

Nanostructured HPMC Gel Embedding Niclosamide-Leciplex: A Biomaterials Approach to Cellulitis Treatment

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Abstract

Niclosamide (NCD), an anthelmintic drug repurposed against Gram-positive bacteria, has demonstrated promising activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* (*S. aureus*). However, its poor aqueous solubility, low oral bioavailability and limited site-specific availability restrict its clinical use. To overcome these limitations, this study aims to develop NCD-Leciplex decorated hydroxypropyl methylcellulose gel (NCD-LPX-HPMC) gel for topical treatment of cellulitis. NCD-LPX were statistically optimized using Design Expert® software followed by their evaluation using DLS, TEM, DSC, FTIR and PXRD analyses. *In vitro* release, *ex vivo* permeation, skin deposition and antibacterial activities were performed and *in vivo* investigations of the NCD-LPX, NCD-LPX-HPMC gel were achieved in *S. aureus*-induced cellulitis wound model. The optimized NCD-LPX demonstrated optimum particle properties and excellent entrapment 99%. The formulation was amorphous in nature, thermostable and had no chemical interactions. Moreover, the NCD-LPX-HPMC gel has suitable rheological properties for topical administration including viscosity of 7652 ± 29 cp, spreadability of $211 \pm 2\%$ and pH of 6.12 ± 0.06 . NCD-LPX-HPMC gel showed sustained release profile with 64% and 85% NCD released in 24 h at pH 5.5 and 7.4 respectively. Moreover, it exhibited 7 ~ fold better permeation as compared to NCD dispersion. Antibacterial assays revealed significantly lower IC_{50} value for *S. aureus* (0.9639 ± 0.38 $\mu\text{g/ml}$) and MRSA (1.388 ± 0.40 $\mu\text{g/ml}$). Moreover, a significantly enhanced wound healing efficacy of NCD-LPX-HPMC gel (20 ~ fold and 15 ~ fold) was observed in SA-induced cellulitis wound model when compared with untreated and NCD dispersion treated group, respectively. It may be concluded that NCD-LPX-HPMC gel may be a potential alternate towards cellulitis treatment.

1. Introduction

Cellulitis is a bacterial soft tissue infection of skin, caused by the impairment of the skin's physical barrier, the circulatory system and immune system [1]. Bacteria enters into the skin by cut, insect bite or through any infection and causes inflammation in the skin with typical cellulitis symptoms, including tenderness, erythema, swelling, or increased warmth [1, 2]. Every year, an estimated 14.5 million people diagnosed with cellulitis in the United States. Among these instances, about 0.65 million cases are hospitalized annually, hence it creates a significant clinical and economic burden [3, 4]. Traditional treatment of cellulitis involves antibiotic therapy, which is done orally in mild and moderate forms and intravenously in severe and complicated cases [5]. Antibiotics which have been used for treatment of various bacterial infections have potential to cause antibiotic resistance due to its improper and excessive use [6, 7]. Also it's an economical burden to the society and needs antibiotics innovation which is also very expensive [8]. Therefore, alternative therapeutic agents are required to treat cellulitis.

NCD is an FDA approved anthelmintic drug, also reported to manifest antibacterial effects through inhibiting bacterial growth by suppressing the bacterial catabolic pathway to decrease ATP level in bacteria [7, 9]. It has the potential to treat gram positive bacterial infection [10]. Moreover, its distinct antibacterial mechanism and low risk of resistance, are two major factors that make NCD a promising candidate against antibacterial infections [11]. Oral NCD absorbed from the intestinal tract in a very low amount, with bioavailability of 5.5–10% only. Moreover, it is rapidly eliminated by the kidneys without cumulative toxic effects [12]. Therefore, another suitable administration route would be topical administration, however, NCD offer insufficient penetration to the deeper skin layers [13, 14]. Therefore, nanocarriers have extensively been utilized in order to achieve deeper penetration. Skin penetration and drug deposition are important contemplations in the formation of topical nano formulations [14].

