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# FORMULATION & EVALUATION OF GENISTEIN NANOEMULSION FOR ENHANCED TOPICAL DELIVERY

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## ABSTRACT

**Purpose**: Genistein is an isoflavone compound having poor water solubility and high membrane permeability. The oral delivery is frequently associated with low bioavailability, high intra- and inter-subject variability and therapeutic failure. Hence an attempt was made by designing the Nanoemulsion (NE) systems through topical application in order to achieve an enhancement in solubility and faster onset of action. **Methods:** Equilibrium solubility studies indicated the choice of paraffin liquid as oily phase, Tween 20 and PEG 400 as emulgents for formulating the NE. From the ternary phase diagram, the surfactant and co-surfactant ratio was selected as 3:1. The NE was systematically optimized by $3^2$ full factorial design. The oil concentration ($X_1$) and Oil: Smix ratios ($X_2$) were selected as independent variables and Droplet size ($Y_1$), Emulsification time ($Y_2$), Polydispersity index (PDI) ($Y_3$) and Entrapment Efficiency ($Y_4$) were selected as dependent variables. **Results:** The optimized formulation F1 showed droplet size 19.46 nm, PDI 0.242, emulsification time 31.7 sec, EE of 94.64% and *in-vitro* release of 80.8% in 12 hrs. Also, the zeta potential was found to be -28.42mV which indicated Formulation F1 was stable. The nanometer size range and the anti-oxidant activity of the optimized F1 formulation was found to be high enough to neutralize the free radicals. **Conclusion:** Hence, it can be concluded that NE formulation of genistein improved the dissolution of genistein with enhanced anti oxidant property.

**KEYWORDS:-**Nanoemulsion (NE), Genistein, Emulgents, full factorial designs, PDI.

## INTRODUCTION

Exposure of skin to ultra violet light induces free radicals oxygen and oxidant formation ($H_2O_2$, OH-radicals, superoxide anion) are referred as Reactive Oxygen Species (ROS). Formation of induced ROS causes oxidative damage to the lipids; proteins and DNA.[1] Under normal circumstances, low levels of ROS are neutralized by skin constitutive anti-oxidant defenses. UV radiation generates abundant of ROS that are free to cause oxidation which contributes to acute (Immuno-suppression, photo sensitive disorder) and chronic (photo aging and skin cancer) form of skin damage.[2] Hence antioxidant in topical products is required to neutralize the reactive oxygen species throughout the epidermis. It is useful to have a method to determine the correct choice and level of anti-oxidant in topical products to provide extra protection against skin damage caused by ROS both at the skin surface and deep in epidermis.

Isoflavones and their metabolites scavenge hydroxyl and superoxide anion radicals. As a result, it protects against lipid per oxidation and DNA damage from oxidative damages caused by hydrogen peroxide. Genistein is the phytoestrogen of the isoflavone class that is found in soya beans and certain other leguminous plant. It acts as anti-oxidant, anti-proliferative, anti-cancer, reduce cholesterol and menopausal complaints.

Genistein is less hydro-soluble than the conjugated form which limits their action in topical formulations. To allow the better actions of Genistein in its topical formulations, a nano-emulsion form is preferred to enhance their penetration through skin. As glycosides form of isoflavone limits the biological action of conjugated form due to low penetration through different skin layers. So, to get desired isoflavone effects, aglycone form such as nano-genistein is required in topical formulations. Among the arrays of nanocarriers, nanoemulsions are best suited option because of the advantages pertaining to topical delivery such as enhanced skin permeation and residence in epidermal layer of the skin thereby, prolonging the drug action and stability of the drug pertaining to its reduced surface tension and increased surface area of nanodroplets.[3-5]

Hence, the aim of the study was to prepare and evaluate the nanoemulsion of genistein.

## MATERIALS AND METHODS

### Chemicals

Genistein was gifted by Somu Chemicals Pvt. Ltd., Bangalore. All other chemicals used were of analytical grade.

### FTIR study

FTIR study was performed to determine the integrity of the drug in the formulation. The FTIR spectrum of various samples such as pure drug, physical mixture with components in formulation and optimized formulation was done using FTIR spectrophotometer (Shimadzu).

