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Formulation optimization and evaluation of nanolipidcarrier containing Withania somnifera for wound healing

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Abstract
This study aimed to develop, optimize and evaluate Withania somnifera Nanolipidcarrier (WS-NLC) for wound healing. Withanolides are the main active constituent of Ashwagandha WS has antioxidant activity, immune system regulation, anti-inflammatory antiageing benefits and wound healing. Thirteen formulations were prepared by high pressure homogenization method according to central composite design. The effects of solid concentration (X1) and liquid lipid (X2) on the particle size (Y1), PDI (Y2), zeta potential(Y3) and entrapment efficiency(Y4) were explored. The optimized nanolipidcarriers exhibited particle size 147.9nm, entrapment efficiency 89.99% and PDI 0.173. NLCs were incorporated into a Carbopol 940 gel base for topical application. The formulation demonstrated high drug content (97.5% w/w) and favourable rheological properties. *In vitro* drug release studies indicated an initial burst followed by sustained release (89.66% at 12 h). The kinetic analysis of drug release obeys zero-order release model. *In vivo* study, 30 Wistar rats in five groups were used and wound was induced by excision method with the help of Biopsy punch tool. Wound healing studies in rats showed reduced healing time and accelerated wound closure in the treatment group than in the control group**.** The developed NLC-based gel presents a promising strategy for localized, sustained delivery in chronic wound management.

Keywords: Withania somnifera, nanostructured lipid carrier, topical, gel, wound healing

Introduction
The human skin is the largest organ in the body, which is responsible for regulating temperature, preventing microbial invasion, and maintaining fluid haemostasis [1]. A wound is characterized as an interruption in the typical composition of the skin and mucous membrane [2]. It is a disruption in tissue lining of skin, leading to the discontinuity in structural integrity of tissue [3]. Wounds are classified as simple and complex depending on their depth, size, wound site, and whether they involve muscles, nerves, or vessels [4]. Wounds can occur due to various types of trauma, including mechanical, thermal, chemical, microbial and radiogenic such as cuts, surgical operations, accidents, bites or abrasions indicate a disruption in the continuity of living tissue [5].
The active constituent of plant Ashwagandha (family Solanaceae) is Withanolides [6, 7]. Withanolides are group of steroidal lactones present in the plant, having therapeutic efficacy against broad spectrum harmful bacteria. Withania Somnifera (WS), is a medicinal plant with great therapeutic importance [8-10]. Withania Somnifera (WS) has antioxidant activity, immune system regulation, anti-inflammatory, antiageing benefits and wound healing [11, 12]. Despite its many benefits the major challenge associated with Withania Somnifera is its poor aqueous solubility, While it belongs to (BCS class II), is also poorly soluble in water, with a solubility of less than 1 mg/ml. Various approaches have been used in the past to enhance their dissolution like nanoparticles, solid dispersion, liposome and liquisolid technology deals with drugs belong to BCS Class-II and Class-IV [13-15]. But various challenges associated with these approaches agglomeration in solid dispersion, high dose not suitable with liquisolid technology.

Nano lipid carrier represents the most effective approach for enhancing therapeutic efficacy when compare to other lipid formulations [16]. NLCs is having ability to entrap and encapsulate high drug, controlled release of drug as compare to other systems like SLNs, liposomes [17, 18]. The improved drug permeation and therapeutic efficacy result from the direct contact between NLC gel and the stratum corneum [19, 20]. The NLC gel has bioadhesive qualities on the skin, it aids in the production of a film, which results in an restrictive effect [21].

Accordingly, the aim of the current study is to enhance water solubility of WS, as a preliminary step, for producing effective topical gel preparations of the drug. The application of central composite design gives a statistically systematic approach for the preparation and optimization of NLC with desired particle size, polydispersity index (PDI), zeta potential and entrapment efficiency. Three levels, low, medium, and high, for each variable were determined. In the following step, the optimized formulation WSNLC was transformed to Carbopol gel (WsNLC gel). The resulting WSNLC gel was evaluated for gel characterization, physical appearance, Transmission electron microscopy, Spreadability, Viscosity Study, pH, % Entrapment Efficiency, *In-Vitro* drug release. *In vivo study*, 30 Wistar rats in five groups were used and wound was induced by excision method with the help of Biopsy punch tool. Wound healing studies in rats showed reduced healing time and accelerated wound closure in the treatment group than in the control group**.** The developed NLC-based gel presents a promising strategy for localized, sustained delivery in chronic wound management.

Materials and Methods

Materials

WS extract was provided in the form of free trials from Gurjar Phytochem Indore Madhya Pradesh.The solid lipid Stearic acid, Glyceryl Monostearate, Cetyl alcohol and Beeswax were purchased from Loba chemicals India. Oleic acid, Coconut Oil, Clove oil, Tween 80, Span 60 were ordered from Moly Chem Pvt. Ltd., New Delhi.

Animals

The Animal experiment study was carried outby following the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines and Institution Animal Ethical Committee (IAEC) with number CCP/IAEC/2025/13 of Chandigarh College of Pharmacy, Landran Mohali Punjab India. The Wistar rats were stored under controlled environmental circumstances (25° ± 2° C, 55 ± 5% RH, 12h light/dark cycle) with normal pellet diet. The rats were divided into 5 groups of 6, which are:

- Control group that did not receive any treatment.
- Standard Control group treated with betadine 10 mg/kg
- Withania Somnifera extract gel 200mg/kg
- Withania Somnifera NLC gel treated group with low dose 200mg/kg
- Withania Somnifera NLC gel treated group with high dose 400mg/kg

Wound healing study of WSNLC gel was done on male Adult Wistar rats weight 180-250gm. Excision wound model was used for study. Biopsy punch ranging 7 mm in diameter is required for creation of an excision wound. The blade was attached to a pencil-like handle which was rotated down through the epidermal and dermal region of skin up to the deep subcutaneous fat layer producing the removal cylindrical core of tissue The injuries were left untreated, and the rats were housed individually to eliminate the risk of cannibalism. From day 1 of wound creation, drug treatment i.e., topical gel application was initiated for 2 weeks (Day 1-Day 14) in treatment groups [22, 23].

Formulation of WSNLC

WSNLCs were synthesized with the help of High pressure homogenization method, using the selected solid lipid (Stearic Acid) and liquid lipid (Oleic acid) (Table 1). The solid lipid (Stearic acid) was melted at a temperature 10°C above its melting point. Subsequently, the determined volume of liquid lipid (5ml) was assimilated into the melted solid lipid, followed by the gradual addition of the drug while maintaining constant stirring to obtain a clear solution. The aqueous phase is prepared by adding measured amount of Span 60 (surfactant) (500 mg) & Tween 80 (1.2gm) were added in distilled water. Subsequently the organic and aqueous phase were heated up to 80 °C under magnetic stirring. Additionally the hot aqueous phase was combined with the organic phase while continuously stirring on magnetic stirrer to produce pre- emulsion [24, 25]. After this the prepared pre- emulsion was Homogenize at 5000 RPM for 15 minutes, further it was probe sonicate for 10 minutes as illustrated in Table 5

Table 1: Variables of CCD

Formulation	Stearic Acid(mg)	Oleic acid (ml)	Particle Size (nm R1)	PDI (R2)	Zeta Potential (mv R3)	Entrapment efficiency (% R4)
1	200	5	147.9	0.173	-31.4	89.99
2	200	5	185.3	0.246	-27.8	80.11
3	200	5	185.3	0.266	-29.6	78.65
4	195	4	167.8	0.282	-25.4	77.52
5	207.071	5	204.4	0.262	-28.7	70.65
6	192.929	5	166.2	0.211	-27.98	73.98
7	200	6.41421	190.9	0.361	-33.4	68.96
8	205	6	202.8	0.344	-30.3	68.54
9	195	6	175.8	0.262	-36.4	70.89
10	200	5	185.3	0.246	-23.8	80.62
11	200	5	185.3	0.223	-29.8	80.54
12	200	3.58579	179.6	0.321	-24.3	79.59
13	205	4	194.8	0.276	-25.2	76.98

Independent Variables: Solid Concentration (Stearic acid), Liquid lipid (Oleic acid), Dependent Variables: Particle Size, PDI, Zeta Potential, Entrapment efficiemcy.

