Section A-Research paper

Formulation and Evaluation of Nano Emulgel Containing Ethanolic Extract of Fruit Rinds of *Garcinia indica* for Wound Healing Activity

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ABSTRACT

A wound is an injury to the skin or to any other portion of the bodily tissues. According to recent estimates, 6 million individuals globally experience chronic wounds. Topical antibiotics are frequently used in clinical settings for wounds, cuts, and burns due to their possible role in localised cutaneous infections. This study aimed to formulate and evaluate a nanoemulgel using a Box-Behnken design with *Garcinia indica* extract, focusing on its wound healing activity. The nanoemulgel was developed to enhance the delivery and efficacy of Garcinia indica extract for wound healing applications. Box-Behnken design was employed to optimize the formulation variables, including the concentration of Garcinia indica extract, oil phase-to-aqueous phase ratio, and emulsifier concentration. The prepared nanoemulgel was characterized for various parameters such as particle size, polydispersity zeta potential, viscosity, and drug content. The in vitro release study was conducted to assess the release pattern of the extract from the nanoemulgel. The wound healing activity of the optimized nanoemulgel was evaluated using an excision wound model in rats. Wound healinhg activity was checked by assessing various parameters in rats. Results showed that For the

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group IV where rats received application of 1.0% nanoemulgel of *Garcinia indica* extract it was observed that on 16th day the wound area shrunk upto 9.5± 1.98 mm^2. For the group IV which were treated with 1 % nanoemulgel of *Garcinia indica* the tensile strength was observed to be 199.85± 10.50 grams which is very appreciable. The developed nanoemulgel using the Box-Behnken design exhibited favorable physicochemical properties and demonstrated significant wound healing activity. The nanoemulgel formulation could serve as a promising approach for enhancing the delivery and therapeutic efficacy of Garcinia indica extract in wound healing applications. Further studies are warranted to explore the underlying mechanisms and conduct clinical trials to validate its effectiveness in human subjects.

Keywords: *Garcinia indica*, Wound healing, Medicinal plants, Nanoemulgel, Box-Behnken design

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Introduction

Wound healing is a complex biological process involving various stages, including inflammation, proliferation, and remodeling, aimed at restoring the integrity of damaged tissues. Impaired wound healing is a significant healthcare concern, leading to chronic wounds and increased morbidity. Natural products derived from medicinal plants have gained attention in recent years due to their potential therapeutic properties, including wound healing activity [1].

Garcinia indica, commonly known as kokum, is a tropical fruit-bearing plant found in India and other Southeast Asian countries. It is well-known for its medicinal properties, including antioxidant, anti-inflammatory, and antimicrobial activities. Garcinia indica fruits ethanolic extract has shown promising effects in promoting wound healing by accelerating the tissue repair process, reducing inflammation, and enhancing collagen synthesis [2]. Nanoemulgel is a hybrid formulation that combines the advantages of nanoemulsion and hydrogel systems. Nanoemulsions are thermodynamically stable, oil-in-water or water-in-oil dispersions with droplet sizes in the nanometer range, providing enhanced drug solubility and bioavailability. Hydrogels, on the other hand, are three-dimensional cross-linked polymer networks that can retain large amounts of water, imparting desirable gel-like properties. The combination of these two systems in a nanoemulgel formulation offers improved drug delivery, prolonged release, and enhanced skin penetration, making it an ideal

choice for topical applications [3].The Box-Behnken design is a statistical experimental design that allows for the optimization of multiple variables simultaneously. It is widely used in formulation development to evaluate the effects of different factors on product characteristics and identify the optimal formulation [4]. By employing the Box-Behnken design, the concentration of *Garcinia indica* fruit extract, oil phase-to-aqueous phase ratio, and emulsifier concentration can be systematically optimized to achieve desirable nanoemulgel characteristics and wound healing activity.

Material and Methods

Formulation of Nanoemulgel

Different formulations with varying amounts of ingredients were prepared by Spontaneous emulsification method (Table 1). The gel component of the nanoemulgel was produced by dissolving carbopol-934 in cold water with consistent stirring at a moderate pace until a uniform mixture was achieved. The pH level was then adjusted to 6-6.5 by using triethanolamine (TEA). To prepare the aqueous phase of the emulsion, Tween 80 was dissolved in distilled water, while for the oil phase of the emulsion, span 80 was dissolved in liquid paraffin. To preserve the emulsion, methyl paraben was dissolved in propylene glycol, and the extract was dissolved in ethanol, and then both solutions were combined with the aqueous phase. The aqueous and oil phases were heated separately in a water bath at 70°C. Next, the oil phase was slowly added to the aqueous phase drop by drop with continuous stirring using a homogenizer (EI) at a speed of 3000 rpm for 10 minutes and then cooled to room temperature. Finally, the gel and emulsion components were mixed in a 1:1 ratio with moderate stirring to produce the nanoemulgel [5].

