ARTICLE

FORMULATION AND EVALUATION OF *CALLISTEPHUS CHINENSIS* LOADED NANOEMULSION FOR ACNE MANAGEMENT

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ABSTRACT

Acne vulgaris is an inflammatory condition of the skin that leads to the formation of acne lesions. Its treatment typically requires long periods of time for the application of topical therapies. The aim of the study was to prepare a nanoemulsion (NE) containing the extract of *Callistephus chinensis* (CC) flowers and to evaluate its potential use in acne vulgaris treatment. The present work focused on extraction of the flower, phytochemical screening, development of NE using tea tree oil, Tween 80, and PEG 400 by using Experimental design (Quality by Design approach) in nine batches NE1-NE9. These were evaluated for physicochemical characteristics that included particle size, Zeta potential, PDI, pH, viscosity, drug content, % entrapment Efficiency (EE), cumulative % in vitro drug release study, and Physical stability. Among all formulations, NE8 exhibited the smallest particle size (135.8±0.65 nm), highest negative zeta potential (−29.7 mV), with PDI (0.142), suitable pH value of 6.02±0.41, good consistency (121.45±0.05) maximum %EE (92.11±0.25) and cumulative % in vitro drug release (94.17±0.66). Statistically validated the response surface methodology applied for NE8, the experimental data were subjected to ANOVA. It confirmed the adequacy and significance of the models developed for each response parameter. Thus, Optimized batch of NE was successfully developed and found stable in the acne management.

Keywords: China aster, Callistephus chinensis, Herbal nanogel, Nanoemulsion, Acne vulgaris,

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INTRODUCTION

Acne Vulgaris, involves the inflammation of the pilosebaceous unit and is characterized by the presence of open and closed comedones, papules, pustules and nodules. The disorder is multifactorial, it results from the overproduction of sebum, hyperkeratinization of the follicle, colonization of cutibacterium acnes and inflammation. Current therapeutic strategies for acne vulgaris are topical antibiotics, retinoids and benzoyl peroxide. These agents control the disease, but all can cause adverse effects and microbial resistance. As such, the research focus has been on developing semi-effective herbal medicines to minimize risk of adverse effects. China Aster CC, belongs to the family Asteraceae and comprises flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, and glycosides. Due to these constituents, the plant has antimicrobial, antioxidant, and anti-inflammatory activities, making it a potential candidate for the management of acne. However, limitations of direct topical application of herbal extracts include poor permeability and short shelf-life the NE was developed. It aimed to control sebum secretion, avoid blockage of the follicle, formation of comedones, prevention of the growth of cutibacterium acnes, reduce inflammation and oxidative stress, induce the healing of acne lesions, avoid post-inflammatory hyperpigmentation and scarring [1]. It may enhanced skin penetration, improve drug retention, provide sustained release, and increase therapeutic efficacy due to colloidal dispersion system consisting of oil phase, aqueous phase, surfactant, and co-surfactant with droplet sizes generally ranging from 20–200nm [2]. NE improve solubility and permeability of active constituents and enhance their penetration through biological membranes. In topically, NE helps to increase drug bioavailability and therapeutic efficacy due to high water content, softness, and excellent spreadability, which improve patient comfort during application [3]. Due to their nanosized structure, it enhance penetration of active constituents through the skin and provide localized therapeutic action with minimal systemic side effects making it suitable for present work in acne management [4,5].

MATERIAL AND METHODS

PROCUREMENT OF CC FLOWERS AND REAGENT

Fresh flowers of CC were procured from local nursery/garden, Pune. PEG-400, Ethanol, Methanol, Ether, 0.1 N NaOH, sesame oil, coconut oil, tea tree oil, Tween 20, Tween 80, span 80, Polyethylene glycol (PG) etc. Analytical grade chemicals were provided by Research-Lab Fine Chem Mumbai, and Freshly prepared distilled water is used throughout the work.