Statistical Experimental Design

The Central Composite design (CCD) software was used for the optimization of WSNLCs [26-28].

Optimization of NLCs

The 3D plot and contour plot in Figure 7-10 illustrate the connection between the independent and dependent variables.

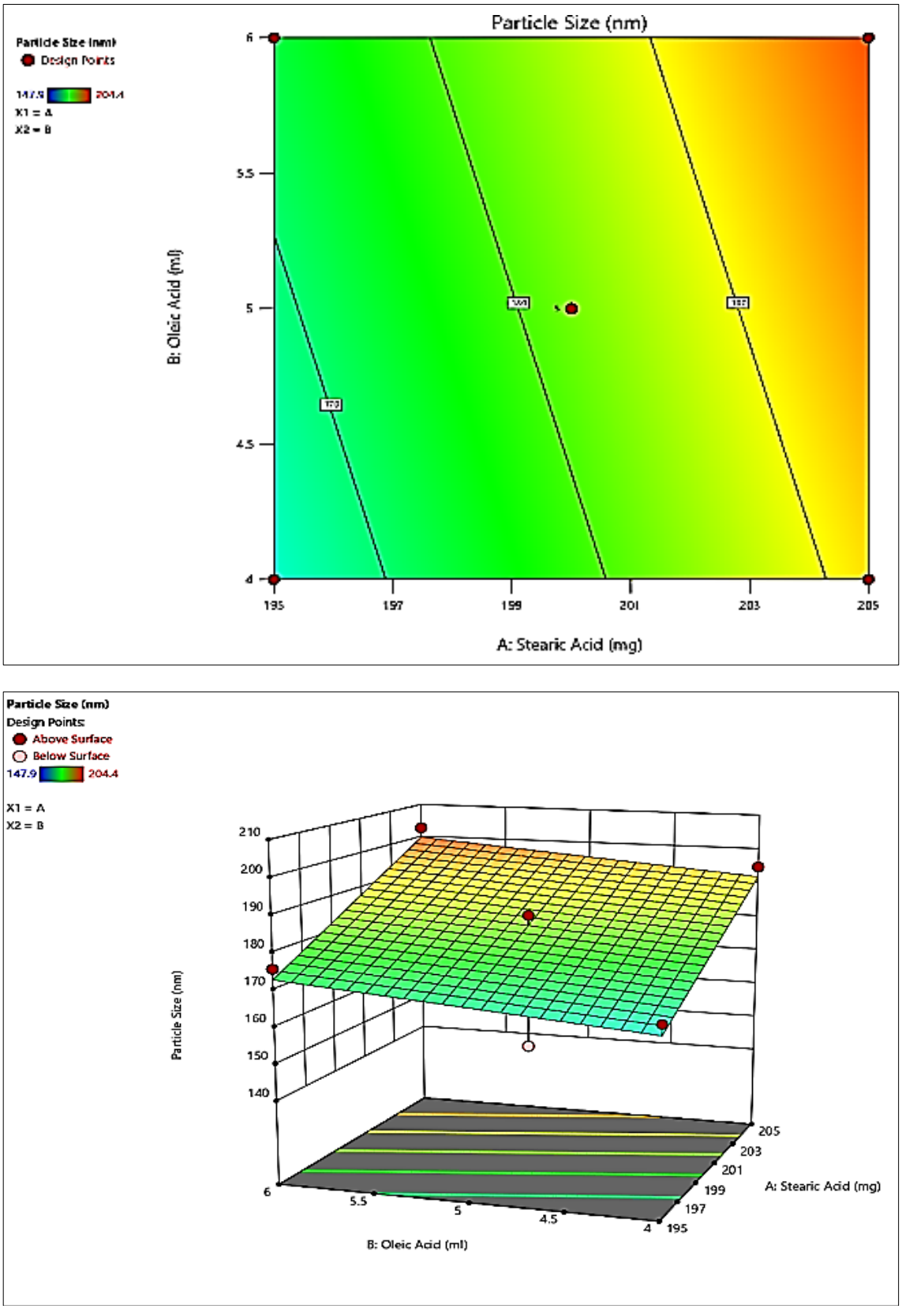
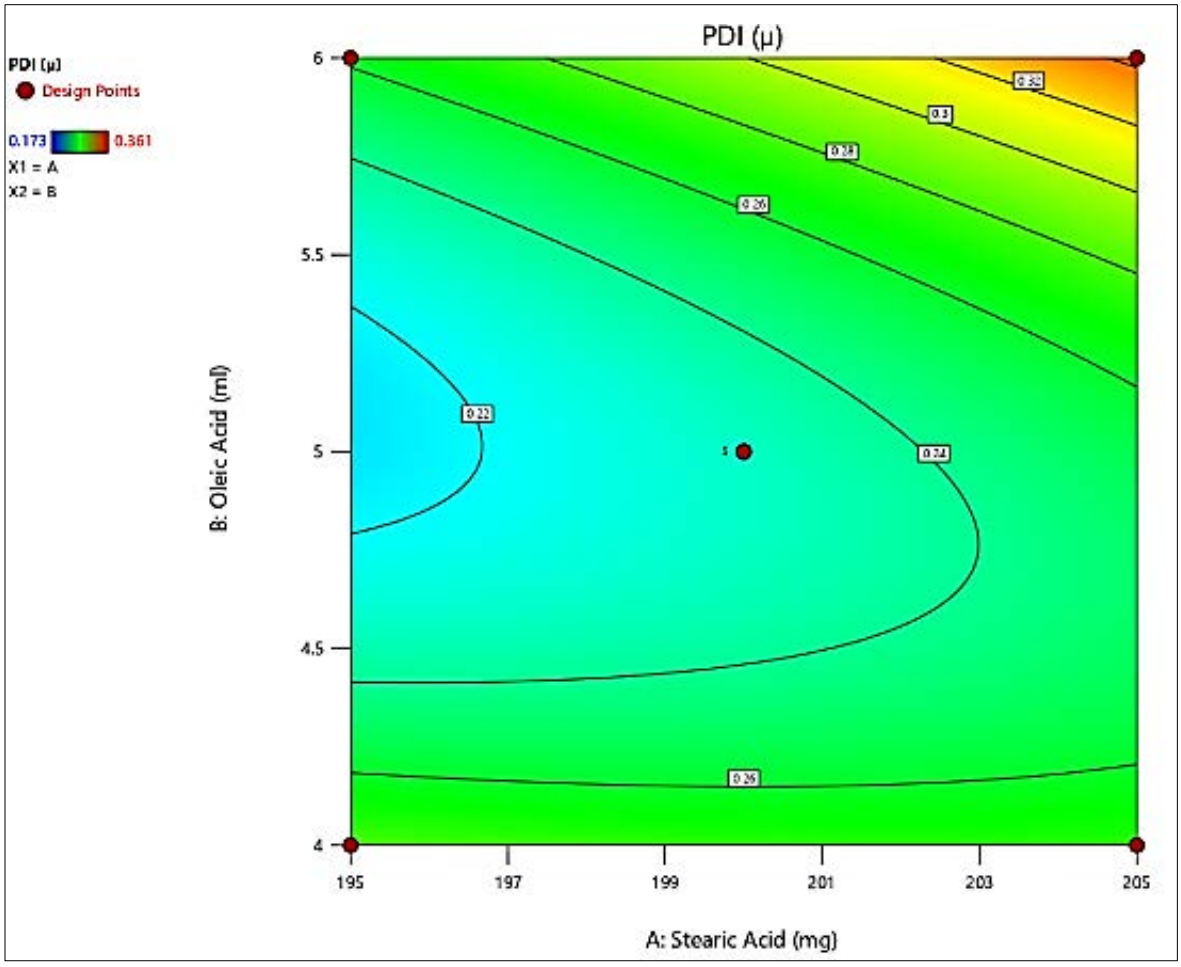


Fig 1: 3D response surface curve and contour plot surface curve depicting the influence of Stearic acid and Oleic acid on particle size of given formulation.