Table 1: Different composition of Formulation of Nanoemulgel

Formulation Code	Extract (%)	Factor 1: Carbopol-934 (%w/v)	Factor 2: Tween 80 (%w/v)	Factor : Span 80 (%w/v)	Liquid paraffin (%w/v)	Propylene glycol (%w/v)	Methyl Parabene (%w/v)	Distilled water (ml)	TEA (ml)
F1	1	1	0.3	1	3.75	3.50	0.01	50	QS
F2	1	1	0.3	0.5	3.75	3.50	0.01	50	QS
F3	1	1	0.1	1.5	3.75	3.50	0.01	50	QS
F4	1	1	0.3	0.75	3.75	3.50	0.01	50	QS
F5	1	0.5	0.5	1	3.75	3.50	0.01	50	QS
F6	1	1	0.3	1	3.75	3.50	0.01	50	QS
F7	1	1	0.5	0.5	3.75	3.50	0.01	50	QS
F8	1	1.5	0.3	1.5	3.75	3.50	0.01	50	QS
F9	1	0.5	0.3	1.5	3.75	3.50	0.01	50	QS
F10	1	0.5	0.1	1	3.75	3.50	0.01	50	QS
F11	1	1.5	0.1	1	3.75	3.50	0.01	50	QS

F12	1	1.5	0.3	0.5	3.75	3.50	0.01	50	QS
F13	1	1.5	0.5	1	3.75	3.50	0.01	50	QS
F14	1	0.5	0.3	0.5	3.75	3.50	0.01	50	QS
F15	1	1	0.3	1	3.75	3.50	0.01	50	QS
F16	1	1	0.1	0.5	3.75	3.50	0.01	50	QS
F17	1	1	0.5	1.5	3.75	3.50	0.01	50	QS

Final Equation in Terms of Coded Factors

Assay = +94.22+0.7488 A-0.2737 B-0.2626 C-0.1000 AB-0.1325 AC-1.78 BC+2.70 A^2+1.89 B^2+0.6390 C^2

Final Equation in Terms of Actual Factors

Assay = +105.09213-19.29309 Carbopol-934-10.85735 Tween 80+0.240180 Span 80-1.00000 Carbopol-934 * Tween 80-0.530000 Carbopol-934 * Span 80-17.82500 Tween 80 * Span 80+10.81029 Carbopol-934^2+47.18933 Tween 80 ^{2}+2.55603 Span 80 ^{2}

Particle size = +233.18+4.29A+6.40 B+4.06 C+10.32 AB+11.85 AC-8.72 BC-29.24 A^2-1.56 B^2+10.71 C^2

Final Equation in Terms of Actual Factors

Particle size = +181.48660+164.10206 Carbopol-934+39.31011 Tween 80-98.79450 Span 80+103.25000 Carbopol-934 * Tween 80+47.40000 Carbopol-934 * Span 80-87.17500 Tween 80 * Span 80-116.95103 Carbopol-934^2-39.00643 Tween 80 ^{2}+42.82872 Span 80^2

Final Equation in Terms of Coded Factors

Experimental data with predicted response

The results of experimental data with predicted response can provide valuable insights into the formulation of nanoemulgel. By using statistical techniques such as response surface methodology (RSM), it is possible to generate a mathematical model that can predict the response of the nanoemulgel to different concentrations of surfactants and polymers.

Table 2: Experimental results with predicted responses

Formulation	Run Order	Composition Carbopol-934/Tween 80/Span 80	Response	Predicted value	Experimental value
ONGF1	11	1.5/0.1/1	% Assay	99.93	99.65
			Particle	189.95	190.65

			Size		
ONGF2	13	1.5/0.5/1	Assay	99.18	99.45
			Particle Size	223.39	220.45

*Formulation ONGF1 and ONGF2 selected as optimized formulation for further evaluation

Characterization of nanoemulgel formulation

Determination of lambda max by UV/Visible spectrophotometer

The lambda max of the plant extract was determined using a UV/Visible spectrophotometer (Labindia 3000+), as reported in previous studies [6]. To prepare the stock solution, 10 mg of extract was dissolved in 10 ml of methanol. 0.1 ml of the stock solution was then taken and dissolved in a volumetric flask, and the volume was made up to the mark with methanol. Aliquots of the 10µg/ml solutions prepared were scanned in the UV range from 190 to 400 nm to determine the wavelength of maximum absorbance for the major constituent of extract, which was found to be herbal extract. Methanol was used as a blank.

Determination of percentage assay

Weigh an accurately 10mg of nanoemulgel and dissolve the 10mg of solvent (methanol) to make a 10ml of methanol (1000µg/ml). Take an aliquot of this solution 0.1ml and transfer it to a 10ml volumetric flask. Dilute the solution to the mark with the same solvent used for the initial dissolution. Mix the solution thoroughly by shaking or stirring. Analyze the solution by a UV-Vis spectroscopy (Labindia 3000+) to determine the amount of extract present in the solution.

Fourier transform infrared spectrophotometer analysis (FTIR)

To assess the compatibility of the extract of *Garcinia indica* with other excipients in the formulation, FTIR analysis was conducted based on a previous study by Burki *et al.* (2020). Infrared spectral analysis of the *Garcinia indica* extract was initially performed using a Fourier Transform Infrared Spectrophotometer (Tencor Series 7, Shimadzu, Germany). Following this, the formulation was also subjected to spectral analysis. The spectra were recorded in the region of 4000 cm^{-1}-400 cm^{-1}.

