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Physicochemical Screening, Formulation Development And Evaluation Of Emulgel Containing Tridax Extract Using Simple Latex Design Approach

Archana G. L[1] , K. Srisailam [2]* , M. Nagulu [3]

[1]Department of Pharmacy, Satavahana University, Karimnagar
[2]Department of Pharmacy, Satavahana University, Karimnagar*
[3]Swami Ramananda Tirtha Institute of Pharmaceutical Sciences, Nalgonda
**Corresponding Author: K. Srisailam *Department of Pharmacy, Satavahana University, Karimnagar*

The present investigation focuses on the development, characterization, and evaluation of a novel nanoemulgel loaded with Tridax procumbens ethanolic extract (TEE) for enhanced topical delivery and anti-inflammatory activity. Preliminary phytochemical screening of TEE confirmed the presence of key bioactive constituents, including alkaloids, steroids, and proteins. An HRBC membrane stabilization assay revealed significant anti-inflammatory potential, with 37.56% protection at 400 mg/mL, comparable to diclofenac sodium (39.22%). Based on solubility studies, PEG-200, Tween 80, and liquid paraffin oil were selected as co-surfactant, surfactant, and oil phase, respectively. A simplex lattice design was employed to optimize the formulation variables, focusing on particle size and viscosity. The optimized batch (F7) exhibited a droplet size of 319.3 nm, PDI of 0.049, and a zeta potential of -30.9 mV, indicating stability. SEM and TEM confirmed the spherical morphology. The nanoemulgel showed a pH in the skin-compatible range (5.51), high drug content (97.64%), good Spreadability (95.89%), and sustained in vitro drug release (95.38% over 360 min). FTIR and DSC studies confirmed extract-excipient compatibility and thermal stability. Accelerated stability studies over 3 months indicated no significant changes in physicochemical parameters. The study concluded that the formulated TEE-loaded nanoemulgel is a promising vehicle for topical therapy, offering enhanced penetration, controlled release, and excellent stability, making it suitable for pharmaceutical and cosmeceutical applications targeting inflammatory skin disorders.

Keywords: Tridax procumbens, Nanoemulgel, Anti-inflammatory, Factorial Design, HRBC Assay.

1. INTRODUCTION

Tridax procumbens, commonly known as coat buttons, is a widely recognized medicinal plant belonging to the Asteraceae family [1,2]. Traditionally used in Ayurvedic and folk medicine, the plant exhibits a diverse range of pharmacological properties such as anti-inflammatory, antimicrobial, wound healing, hepatoprotective, and antioxidant activities due to the presence

of flavonoids, alkaloids, carotenoids, and sterols [3,4]. However, its poor aqueous solubility and permeability pose challenges for effective topical delivery. Emulgels, which are emulsions incorporated into a gel base, serve as an ideal vehicle to enhance the skin permeability of lipophilic herbal extracts like Tridax procumbens[5-7]. These systems offer dual benefits: the stability and Spreadability of gels and the enhanced drug release of emulsions [8,9].
Emulgel is an innovative and versatile topical drug delivery system that merges the advantages of emulsions and gels, making it particularly effective for the delivery of lipophilic drugs and phytoconstituents [10]. While traditional gels are preferred for their ease of application and patient compliance, they cannot often incorporate hydrophobic compounds. Emulgels overcome this limitation by forming an emulsion base (either oil-in-water or water-in-oil), which is then gelled using suitable gelling agents such as Carbopol or HPMC. This hybrid system enhances the solubility, Spreadability, and skin penetration of active pharmaceutical ingredients [11-13]. Additionally, Emugels provide a non-greasy feel, improved drug release kinetics, and better patient acceptance, especially for conditions requiring prolonged application such as inflammation, wounds, fungal infections, and dermatological disorders. Due to these benefits, Emugels have gained prominence in both pharmaceutical and cosmeceutical applications and are increasingly being used to formulate herbal extracts and bioactives for topical therapy [14].

2. MATERIALS AND METHODOLOGY

Materials

Tridax extract was purchased from Herb Sky Bio-tech Co. Ltd., China; Liquid paraffin oil was purchased from Moychem Pvt. Ltd., Methanol, Ethanol, Chloroform, Acetone, Potassium dihydrogen phosphate (KH_2PO_4), Sodium chloride, HPMC K4, PEG-200, and Tween80 were obtained from Loba Chem Pvt. Ltd., Mumbai. All materials used were of analytical grade. Double-distilled water was used as an aqueous solvent.

Preliminary Phytochemical Screening

Standard qualitative chemical tests were carried out to identify phytoconstituents such as carbohydrates (Molisch's test), proteins (Biuret, Xanthoprotein, Cystein tests), steroids (Salkowski and Liebermann-Burchard), alkaloids (Dragendorff's, Mayer's, Wagner's, Hager's), flavonoids (Shinoda, Lead acetate), and tannins (Ferric chloride, Gelatin, Acetic acid). Glycosides and fats were also screened using their respective methods.

In Vitro Anti-inflammatory Activity

The HRBC membrane stabilization method was used. Blood was collected from healthy volunteers, mixed with Alsever's solution, centrifuged, and washed with isotonic saline. A 10% RBC suspension was prepared. 0.5 mL of this suspension was mixed with phosphate buffer, hyposaline, and TEE extract at 200 and 400 mg/mL. The mixture was incubated at 37 °C for 30 minutes, followed by centrifugation at 3000 rpm. The absorbance of the supernatant was recorded at 560 nm. The percentage protection was calculated in comparison to the control using diclofenac sodium (5 mg/mL) as a standard.