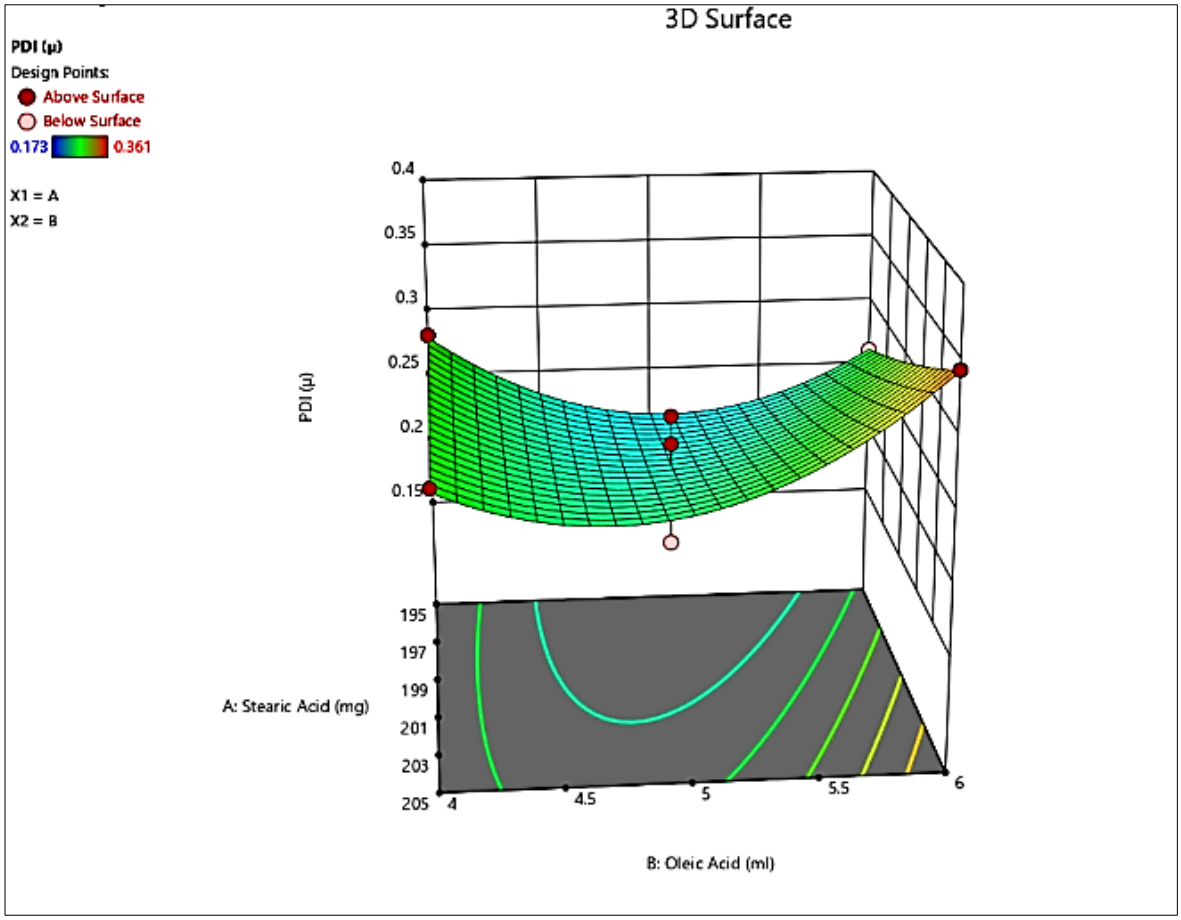
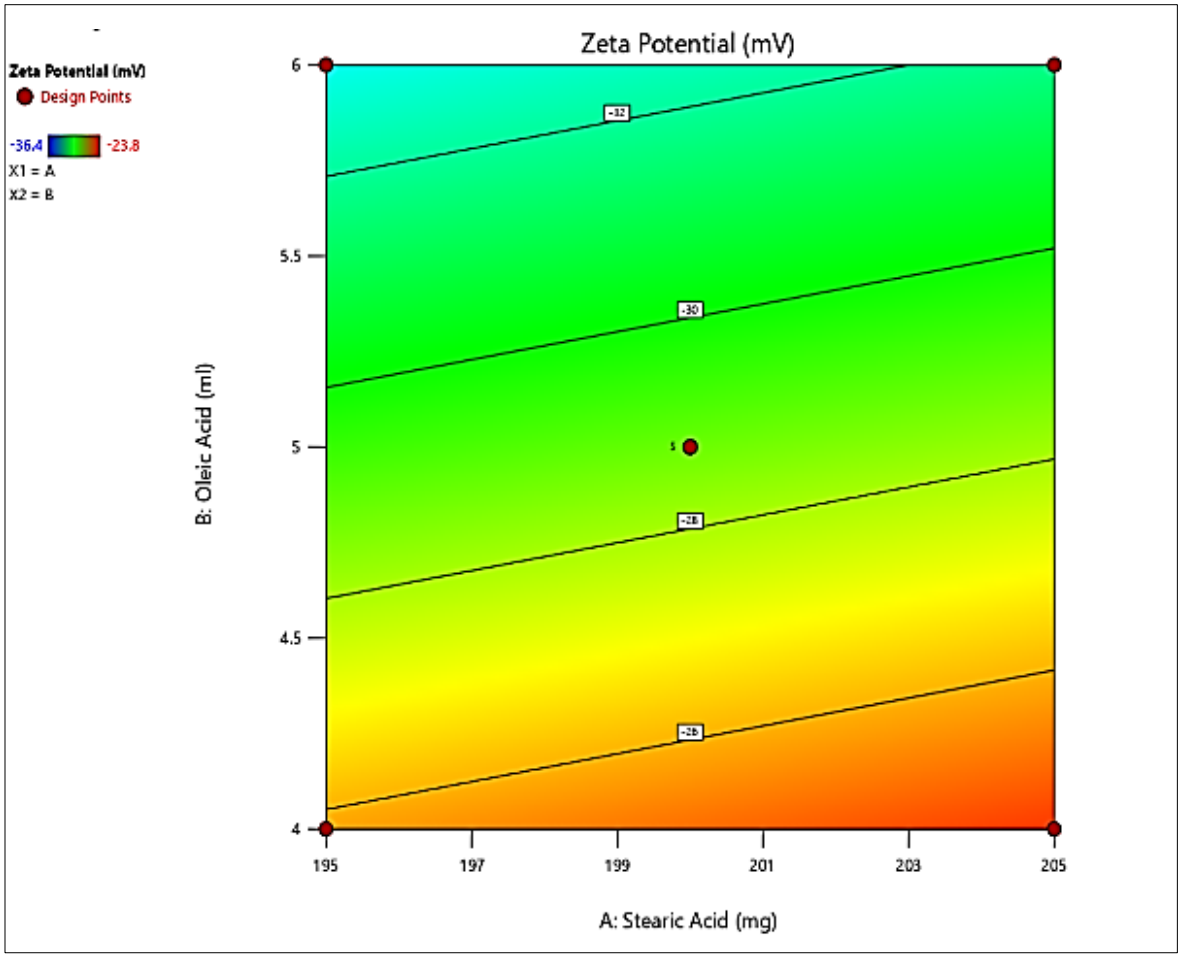


Fig 2: 3D response surface curve and contour plot surface curve depicting the influence of Stearic acid and Oleic acid on PDI of given formulation.