Spreadability study

To determine the spreadability of the nanoemulgel, an apparatus suggested by Mutimer et al. was utilized. The apparatus consisted of a wooden block with a pulley connected to one end. A ground glass slide was fixed onto this block. The spreadability was determined using the 'drag' and 'sleep' method. Specifically, 2 g of the test nanoemulgel was placed on the ground glass slide, and then sandwiched between another glass slide of the same size and a slide provided with a hook. A weight of 40 g was placed on top of the slide. The time (in seconds) taken by the top slide to cover a distance of 6 cm was recorded, and spreadability was calculated using the following formula.

$S = M.L/T$Where S = spreadability, M = Weight tied to upper slide, L = Length of glass slides T = Time taken to separate the slides completely from each other.

Viscosity/Rheology

To determine the viscosities of the freshly prepared formulations (ONGF1- ONGF2), a Brookfield viscometer with spindle no. 04 was used. The spindle was lowered perpendicular into the center of the Nanoemulgel formulation placed in a beaker, taking care that the spindle did not touch the bottom of the beaker, and was rotated at a speed of 2.5 rpm for 5 minutes [7].

Surface charge and particle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the transfersomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm.

pH determination

The digital pH meter was used for the determination of the pH of Nanoemulgel. Nanoemulgel was weighed (2g) and the dispersion medium was purified water (20 ml). The 4, 7, and 9.2 pH buffer solution was used for calibration of pH meter. The samples were repeated in the triplicate manner and mean values were calculated [8].

Extrudability

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of

certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube. Extrudability of gel required weight to extrude a 0.6cm ribbon of gel in 6 seconds [9].

***In-vitro* drug release study**

According to a study conducted with slight modification [10], in vitro drug release studies were performed using the Franz diffusion cell method, with a dialysis membrane separating the donor and recipient compartments. The nanoemulgels were placed in the donor compartment, while phosphate buffer of pH 7.4 was added to the recipient compartment. To ensure proper mixing, a magnetic bar was rotated at 75 rpm in the recipient compartment. The temperature of the system was maintained at 37°C using circulating hot water in the outer jacket. Samples were taken at predetermined time intervals, including time 0.5hrs, 1hrs, 2hrs, 4hrs, 6hrs, 8hrs, 10hrs, and 12hrs and 24hrs. with each sample being replaced with fresh buffer. The absorbance was then measured using a UV visible spectrophotometer at 338 nm.

***In vivo* wound healing activity**

Animals

Wistar rats (180±20 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC, PCP/IAEC/2023/JAN/16),constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute dermal toxicity

The acute dermal toxicity testing was carried out based on the methods described in the OECD draft guideline number 434. Animals that show normal skin texture were housed individually in a cage and acclimatized to the laboratory condition for 1 week prior to the test. Following acclimation, 10% of the body surface area fur was shaved 24 hours before the study on the dorsal area of the trunk of the test animals. A lower and upper dose of 0.5% and 1% nanoemulgel of *Garcinia indica* extract was

applied evenly over the shaved area for 24 hours. The purpose of the sighting study is to check whether the larger dose selected is toxic or not for the main study. Observations for any signs of adverse skin reactions, inflammation, or edema were made for 24 hours with special attention within the first 4 hours [11].

Experimental designs

Group I: Received no treatment and served as control

Group II: Received application of standard drug ointment i.e. 0.2% nitrofurazone topical ointment (N-Zone cream*)

Group III: Received application of 0.5% nanoemulgel of *Garcinia indica* extract

Group IV: Received application of 1.0% nanoemulgel of *Garcinia indica* extract

Excision wound model

Excision wounds were used for the study of the rate of contraction of wounds and epithelization. Animals were initially anaesthetized using ketamine (100mg/mL/i.p.) and xylazine (20 mg/mL/i.p.) at a 3:1 v/v ratio and subsequently, a 1 cm^2 piece of skin was surgically removed from the dorsal region of each rat. The back of the rats was further shaved. After skin excision, the wound was cleaned initially with diluted soap 50% in saline and rinsed with saline solution. The area was marked and the surface of the marked area was carefully excised by using sharp sterilized scissors. Excision wounds sized 300 mm^2 and 2 mm in depth were made by cutting out layers of skin from the shaven area. The entire wound was left open. The treatment was done topically in all the cases. The nanoemulgel of *Garcinia indica* extract was applied at a dose of 0.5% and 1.0% for 16 days. Wound areas were measured on days 1, 4, 8, and 16 for all groups, using a transparency sheet [12].

The rats were maintained in individual cages under a warming lamp and were monitored until fully recovered from the anesthesia.