Physical Characterization and Identification

Physical examination

In the pre-formulation study, the physical inspection of Tridax extract is done first for its appearance, Colour, and taste for identification.

FTIR Analysis

FTIR analysis was done on FTIR spectrometer (Ver. 7.03 Shimadzu, Japan) with KBr disc. In the FTIR infrared spectroscopy, the spectrum was recorded in the wavelength region of 4000-400 cm-1. 10 mg of the drug was mixed with KBr and triturated, then it was placed in a holder and pressed to form a pellet. Then it was placed under an IR beam and a spectrum was obtained on a computer.

Differential Scanning Calorimetry

Thermogram for Tridax extract was obtained using DSC (Mettler DSC 1-star system, Mettler-Toledo, Switzerland). The drug was sealed in **a** perforated aluminum pan and heated at a constant rate of 10°C/min over the temperature ranges of 30-350°C at 20ml/min nitrogen purging.

Extract-Excipients Compatibility Study

FTIR Analysis

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Differential Scanning Calorimetry

Thermogram for Tridax extract and other excipients was obtained using DSC (Mettler DSC 1 star system, Mettler-Toledo, Switzerland). The drug was sealed in **a** perforated aluminum pan and heated at a constant rate of 10°C/min over the temperature ranges of 30-350°C at 20ml/min nitrogen purging.

Screening of Oil, Surfactant, and Co-surfactant (Solubility Study)

Based on the solubility of Tridax extract, oils, surfactants, and co-surfactants were screened out. The solubility of Tridax extract was determined in various oils (oleic acid, liquid paraffin oil, olive oil) Surfactants (tween80, span20, tween 20), and co-surfactants (polyethylene glycol (PEG-200, ethanol, propylene glycol). The Tridax extract was taken in excess in centrifuge tubes, and 5 mL of each of the oil, surfactant, and co-surfactant was added. The mixtures were shaken in a vortex mixer for 15 minutes. After 24 hrs, samples were centrifuged for 15min at 3000 rpm. The supernatant was then filtered through a Whatman filter and diluted with methanol and analyzed in a UV-visible spectrophotometer at 415 nm; all samples were repeated thrice.

Preparation of Tridax extract loaded nanoemulsion

On the basis of their visual observation like transparency and viscosity, 14 formulations were selected out as per factorial design for preparing Tridax extract loaded nanoemulsion. The required amount of Tridax extract was dissolved in the calculated quantity of oil phase for the said volume of nanoemulsion. The calculated quantity of Smix (surfactant and co-surfactant) were added and mixed thoroughly in beaker using magnetic stirrer at room temperature. Then double distilled water was added drop wise drop till a clear and transparent liquid was obtained. The prepared nanoemulsion was stored in tightly closed suitable container at ambient temperature.

Preparation of Tridax extract loaded nanoemulsion in gel (nano-emulgel)

For the preparation of nanoemulgel HPMC K4 was used as gelling agents in ratio 1.0% (gels made with specified concentration range in the water). The mixed mass was stirred for the time, gels were added slowly into nanoemulsion, mixed and kept aside to settle the air entrapment and assessed next day for visual property. The gelling agent was selected for further preparation of nanoemulgel. The gel made with selected polymer concentration of at various concentrations (1.0%).

Factorial Design

Construction of and Formula optimization using SLD

TW80:TP (Smix) were blended in various proportions, and a mixture of LPO with Smix(s) was created, resulting in LPO: Smix (TW80:TP) ratios as outlined in Table 5 and 6. The optimization of Tri-NEs was carried out using Design-Expert (Stat-Ease Inc., USA), where the variables included concentrations of water (X1), LPO (X2), and Smix (X3), and the corresponding response measured was particle size (Y1) and viscosity (Y2). The statistical relationships between independent variables and the 3D Response surface plot were also generated. The formulation layout for the factorial design batches is shown in Tables 1 and 2. [15].

Table 1: Levels of variables for optimization

Batch Code	X1: Water (%)	X2: Paraffin Oil (%)	X3: Smix (%)
F1	0	0	1
F2	1	0	0
F3	0.5	0.5	0
F4	0.5	0	0.5
F5	0.16	0.66	0.16
F6	0.5	0.5	0
F7	0.33	0.33	0.33

F8	0	0	1
F9	0.66	0.16	0.16
F10	1	0	0
F11	0.16	0.16	0.66
F12	0	0.5	0.5
F13	0	1	0
F14	0	1	0

Table 2: Simple Latex Design (SLD)

Batch Code	X1: Water (%)	X2: Paraffin Oil (%)	X3: S_{mix} (%)	Particle Size (nm)	Viscosity (cps)
F1	62	2	34	432	1531
F2	64	2	32	471	1630
F3	66	2	32	349	1735
F4	63	2	33	377	1821
F5	62.21	2.64	32.16	363	1732
F6	63	2	32	270	1625
F7	**62.36**	**1.32**	**32.64**	**319**	**1685**
F8	62	2	34	456	1651
F9	63.56	0.64	32.16	345	1765
F10	64	2	32	430	1845
F11	62.21	0.64	33.56	470	1525
F12	62	2	33	339	1809
F13	62	4	32	351	1715
F14	62	4	32	333	1678

Characterization of Nanoemulsion

Physical characterization

The prepared nanoemulsion formulations were visually inspected for their colour, transparency, homogeneity and consistency [16-18].