### Screening studies for selection of oil, surfactant and co-surfactant (Saturation solubility studies)

Solubility of Genistein was determined in various oils (liquid paraffin, isopropyl myristate and oleic acid) surfactants (Tween 20 & Tween 80) and co-surfactants (PEG-400, PEG-200, and Transcutol-P & Span 20). Known quantity of excess drug was added in screw cap vials containing 2mL of each component. A vortex mixer was used to facilitate solubilization. Sealed vials were kept on water bath shaker for 72 hours. After equilibrium, each vials were transferred to eppendroff`s tubes and were centrifuged at 6000 rpm for 20 min using centrifuge. Aliquots of supernatant were diluted with methanol and drug content was quantified using a UV spectrophotometric technique at 260nm.[6]

### Construction of pseudo ternary phase diagram

The existence of micro emulsion region was determined by constructing pseudo ternary phase diagram using CHEMIX School v3.61 software for each Smix depicting clear micro emulsion region. Pseudo ternary phase diagrams were constructed by aqueous titration method by titrating the blend of oil and surfactant: co-surfactant mixture (Smix) in different weight ratios (1:1, 2:1, 3:1, 4:1, 1:2) with and without drug by incremental amounts of water. Smix and Oil (Oil: Smix) were mixed in the different weight ratios as 1:9,1:8,1:7,1:6,1:5,1;4 and 1:3. Each mixture of oil and Smix was subjected to vortex to form homogenous mixture before titration with water. During titration the aqueous phase was added in increments of 5% to 95% with vortexing to ensure proper mixing.[7]

### Preparation of Nanoemulsion (NE) of genistein:

NE formulation was prepared by spontaneous emulsification method. Briefly the procedure involves addition of Genistein to accurately weighed amount of oil and the oil mixture was kept on a magnetic stirrer (50 rpm) at room temperature. To this surfactant and co-surfactant mixture was added gradually with continuous stirring. These formulations were further sonicated for 15 min and stored at room temperature until they were used in subsequent studies.[8-9]

### Optimization of Formulation by $3^2$ Factorial design

The optimization of the oil concentration and the oil: Smix ratio was carried out by $3^2$ factorial design. The formulation variables, oil concentration ($X_1$) and the oil: Smix ratio ($X_2$) was selected as the independent variables (table 1 & 2), particle size ($Y_1$), emulsification time ($Y_2$), PDI ($Y_3$) and Entrapment efficiency (EE) ($Y_4$) were selected as the dependent variables. Based on the factorial design, nine batches of Genistein loaded NE was prepared in triplicate. Various response surface methodology (RSM) computations for the current optimization study were performed employing Design Expert software Trial version 7.0. Multiple linear regression analysis (MLRA) approach was used to generate polynomial models including interaction and quadratic terms for all the response variables.[10-11]

**Table 1: Coded values and actual values for Independent variables**

| Coded Values | Actual Values | |
|---|---|---|
| | $X_1$ (%) | $X_2$ |
| **-1** | 3 | 1:1 |
| **0** | 6 | 2:1 |
| **+1** | 9 | 3:1 |

**Table 2: Factorial Design Batches for NE Formulations.**

| | Oil % | | Oil: Smix ratio | |
|---|---|---|---|---|
| **Runs** | **Coded** | **Actual %** | **Coded** | **Actual %** |
| 1 | -1 | 3 | -1 | 1:1 |
| 2 | -1 | 3 | 0 | 2:1 |
| 3 | -1 | 3 | 1 | 3:1 |
| 4 | 0 | 6 | -1 | 1:1 |
| 5 | 0 | 6 | 0 | 2:1 |
| 6 | 0 | 6 | 1 | 3:1 |
| 7 | 1 | 9 | -1 | 1:1 |
| 8 | 1 | 9 | 0 | 2:1 |
| 9 | 1 | 9 | 1 | 3:1 |

### Evaluation Studies

The prepared NE was evaluated for both morphological and technological properties.

### Droplet size and PDI analysis

1 mL of NE formulation was diluted to 100 mL in a beaker and gently mixed using a glass rod. The resultant

emulsion was then subjected to particle size and PDI analysis by using Malvern zetasizer. All droplet analysis was repeated in triplicate.

**Emulsification time**

The emulsification time is the time taken for a pre-concentrate to form a homogeneous mixture upon dilution. It was monitored visually observing the disappearance of NE and the final appearance of the micro emulsion. In this method, a predetermined volume of formulation (1mL) was introduced into 100 mL of water in a glass beaker with stirring continuously on a magnetic stirrer at 100rpm. The time to emulsify spontaneously and progress of emulsion droplets were observed.[12]

**Entrapment Efficiency**

Entrapment efficiency of NE formulations was determined by centrifugation method. NE was poured into a stopper test tube and centrifuged by using cold centrifuge at 10,000 rpm maintained at 4°C for 60 minutes and then filtered by using whattman filter paper to obtain clear fraction. The clear fraction was used for the determination of free drug by UV spectrophotometer at 260 nm. The entrapment efficiency was calculated using the formula.