Preparation of CC Extract

The Methanolic extract was prepared by maceration method using fresh and shade dried flowers of CC at room temperature. Coarsely powdered using mechanical grinder and passed through sieve No.40. Approx 15 gm dried petals powder was soaked in 150 ml methanol in closed container for 48 h with occasional shaking for extract preparation. Filtered and stored for further work.

Preformulation Studies

Physicochemical characteristics

The colour, odor, texture was done by visual inspection of flower and herbal extract.

Solubility Study

The solubility of CC extract was determined in different solvents (Distilled water, Ethanol, Methanol, Ether, 0.1 N NaOH, Phosphate buffer pH 7.4), oils (sesame oil, coconut oil, tea tree oil), surfactants (Tween 20, Tween 80, span 80), and co-surfactants (PEG 400, PG). An excess amount of the extract was added separately to each vehicle and shaken until equilibrium was achieved. The mixtures were centrifuged at 3,000 rpm for 15 minutes., and the supernatant was analyzed by UV-spectrophotometer (UV- Jasco V-630) method (n=3)[6].

Partition Coefficient

The shake flask procedure was used to determine the partition coefficient of extract of CC. To each 10 ml solution of n-octanol and distilled water (1:1) system, 100 mg extract was added. This was shaken for 24h in a separating funnel till equilibrium. UV method was used to determine the concentration of extract in both phases and the partition coefficient was calculated (n=3) using the equation:

Partition coefficient = Concentration of extract in the organic phase / Concentration of extract in the aqueous phase

UV-Visible Spectroscopy

Preparation of stock solution : To make a primary stock solution of extract of CC, was dissolved 10 mg of extract in 100 ml of methanol.

Preparation of standard curve: A standard solution of extract (10ug/ml) was prepared, diluted suitably for concentration of 10, 20, 30,40, 50 and 60 µg/ml to prepare standard curve. Scanned across the wavelength range of 200-400nm for absorption maxima (λmax) and reported.

Assay of CC

A known amount 10 ml of extract was dissolve in methanol, filtered and measured the absorbance against methanol as blank by UV and Drug content was calculated using calibration curve (n=3) [7].

(Drug content = Absorbance /Slope)

Phytochemical Screening

The methanolic extract was subjected to screening using standard qualitative tests to detect the presence of Flavonoids - Shinoda Test, Phenolic Compounds- Ferric Chloride Test, Anthocyanins-Sodium Hydroxide Test , Alkaloids- Dragendorff's Test, Tannins-Ferric Chloride Test, Terpenoids- Salkowski Test, Glycosides- Keller–Killiani Test.

Drug excipient compatibility study

Preparation of Physical Mixture

Suitable amount of Extract, Tree tea oil,Tween-80 (in g) and PEG 400 (in g) were mixed in 1:1:1:1 ratio and analyzed by FTIR method to check the interaction of drug with excipients.

Experimental design

The response surface methodology was employed for constructing and investigating the polynomial models, using fewer experimental runs. Central composite design comprising of 2-factors and 3-levels was employed to examine the quadratic response surfaces by assessing the effect of pre-defined independent variables on different response dependent variables Particle size (nm), pH and Entrapment efficiency (%) was coded as Y1, Y2 and Y3. Two independent variables namely Tea tree oil concentration (%) (A) and Stirring speed (RPM) (B) were chosen. Each of the variables was varied at two different levels, known as high, and low levels [8].

Formulation of CC loaded NE

Oil, surfactant and co-surfactant was selected on the basis of maximum solubility studies as components for preparing further formulation development. CC loaded NE was prepared by Spontaneous Emulsification followed by sonication method using Experimental design (response surface methodology) to perform Quality by Design approach. Accurately 5g extract was dissolve in 5 ml methanol and sufficient water was mixed with stirring to make water phase. Required amount of tea tree oil was mixed in Smix Tween 80 as a surfactant and PEG 400 and as co-surfactant (oil phase). Water phase was slowly added in oil phase to make O/W emulsion. Stirred continuously and Sonicated using ultra sonication to form NE and stored [9]. The same method was adopted for all formulation batches NE1-NE9 as shown in Table 1.