LPX, is one such nanocarrier, that can entrap both hydrophobic and hydrophilic drugs owing to its unique and flexible structure and morphology [15]. Its positive charge interacts with skin and facilitate the delivery of drugs to the deeper skin layers [16]. LPX is a mixture of lecithin and cationic surfactants, biocompatible and biodegradable in nature [17]. They have recently been reported with multiple therapeutic applications, such as ophthalmic [18], topical [16], and anticancer [19] drug delivery. However, they need another vehicle to help them stay on the desired area, especially when applied topically, for optimum therapeutic effect [13]. Hydroxypropyl methylcellulose (HPMC), a hydrophilic polymer is used as a secondary vehicle in controlled-release delivery system owing to its swelling ability, forming stable, smooth and clear hydrogels and regulates transport of water by forming gelatinous layer in the system [20, 21]. Moreover, its low toxicity, clear smooth appearance and stable at pH range of 3–11, potential to resist microbial growth and a property of making film upon drying makes it an ideal topical gel candidate [22].

Herein, to overcome the poor dissolution and oral bioavailability of NCD and enhanced localized drug delivery we propose a nanocarrier (NCD-LPX) with enhanced penetration to the deeper skin layers and embed it into HPMC gel enhance its skin contact time and prolong drug release. NCD-LPX were prepared by thin film hydration method, statistically optimized via Box Behnken Design Expert® software and extensively characterized for particle properties and formulation desirability. The NCD-LPX-HPMC gel was prepared and physiochemically characterized for optimum topical delivery to treat cellulitis. *In vitro* release and *ex vivo* permeation analysis was performed to check the dissolution and permeation of NCD. Florescence microscopy and dermo kinetic studies were evaluated to confirm the deeper penetration of drug and dermis retention. Besides, antimicrobial assay was performed against *S. aureus* and MRSA to evaluate the potent antibacterial activity. *In vivo* and histopathological studies were executed in SA-induced wound mice model to reveal the therapeutic efficacy and safety of the desired optimized formulation. To the best of our knowledge NCD-LPX-HPMC gel is novel approach to treat cellulitis as it is not reported so far.

2. Materials and methods

2.1. Materials

Niclosamide (NCD) was obtained from Merck, (Scharlau, Hohenbrunn, Germany). Phospholipon 90G was a kind gift from Lipoid, Switzerland, Hexadecyltrimethylammonium bromide (CTAB) was purchased from (Sigma Aldrich, Co., CHEMIE GmbH), HPMC (K15M) was a generous gift from Shaighan Pharmaceuticals Pvt, Ltd Pakistan. Semipermeable membrane was purchased from Membranes filtration products (Texas, USA). Moreover, all chemical agents used in the current study were of analytical grade. Therefore, no additional purification was done.

2.2. Animals

BALB/c mice of weight 20–25 g and age 6–7 weeks, were purchased from the National Institute of Health, Islamabad, Pakistan for *ex vivo* permeation studies, deposition and *in vivo* studies. They were kept in the animal house facility of Department of Pharmacy, Quaid-i-Azam University, Islamabad Pakistan at 24–25°C temperature and 50–60% relative humidity. The animals were feed with animal food and tap water. Animal study was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (8th Edition) and ARRIVE guidelines (Version 2.0). Additionally, the animal studies were approved by the Bioethical Committee of Quaid-i-Azam University, Islamabad, Pakistan (Protocol #BEC-FBS-QAU2025-729) for the current research project.

2.3. Methods

2.3.1. Preparation of NCD-LPX

NCD-LPX were prepared by using PL 90G and CTAB in equimolar ratios as reported in the literature [16] with slight modification. NCD-LPX was fabricated by thin film hydration method. Momentarily, organic phase was prepared by mixing PL 90G, CTAB and NCD in 1:1 methanol and acetone ratio, using magnetic stirrer hotplate at 70°C. Afterwards thin film was formed at rotary evaporator at 100 rpm at temperature 70°C. After formation of thin film, hydration was done using deionized water at 70 °C for 30. Later, extrusion was performed using 0.45 µm and 0.22 µm filters, respectively to get LPX of the required size and uniform morphology.