Droplet Size and Size Distribution
Droplet size was determined by photon correlation spectroscopy (PCS) that analyses the fluctuations in light scattering due to Brownian motion of the droplets using a Zetasizer (1000 HS, Malvern Instruments, Italy). 0.1ml nanoemulsion was dispersed in 50ml of water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25°C a 90° angle.

Zeta potential analysis
Zeta potential of a droplet is the overall charge that the particle acquires in a particular medium. Knowledge of the zeta potential of nanoemulsion helps to assess the stability of the formulation during storage.

Surface Morphological Study
Surface morphology of the nanoemulsion was performed by using SEM. A nanoemulsion was placed on Formvars coated copper grids and allowed to equilibrate. Excess liquid was removed with a filter paper and dried at room temperature for about half an hour. The dried grid containing the nanoemulsion was visualized using SEM.

Characterization of Nanoemulsion Gel

Measurement of pH
The pH values of the nanoemulsion were measured at 25ºC using digital pH meter. 10% w/w dispersion (1gm of nanoemulsion was dispersed in 10 ml of distilled water. At first the reading of pH- meter was adjusting with a known pH solution (pH 4 and pH 7). Then the prepared formulations were subjected for pH analysis.

Measurement of Viscosity
The viscosities of nanoemulsion formulation were measured at 25ºC using Brookfield viscometer (Brookfield DV-E Viscometer) using spindle no 6 at 10, 20.30 and 60 rpm.

Drug Content Determination
The drug content of Tridax extract in nanoemulsion gel was determined by UV-Spectrophotometer. 1.0 g of formulation was accurately weighed, dissolved in 100 ml of methanol: phosphate buffer (2:8). It was filtered and diluted if required. Absorbance was determined using UV spectrophotometer at 415 nm.

Spreadability
1g emulgel preparation was placed above ground slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. A weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the emulgel between the two slides. Measured quantity of weight (35g) was placed in the pan attached to the pulley with the help of hook. Time in seconds taken by two slides to slip off from emulgel and placed in between the slides under the direction of certain

load. Lesser the time taken for separation of two slides, better the Spreadability. It is calculated by using the formula.

S = m X t/l x 100

Where S is spreadability, m is weight placed on upper slide, l is length of upper slide, and t is the time taken.

In-vitro Release Study of Tridax Extract Loaded Nanoemulgel Formulations

The in-vitro permeation studies were carried out using Franz diffusion cell, which is a reliable method for prediction of drug transport across the skin. These studies were conducted employing dialysis membrane. The receptor compartment of the diffusion cell was filled with 25 ml of phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 100 rpm and the temperature was maintained at 37±0.50°C throughout the experiments.

Accelerated Stability Study

Stability studies were done as per ICH guidelines for 3 months. The optimized micro emulgel was kept in an amber color glass bottle then placed in an accelerated stability chamber at 40°C±5°C temperature and 70%±5% RH. After three months, gel was tested for pH, Viscosity, and drug content [19].

RESULT AND DISCUSSION

Phytochemical Screening

As presented in Table 3, TEE tested positive for carbohydrates, proteins, steroids, and alkaloids. Notably, proteins were confirmed by multiple tests (Biuret, Xanthoprotein, and Cystein), suggesting the presence of enzymatic or structural protein fractions. The consistent presence of all alkaloids and steroid markers indicates significant pharmacological potential. Flavonoids and tannins were less prominent, but their presence in trace amounts may still contribute to antioxidant and anti-inflammatory actions.

Table 3: Phytochemical test results for TEE (Tridax Ethanol Extract)

Chemical Test	Observation in TEE
Test for Carbohydrates	
Molisch Test	Present
Test for Reducing Sugars	
i) Benedict's Test	Absent
ii) Fehling's Test	Absent
Test for Monosaccharides	
i) Barfoed's Test	Absent
Test for Proteins	
i) Biuret Test	Present
ii) Million's Test	Absent

iii) Xanthoprotein Test	Present
iv) Test for Sulphur-containing Protein	Present
v) Precipitation Test	Present
Test for Amino Acids	
i) Ninhydrin Test	Present
ii) Tyrosine Test	Absent
iii) Tryptophan Test	Absent
iv) Cystein Test	Present
Test for Fats and Oils	
i) Solubility Test	Absent
ii) Saponification Test	Absent
Test for Steroids	
i) Salkowski Test	Present
ii) Liebermann-Burchard Reaction	Present
iii) Liebermann's Reaction	Present
Test for Cardiac Glycosides	
i) Keller-Killiani Test	Absent
ii) Legal's Test	Absent
Test for Anthraquinone Glycosides	
i) Borntrager's Test	Absent
ii) Modified Borntrager's Test	Absent
Test for Saponin Glycoside	
i) Foam Test	Absent
ii) Test for Cyanogenetic Glycoside	Absent
Test for Alkaloids	
i) Dragendorff's Test	Present
ii) Mayer's Test	Present
iii) Wagner's Test	Present
iv) Hager's Test	Present
Test for Tannins and Phenolic Compounds	
i) Ferric Chloride Test	Absent
ii) Lead Acetate Test	Absent
iii) Dilute Iodine Test	Absent
iv) Dilute Nitric Acid Test	Absent
v) Potassium Permanganate Solution Test	Absent
vi) Gelatin Test	Absent
vii) Bromine Test	Absent
viii) Acetic Acid Test	Absent
Test for Flavonoids	
i) Shinoda Test	Absent
ii) Lead Acetate Test	Absent
iii) Ferric Chloride Test	Absent