Table 1: DOE suggested experimental batches

Formulation code	Extract (%)	Tea tree oil conc. (%)	Methanol (mL)	Tween 80 (%)	PEG 400 (%)	Water (mL)	Stirring Speed
NE1	5	9	5	15	15	25.5	1000
NE2	5	6	5	15	15	27.0	1000
NE3	5	9	5	15	15	25.5	1500
NE4	5	3	5	15	15	28.5	2000
NE5	5	3	5	15	15	28.5	1000
NE6	5	6	5	15	15	27.0	2000
NE7	5	3	5	15	15	28.5	1500
NE8	5	6	5	15	15	27.0	1500
NE9	5	9	5	15	15	25.5	2000

Evaluation of NE formulation

Measurement of particle size and polydispersity index (PDI)

The particle size analyzer was used to examine all nine batches the mean particle size and degree of particle size distribution of the improved NE after appropriate dilution with distilled water. The same equipment was used to measure the surface charge of globules. All measurements were performed at a temperature of 25±1^{0}C and a scattering angle of 900 (n=3).

Measurement of zeta potential

All batches were measured for zeta potential using a zeta sizer,using a laser beam, which acts as a light source to illuminate the particles inside the sample. Using zeta sizer software, a frequency spectrum is created, and zeta potential are calculated from this spectrum statistically (n=3).

Measurement of pH

The pH of Suitably diluted samples of all batches with distilled water was measured using calibrated pH meter. Readings were recorded in triplicate.

Measurement of viscosity

All batches of NE were evaluated for viscosity using Brookfield viscometer and recorded the readings (n=3).

Dilution test

Accurately weighed 1 ml NE for all batches in a beaker and 5 ml distilled water was added and mixed gently to check o/w emulsion [10]. same procedure were adopted for all batches.

% Entrapment efficiency (EE)

The %EE of NE all formulations was calculated by using an indirect method reported previously with some modifications. All formulations were ultra-centrifuged at 12000 rpm for 1 h, supernatant was collected separately and diluted with methanol to calculate the concentration of free drug refers to the aster extract. The supernatants were analyzed by UV at 271 nm. The %EE was calculated (n=3) as:

% EE = Total drug - free drug/Total drug × 100

Cumulative % In vitro release study

It was carried out for all nine batches by using a Franz diffusion cell apparatus at 37± 0.5°C. by previously soaked dialysis membrane in the phosphate buffer of pH 7.4 (PBS). A weighted amount (0.5 g) of NE sample was placed in the donor compartment and PBS was filled into the receptor compartment. The dialysis membrane was positioned between both compartment. At each prefixed time interval of 1, 2, 3, 4, 5,6,7 and 8h, 2ml aliquots were collected and sink condition was preserved over a period of full diffusion analysis. Using UV at wavelength 271nm, the collected aliquots were scanned and the percentage of cumulative drug release of NE was calculated (n=3) [11, 12,13].

Physical stability study

Short term stability study conditions at refrigeration temperature (4±2)ºC, room temperature (25±2)ºC and 40ºC, 75% RH as per ICH guidelines for 3 months was conducted on NE8 batch. After every month time interval % residual drug content and physical appearance was determined and reported (n=3). Centrifugation test was done to check the phase separation of emulsion. 5 ml of NE was centrifuged at 3000 rpm for 15 min. Observation were reported for stability [14].

Statistical validation

It was done by ANOVA using surface response method applied for optimized batch formulation. The contour plots were plotted for particle size, pH, and %EE for statistical validation [15].