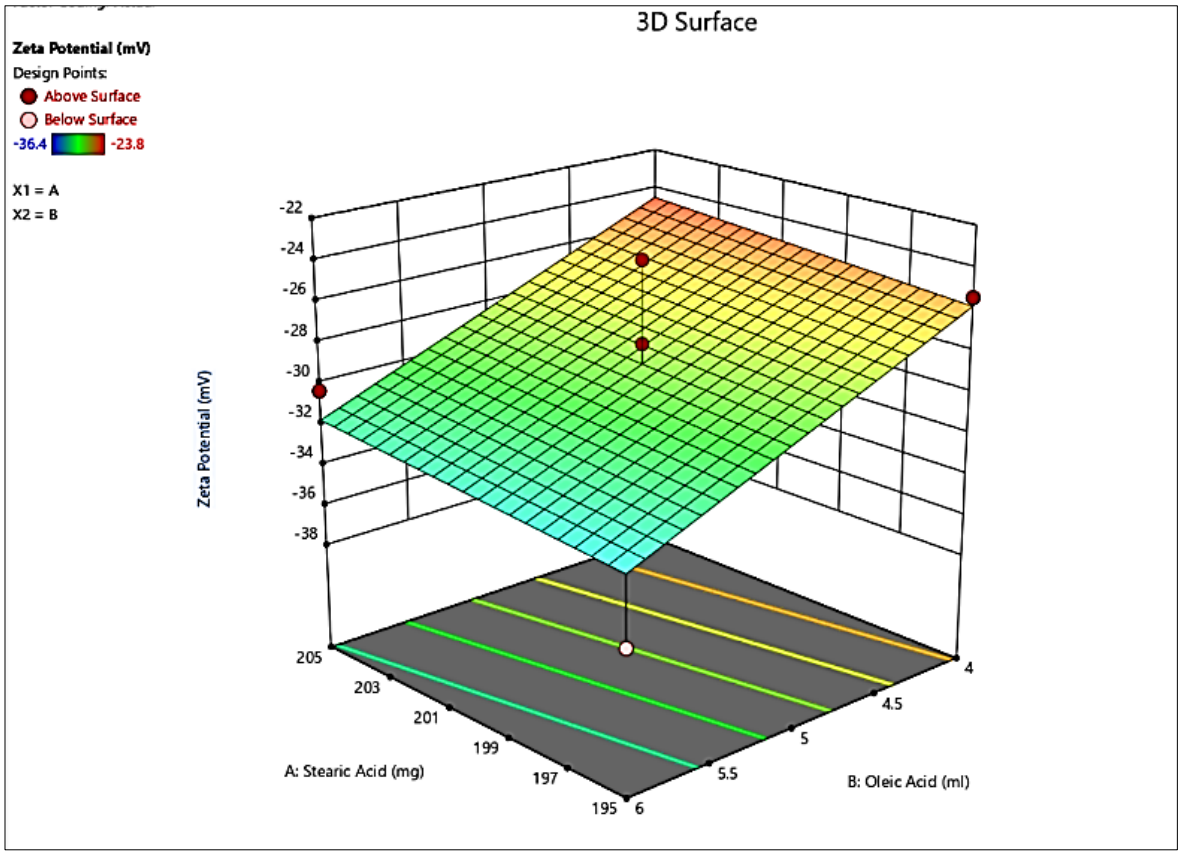
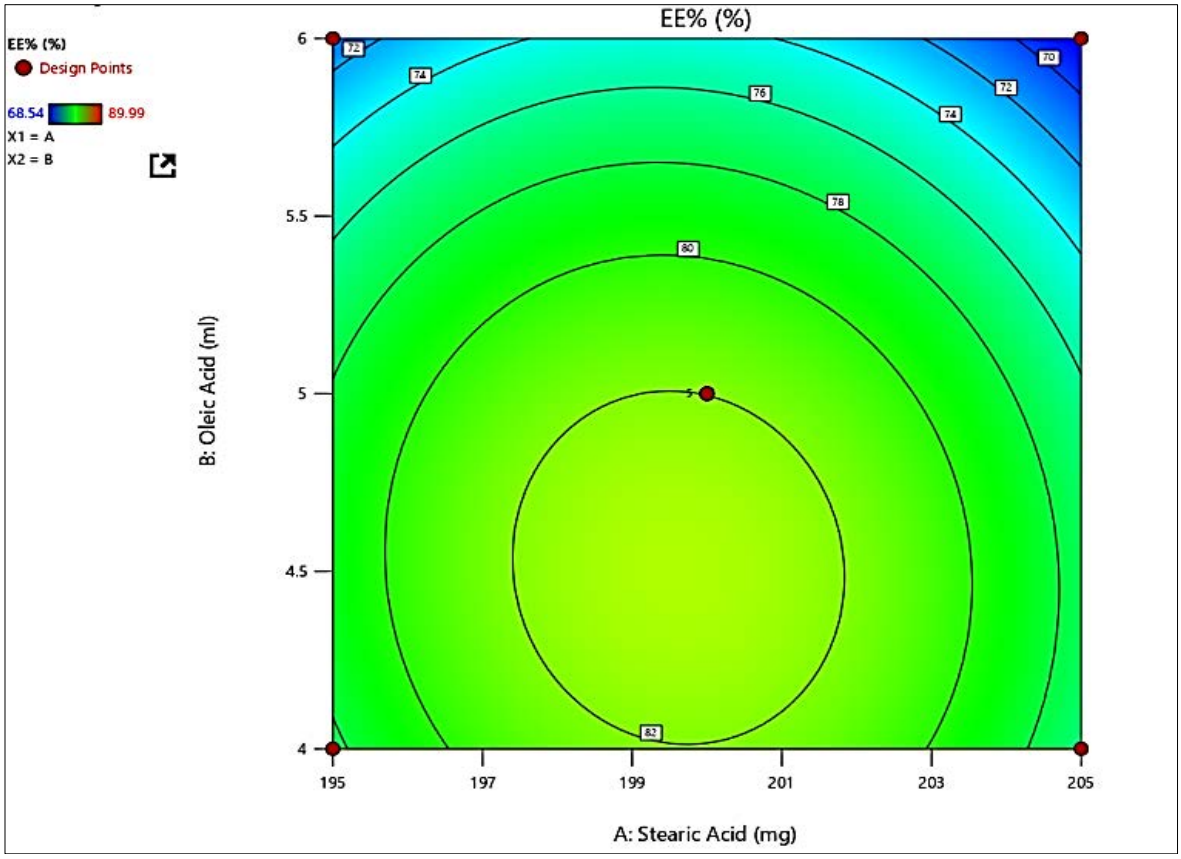


Fig 3: 3D response surface curve and contour plot surface curve depicting the influence of Stearic acid and Oleic acid on Zeta Potential of given formulation.