iv) Alkali Test	Absent

In Vitro Anti-inflammatory Activity

TEE exhibited notable membrane stabilization in HRBC assays. At 200 mg/mL and 400 mg/mL, it showed 29.89% and 37.56% protection, respectively, closely approaching the standard drug diclofenac sodium (39.22%). The results validate the anti-inflammatory potential of TEE, likely due to synergistic effects of steroids, alkaloids, and minor flavonoids, which contribute to membrane integrity and reduce hemolysis under hypotonic conditions.

Preformulation Studies

The pre-formulation studies of tridax extract were performed, and the tridax extract was found as yellow-brown resinous amorphous powder with a pungent characteristic taste of ginger.

FTIR Analysis

In Figure 1, the FTIR spectra of tridax extract is given, which show the characteristic band at 3472.21 cm-1 (O-H stretching), 2990.28 cm-1 (CH2 methylene stretch), 1634.81 cm-1 (C=O stretch) 1448.24 cm-1 (C=C aromatic ring stretch), 1364.82 cm-1 (CH3 methyl bond), 1017.87 cm-1 (C-OH stretch CH2OH) and 711.94 cm-1 (Phenolic O-H bond).

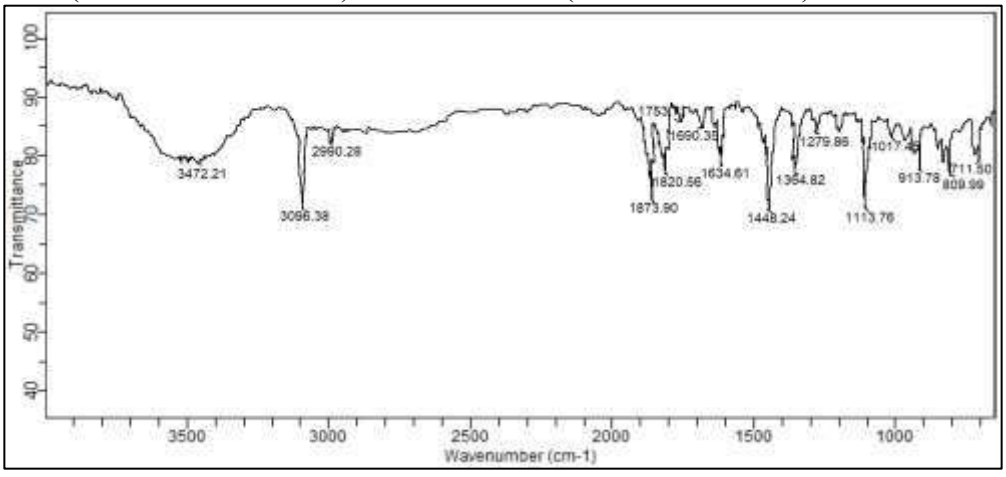


Figure 1: FTIR spectra of Tridax Extract

Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) analysis of tridax extract at a scanning rate of 10°C/min revealed a distinct melting endothermic peak at 123.47ºC, as depicted in Figure 36. This peak indicates the transition from a semi-solid to a liquid state, showing the heat flow associated with this phase change. Furthermore, as the temperature increases, certain components of the plant extract may undergo decomposition, releasing energy in the process. Show small endothermic peak. This decomposition is evident on the DSC curve as an endothermic peak, corresponding to the heat absorbed during this specific thermal event (Figure 2).

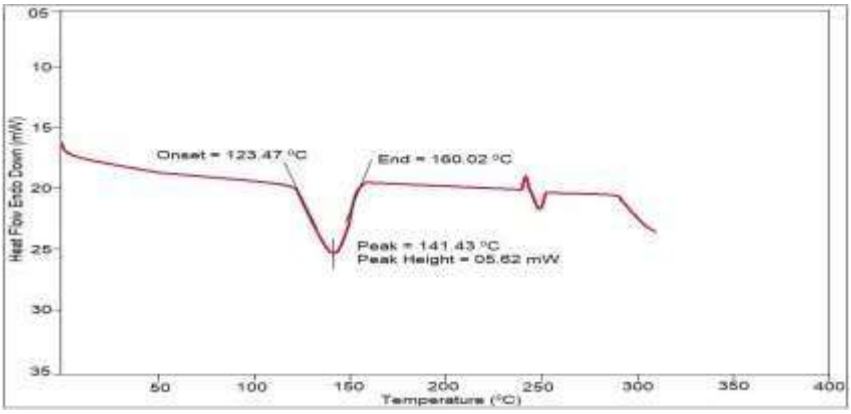


Figure 2: DSC spectra of Tridax Extract

Extract-Excipients Compatibility Study

FTIR Analysis

In Figure 3, the FTIR spectra of tridax extract is given, which show the characteristic band at 3472.21 cm-1 (O-H stretching), 2990.28 cm-1 (CH2 methylene stretch), 1634.81 cm-1 (C=O stretch) 1448.24 cm-1 (C=C aromatic ring stretch), 1364.82 cm-1 (CH3 methyl bond), 1017.87 cm-1 (C-OH stretch CH2OH) and 711.94 cm-1 (Phenolic O-H bond). All the peaks were found intact and there no significant changes were observed in characteristics peaks of pure extract when compared with pure extract. This indicates the extract is compatible with other excipients.