RESULTS AND DISCUSSION

The methanolic extract of the CC flower was Faint purple in colour, having characteristic herbal odour, smooth and viscous consistency. Extract was found suitable for further formulation studies.The solubility study revealed that CC extract exhibited the highest solubility in methanol (3.56±0.85 mg/ml) and the lowest solubility in distilled water (0.62±0.13 mg/ml). The drug showed appreciable solubility in organic solvents. Tween 80,Tea tree oil and PEG 400 were selected on the basis of maximum solubility (mg/ml) as 0.515±0.07; 2.491±0.58 & 1.263±0.81 respectively, as component for preparing NE. The partition coefficient (log10 P) of extract was found to be 1.36. The positive log P value suggests that the phytoconstituents of the extract have a greater affinity for the organic phase than for the aqueous phase and exhibited a moderate lipophilic character. The UV spectrum CC extract in methanol showed a λmax of 271nm and the regression equation of y=0.0049x- 0.0024 with a very high correlation coefficient (R^2=0.9932), indicating almost perfect correlation between concentration and absorbance as shown in Fig.1. This showed a linear correlation in conc. range of 10-50µg/ml and strong adherence to Beer–Lambert's law.

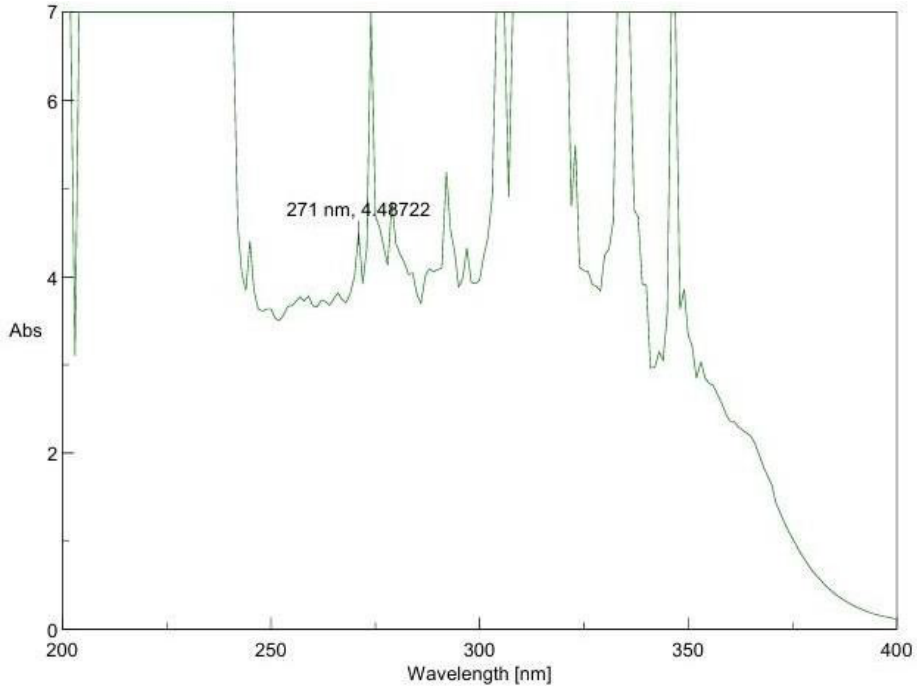

Figure 1: UV spectrum of CC extract

The assay of extract was reported and drug content was calculated using calibration curve equation and was found to be 85.91±0.65%. Preliminary phytochemical screening of CC extract revealed the presence of important bioactive constituents such as alkaloids, steroids, and tannins & phenolic compounds. These phytoconstituents are known for their potential antimicrobial, antioxidant, and therapeutic activities. The study also indicated the absence of glycosides and triterpenoids in the extract. The FTIR of pure drug and the drug excipient compatibility study by FTIR confirmed the presence of characteristic functional groups of drug and formulation excipients without any significant shift or disappearance of major peaks. It suggested good compatibility between the drug and excipients.