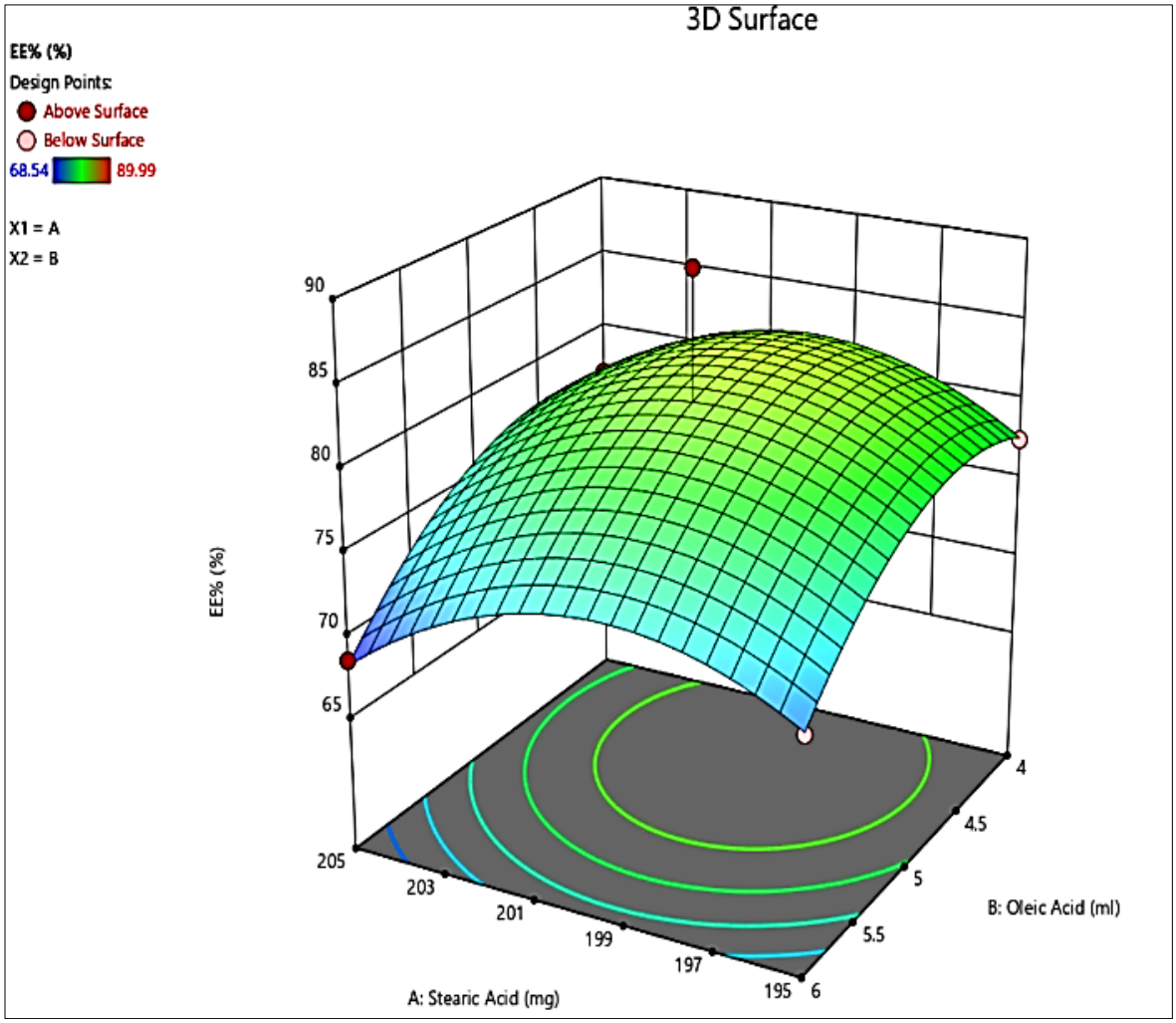


Fig 4: 3D response surface curve and contour plot surface curve depicting the influence of Stearic acid and Oleic acid on Entrapment efficiency of given formulation

Preparation of WSNLC Gel

The gel was formulated using the Dispersion method, with Carbopol 940 serving as the gelling agent. Carbopol 940 was immersed overnight in distilled water that contained Sodium benzoate (0.2% w/v). An HPMC solution was created and homogenized at 3000 RPM. Subsequently, a drug solution was prepared using ethanol and Propylene glycol in a glass vial. The drug solution was subsequently mixed into the HPMC solution and thoroughly homogenized. In the end, the polymer drug solution was combined with the Carbopol solution and neutralized with Triethanolamine [29, 30].

Identification of WSNLCs

a. FTIR analysis of WSNLCs: The FTIR spectra of WsNLC were examined utilizing an FTIR spectrophotometer within the range of 4000-400 cm^{-1} [31, 32].

b. Particle Size, PDI & Zeta potential Determination: Malvern Nano ZS-90 Zetasizer was used to measure the particle size and poly-dispersibility index of the WSNLCs. The range of particle size distribution is quantified by the PDI. Zeta potential (ZP), which is also known as surface charge, is a key factor in the physical stability of nanostructured lipid carriers (NLCs). The experiment was conducted at 25°C [33].

Drug entrapment: The complex was precipitated from the new WSNLCs by centrifuging them for three minutes at 13,000 rpm. The supernatant was composed, combined with ethanol in a 1:4 ratio, and the absorbance of the combination was sustained at 295 nm [34].

Statistical Analysis: The results obtained from the recovery percentage in the studied groups were analyzed, and P< 0.005 were considered significant.

Results

FTIR of WsNLC

FTIR is done to check the compatibility between the solid lipid, liquid lipid and Ws. FTIR spectra of formulation is shown in Figure: 1

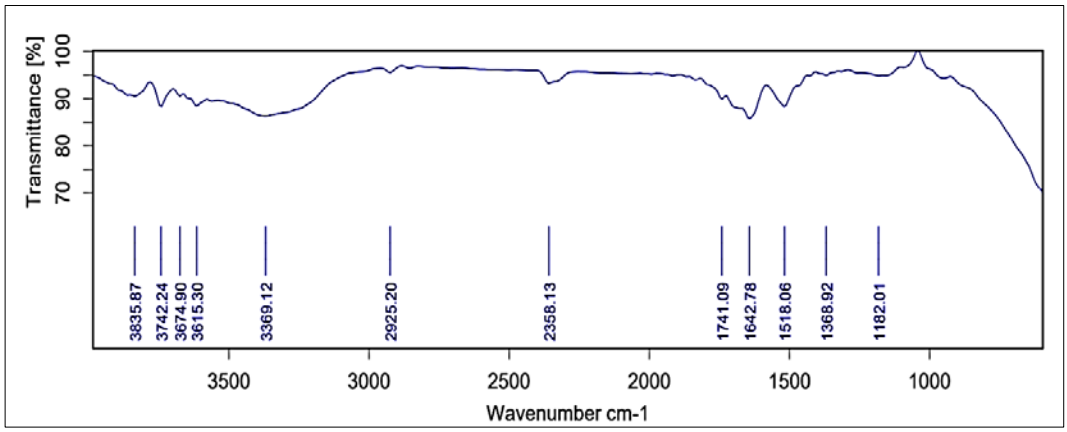


Fig 5: FTIR spectra of WsNLC

Particle Size and PDI

The optimized formulation exhibited a particle size of 147.9±1.43nm (Figure:2), the PDI of formulation was 0.173. The low PDI value indicates that the uniformity between the particles [35].