**Entrapment Efficiency** (%) = $[(Ct - Cf)/Ct \times 100]$

Where, $Ct$ is concentration of total drug and $Cf$ is concentration of unentrapped drug.[13]

**Determination of drug content**

A measured quantity of nanoemulsion was added in 100 mL of methanol. The resulting mixture was kept for 24hrs in a dark place. Then the solution was filtered and 1ml of this solution was diluted to 10 mL of methanol. After further dilution the samples were analyzed for drug content by developed UV method.

**Determination of Zeta potential**

1 mL of NE formulation was diluted to 100 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to zeta potential analysis by using Malvern zetasizer.

**Precipitation and Clarity assessment**

1mL of NE containing Genistein was taken in a small beaker and was diluted with 100mL of distilled water at 37ºC to check visual appearance and the diluted preparation was vortexed for 1 min, then the mixtures was stored for a period of 24 hrs, and observed for phase separation and precipitation with naked eye.

**Robustness to dilution**

Robustness of the optimized formulation for dilution was assessed by exposing it to 50, 100 and 1000 fold dilution with water, gastric fluid (0.1N HCl) and physiological fluid (phosphate buffer pH 7.4). The diluted nanoemulsion was stored for 24 hours and monitored for any physical changes such as precipitation or phase separation.

**Thermodynamic stability studies**

The optimized formulation was subjected to various thermodynamic stability studies by centrifugation and freeze thaw cycling method in order to assess the phase separation and stability of the nanoemulsion.

**Centrifugation study**

The optimized formulation was centrifuged at 15000 rpm for 30 min. The resultant formulation was then checked for any instability problem, such as phase separation, creaming or cracking.

**Freeze thaw cycling test**

The optimized formulation was subjected to freeze-thaw cycling. One freeze-thaw cycle consist of storing of nanoemulsion at -20ºC for 2 days followed by 40°C for 2 days. Three such freeze thaw cycles were carried out and then they were assessed for their physical instability like phase separation and precipitation of the nanoemulsion.[14]

***In-vitro* drug permeation study**

The permeation studies of pure Genistein and optimized nanoemulsion were carried out with Franz diffusion (FD) cell using cellophane membrane. The membrane was placed between donor and receptor compartments of the FD cell. Pure drug solution or optimized formulation was placed in donor compartment and PBS pH 7.4 was taken in receptor compartments as media. Temperature was maintained at 37°C ± 0.5°C. The assembly was kept on magnetic stirrer and samples were withdrawn at time intervals of 2, 4, 6, 8, 10 and 12h and replaced with equal volume of fresh media. Samples were analyzed by UV spectrophotometer at 260 nm and % cumulative drug release was calculated. The % cumulative drug released of pure drug solution was compared to the corresponding optimized nanoemulsion.[15]

**Anti oxidant activity determination of optimized Formulation (F1)**

Antioxidant potential of prepared nanoemulsion was assessed using 2,2-diphenyl-1-picyrlhydrazyl (DPPH) free radical capture assay. One milliliter of DPPH 0.1 mM methanolic solution was added to 2.5 mL of nanoemulsion and 1mL of DPPH 0.1 mM methanolic solution plus 2.5 mL of methanol was used as control. The reactions took place at 25°C under dark conditions for 1 h, the absorbance (Abs) of the solutions were measured in the wavelength of 518 nm (UV spectrophotometer 1700). Antioxidant activity (AA) was assessed using equation given below.

% Antioxidant activity =

$$\frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} X100$$

The ability to scavenge DPPH radical was measured by the discoloration of the solutions prepared using spectrophotometer. The higher the antioxidant activity

more will be the intensity of solution discoloration. IC50 (half maximal effective concentration) of the nanoemulsion and the ascorbic acid were determined using linear regression graph. The assay was performed in triplicate and the mean ± SD was assessed.[16-17]

## RESULTS AND DISCUSSION

### FTIR study of the pure Genistein, its physical mixture and optimized F1 formulation

The IR spectrum (Fig. 1A) of Genistein revealed the presence of peak at 3413.39 $cm^{-1}$ due to benzene ring, 2954.41 $cm^{-1}$ indicates the C-H stretching, 1064.51 $cm^{-1}$ C-O bond, 1653.68 $cm^{-1}$ C=O stretching, 3413.39 $cm^{-1}$ O-H stretching and 1616.27 $cm^{-1}$ C=C stretching. It has been observed that there were no major shifts in the spectral values of drug in physical mixture and optimized formulation (Fig.1B & Fig.1C), indicating no chemical interaction between the drug and components of formulation used. Hence, it can be concluded that there is compatibility between drug and the components of formulation used.