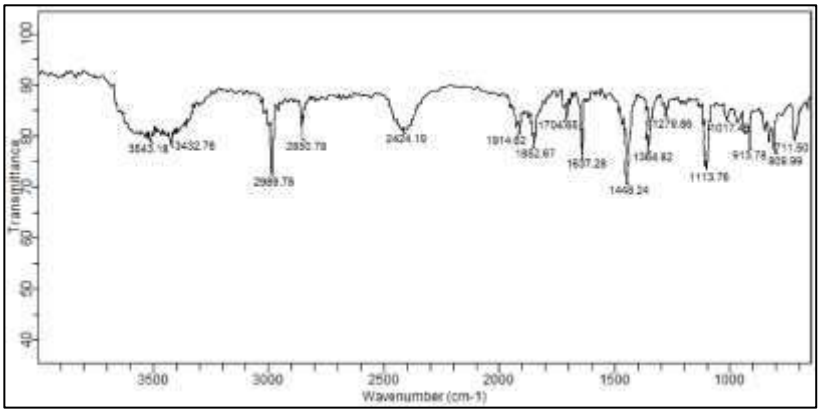


Figure 3: FTIR spectra of Tridax Extract with Physical Mixture

Differential Scanning Calorimetry

In this context, when examining the DSC thermograms, the drug displayed a distinct melting point at 123.47°C. HPMC K4 and liquid paraffin oil exhibited their respective melting points at 78.18°C and 62.19°C in their DSC thermograms, with no observable shifts in these peaks when mixed with extract. This lack of shift suggests compatibility between the drug and both

HPMC K4 and liquid paraffin oil. The comparison of DSC thermograms for tridax extract, individual excipients, and drug-excipient mixtures can be seen in Figures 4 .

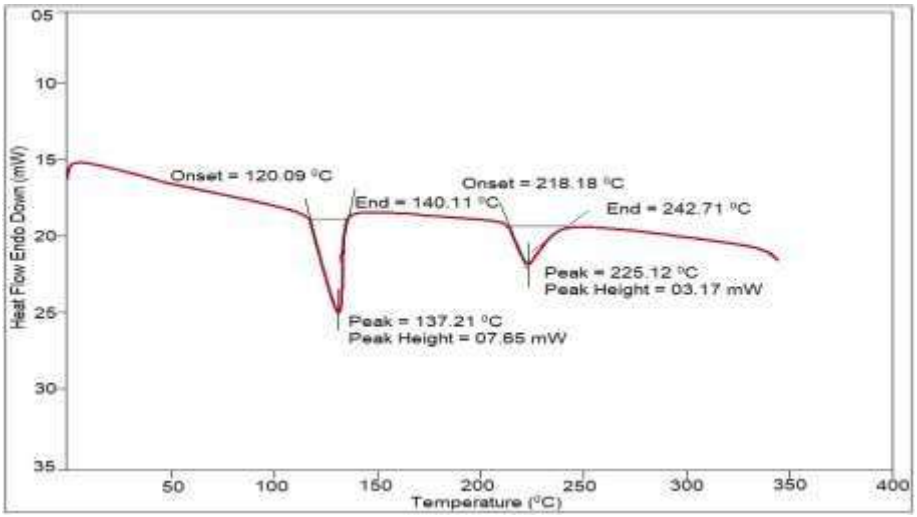


Figure 4: DSC spectra of Tridax Extract with Physical Mixture

Screening of Oil, Surfactant, and Co-surfactant (Solubility Study)

Screening of oils, surfactants, and co-surfactants is based on their solubility profile for ginger extract as shown in Table 4. The LPO was selected as oil, Tween 80 as surfactant, and PEG-200 as co-surfactant.

Table 4: Solubility of Ginger extract

Name of Excipients	Solubility (mg/ml)
Liquid paraffin oil	36.12
Tween 80	37.02
PEG-200	38.58

Formulation and selection of Nanoemulsion and Nanoemulgel

On the basis of their visual observation like transparency and viscosity, 14 formulations were selected out as per factorial design for preparing ginger extract loaded nanoemulsion. For the preparation of nanoemulgel HPMC K4 was used as gelling agents in ratio 1.0% (gels made with specified concentration range in the water).

Formulation Design

The two factors with lower, middle and upper design points in coded and un-coded values are shown in table. The ranges of responses Y1 and Y2 were 270-471 d.nm and 1525-1845 cps respectively. All the responses observed for nine formulations prepared were fitted to main effect model, which was found as the best fitted model for Y1 and Y2, using Design Expert® software. The values of R^2 SD and % CV are given in (Table 5), along with the regression equation generated for each response. The results of ANNOVA in (Table 6), for the dependent

variables demonstrate that the model was significant for all the response variables. It was observed that independent variables X1, X2 and X3 had a positive effect on the entrapment efficiency and an desired particle size of nano-formulation i.e. nano-emulsion was achieved.