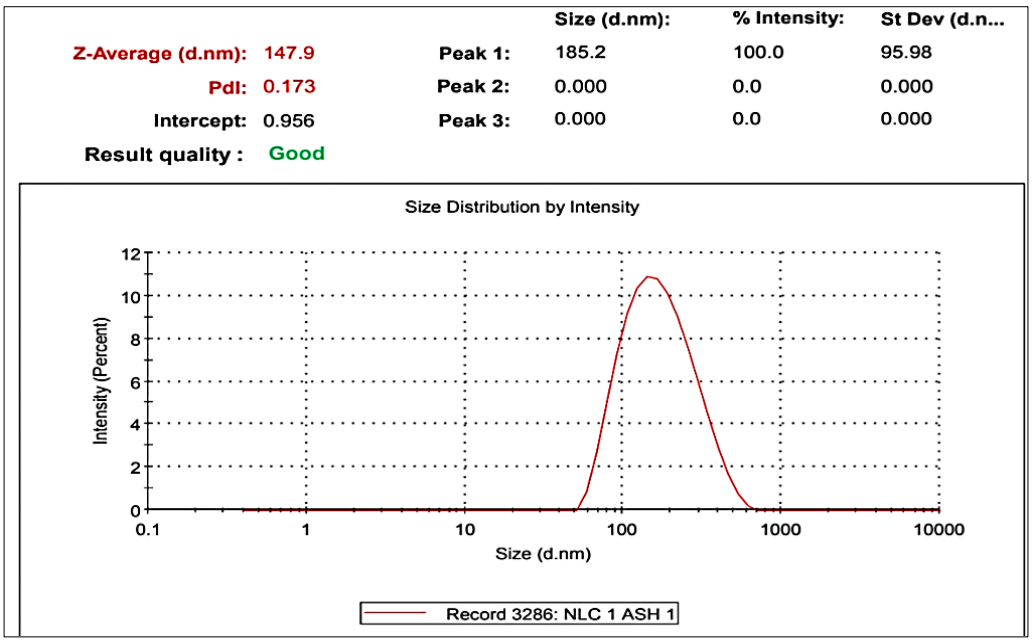


Fig 6: Particle Size & PDI of WsNLC

Zeta Potential

The optimized formulation exhibited a zeta potential of -31.4 mV (Figure:3). The negative value of zeta potential suggests a greater electrostatic repulsion between the particles in the dispersion, thus fostering stability by avoiding aggregation [36].

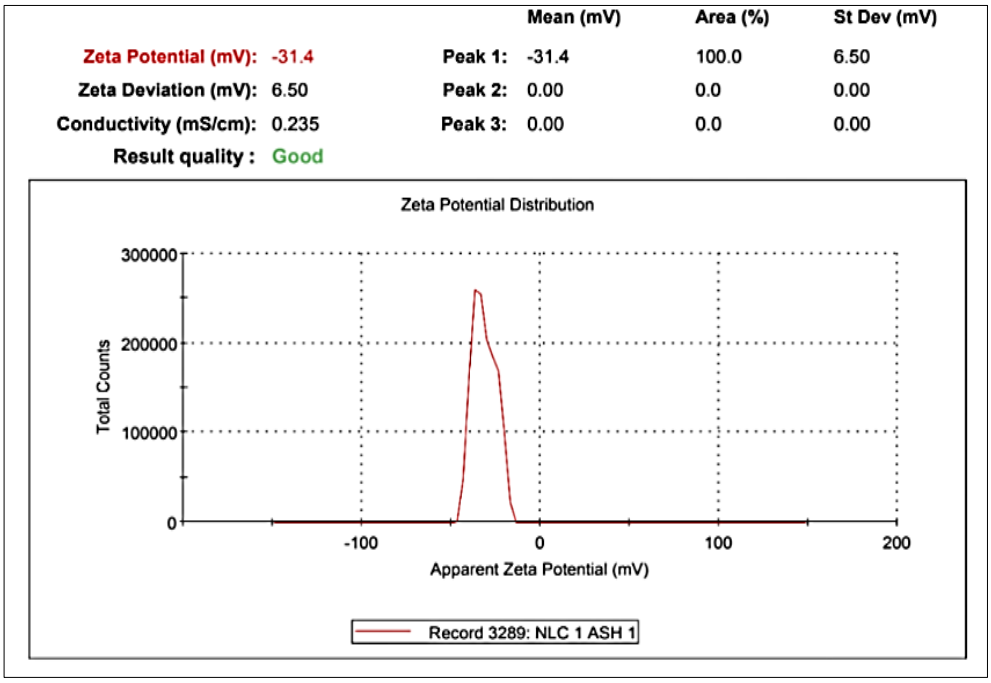


Fig 7: Zeta Potential of WsNLC

Entrapment Efficiency

The optimized formulation explicates an entrapment efficiency of 89.99 ± 0.86%.

Characterization of WsNLC Gel

HR-TEM analysis of WSNLC

The HR-TEM images of optimized WSNLC wasshown in Figure:4

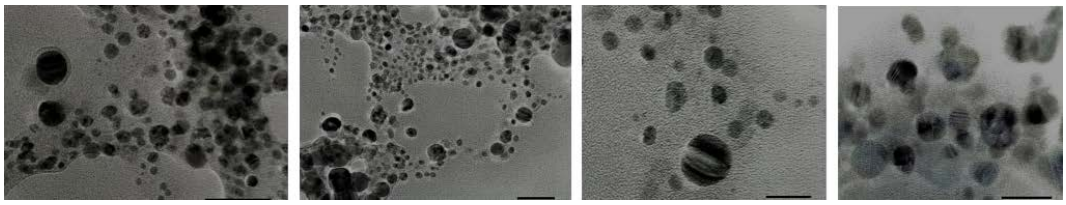


Fig 8: HR-TEM image of WsNLC Gel

Determination of % Entrapment Efficacy (EE)

The entrapment efficacy of WsNLC gel was found to be 86.82%. The method used to determine percentage entrapment efficacy was described earlier.

***In vitro* drug release study:** *In vitro* study revealed that the prepared WsNLCgel demonstrated sustained drug release with 1.48% release in the first 0.5 hours and 89.66% releases in 12hrs.The percent cumulative drug release (%CDR) of WsNLC gel have been illustrated in Figure: 5.

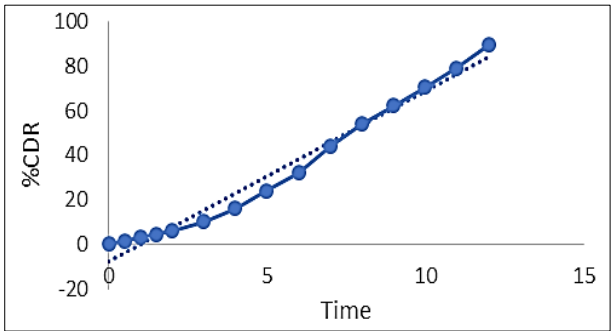


Fig 9: In-Vitro drug release plot of WsNLC gel

Drug release kinetic study: Regression analysis was employed to compute correlation coefficients for linear fits. The Zero order model showed the highest regression coefficient (R^2 = 0.9778)) as shown in Figure 6