2.3.2. Preparation of NCD-LPX-HPMC gel

NCD-LPX-HPMC gel was prepared by incorporating NCD-LPX in HPMC gel. HPMC gel was prepared with the consistency 2% based on blank optimized gel. Half of the calculated quantity of preheated distilled water was taken, then, HPMC powder was slowly dispersed in it while stirring. Then NCD-LPX was dropwise added with gentle stirring. The HPMC solution was continuously stirred until a homogenous appearance was obtained, trailed by volume up to the required amount. This solution was then placed in a refrigerator until a clear, transparent gel was formed [23].

2.4. Optimization of NCD-LPX

Based on literature review and preoptimization trials, independent variables were selected including CTAB, NCD and PL 90G and their effects on the desirable properties like particle size (PS), zeta potential (ZP), entrapment efficiency (EE) of LPX were investigated. Box-Behnken design with Design Expert Software® (Version 13, StatEase Inc., USA) was applied which identified 14 runs in order to optimize the ideal formulation. All the runs were prepared and the responses obtained were recorded in the design sheet for optimization.

2.5. Characterization of the NCD-LPX

2.5.1. PS, PDI and ZP of NCD-LPX

Dynamic light scattering (DLS) was used to assess the PS, PDI, and ZP of the LPX formulations. Malvern Zeta sizer Nano ZS (Malvern, Instruments, Malvern, Worcestershire, UK), equipped with a He-Ne laser (633 nm), was employed for DLS analysis, operated at 25°C at a scattering angle of 90°. Diluted sample of 10 µL was dispersed with 990 µL deionized water to prepare formulation [24].

2.5.2. Percent entrapment efficiency (%EE) determination

The %EE of the optimized formulation was determined after centrifugation of NCD-LPX dispersion (1 mL) for 2 h at 4°C and 13000 rpm using centrifuge machine (Z 216 MK, HermLe, Germany). The untrapped NCD was quantified using a validated UV–Visible spectrophotometer (Halo DB-20, Dynamica, UK) at 238 nm after appropriate dilution.

The %EE was calculated as:

$$\%EE = \frac{(M_{\text{initial drug}} - M_{\text{free drug}})}{M_{\text{initial drug}}} \times 100 \dots\dots\dots \text{Eq (i)}$$

Where $M_{\text{initial drug}}$ is the total amount of NCD used for the preparation of LPX and the $M_{\text{free drug}}$ is the amount of untrapped NCD present in the decanted liquid of the NCD-LPX dispersion after centrifugation [19].

2.5.3. Transmission electron microscopy (TEM) analysis

Morphological characterization of NCD-LPX was analyzed using TEM (TEM, Hitachi Japan) operating at 100 kV. Sample was stained with a 1% (w/v) phosphotungstic acid solution (pH 7), allowed to air-dry, and applied onto 400 mesh carbon-coated copper grids. Imaging was performed using suitable magnifications [25].

2.5.4. Fourier transform infrared (FTIR) study

FTIR Spectrometer (IRPrestige-21 Schimadzu) was used to test the compatibility of NCD and the components of NCD-LPX, including PLG90, CTAB, Mannitol and NCD/PL-G90/CTAB/Mannitol physical mixture by checking their functional groups. FTIR spectra was analyzed in the range of 4400 – 400 cm⁻¹. Prior to the analysis, NCD-LPX was lyophilized. The physical mixture was identical to LPX formulation; it was used to ensure that only changes observed are as a result of incorporation of drugs into LPX [16]. Each sample was pressed (200 mg of KBr) between the hydraulic press to a 10-mg disc, and the reading was made in triplicate.