Model Assessment

After putting the data in Design Expert® software for, Fit summary applied to data in that Main Effect Model had been suggested by the software for all the responses. The statistical evaluation was performed by using ANNOVA. Results are shown in (Table 5and 6). The coefficients with more than one factor term in the regression equation represent interaction terms. It also shows that the relationship between factors and responses is not always linear. When more than one factor are changes simultaneously and used at different levels in a formulation, a factor can produce different degrees of responses.

Table 5: Results of Analysis of Variance for Measured Response (Particle Size)

Parameters	Values
Model	Quadratic Model (Significant)
Model p-value	0.045
Standard Deviation	7.82
Mean	77.67%
CV	10.07%
R^2	0.8179
Adequate Precision	7.7072
Regression Equation	Y1 = 764.63 X1 + 733.37 X2 + 643.37 X3 - 2789.60 X1 X2 – 2447.20 X1 X3 - 2477.87 X2 X3 +5494.40 X1 X2 X3

Table 6: Results of Analysis of Variance for Measured Response (Viscosity)

Parameters	Values
Model	Quadratic Model (Significant)
Model p-value	0.039
Standard Deviation	7.19
Mean	77.07%
CV	10.39%
R^2	0.8079
Adequate Precision	7.6903
Regression Equation	Y1 = 734.63 X1 + 723.37 X2 + 623.37 X3 - 2879.60 X1 X2 – 2437.20 X1 X3 - 2837.87 X2 X3 +5954.40 X1 X2 X3

Response Surface Plot Analysis

From the 3D response surface plot (Figure 5), and Nanoparticles being nanoparticulate structures, the formulation batch amongst all the design batches giving the least particle size will be preferred more and selected as an optimized batch. Where F7 Design Batch, with a Smix concentration of about 32.64% and paraffin oil concentration 1.32%, shows the least particle size i.e., 319.3 nm.

From the 3D response surface plot (Figure 5) and the regression coefficient values of factors, it was concluded that the viscosity of nanoemulgel increases with an increase in the concentration of liquid paraffin oil and HPMC K4M. Interaction and nonlinearity was not observed. The results also indicated that the HPMC K4M was given a more significant effect on viscosity as compared to liquid paraffin oil.

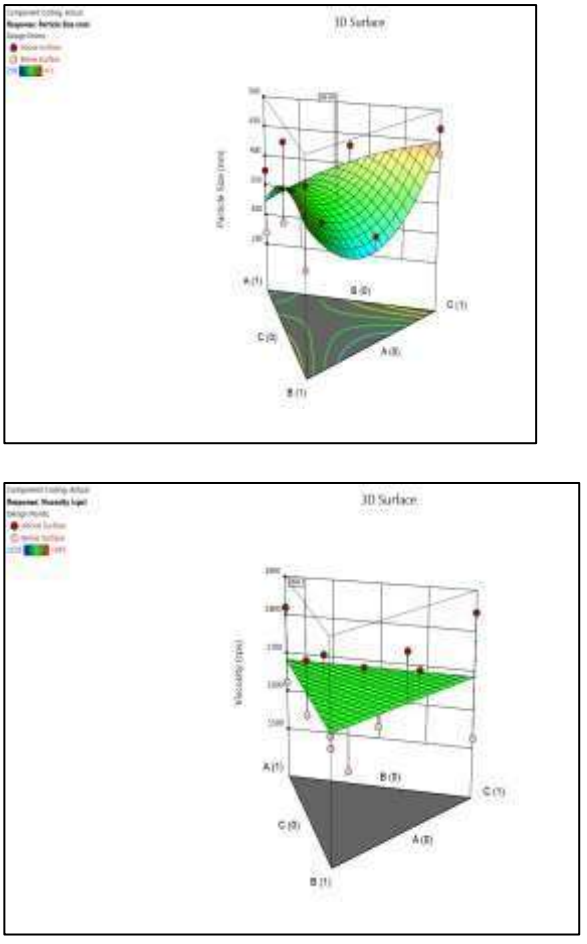


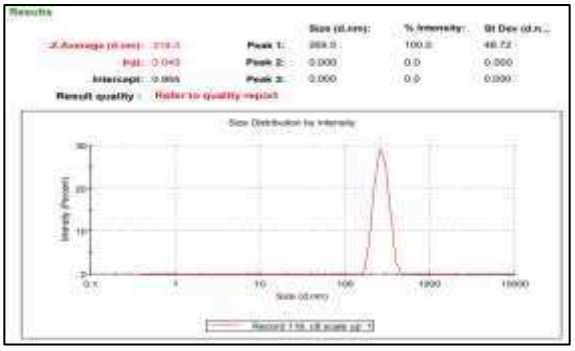
Figure 5: Response surface plots for X1, X2 and X3 on Mean Particle Size (Y1) and Viscosity (Y2)

Physical characterization

All formulations are clear, transparent, and homogenous and no grittiness and no clogs were found and suitable consistency.