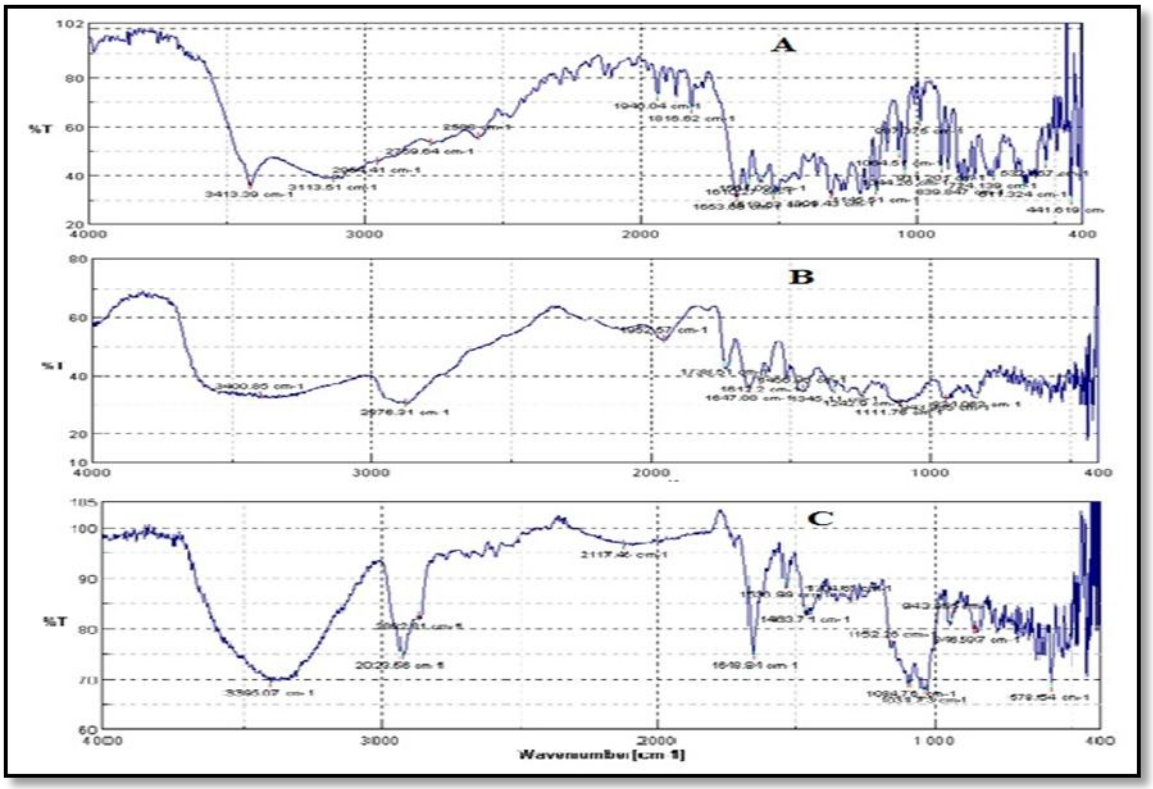


**Fig. 1: FTIR spectra A) Pure drug –Genistein B) Physical mixture C) Optimized F1 formulation.**

### Screening studies for selection of oil, surfactant and co-surfactant (Saturation solubility studies)

Solubility of Genistein in oils, surfactants, co-surfactants is depicted in the table 4. Based upon the maximum solubility of Genistein in tested oils and surfactants, Paraffin liquid was selected as oil phase, Tween 20 and PEG 400 was selected as surfactant and co-surfactant respectively.

**Table 4: Selection of NE Components.**

| Vehicle | Function in NE | Solubility (mg/mL) |
|---|---|---|
| Paraffin liquid | Oil | 10.50 |
| Isopropyl myristate | Oil | 8.109 |
| Oleic acid | Oil | 3.698 |
| Tween 20 | Surfactant | 9.095 |
| Tween 80 | Surfactant | 7.027 |
| PEG 400 | Co-surfactant | 7.589 |
| PEG 200 | Co-surfactant | 3.22 |
| Transcutol P | Co-surfactant | 2.49 |
| Span 20 | Co-surfactant | 4.273 |

### Construction of pseudo ternary phase diagram

The nature and amount of surfactant and co-surfactant plays an important role in influencing the phase properties such as size distribution and position of microemulsion region.