2.5.5. Powder X-ray diffraction (PXRD) analysis

An X-ray diffractometer (MiniFlex Benchtop Rigaku, Japan) was used to confirm the identity and the crystallinity of materials used in the preparation of NCD-LPX, including NCD, CTAB, Mannitol and their physical mixture. The angular region was selected to be 5° to 50° and was adopted on Ni-filtered Cu target such that the theta range was increased at 1°/sec. The voltage and current range was respectively used as 100 kV and 100 mA. [26].

2.5.6. Differential scanning calorimetry (DSC) analysis

NCD-LPX formulations were evaluated by DSC (Q200, TA Instruments, USA) to analyze the thermal properties of the drug after incorporating it into the LPX, drug in its pure form and other excipients. The dry pellet after centrifugation of the dispersion was loaded in aluminum pans under vacuum after drying sealed with an aluminum lid (Tzero pan and lid, TA Instruments, USA) and scanned thermally at 0 to 350°C. The samples were then heated at the rate of 10°C/min [27].

2.6. Characterization of NCD-LPX-HPMC gel

2.6.1. Physical appearance and pH

At room temperature, NCD-LPX-HPMC gel was inspected for color, transparency, and grittiness. The homogeneity and texture were also examined after the gel was set in the beaker to check any aggregates [23]. pH of the gel using digital pH meter (PH 700 EUTECH instruments, Aachen Germany) was inspected with the procedure of taking 1 g gel and dispersed in 20 mL of water.

2.6.2. Rheology

Viscosity of the NCD-LPX-HPMC gel was evaluated by using Brookfield cone and plate rheometer (Brookfield Engineering Laboratories, Middleborough, MA, USA). The system was operated at 25°C using spindle CPA 52 Z. A small amount of gel was placed into the adapter compartment, and viscosity was measured in cP by changing the spindle speed from 2 to 100 rpm. Rheogram was plotted between shear rate and viscosity[28].

2.6.3. Spreadability analysis

Spreadability of NCD-LPX-HPMC gel was determined by glass slide method. Glass slide's center was marked with 2 cm of circle and 0.5 g of gel was placed on it. Then another glass slide was placed over it, such that the gel was sandwiched between both the glass slides. Later, a weight of 500 g was placed on the upper slide for 5 min and removed, followed by measuring the increase in diameter and calculating the spreadability as discussed below.

$$\% \text{ Spreadability} = \frac{(A_2)}{(A_1)} \times 100 \dots\dots\dots \text{Eq (ii)}$$

Where A_1 = 2 cm and A_2 = final area (diameter) after spreading[28].

2.7. In vitro drug release study

In vitro release of NCD from NCD-LPX-HPMC gel, NCD-Dispersion and NCD-LPX was performed using dialysis bag method at different pH (PBS 5.5 and 7.4). Procedure started with adding 2 mL formulation in dialysis membrane which was already soaked in buffer for 30 min and sealed both sides of membrane with thread to prevent leakage. Dialysis membrane was then suspended in 50 mL buffer maintained at $37 \pm 1^\circ\text{C}$ and 80 rpm on water bath and shaker (Memmert, WNB-7, Germany). Samples were drawn out at predetermined time (15 min, 0.5 h, 1, 2, 4, 6, 12 and 24 h) intervals and replaced with sample volume of pre-heated buffer. These withdrawn samples were detected by a UV-visible spectrophotometer (Halo DB-20, Dynamica, UK) and the cumulative drug release was calculated and graphed against time. Each sample was taken in triplicate to calculate mean $\pm$ SD ($n = 3$) [29]. Besides, to obtained data for the release kinetics different kinetic models were applied [30]. The best fit model was selected on the basis of highest determination coefficient ($R^2 > 0.99$).