Droplet Size and Size Distribution, Zeta potential analysis

Low polydispersity index values might be associated with a high homogeneity in the particle population, whereas high polydispersity index values suggest a broad size distribution or even several populations. The optimized formulation batch (F7) showed a mean particle size of 319.3 nm with PDI 0. 049.The zeta potential values of extract extract-loaded nanoemulsion that was found to be -30.9 mV. The high value of zeta potential confirms the stability of the nanoemulsion (Figure 6).



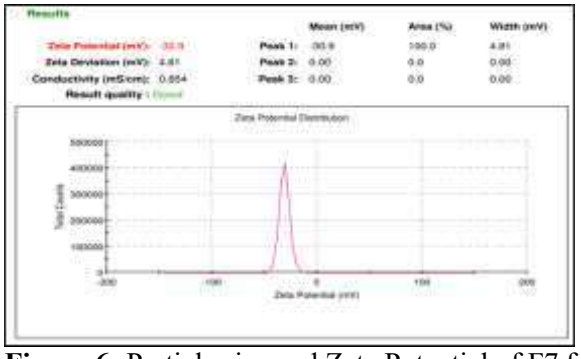


Figure 6: Particle size and Zeta Potential of F7 formulation

Surface Morphological Study

Surface morphology of the nanoemulsion was evaluated using SEM/TEM, from which it can be seen that the droplets have smooth surfaces. Droplets show a spherical shape with a size 200 nm (Figure 7).

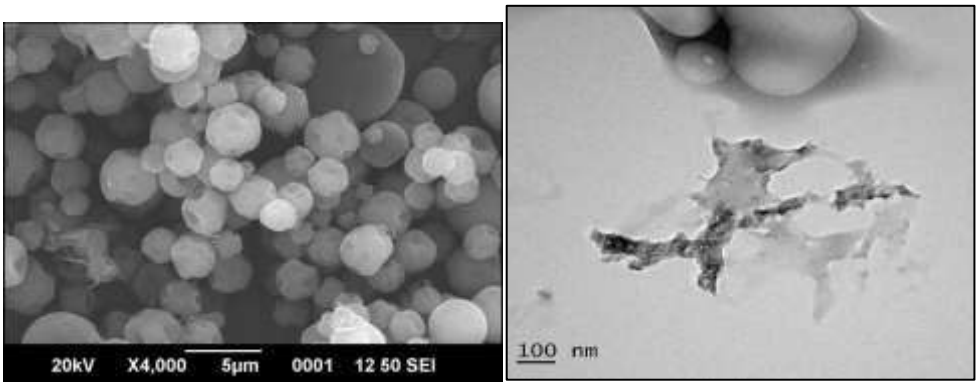


Figure 7: SEM and TEM image of F7 formulation

Characterization of Nanoemulsion Gel

Measurement of pH

The pH value for the selected NE and its gel (F1-14) formulation was found to be 6.18-6.72 and 5.07-5.87 as shown in Table 7. The pH of the NE was found to be within the range of pH of skin (5-7) and would not cause any irritation to the skin. Thus, prepared NE formulations are suitable for skin application and the formulated nanoemulgel are also suitable for skin application.

Table 7: Characterization of Nanoemulsion Gel

Sr. No.	Formulation code	pH value of NE	pH value of NE gel	Viscosity (cps)
1	F1	6.22±0.02	5.11±0.03	1531±2.33
2	F2	6.46±0.03	5.13±0.07	1630±2.28
3	F3	6.48±0.04	5.15±0.04	1735±2.21
4	F4	6.68±0.01	5.25±0.01	1821±2.29
5	F5	6.27±0.03	5.29±0.03	1732±2.31
6	F6	6.32±0.02	5.33±0.05	1625±2.35
7	F7	6.50±0.01	5.51±0.02	1685±2.36
8	F8	6.72±0.04	5.53±0.04	1651±2.39
9	F9	6.53±0.06	5.57±0.07	1765±2.33
10	F10	6.30±0.03	5.07±0.01	1845±2.27
11	F11	6.69±0.02	5.11±0.05	1525±2.26
12	F12	6.29±0.04	5.63±0.03	1809±2.21
13	F13	6.18±0.05	5.87±0.02	1617±2.28
14	F14	6.39±0.03	5.15±0.03	1778±2.31

Measurement of Viscosity

Brookfield viscometer was used to measure the viscosity of nanoemulsion and nanoemulsion gel (NE gel) at different spindle speeds. Viscosity reveals the rheological properties of all

nanoemulsion formulation. All formulation shows shear thinning effect as the shear stress increased the viscosity was decreased. Formulation F7 was found more viscous than other formulations.

Drug Content of Nanoemulsion Gel

Drug content in the nanoemulgel (which is made by adding gelling agent in NE) is supposed to be decreased in some extent because of gelling agent which occupies some volume as it swells in formulations so it was determined by UV spectrometer at 415 nm for the same. The range of percentage drug content of nanoemulsion Gel was 85.19% to 97.64% as shown in Table 19. The percentage drug content of formulations was within a permissible range.