Pseudo ternary phase diagrams were constructed to identify the micro emulsion regions and to optimize the concentration of oil, surfactant, and co-surfactant in the NE formulations. A series of the mixtures of oil and Smix were prepared with and without drug and their emulsifying properties were observed visually for micro-emulsions, cloudiness, milky emulsion, microemulsion gel and phase separation by adding incremental amounts of water. The phase diagrams were constructed at surfactant/co-surfactant ratios of 1:1, 2:1, 3:1, 4:1 and 1:2 (w/w) (fig.2). The gel- like region was found to become large with the increasing concentration of Tween 20, while the micro emulsion region decreased. The maximum micro emulsion region was observed visually at a Smix ratio of 3:1 in most of the oil and Smix ratio. However, the drug precipitation was observed after several hours at ratios of 4:1 and 1:2. Co-surfactants are beneficial to form a micro emulsion at a proper concentration range. On the other hand, an excessive amount of the co-surfactant will lead to the increase in droplet size as a result of the expanding interfacial film which reduces stability of the system due to its high intrinsic aqueous solubility. Hence, the optimal ratio of surfactant to co-surfactant was selected to be 3:1. Based on above results, a three-component NE formulation was established containing 6% Paraffin liquid as oil (on the basis of the solubility study), 70% Tween 20 as the surfactant and 24% PEG 400 as the co-surfactant (on the basis of phase diagrams).[18]

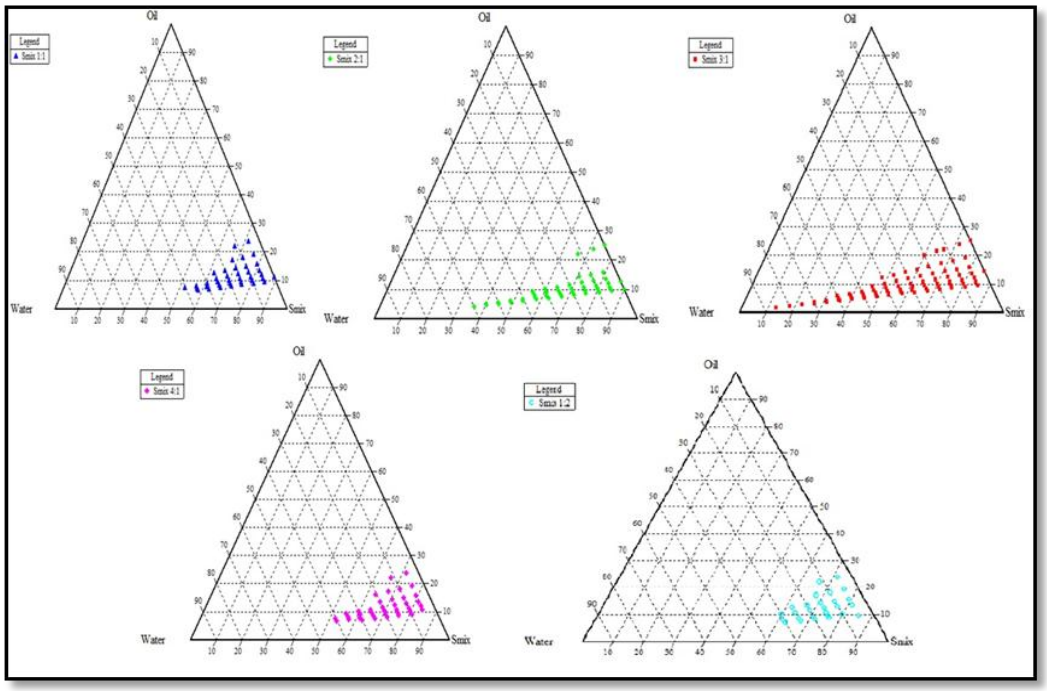


**Fig. 2: Pseudo ternary phase diagrams in presence of drug with different Smix ratios.**

**Optimization of formulation By $3^2$ Factorial design**

The nine formulations of genistein nanoemulsion were prepared by spontaneous emulsification technique using $3^2$ factorial design for optimization of two variables as oil concentration % and Oil:Smix ratio concentration. All batches of nine formulations of genistein NE were analyzed for droplet size ($Y_1$), Emulsification time ($Y_2$), PDI ($Y_3$) and % EE ($Y_4$) as shown in Table 5. The effect of independent variables and their interactions were studied based on the 3D response surface graph, polynomial equations, p-value and F-value generated by $3^2$ full factorial design.

For droplet size ($Y_1$), Emulsification time ($Y_2$), PDI ($Y_3$) and % EE ($Y_4$), the analysis of variance for these responses showed statistical significance and the model F value was found to be 3844.02, 340.91, 60.16 and 48.33 respectively which implies that the model is significant. The p-value was found to be less than 0.05 indicating significance of each variable affecting the response.