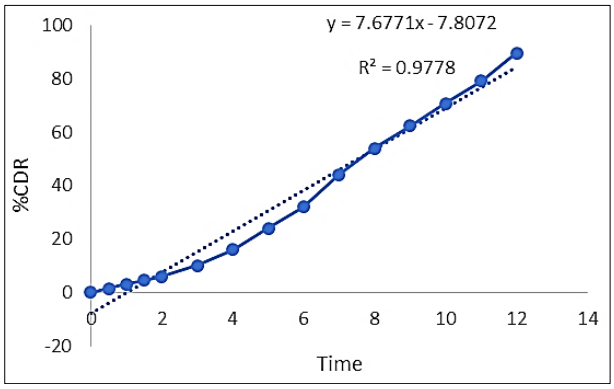
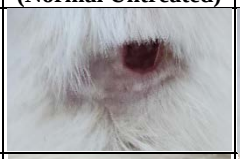
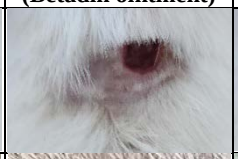
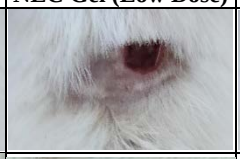
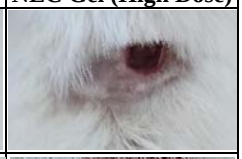
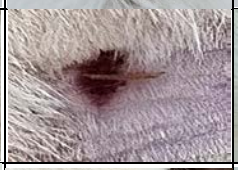
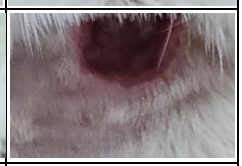
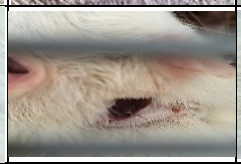
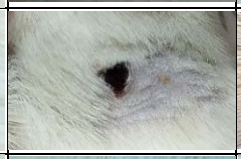
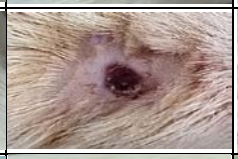
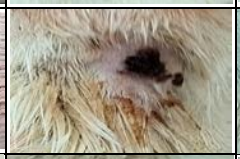
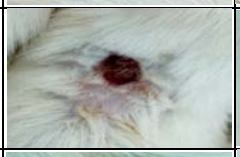
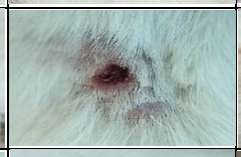
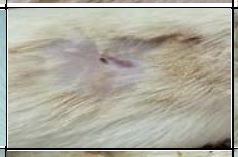
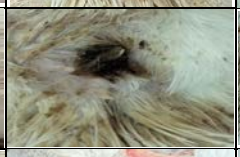
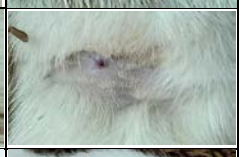
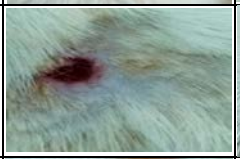
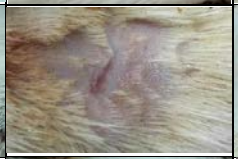
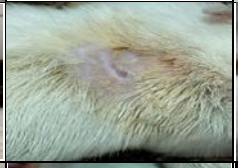
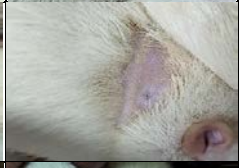
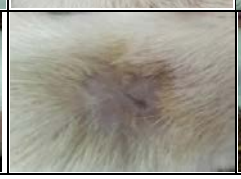
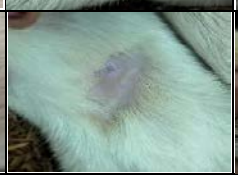


Fig 10: Zero Order Release

***In Vivo* study**

The present study focuses on a 14-day *in vivo* evaluation of wound healing using an excision wound model. The healing process was monitored by measuring wound diameter and calculating the percentage of wound contraction, which serve as critical indicators of tissue repair and epithelial regeneration. These parameters were systematically recorded on days 0, 2, 4, 6, 8, 10, 12, and 14 post-wounding, allowing for a detailed assessment of healing kinetics over time as represented in Table 2

Table 2: Visual observation of Wound

Day	Group 1 Negative Control (Normal Untreated)	Group 2 Standard Group (Betadin ointment)	Group 3 Withania Somnifera Extract Gel	Group 4 Withania Somnifera NLC Gel (Low Dose)	Group 5 WithaniaSomnifera NLC Gel (High Dose)
0					
2					
4					
6					
8					
10					
12					
14					

A. Wound Diameter

In negative control group, the diameter of the wound found to be 0.36 cm on Day 14. The standard, betadine ointment was found to be 0.23 cm on Day 14. Treated group was found to be more effective than standard group. Diameter of wound in treated group is 0.06cm on day 14.

Table 3: Wound diameter of different groups (in cm)

Day	Group 1 Negative Control (Normal Untreated)	Group 2 Standard Group(Betadin ointment)	Group 3 WsExtract Gel	Group 4 WsNLC Gel (Low Dose)	Group 5 Ws NLC Gel (High Dose)
0	0.7±0.00	0.7±0.00	0.7±0.00	0.7±0.00	0.7±0.00
2	0.7±0.00	0.68±0.05	0.63±0.05	0.58±0.05	0.50±0.05
4	0.66±0.05	0.63±0.05	0.61±0.05	0.47±0.05	0.41±0.05
6	0.63±0.05	0.60±0.05	0.55±0.05	0.36±0.05	0.30±0.05
8	0.60±0.00	0.56±0.05	0.50±0.05	0.28±0.05	0.18±0.05
10	0.56±0.05	0.49±0.05	0.42±0.05	0.21±0.05	0.12±0.05
12	0.46±0.05	0.38±0.05	0.35±0.05	0.15±0.05	0.10±0.05
14	0.36±0.05	0.23±0.05	0.21±0.05	0.10±0.05	0.06±0.05

B. Wound area and wound contraction

Semi-transparent tracing paper was used to inspect the wound and assess the healing process. Tracing paper was placed on a 1mm^2 graph sheet and sketched out. The area was frequently checked, and % wound closure was estimated using the following method illustrated in Table 3

Table 4: Wound Area of different groups (in mm^2)

Day	Group 1 Negative Control (Normal Untreated)	Group 2 Standard Group(Betadin ointment)	Group 3 WsExtract Gel	Group 4 WsNLC Gel (Low Dose)	Group 5 Ws NLC Gel (High Dose)
0	38.47	38.47	38.47	38.47	38.47
2	38.47	36.30	29.22	26.41	19.63
4	34.19	31.10	23.75	17.35	13.2
6	31.16	28.27	18.09	10.17	7.07
8	28.26	24.61	19.63	6.15	2.54
10	24.62	18.90	13.85	3.46	1.13
12	16.61	11.31	9.62	1.76	0.75
14	10.17	4.15	3.46	0.78	0.28

Table 5: Wound contraction of different groups (%)

Day	Group 1 Negative Control (Normal Untreated)	Group 2 Standard Group(Betadin ointment)	Group 3 WsExtract Gel	Group 4 WsNLC Gel (Low Dose)	Group 5 Ws NLC Gel (High Dose)
0	0	0	0	0	0
2	0	5.64	24.04	31.34	48.97
4	11.13	18.37	38.26	54.89	65.68
6	19.00	26.51	52.97	73.56	81.62
8	26.54	36.02	48.97	84.01	93.39
10	36.00	50.87	63.99	91.01	97.06
12	56.82	70.61	74.93	95.42	98.05
14	73.56	89.21	91.02	97.97	99.27

Discussion

Excision wound healing model is often used for wound healing evaluation because, it represents a true wound that could be reproducibly analysed in non-subjective, highly controlled manner. The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing process. The enhanced rate of wound contraction and significant reduction in healing time might be due to enhanced epithelialisation In a study regarding the use of WsNLC loaded gel for local wound coverage, the results show an improvement in wound healing due to the antibacterial properties. Also, in the study of Kamble *et al*., the use of nano drug delivery systems due to the reduction of particle size and high porosity leads to skin penetration and increased drug effectiveness *In vitro studies*, zero order release implies that the drug is produced at a steady rate, independent of the concentration of the drug remaining in the dosage form. Such a release mechanism is ideal for maintaining a consistent drug level in the system over time and suggests a controlled release behaviour. It has been demonstrated that the concentrations of both Stearic Acid (A) and Oleic Acid (B) directly influence particle size. A higher concentration of lipids increases viscosity and surface tension, which causes the particle size to increase. Below is the linear polynomial equation that describes the effect of solid and liquid lipids on particle size. It reveals that both Stearic Acid (A) and Oleic Acid (B), along with their interaction and quadratic terms, exert a direct and nonlinear influence on PDI, indicating that increased concentrations result in heightened polydispersity, with optimal values observed at moderate levels. Stearic Acid (A) has a minor positive effect, while Oleic Acid (B) exerts a strong negative effect on zeta potential, suggesting that an increase in Oleic Acid significantly reduces surface charge and may affect stability. Below is the linear polynomial equation that describes the effect of solid and liquid lipids on zeta potential and increased lipid concentrations diminish drug encapsulation efficiency. Therefore, the WsNLCgel exhibits a sustained and predictable drug release profile, making it a suitable formulation for effective wound healing

Conclusion

WSNLCs were formulated using Stearic acid as the solid lipid, Oleic acid as the liquid lipid, and Span 60 and Tween 80 as surfactants, with optimization achieved through CCD design. The optimized formulation (WSNLC) has a particle size of 147nm, an entrapment efficiency of 89.99 %, a PDI of 0.173, and a Zeta Potential of -31.4mV. The In-vitro drug release study conducted over 12 hours indicated asteady release of the medication. The In-vivo wound healing study shows that the WsNLCgel enhances wound healing. The gel containing 400mg demonstrates superior wound healing activity, indicating its potential as an effective topical therapeutic formulation.