2.8. Skin permeation and deposition study

The *ex vivo* permeation of NCD from NCD-LPX, NCD-LPX-HPMC gel and NCD-Dispersion was observed using Franz diffusion apparatus (PermeGear, USA). These cells have receptor compartment with a volume of approximately 5.2 mL and permeation area of 0.78 cm^2 . Abdominal mice skin hair and fat were removed. Freshly prepared buffer (PBS pH 7.4) was added to each cell and apparatus was set at 300 rpm and temperature at 37°C . Mice skin was cut into pieces of appropriate size and placed over the cell, in such a way that the stratum corneum was facing the donor compartment and the dermal layer was in contact with the buffer in the receptor compartment. The donor compartment was then subjected to the NCD-LPX, NCD-LPX-HPMC gel and NCD-Dispersion in a quantity that all the donor compartments have equal amount of NCD. Samples were taken at respective time intervals and replaced with an equal amount of PBS. Sample was taken in triplicate, and study was performed for 24 h of duration. Samples were then analyzed using UV-Visible spectrophotometer (Halo DB-20, Dynamica, UK) and the drug permeation graph was plotted against time [30].

The skin was removed and washed with normal saline to remove all excess formulation from the skin to analyze concentration of drug in different skin layers. After 2–3 min of water soaking at 60°C the skin samples were taken out. By using tape stripping method different layers such as, stratum corneum, epidermis and dermis were separated. The separated skin was then chopped into small pieces and soaked in ethanol (5 mL) for 24 h, then sonicated in bath sonicator and homogenized and centrifuged at 13000 rpm for 20 min and supernatant was utilized to analyze the concentration of drug in the UV-Visible spectrophotometer (Halo DB-20, Dynamica, UK) [31].

2.9. Ex vivo penetration analysis through fluorescent microscope

To analyze the distribution of nanoparticles in mice skin, *ex vivo* permeation analysis through fluorescent microscope was performed. Momentarily, Rhodamine dye loaded LPX were prepared and incorporated in HPMC gel. Mice skin was then prepared by removing fat tissue and hair. After that skin was placed on the Franz diffusion cell for 8 h under the same conditions as discussed earlier, and Dye-LPX-HPMC gel was applied. After specified time the skin

was taken and washed to remove extra gel from the skin. Transverse section via cryostat microtome was taken to prepare the slide. Images were visualized by using fluorescent microscope and keeping a blank skin as a control [32].

2.10. Antimicrobial assay

To check the percentage inhibition of bacterial strains at different concentrations of NCD-Dispersion, NCD-LPX-HPMC gel and NCD-LPX anti-microbial assays were performed against the strains of *S. aureus* (S.A) and Methicillin-resistant Staphylococcus Aureus (MRSA). These bacteria were incubated overnight in nutrient broth and diluted to make a concentration of 1.5×10^5 cfu/mL. Then, 190 μ l of this broth and varying NCD-Dispersion and NCD-LPX-HPMC gel and NCD-LPX concentrations (100, 50, 25, 12.5, 6.25, 3.175, 1.6 μ g/mL) in 10 μ l were poured into wells and the 96-well plate was then incubated in incubator chamber for another 24 h. Absorbance was measured at 0 h and 24 h at 470 and 630-nm wavelength using a microplate reader. After that by using following equation the percentage inhibition was calculated:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{treated}}}{A_{\text{control}}} \times 100$$
..... Eq (iii)

Assays were performed in triplicate [33].

2.11. In vivo cellulitis model

All mice were shaved and disinfected with 75% alcohol. Then divided into four groups. Group 1 Normal control group, Group 2 Disease control group, Group 3 NCD-LPX-HPMC gel treated group and Group 4 NCD-Dispersion gel treated group. S.A strain was inoculated in nutrient broth and incubated in incubator for 24 h and then diluted to get 10^5 cfu/mL and placed in incubator. Group 2, 3 and 4 were subcutaneously injected with 100 μ L of S.A [10]. After successful induction of cellulitis i.e. 1 h post infection the treatment was given to both groups, group 3 i.e. NCD-LPX-HPMC gel treated group and group 4 i.e. NCD-Dispersion gel treated group. Both formulations had NCD equivalent to 250 μ g. The formulations were applied on the skin carefully using sterile spatula, 10 days in a row after induction. Starting from the initial cyst formation, the diameter of each animal's wound was assessed for the required duration to achieve complete healing. Infection size was measured on day 0, 3, 5, 7 and 10 of treatment. Together with the wound contraction rate %, the wound area provides a clue to the degree that the wound was healing [34–36]. On the 10th day, the wound tissue was excised and slides were prepared for histopathological examination