The polynomial equation generated helped in understanding the effect of selected independent variables on the.

**Droplet size ($Y_1$)** =
$48.04+18.41*X_1+5.10*X_2+0.42*X_1*X_2-7.42*X_1^2+2.18*X_2^2$

**Emulsification time ($Y_2$)**
$=47.04+14.37*X_1+4.48*X_20.50*X_1*X_2+3.82*X_1^2+0.76*X_2^2$

**PDI ($Y_3$)** =
$0.36+0.14*X_1+0.048*X_2+0.017*X_1*X_2+0.053*X_1^2-5.500E-003*X_2^2$

**% EE ($Y_4$)** = $86.40-5.52*X_1-2.11*X_2+0.17*X_1*X_2-0.013*X_1^2-0.21*X_2^2$

**Table 5: Droplet size, Emulsification time, PDI & % EE of Genistein NE.**

| Formulation Code | Run | Factor 1($X_1$) Oil Concentration (%) | Factor 2 ($X_2$) Oil: Smix ratio (3:1) | Response 1 ($Y_1$) Droplet Size (nm) | Response 2 ($Y_2$) Emulsification time (sec) | Response 3 ($Y_3$) PDI | Response 4 ($Y_4$) % EE |
|---|---|---|---|---|---|---|---|
| F1 | 1 | 3 | 1:1 | 19.46 | 31.7 | 0.242 | 94.64 |
| F2 | 2 | 3 | 2:1 | 22.56 | 37.5 | 0.256 | 90.98 |
| F3 | 3 | 3 | 3:1 | 28.96 | 41.8 | 0.308 | 89.67 |
| F4 | 4 | 6 | 1:1 | 45.41 | 43.6 | 0.314 | 87.45 |
| F5 | 5 | 6 | 2:1 | 47.89 | 46.9 | 0.356 | 86.82 |
| F6 | 6 | 6 | 3:1 | 45.78 | 55.8 | 0.426 | 84.66 |
| F7 | 7 | 9 | 1:1 | 55.64 | 62.3 | 0.468 | 82.76 |
| F8 | 8 | 9 | 2:1 | 58.96 | 64.5 | 0.578 | 80.95 |
| F9 | 9 | 9 | 3:1 | 66.83 | 70.4 | 0.604 | 78.46 |

The positive sign of the coefficient in the equations generated for the selected responses indicates increase in independent variable concentration increases the dependent variable of NE; whereas negative sign of the coefficient indicates increase in independent variable concentration decreases the dependent variable of NE. Further, higher value of coefficient suggests greater effect of that variable on the response.

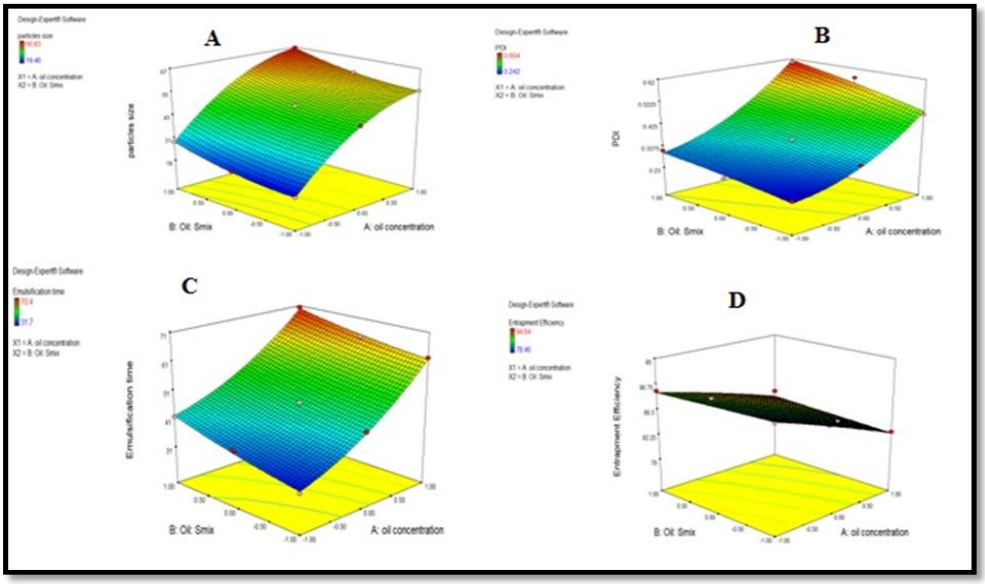


**Fig 3: Response surface 3D plot showing effect of factorial variables (A) Particle Size (B) PDI (C) Emulsification time (D) Entrapment Efficiency.**

**Droplet size analysis**

The droplet size of the emulsion is a crucial factor in nanoemulsion performance and also it determines the rate and extent of drug release as well as absorption. As depicted in the table 5, average droplet size ranges from 19.46 to 66.83nm indicating all the particles were in the required size range. As depicted in the Fig.3A, the droplet size increased with increase in oil and oil: Smix concentration due to increase in viscosity of the solution, that hinders the process of breaking the globules into smaller size.