Conflict of Interest

Nil.

Funding

Nil.

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Data Availability

Data generated while conducting the research are available to the corresponding author upon reasonable request

References

1. McKnight G, Shah J, Hargest R. Physiology of the skin. Surgery (Oxford). 2022 Jan 1;40(1):8-12.
2. Nagle SM, Stevens KA, Wilbraham SC. Wound assessment. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
3. Kujath P, Michelsen A. Wounds - from physiology to wound dressing. Deutsches Ärzteblatt International. 2008 Mar 28;105(13):239-248.
4. Percival NJ. Classification of wounds and their management. Surgery (Oxford). 2002 May 1;20(5):114-117.
5. Geetha S, Gupta VN, Dv G, Pk K. Research and development in wound management products: a brief review. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(1):1-6.
6. Sangwan RS, Chaurasiya ND, Lal P, Misra L, Tuli R, Sangwan NS. Withanolide A is inherently de novo biosynthesized in roots of the medicinal plant *Ashwagandha* (*Withania somnifera*). Physiologia Plantarum. 2008 Jun;133(2):278-287.
7. Zahiruddin S, Basist P, Parveen A, Parveen R, Khan W, Ahmad S. *Ashwagandha* in brain disorders: a review of recent developments. Journal of Ethnopharmacology. 2020 Jul 15;257:112876.
8. Verma SK, Kumar A. Therapeutic uses of *Withania somnifera* (*Ashwagandha*) with a note on withanolides and its pharmacological actions. Asian Journal of Pharmaceutical and Clinical Research. 2011 Jul 4;4(1):1-4.
9. John J. Therapeutic potential of *Withania somnifera*: a report on phyto-pharmacological properties. International Journal of Pharmaceutical Sciences and Research. 2014;5(5):2131-2138.
10. Punetha H, Singh SO, Gaur AK. Antifungal and antibacterial activities of crude withanolides extract from the roots of *Withania somnifera* (L.) Dunal (*Ashwagandha*). Environment Conservation Journal. 2010 Jun 18;11(1-2):65-69.
11. Bhattacharya SK, Satyan KS, Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera*. Indian Journal of Experimental Biology. 1997;35(3):236-239.
12. Umadevi M, Rajeswari R, Rahale CS, Selvavenkadesh S, Pushpa R, Kumar KS, *et al*. Traditional and medicinal uses of *Withania somnifera*. The Pharma Innovation. 2012 Nov 1;1(9 Pt A):102-106.
13. Sharma S, Arora V, Sharma T. An effective approach to enhance the dissolution profile of curcumin and quercetin: liquisolid compacts. Letters in Drug Design & Discovery. 2024 Jun 1;21(7):1172-1184.
14. Sharma S, Arora V. Powder solution technology review. International Journal of Current Pharmaceutical Research. 2021;13(4):32-35.
15. Sharma S. Application of liquisolid technology in anti-diabetics. CGC International Journal of Contemporary Technology and Research. 2021 Dec;4(1):1-6.
16. Mall J, Naseem N, Haider MF, Rahman MA, Khan S, Siddiqui SN. Nanostructured lipid carriers as a drug delivery system: a comprehensive review with therapeutic applications. Intelligent Pharmacy. 2024 Sep 23;4(3):1-12.
17. Sanna V, Caria G, Mariani A. Effect of lipid nanoparticles containing fatty alcohols having different chain length on the ex vivo skin permeability of econazole nitrate. Powder Technology. 2010;201(1-2):32-36.
18. Khan S, Sharma A, Jain V. An overview of nanostructured lipid carriers and its application in drug delivery through different routes. Advanced Pharmaceutical Bulletin. 2022 Sep 18;13(3):446-455.
19. Jenning V, Schafer-Korting M, Gohla S. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. Journal of Controlled Release. 2000;66(2-3):115-126.
20. Müller RH, Petersen RD, Hommoss A, Pardeike J. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Advanced Drug Delivery Reviews. 2007;59(6):522-530.
21. Puglia C, Bonina F. Lipid nanoparticles as novel delivery systems for cosmetics and dermal pharmaceuticals. Expert Opinion on Drug Delivery. 2012;9(4):429-441.
22. Shailajan S, Menon S, Pednekar S, Singh A. Wound healing efficacy of *Jatyadi Taila*: *in vivo* evaluation in rat using excision wound model. Journal of Ethnopharmacology. 2011 Oct 31;138(1):99-104.
23. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. Evaluation of *in vivo* wound healing activity of *Bacopa monniera* on different wound model in rats. BioMed Research International. 2013;2013(1):972028.
24. Wirawan W, Raharjo S, Supriyadi S. Formulation and characteristics of nanostructured lipid carrier (NLC) red palm oil (RPO) prepared by high-pressure homogenization and its applications in orange juice. Indonesian Food and Nutrition Progress. 2022;19(1):31-40.

25. Luedtke FL, Stahl MA, Grimaldi R, Forte MB, Gigante ML, Ribeiro AP. Optimization of high-pressure homogenization conditions to produce nanostructured lipid carriers using natural and synthetic emulsifiers. Food Research International. 2022 Oct 1;160:111746.
26. Bhattacharya S. Central composite design for response surface methodology and its application in pharmacy. In: Response Surface Methodology in Engineering Science. London: IntechOpen; 2021 Jan 28. p. 45-60.
27. Ahmadi M, Vahabzadeh F, Bonakdarpour B, Mofarrah E, Mehranian M. Application of the central composite design and response surface methodology to the advanced treatment of olive oil processing wastewater using Fenton's peroxidation. Journal of Hazardous Materials. 2005 Aug 31;123(1-3):187-195.
28. Kaur DA, Raina AP, Singh NI. Formulation and evaluation of carbopol 940-based glibenclamide transdermal gel. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(8):434-440.
29. Somadasan S, Subramaniyan G, Athisayaraj MS, Sukumaran SK. Central composite design: an optimization tool for developing pharmaceutical formulations. Journal of Young Pharmacists. 2024 Jul 1;16(3):311-320.
30. Lin X, Li X, Zheng L, Yu L, Zhang Q, Liu W. Preparation and characterization of monocaprate nanostructured lipid carriers. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2007 Dec 1;311(1-3):106-111.
31. Thatipamula RP, Palem CR, Gannu R, Mudragada S, Yamsani MR. Formulation and *in vitro* characterization of domperidone-loaded solid lipid nanoparticles and nanostructured lipid carriers. DARU: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2011;19(1):23-30.
32. Thatipamula RP, Palem CR, Gannu R, Mudragada S, Yamsani MR. Formulation and *in vitro* characterization of domperidone-loaded solid lipid nanoparticles and nanostructured lipid carriers. DARU: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2011;19(1):23-30.
33. Sareen R, Kumar S, Gupta GD. Meloxicam carbopol-based gels: characterization and evaluation. Current Drug Delivery. 2011 Jul 1;8(4):407-415.
34. Pezeshki A, Ghanbarzadeh B, Mohammadi M, Fathollahi I, Hamishehkar H. Encapsulation of vitamin A palmitate in nanostructured lipid carrier (NLC): effect of surfactant concentration on the formulation properties. Advanced Pharmaceutical Bulletin. 2014 Dec 31;4(Suppl 2):563-570.
35. Danaei MR, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, *et al*. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018 May 18;10(2):57-68.
36. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - a review (Part 1). Tropical Journal of Pharmaceutical Research. 2013 May 9;12(2):255-264.