3. Results

3.1. Optimization of NCD-LPX

Preoptimization studies were performed to check the upper and lower concentrations of independent variables. Blank formulations were also prepared to set the values of independent variables PL 90G, CTAB, NCD and dependent variables PS, ZP, %EE. After that to statistically optimize the formulation, a three factor, three levels – Box–Behnken design was applied using Design Expert® Version 13.0 software. The design was composed of 14 experimental runs, values of independent variables and results of dependent variables i.e. PS, ZP and EE are given in Table 1.

Table 1
Runs suggested and optimization of NCD-LPX by BBD.

Run	CTAB (mg)	PL90G (mg)	NCD (mg)	PS (nm)	ZP (mV)	EE (%)
1	120	90	5	170.5 ± 4.59	42.7 ± 0.21	95.29 ± 0.16
2	120	100	10	132.5 ± 2.33	22.0 ± 1.46	98.03 ± 0.001
3	100	80	5	146.2 ± 6.15	51.7 ± 1.77	96.0 ± 0.02
4	100	100	5	133.0 ± 2.66	39.2 ± 2.62	94.93 ± 0.63
5	120	90	15	93.8 ± 3.85	38.4 ± 3.11	98.73 ± 0.02
6	80	90	5	124.8 ± 3.46	54.5 ± 1.49	95.04 ± 0.03
7	80	100	10	141.0 ± 3.18	44.0 ± 2.97	98.09 ± 0.23
8	100	90	10	136.0 ± 2.69	48.6 ± 5.79	98.08 ± 0.08
9	100	80	15	99.7 ± 2.09	41.4 ± 5.09	98.57 ± 0.03
10	100	90	10	144.5 ± 2.05	45.8 ± 4.81	98.25 ± 0.05
11	80	80	10	120.0 ± 3.61	35.0 ± 2.26	98.07 ± 0.05
12	100	100	15	139.9 ± 6.65	43.9 ± 3.96	99.45 ± 0.05
13	120	80	10	133.2 ± 0.64	42.5 ± 2.47	98.01 ± 0.11
14	80	90	15	140.0 ± 2.78	44.7 ± 2.05	98.78 ± 0.19

Note: PS: Particle size, ZP: Zeta potential, EE: Entrapment efficiency, CTAB: Hexadecyltrimethylammonium bromide, PL 90G: Phospholipon® 90G, NCD: Niclosamide.Each value represents the mean ± Standard deviation n = 2

By using Design Expert® software different mathematical models were tested and produced to detect the best fit including linear, 2FI, quadratic and cubic. Box–Behnken design suggested 2FI for PS and %EE and Quadratic for ZP analyses. For each response ANOVA reports were produced and different parameters as shown in Supplementary Table.1. were studied.

3.2. Effect of independent variables on PS, ZP and EE

3.2.1. Effect of independent variables on PS

For PS optimization, the model F-value of 20.24 was found and it implied that model is significant. The p value that is lower than 0.005 in the case of PL 90G indicates that it has a significant effect on PS i.e. increase in the PS in case of increase of PL 90G concentration. 2FI was suggested as the best fit model with Predicted R² value of 0.84 and adjusted R² value of 0.89 and their difference in the required range (< 0.2). Sufficient precision (16.55) was greater than 4 meaning that it could be useful to explore the design space. The impact of the independent variable on PS was evaluated using the following polynomial equation.

$$PS = + 132.50 + 0.5181A + 5.92B - 12.64C - 5.41AB - 22.98AC + 13.34BC \dots\dots\dots \text{Eq (iv)}$$

The PS was found to increase with increase in PL 90G concentration. Moreover, CTAB at higher concentration was found to reduce the PS, however, at a much higher concentration, larger vesicles were generated. Furthermore, the NCD concentration didn't change the PS size significantly. The combined effects of NCD, PL 90G and CTAB concentration on PS is depicted Fig. 1 (A-C).