**Emulsification time**

The efficiency of nano-emulsification is primarily estimated by determining the rate of emulsification which is an important index for the assessment of the efficiency of emulsification to form stable nanoemulsion. As depicted from Fig.3B, the emulsification time increased with increase in oil and oil:Smix proportion as the amount of surfactant and co-surfactant present was less to emulsify the larger proportion of oil.

**PDI analysis**

Polydispersity index (PDI) was found to be between 0.242 to 0.604. PDI below 0.3 indicates uniformity in the size distribution. As depicted in the Fig.3C, the PDI increased with increase in oil and oil:Smix concentration due to increase in viscosity of the solution, that hinders the process of breaking the globules into uniform size.

**Entrapment Efficiency**

The entrapment efficiency was found to be between 78.46 to 94.64%. As depicted in the Fig. 3D, the entrapment efficiency decreased with increase in oil and oil:Smix concentration.

From the $3^2$ factorial designs, it was evident that the formulation F1 showed satisfactory results with good emulsification time, droplet size, PDI and Entrapment efficiency. Hence, F1 formulation was selected as the optimized formulation and evaluated further for different technological properties.[19-21]

**Characterization of optimized formulation F1**

The F1 formulation was assessed visually and no sign of drug precipitation was observed even upon incremental dilution of formulation and the formulation was clear and stable. The zeta potential of the F1 formulation was found to be -28.42 mV (Fig. 4). High values of zeta potential construe an increase in electrostatic repulsive forces, thus ruling out the plausibility of coalescence. As such, the results clearly showed that phase separation did not occur which indicate the formation of stable NE. The drug content of the F1 formulation was found to be 93.32±0.206 %

**Table *6*: Evaluation of the F1 formulation.**

| Precipitation* | Clarity* | Zeta Potential (mV)* | % Drug content* |
|---|---|---|---|
| Stable | Clear | -28.42 | 93.32±0.206 |

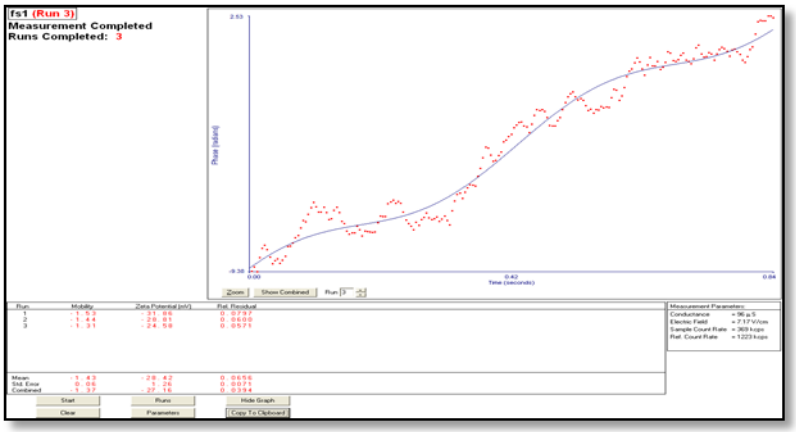


**Fig.4: Zeta potential of Optimized formulation F1.**

**Robustness to dilution**

Micro-emulsions resulting from dilution with various dissolution media, must be robust to all dilutions, and should not show any phase separation or dug precipitation even after 24h of storage. F1 was subjected to 50, 100 and 1000 fold dilution in distilled water, physiological fluid (pH 7.4 phosphate buffer) and F1 dispersions showed no signs of precipitation, cloudiness and separation after 24 h.

**Thermodynamic stability studies**

In order to understand kinetic stability of the formulation and to examine the chemical reaction between the excipients, thermodynamic stability studies were performed. As such, formulation should possess considerable stability in order to prevent precipitation, creaming or cracking. The formulation F1 was subjected to centrifugation studies and freeze thaw cycling test. The F1 did not show any signs of precipitation, creaming thereby establishing the kinetic stability of the system.

***In-vitro* permeation studies of the optimized formulation F1 and the pure drug**

The *in-vitro* permeation of the pure drug was found to be 65.34% at $12^{th}$ hour whereas the optimized formulation F1 exhibited 80.8% of drug permeation at $12^{th}$ hour (Fig. 5) which indicates that nanonization of the genistein enhanced the permeation of the drug.