Measurement of the tensile strength

The measurement of the tensile strength of skin wound is possible realize by biomechanical tensile strength test by means of tensile strength tester adapted to that specific purpose. The tensile strength of the wound on each animal was measured by using a constant water flow method. In this method, the animals were first anesthetized in the same manner described above and positioned on a table between two metal stands. To both stands, allice forceps of equal size were suspended with strings,

one of which passed on a small wheel with the stands. One of the forceps served as a support and the other forceps holed a plastic of volume 1,000 mL (plastic bottle).Then, both forceps were allowed to grip the edges of the wound 3 mm away on both sides. After this, constant water flow was allowed to enter the plastic bottle which was suspended with the string that passed on the wheel until the wound opened up (broken). The flowing water was stopped immediately as the wound breaks in each rat. Then the volume of the waterin the plastic bottle was measured by using a measuring cylinder which was taken as the wound breaking strength for the individual rat. The breaking strength of the wound on each animal was measured by using the constant water flow method [13].

Skin irritation test

Acute dermal toxicity study

Skin irritation test was carried out using an occluded dermal irritation test [14] with slight modification. Two groups of rats (each group containing six animals) were employed for this test and two areas on the dorsal side of each rat on each side of the vertebrae (1 cm from the midline of the vertebral column) were shaved and marked before the experiment.

The animals were put into two groups of five animals each. One group was for test sample ointment and the other group was control for comparison. Topical extract preparation was applied to the respective group. Immediately after, the area was covered by dressing gauze over which a plastic sheet (occlusive material) was placed. The covering was loosely held in contact with the skin by means of a non-irritating adhesive tape and tied across the diameter of the back of the rats with an elastic bandage. After 24 h of the exposure period, the elastic bandage, the adhesive plaster, the plastic sheet and the gauze were removed by taking care not to damage the skin, and the test site was rinsed with distilled water. The animals were examined for the presence of erythema and oedema according to Draize dermal irritation scoring system at intervals of 1, 24, 48 and 72 h.

The Appearance of eschar without any raw wound area was treated so that the wound is completely healed. The number of days required for the appearance of eschar without any leftover raw wound was calculated as epithelization period.

Statistical Analysis

All analysis was performed using Graph Pad Prism version 8.0.1. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one-way ANOVA, where

applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Tukey's *Post hoc* test.

Results and Discussion

The results of the % assay and particle size analysis of the prepared nanoemulgel formulation by Box-Behnken Design (BBD) provide important information about the relationship between the formulation variables and the quality and performance of the product. Percentage assay of different formulation was found between 93.32 to 99.65 percentages for F1 to F17 respectively. The particle Size of Formulation F1 to F17 was found between 190.65 to 246.65nm respectively.

The use of DOE and statistical software can help to streamline the formulation process and optimize the performance and stability of the final product. The use of experimental data and predicted responses is critical in the development of a nanoemulgel formulation. These data help to identify the optimal formulation that meets the desired response values, and can streamline the formulation process. However, it is important to consider the sources of variation and limitations of the data, and to validate the predicted responses with additional experimental data. The particle size of optimized formulation ONGF1 and ONGF2 was found to be 190.65 and 220.45nm respectively. Optimizing the particle size of nanoemugel formulations is crucial to achieving their desired performance and properties. Therefore, it is important to carefully consider the factors that influence particle size and to select appropriate measurement techniques to optimize the particle size of nanoemugel formulations. *Garcinia indica*, also known as kokum, is a fruit-bearing tree found in India that contains bioactive compounds with various medicinal properties. Determination of lambda max by UV/Visible spectrophotometer can be used to study the absorption spectrum of *Garcinia indica* extracts and identify the specific wavelength at which its bioactive compounds exhibit maximum absorption. UV/Visible spectrophotometry of *Garcinia indica* extracts has shown that the lambda max occurs at 338 nm, which is characteristic of polyphenolic compounds such as flavonoids and phenols. The percentage assay of optimized formulations ONGF1 and ONGF2 was found to be 99.12 and 98.78 percentage respectively. The maximum percentage assay and minimum particle size was found in formulation ONGF1, select as optimized formulation for further evaluation. FTIR analysis of *Garcinia indica* has shown the presence of various functional groups, such as hydroxyl, carbonyl, and carboxyl groups. These functional groups are associated with compounds such as polyphenols,

flavonoids, and organic acids, which are known to have antioxidant and anti-inflammatory properties. FTIR analysis can also be used to study the interactions between bioactive compounds in *Garcinia indica* and other compounds, such as proteins or lipids, which may be relevant to its medicinal properties.The spreadability of formulation ONGF1 and ONGF2 was found 11.25 and 12.12g/cm respectively. The extrudability of prepared ONGF1 and ONGF2 formulation was found to be 165±3 and 158±2 respectively. The pH values of the prepared nanoemulgel formulations ONGF1 and ONGF2 were measured and found to be 6.72 and 6.81, respectively. The pH of a formulation is an important parameter as it can affect various factors such as stability, skin compatibility, and drug release. The Zeta Potential and Particle Size of optimized formulation ONGF1 and ONGF2 were found to be 189.95 and 223.39nm and -38.80 and -39.12mV respectively.