Spreadability

Spreadability determined as % increase in area of gel upon pressing with certain weight. All formulations have shown good Spreadability (Table 8)

Table 8: Characterization of Nanoemulsion Gel

Sr. No.	Formulation code	Drug Content (%)	Spreadability (%)
1	F1	85.19±0.27%	89.18±1.10%
2	F2	87.91±0.29%	88.45±1.14%
3	F3	85.92±0.30%	87.36±1.16%
4	F4	86.38±0.31%	85.91±1.22%
5	F5	86.91±0.32%	92.21±1.25%
6	F6	87.28±0.26%	93.35±1.27%
7	F7	97.64±0.28%	95.89±1.29%
8	F8	91.47±0.32%	92.26±1.28%
9	F9	92.21±0.31%	91.65±1.32%
10	F10	93.38±0.30%	90.78±1.26%
11	F11	94.36±0.28%	91.89±1.24%
12	F12	88.38±0.29%	89.65±1.22%
13	F13	89.65±0.35%	90.45±1.29%
14	F14	90.35±0.39%	88.36±1.32%

In-vitro Release Study

Tridax extract-loaded emulgel of all batches were studied in-vitro for drug release using the Franz diffusion cell. For batches F1-F14, the maximal drug release was found to be about 85.64±2.11%-95.38±3.16% as shown in Table 20. The in-vitro release of produced emulgel in phosphate buffer saline (PBS) (PH 7.4) at 37ºC was studied. Nanoemulgels were dialyzed for 60 min. The quantity of drug released was measured by using a UV-visible spectrophotometer to measure absorbance. A burst drug release was observed in the beginning, which may be due to the smaller particle size attributed to the large surface area of the emulgel; apart from it, diffusion of the drug from the outer shell of the emulgel may be responsible for the initial burst release (Table 9& Figure 8).

Table 9: In-vitro release profile of nanoemulgel

Sr. No.	Time (Min.)	nanoemulgel (F7)
1	0	0
2	60	21.49±1.63
3	120	42.42±2.14
4	180	66.89±1.96
5	240	79.98±2.23
6	300	89.29±2.65
7	360	95.38±2.69

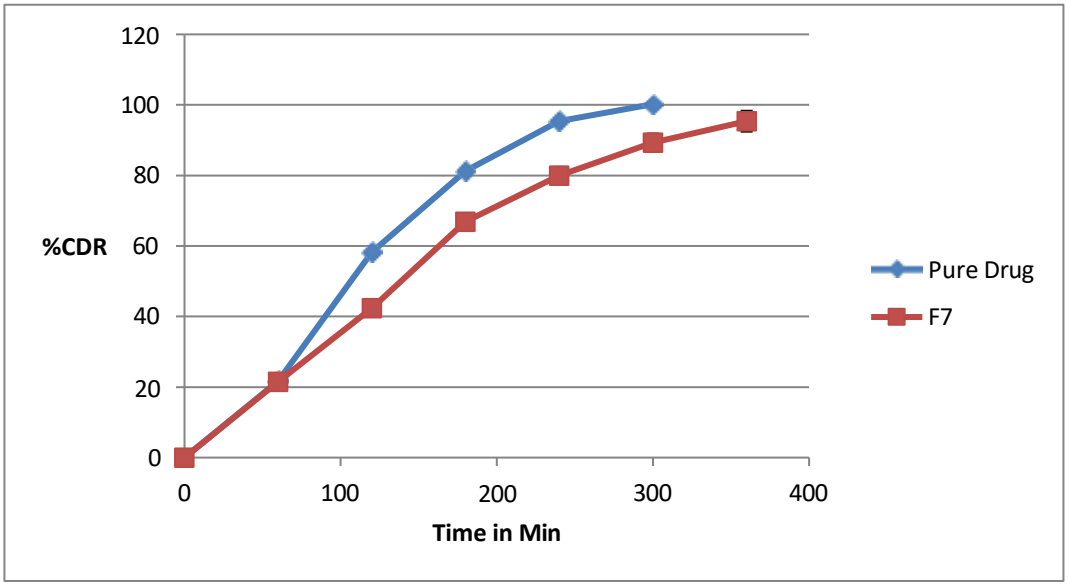


Figure 8: In-vitro drug release study of F7

Accelerated Stability Study

The optimized emulgel were subjected to stability studies and the results are given in Table 10. Based on these results it is revealed that, Tridax loaded nanoemulgel (Formulation batch F7) were found to be stable formulation at the given temperature and humidity condition.

Table 10: Stability study of parameters of the optimized formulation (F7)

Parameters	Initial Month	1st Month	2nd Month	3rd Month
pH	5.51 ± 0.03	5.53 ± 0.04	5.59 ± 0.02	5.53 ± 0.03
Viscosity (cps)	1685 ± 2.31	1675 ± 2.28	1695 ± 2.33	1687 ± 2.30
Drug content (%)	97.64 ± 0.32	97.49 ± 0.35	97.68 ± 0.31	97.60 ± 0.39

Conclusion

In summary, the in-depth pre-formulation investigations, encompassing FTIR and DSC analyses, offered vital insights into both the physical and molecular traits of tridax extract. Compatibility assessments with excipients, followed by the formulation of nanoemulsion and nanoemulgel, demonstrated the practicality of establishing a reliable delivery system for tridax extract. The optimized formulation (F7) displayed positive attributes, including a mean particle size of 319.3 nm, a notable zeta potential, and sustained release characteristics. The comprehensive assessment, covering pH, viscosity, drug content, and stability, collectively lays a sturdy foundation for developing a dependable and efficient delivery system for tridax extract, with potential applications in both therapeutic and cosmetic formulations.

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