CC loaded NE was prepared by Spontaneous Emulsification followed by sonication method using Experimental design to perform Quality by Design approach in nine batches NE1-NE9. These were evaluated for quality control parameters. The particle size were ranged from 135.8±0.65 to 182.3±0.13 nm confirming successful formation of nanosized emulsions. Zeta potential values varied from −20.6mV to -29.7mV demonstrating adequate electrostatic stability of NE system. The pH evaluation of formulations NE1–NE9 showed values ranging from 4.08±0.47 to 6.12±0.43. Overall, the pH results confirmed the suitability of the developed formulations for oral delivery as well as for topical delivery. The formulations exhibited near slightly acidic pH, which may help maintain formulation stability and minimize irritation. Viscosities found in the range of 113.27±0.01 to 160.39±0.54 cps, that showed that developed NE has flowable

consistency. Percentage EE was optained in the range of 81.70±0.02 to 92.11±0.25, suggesting efficient drug entrapment and minimal drug loss during formulation. The cumulative % in vitro drug release study of all formulations, demonstrated a gradual and sustained release pattern over a period of 8 h. It was showed an increase in cumulative drug release with increasing time, indicating effective diffusion of the drug from emulsion base. It varied in the range from 83.95±0.91 to 94.17±0.66. The overall evaluation data confirmed that among all formulations, NE8 exhibited the smallest particle size (135.8±0.65 nm), highest negative zeta potential (−29.7 mV), with PDI (0.142), suitable pH value of 6.02±0.41, good consistency (121.45±0.05) to follow the newtonian law. The maximum %EE was observed in NE8 (92.11±0.25) and cumulative % in vitro drug release was 94.17±0.66 (Fig. 2). The finding suggested that the batch NE8 was the optimized formulation batch and suitable formulation for CC extract delivery for acne management (Table 2).

Table 2: Evaluation of NE formulations

Formulation code	Particle size (nm) (Mean±SD)	PDI	Zeta potential potential (mV) (Mean±SD)	pH (Mean±SD)	Viscosity (cp) (Mean±SD)	%EE (Mean±SD)	Cumulative % in vivo drug release after 8h (Mean±SD)
NE1	167.6±0.12	0.245	-23.4	6.07±0.31	113.27±0.01	82.75±0.02	78.15±0.36
NE2	172.4±0.78	0.163	-22.8	5.11±0.11	137.96±0.35	80.34±0.53	81.39±0.12
NE3	180.6±0.11	0.355	-28.1	5.28±0.25	141.66±0.21	84.20±0.11	82.14±0.71
NE4	144.2±0.54	0.286	-26.1	6.09±0.02	160.39±0.54	81.82±0.15	85.23±0.22
NE5	182.3±0.13	0.328	-25.7	6.12±0.43	115.42±0.09	89.27±0.91	87.16±0.01
NE6	167.1±0.22	0.189	-20.6	4.08±0.47	132.51±0.06	84.09±0.24	88.95±0.91
NE7	158.3±0.98	0.274	-25.9	4.71±0.22	141.94±0.72	81.70±0.02	90.98±0.87
NE8	135.8±0.65	0.142	-29.7	6.02±0.41	121.45±0.05	92.11±0.25	94.17±0.66
NE9	148.3±0.14	0.204	-28.9	4.61±0.27	148.04±0.77	82.40±0.72	89.08±0.85