In-vitro drug release studies are critical in determining the efficacy of a drug delivery system, and the results obtained can help in optimizing the formulation. The drug release profile of the optimized nanoemulgel formulations (ONGF1 and ONGF2) can provide insights into their behavior in the target environment and can guide further development and optimization. The *in-vitro* drug release study of ONGF1 and ONGF2 conducted using Franz diffusion cell method. The samples are analyzed using UV-Vis spectrophotometry. The results of the in-vitro drug release study can be presented in the form of a drug release profile, which shows the percentage of drug released from the formulation over time. The results of the study can also be used to optimize the formulation by adjusting the composition and concentration of the emulsion and gel components, the surfactant type and concentration, and the processing conditions. The drug release from nanoemulgel formulation ONGF1 was found 12.35, 26.65, 38.89, 46.65, 53.32, 68.38, 76.65, 89.98, 96.65 and 99.05 percentages after 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. respectively. When compare the data obtained by ONGF1 with ONGF2, the drug release from formulation ONGF2 showed the drug release 23.36, 45.65, 52.23, 65.65, 72.23, 86.65, 98.85, 98.92, 99.12 and 99.021 percentages after 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs. respectively. The (r2) value of optimized formulation ONGF1 was found 0.721, 0.949, 0.901 and 0.944 for Zero order, First order, Higuchi and Korsmeyers Peppas respectively. The maximum (r2) value was found maximum 0.944 in optimized nanoemulgel formulation ONGF1, so the formulation follows Korsmeyers Peppas release kinetics. In excision wound model, extract showed varying degrees of wound area contraction compared to the negative control at both dose levels, better activity was

observed for 0.5 and 1.0% w/w nanoemulgel of *Garcinia indica* extract. In the excision model, the 1.0% extract treated group showed significant wound contraction from day 4 to day 16, whereas the 0.5% extract treated group showed significant wound area contraction beginning from the 8thday on wards. Hence, a faster rate of wound contraction and shorter period of epithelialization of the extract may be due to the effects of different secondary metabolites present in the plant.

Table 3: Results of % assay and particle size of prepared nanoemulgel formulation

Formulation Code	% Assay	Particle Size (nm)
F1	94.12	230.52
F2	95.65	242.12
F3	98.78	246.65
F4	94.85	235.74
F5	98.32	194.15
F6	94.65	235.45
F7	98.12	254.74
F8	98.05	236.65
F9	96.65	201.45
F10	98.12	205.65
F11	99.65	190.65
F12	98.58	203.45
F13	99.45	220.45
F14	96.65	215.65
F15	93.32	230.74
F16	95.65	220.87
F17	94.12	245.65

Normal plots of residuals for percentage assay

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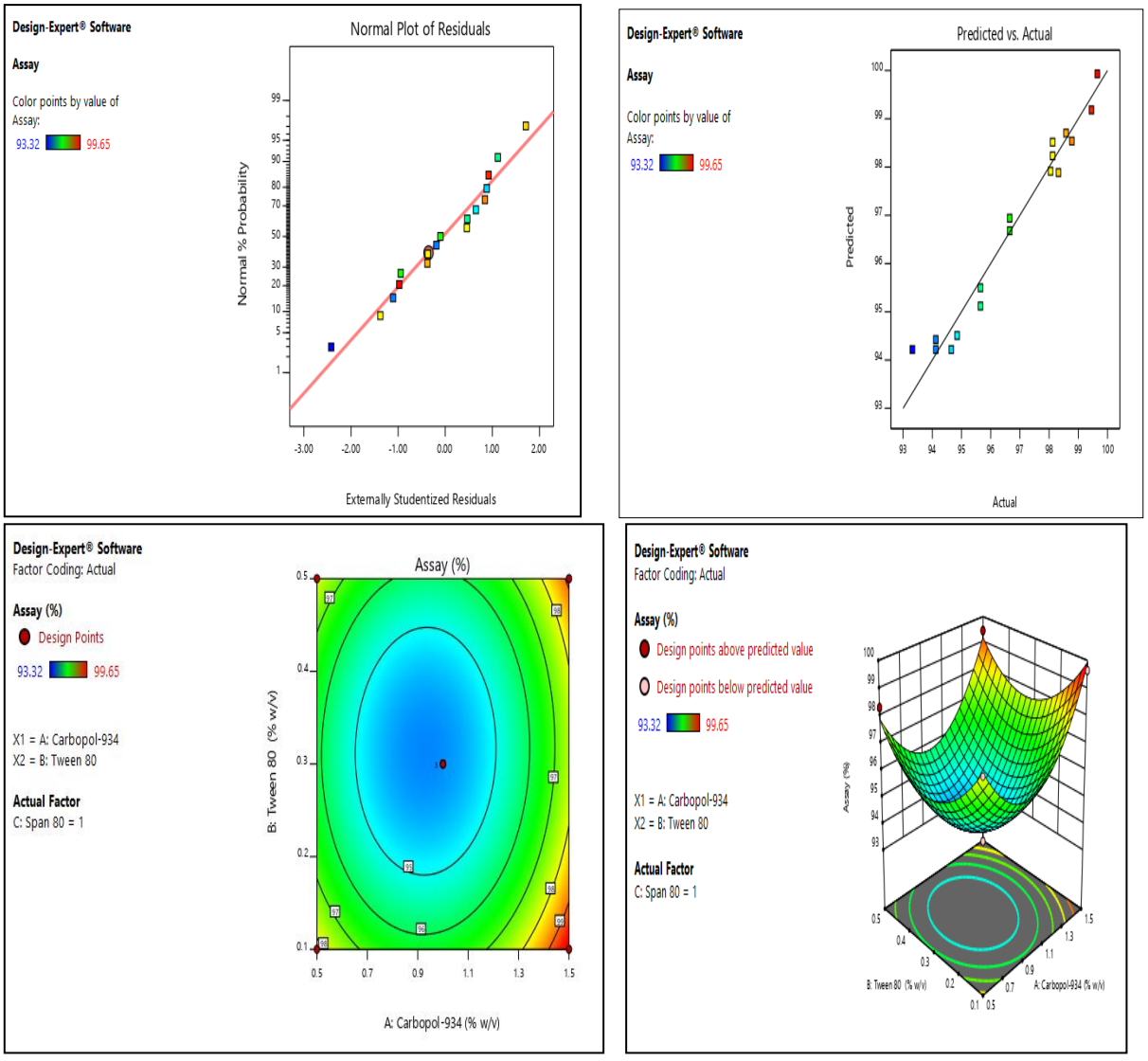

