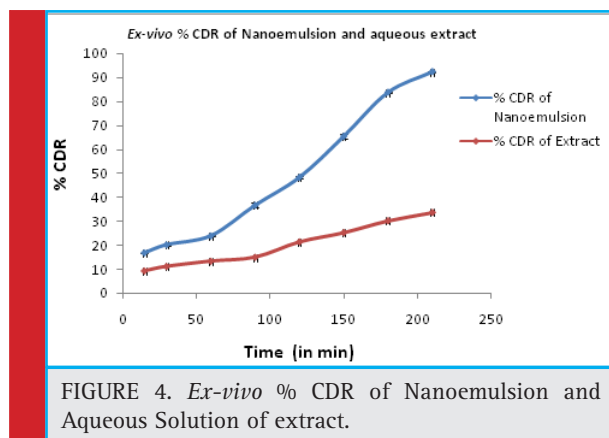


FIGURE 4. Ex-vivo % CDR of Nanoemulsion and Aqueous Solution of extract.

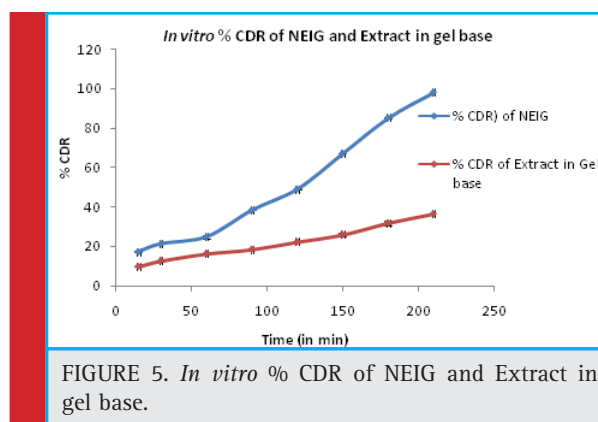


FIGURE 5. In vitro % CDR of NEIG and Extract in gel base.



Formulation	Homogeneity	pH	Viscosity	Spreadability	Extrudability
NEIG	Homogeneous	5.65±0.02	118.66±0.30	5.36±0.11	Good

Time (Minutes)	% Cumulative Drug Release (Mean ± SD) of NEIG	% Cumulative Drug Release (Mean ± SD) of Extract in Gel base
15	17.30±0.10	9.75±0.08
30	21.32±0.42	12.75±0.09
60	24.88±0.26	16.31±0.31
90	38.43±1.02	18.48±0.13
120	49.10±0.15	22.31±0.43
150	67.22±0.19	25.97±0.42
180	85.24±0.21	31.92±1.07
210	98.19±0.22	36.54±1.25

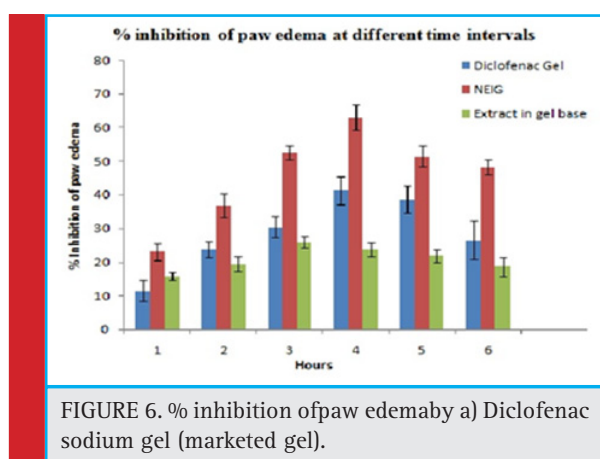


FIGURE 6. % inhibition of paw edema by a) Diclofenac sodium gel (marketed gel).

of nanoemulsion was very less as compared to extract it means better antimicrobial activity. Nanoemulsion further incorporated in Carbopol 934 (NEIG) with good homogeneity. *In-vitro* diffusion study revealed that the nanoemulsion incorporated in Carbopol gel enhances its permeation than nanoemulsion alone. Drug delivery through the skin to the systemic circulation is convenient for a number of clinical conditions due to which there has been a considerable interest in this area. NEIG has significant anti-inflammatory and antimicrobial effect attributed to nanosize of extract in responsible for rapid and complete absorption improving its therapeutic effect. Use of nanoemulsion in transdermal drug delivery represents an important area of research in drug delivery, which enhances the therapeutic efficacy and also the bioavailability of the drugs without any adverse effects. It is also regarded as a promising technique with many advantages including, high storage stability, low preparation cost, thermodynamic stability, absence of organic solvents, and good production feasibility.

## CONCLUSION

*In-vitro* drug diffusion and *Ex-vivo* permeation study was concluded that permeation rate was faster in nanoemulsion as compared to solution of extract; also MIC value

Micro-organism	MIC of Extract mg/ml	MIC of nanoemulsion mg/ml
Escherichia coli	5+0.3	2.5
Staphylococcus aureus,	10+1.00	2.5
Pseudomonas aeruginosa,	15+1.74	2.0
Bacillus Substalis	15+1.52	2.5
Proteus species	20+1.98	1.25
Shigella soni	15+1.02	3.75
Aspergillus niger	5+0.25	3.75
Candida Albicans	20+1.06	3.75

Table 10. Mean paw volume and % inhibition at different time interval							
Paw volume at different time after carrageenan injection							
Treatment	Initial	1h	2h	3h	4h	5h	6h
Group I (Control)	1.19±0.020**	1.43±0.021***	1.62±0.018***	1.67±0.017***	1.71±0.015***	1.63±0.013***	1.61±0.010***
GroupII Standard (Diclofenac)	1.17±0.003** ---	1.23±0.009*** (13.80)	1.29±0.008*** (20.43)	1.31±0.002*** (21.28)	1.39±0.01*** (18.74)	1.36±0.013*** (16.55)	1.32±0.019*** (17.67)
Group III Test1 (NEIG)	1.08±0.021** ---	1.18±0.02*** (16.85)	1.26±0.01*** (22.55)	1.286±0.01*** (23.04)	1.345±0.01*** (21.52)	1.33±0.01*** (18.26)	1.32±0.001*** (17.88)
Group IV Test2 (Extract gel)	1.12±0.025** ---	1.29±0.039*** (9.42)	1.36±0.038*** (15.90)	1.44±0.04*** (13.78)	1.50±0.04*** (11.92)	1.48±0.04*** (8.60)	1.48±0.038*** (8.01)

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