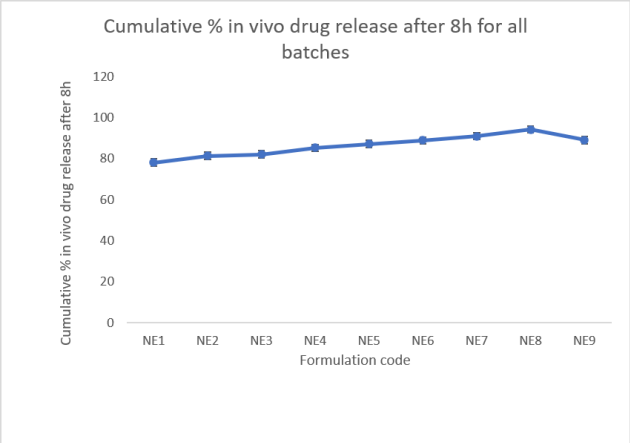

Figure 2: Cumulative % in vivo drug release after 8h for all batches

The optimized batch NE8 was chosen for short term stability study conditions at refrigeration temperature (4±2)ºC, room temperature (25±2)ºC and 40ºC, 75% RH as per ICH guidelines for 3 months. After every month time interval, dilution test, % residual drug content and physical appearance was determined and reported (n=3). Also, Centrifugation test was done to check the phase separation of emulsion. Data revealed that NE8 emulsion was found stable having no cracking, No significant changes in appearance and observed 97.08±0.17 % drug content indicating acceptable formulation homogeneity. The dilution and centrifugation test revealed that formulation mixed easily and uniformly with water without any phase separation or instability (Fig. 3).

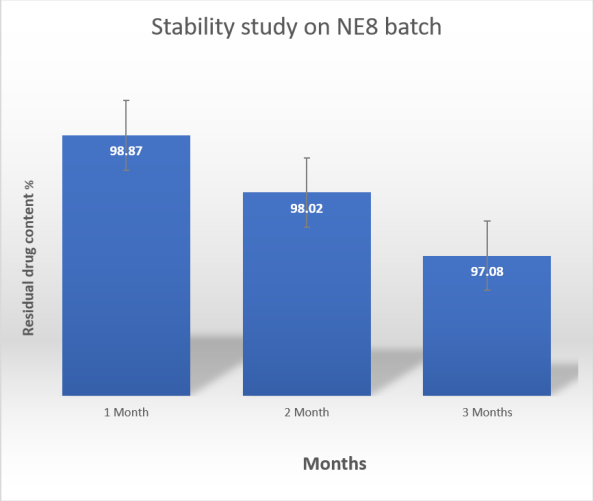

Figure 3: Stability study on NE8 batch after 3 months

ANOVA (Analysis of Variance) for NE8 : contour plots for particle size, pH, and %EE In order to statistically validate the response surface methodology applied for NE8, the experimental data were subjected to ANOVA. It confirmed the adequacy and significance of the models developed for each response parameter. The contour plots for particle size (Fig. 4), pH (Fig. 5), and %EE (Fig. 6), visually represent the interaction effects of independent variables, while the ANOVA tables provide the statistical evidence supporting these plots (Table 3).

Table 3: ANOVA for NE8 optimized batch

Response Parameter	Source of Variation	DF	Sum of Squares	Mean Square	F-value	p-value	Significant
Particle size	Model ,residual	5,3	2097.71	419.54	21.33	0.0150	Significant
pH	Model,residual	2,6	0.0508	0.0254	15.34	0.0044	Significant
%EE	Model	5,3	85.69	17.14	41.40	0.0057	Significant