Figure 1: Graph of Normal plots of residuals, predicted vs. actual, Contour plots and 3D plots of nanoemulgel for percentage assay

Normal plots of residuals for particle size

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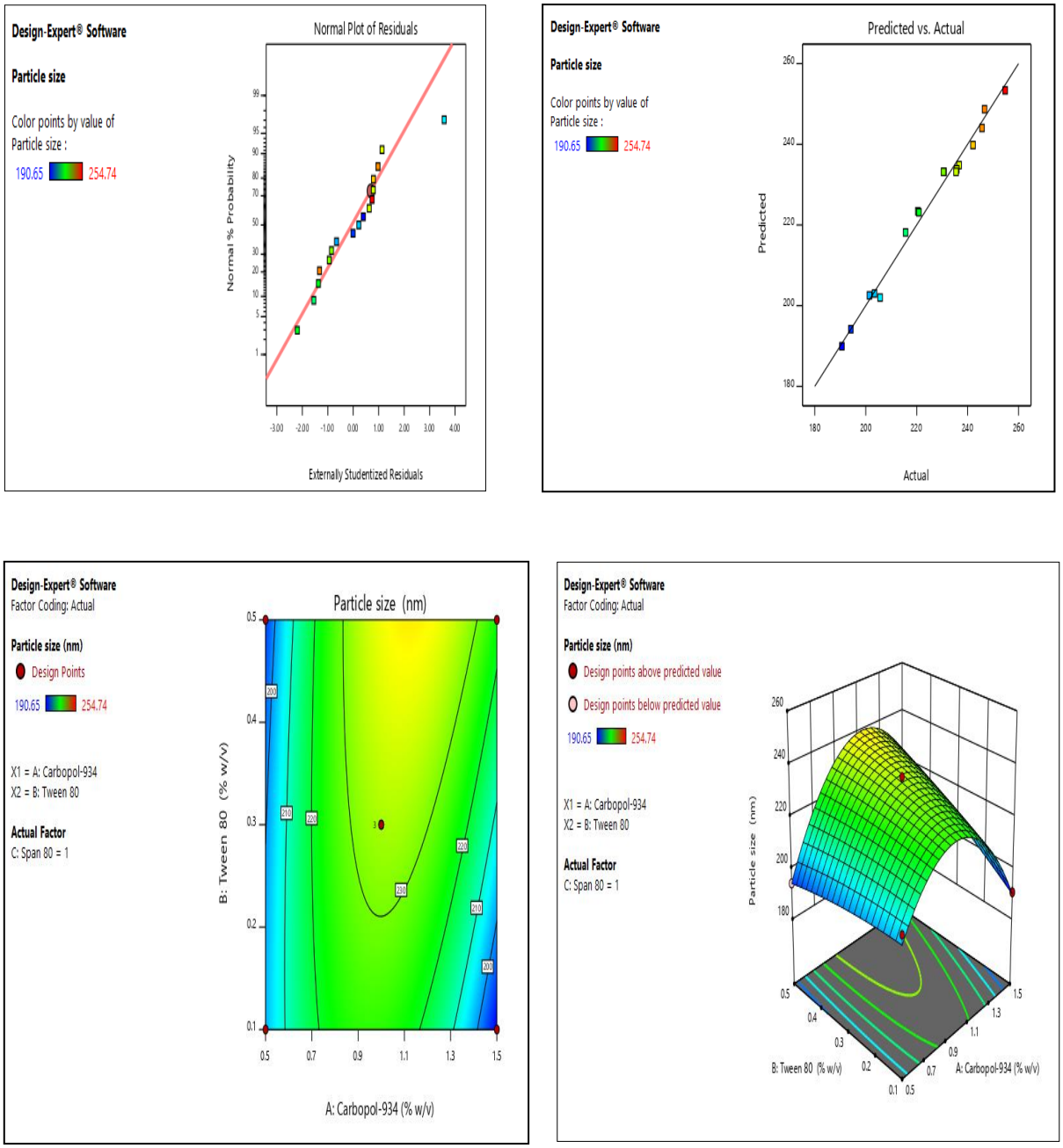