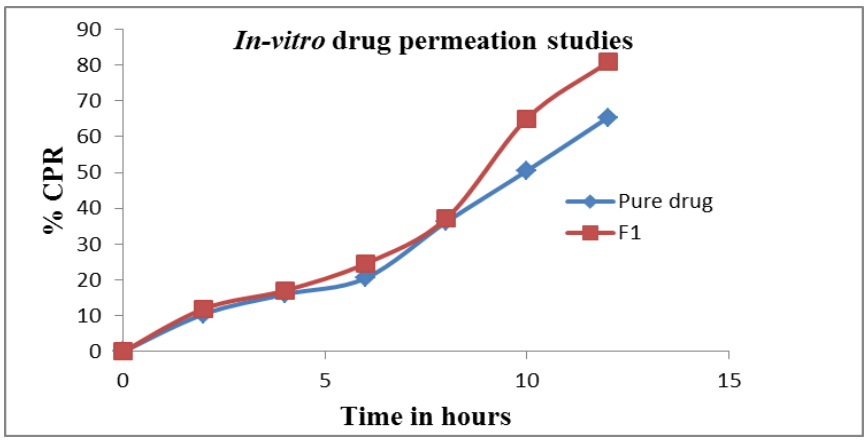


**Fig.5: *In-vitro* permeation study of optimized NE and pure Genistein in the phosphate buffer pH 7.4.**

**Anti oxidant Activity of Ascorbic Acid, Pure genistein solution and Genistein nanoemulsion**

The antioxidant activity of pure genistein solution and genistein nanoemulsion was compared with the control ascorbic acid solution at different concentrations as shown in Fig.6. The IC50 value of Ascorbic Acid, Pure genistein solution and Genistein nanoemulsion was found to be 44.31µg/mL, 47.90µg/mL and 53 µg/mL respectively. Hence antioxidants can be added to photo protective products to protect the skin from UV exposures which involves oxidation reactions and consequently increase their period of action. Antioxidants can also protect skin against Reactive Oxygen Species, decreasing skin aging.

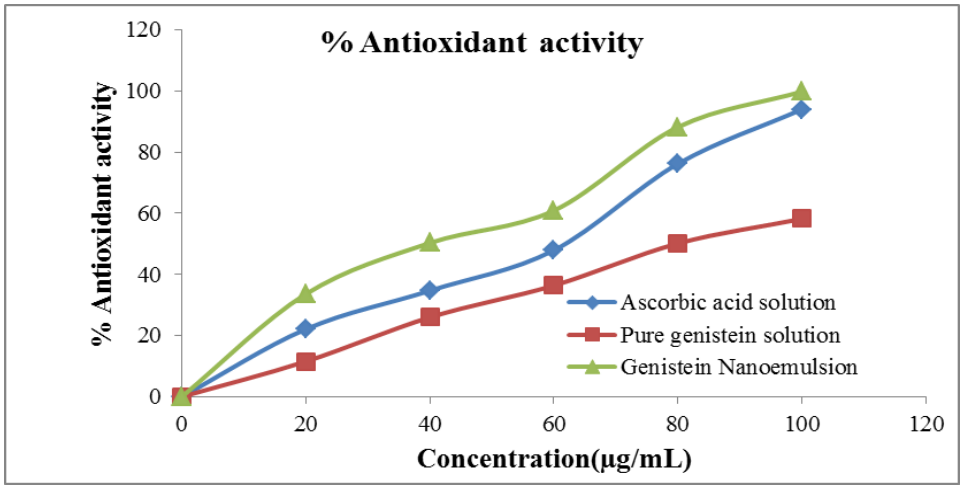


**Fig.6: Antioxidant activity of Ascorbic Acid, Pure genistein solution and Genistein nanoemulsion.**

## CONCLUSION

Nanoemulsion is promising drug delivery system for topical delivery of hydrophobic drugs, as it enhances the aqueous solubility of BCS class-II drugs. In the current study, after through screening and optimization study, F1 formulation was selected as optimized formulation based on droplet size, PDI and entrapment efficiency. The prepared nanoemulsion formulation showed enhanced and sustained delivery of drug and better anti-oxidant activity in comparison to pure drug. Thereby, proving that nanoemulsion formulation of genistein has overcome the shortcomings of the pure drug by maintaining drug concentration for a prolonged period, indirectly minimizing drug administration frequency. Hence, it can be concluded that NE formulation of genistein improved the dissolution of Genistein with enhanced anti oxidant property when exposed to the UV radiation.

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