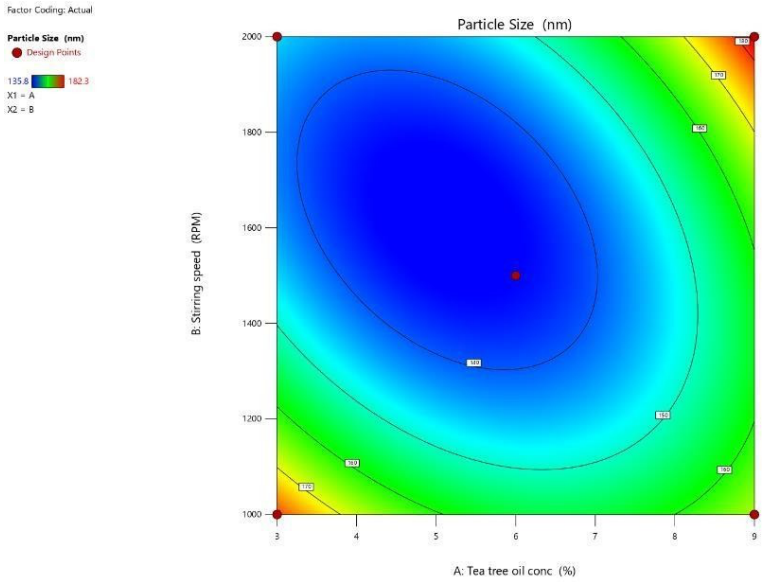

Figure 4: Contour plot for Particle size

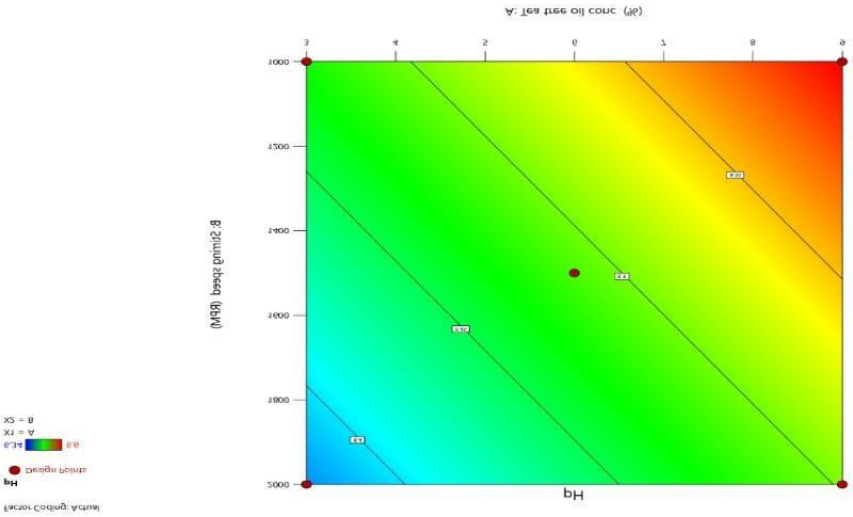

Figure 5: Contour plot for pH

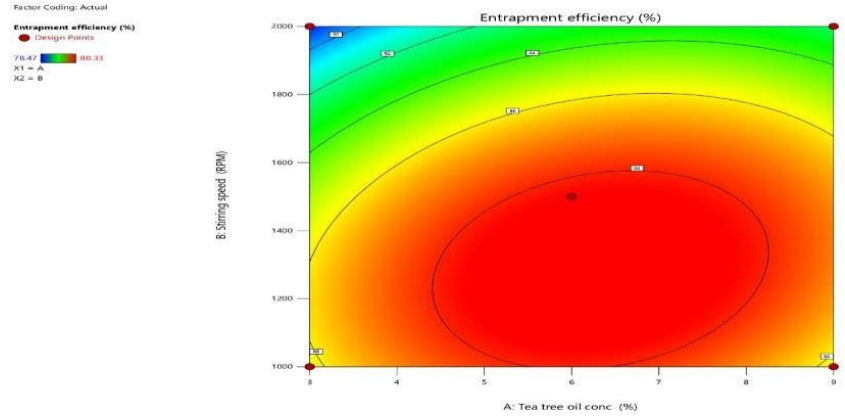

Figure 6: Contour plot for %EE

CONCLUSION

The current study was successful in developing and evaluating a CC extract loaded NE for the local treatment of Acne Vulgaris. The system was utilized including Tea Tree Oil, Tween-80, PEG 400 to be incorporated into a emulsion base. Among all the developed formulations, NE8 was identified as the optimized formulation based on its superior performance. Therefore the formulation exhibited all desired physical/chemical characteristics like transparent, smooth texture, stable pH, correct viscosity, high amount of drug entrapment, acceptable nanosized NE and drug stability. *In vitro* diffusion experiments were able to confirm a controlled release of the active ingredient. Stability were performed according to ICH guidelines confirming that there would be no phase separation and/or chemical decomposition of the optimized NE during three months. ANOVA for Quadratic model demonstrated that model was fitted significantly for particle size, pH, and %EE contour plots. Thus, as the foundation for herbal nanomedicines, this research provides new avenues for future preclinical and clinical trials to demonstrate therapeutic efficacy and provide expanded pharmaceutical uses.

CONFLICT OF INTEREST

Nil

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