Figure 2: Graph of Normal plots of residuals, predicted vs. actual, Contour plots and 3D plots of nanoemulgel for percentage assay

Determination of lambda max by UV/Visible spectrophotometer

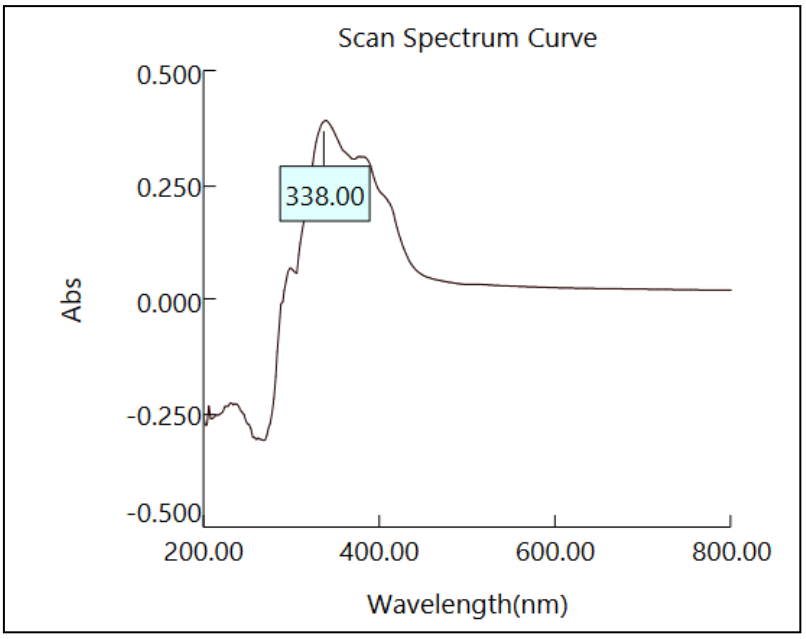

Figure 3: Graph of determination of λ_{max} by UV/Visible spectrophotometer

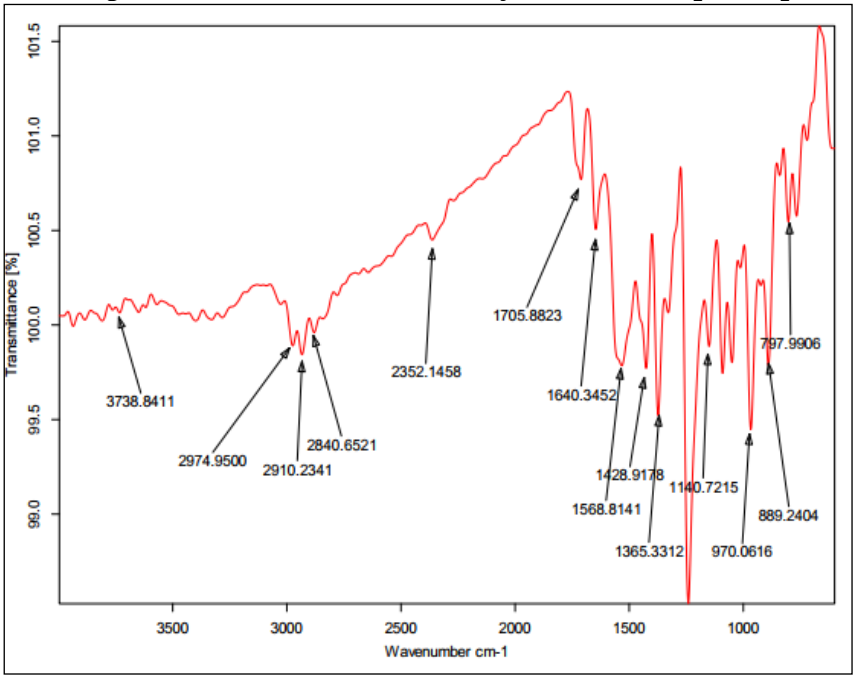

Figure 4: Fourier transform infrared spectra of ethanolic extract

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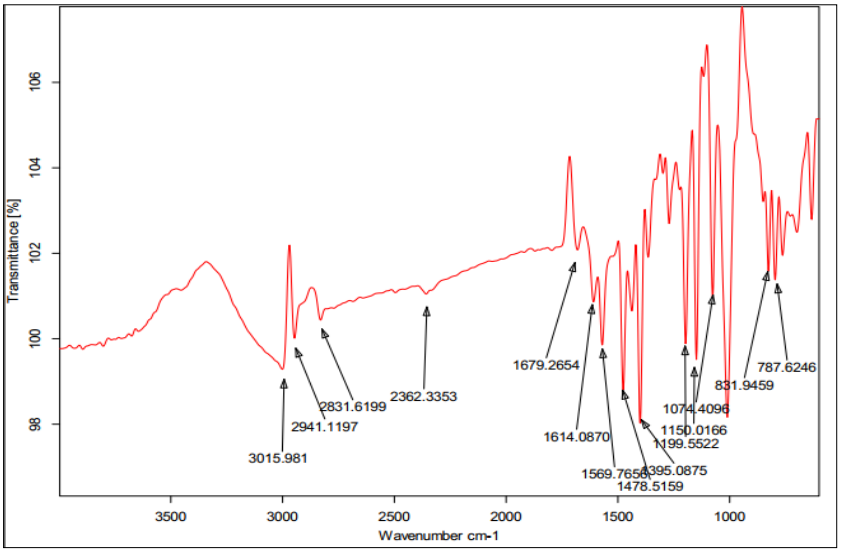

Figure 5: Fourier transform infrared spectra of formulation ONGF1

Table 4: Results of Determination of percentage assay of optimized formulations

Formulation Code	Percentage Assay*	Spreadability (g/cm)	Extrudability (g)	Viscosity* (cp)	pH*	Zeta Potential (mV)
ONGF1	99.12±0.15	11.25±0.15	165±3	6856±8	6.72±0.02	-38.80
ONGF2	98.78±0.25	12.12±0.12	158±2	6712±12	6.81±0.01	-39.12

Average of three determination (n=3)

Table 5: *In-vitro* drug release Study

Time (Hrs.)	Herbal Extract	Formulation Code	
		ONGF1	ONGF2
0.5	6.85	12.35	23.36
1	16.65	26.65	45.65
2	28.85	38.89	52.23
3	39.98	46.65	65.65
4	40.12	53.32	72.23
6	-	68.38	86.65
8	-	76.65	98.85
10	-	89.98	98.92
12	-	96.65	99.12
24	-	99.05	99.02

Table 6: Regression analysis data of nanoemulgel optimized formulation ONGF1

Batch	Zero Order	First Order	Higuchi's Model	Korsmeyers Peppas Equation

	R^2			
ONGF1	0.721	0.949	0.901	0.944

Table 7: Effect of pre-treatment of nanoemulgel of *Garcinia indica* extract on Excision Wound [Wound Area (mm^2)]

Days	1	4	8	16
Group-I	240.60 ± 6.1	215 ± 8.20	170.87 ± 9.52	71.01 ± 5.20
Group-II	210.38 ± 7.12	157.12±14.00*	90.12 ± 10.89**	4.52 ± 1.94***
Group-III	205.90 ± 10.90	185.32±10.00*	118.41 ± 10.97**	15.16 ± 3.10***
Group-IV	207.12 ± 9.50	160.12±9.00*	65.87 ± 9.00**	9.5± 1.98***

Values are expressed as the mean ± SEM of six observations.*P<0.05;**P<0.001;***P<0.0001 vs. control treatment (One-way ANOVA followed by Tukey's post hoc test)

Table 8: Effect of pre-treatment of nanoemulgel of *Garcinia indica* extract on Tensile Strength of Incision Wound Model in rats

Group	Tensile Strength(Grams) (mean±SEM)
Group-I	134.50± 10.20
Group-II	242.83± 7.12***
Group-III	175.17± 9.90**
Group-IV	199.85± 10.50***

Values are expressed as the mean ± SEM of six observations.**P<0.001;***P<0.0001 vs. control treatment (One-way ANOVA followed by Tukey's post hoc test)

Table 9: Erythema and edema scores used to determine the primary irritation index

Erythema	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2

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Moderate to severe erythema	3
Severe erythema (beef redness)	4
Oedema formation	**Value**
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (extending beyond the area of exposure)	4

Conclusion

The formulation and characterization of a nanoemulgel loaded with *Garcinia indica* extract for wound healing activity were conducted, and the following conclusions can be drawn: The nanoemulgel formulation of *Garcinia indica* extract was successfully developed using appropriate emulsifiers, gelling agents, and optimization techniques. The combination of a nanoemulsion and a gel matrix provides a stable and easily applicable formulation. The particle size analysis demonstrated that the nanoemulgel formulation achieved a small and uniform particle size distribution. This small particle size is advantageous for enhanced drug delivery and improved penetration into the wound site. The nanoemulgel formulation exhibited a negative zeta potential, indicating good physical stability and resistance to particle aggregation. This is crucial for maintaining the stability of the formulation during storage and application. The loaded Garcinia indica extract in the nanoemulgel formulation holds promise for wound healing activity. *Garcinia indica* extract is known for its antimicrobial, anti-inflammatory, and antioxidant properties, which can aid in wound healing processes. The nanoemulgel formulation offers controlled and sustained drug release, allowing for prolonged therapeutic effects at the wound site. This controlled release profile is beneficial for wound healing, as it promotes continuous drug exposure to the wound area. The developed nanoemulgel formulation of *Garcinia indica* extracts shows potential for clinical application in wound healing. Its favourable physicochemical properties, drug release profile, and wound healing activity make it a promising candidate for further evaluation in *In vivo* and clinical studies.

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