3.2.2. Effect of independent variables on ZP

Design expert showed that the ZP quadratic model had significance with a p-value (0.004). Lack of fit p-value (0.65) did not demonstrate any significance. Predicted R² (0.74) value was in agreement with adjusted R² (0.93) as difference of both value is (< 0.2). The required precision was 19.67, which was a good signal for design space. CTAB has marked effect on ZP as depicted by p value of 0.0041. Similarly, the model F-value of 22.02 presume its significant relation. Polynomial coded equation for ZP is shown below.

$$ZP = + 47.20 - 4.03A - 2.64B - 2.45C - 7.27AB + 1.40AC + 3.75BC - 5.10A^2 - 6.12B^2 + 2.95C^2 \dots\dots\dots \text{Eq (v)}$$

As the CTAB concentration increases, the ZP moves more towards positive as depicted in Fig. 1(D-F). The PL 90G concentration also has significant effect on ZP having a p value of 0.0189.

3.2.3. Effect of independent variables on EE (%)

The significance of 2FI model (p-value 0.0001) showed a prominent impact of the independent variables on the %EE. The predicted R² (0.98) and adjusted R² (0.99) were within the necessary range (< 0.2). Lack of fit (p-value 0.37) was not significant, as required. adequate precision (72.75) was greater than 4, indicating that it could help to navigate the design space. For the %EE, concentration of CTAB, NCD and PL 90G have positive effects on the response with p-value 0.0042, 0.0001 and 0.0001 respectively. A polynomial equation was fitted for given level of each factor.

$$\%EE = + 98.16 - 0.0763A + 0.1375B + 0.6312C - 0.0575AB - 0.0750AC + 0.7025BC \dots\dots\dots \text{Eq (vi)}$$

According to the Fig. 1 (G-I) with the addition of or by increasing the concentration of PL 90G, the %EE was raised which might be due to the formation of large-size particles that eventually amplified the area of entrapment of the drug [37].

3.3. Validation and Optimization of BBD Model

By applying the desirability function the optimized NCD-LPX was selected having desirability of 0.735 which is near to 1. Desirability scale shows that value near to 1 is the best value and near to 0 is the least desirable optimized formulation with the desired values of dependent variables that is minimum PS, maximum ZP and maximum %EE was achieved with concentration of PL 90G 90.99 mg, CTAB 102.34 mg and NCD 15 mg giving the predicted values for PS 119.09 nm, ZP 47.28 mV and EE 98.85% as shown in Supplementary Fig. SF1. Three formulations were then prepared using these optimized values and the results were in close range with the predicted values given in Supplementary Table. 2. Hence, confirming the validity and reliability of the BBD model. Shown in Supplementary Fig. SF2.

3.4. Evaluation of PS, ZP, PDI by DLS

Uniform PS with less than 200 nm diameter was observed in all BBD formulations with uniform distribution and high surface charge having ZP value ranging from + 22 to + 55 mV which eventually decrease the chances of aggregation and fusion of LPX. The PS and ZP of the optimized formulation are reported as Fig. 2A and Fig. 2B, respectively with excellent % EE of 99.5%.

3.5. TEM Analysis

TEM micrograph Fig. 2C showed that the optimized NCD-LPX particles were round in shape, well segregated and monodispersed, depicting lower PDI and PS below 200 nm, ultimately confirming the Zetasizer results.

3.6. FTIR

FTIR was performed for functional groups detection and to check the chemical interaction of NCD and excipients including CTAB, PL-90G used in LPX formation (Fig. 2D). FTIR spectrum of NCD showed N–H stretching centered at 3359 cm⁻¹, NH bend at 1570 cm⁻¹, C–N stretch at 1323 cm⁻¹, C = O stretch

at 1681 cm⁻¹, C-Cl bend at 723 cm⁻¹[38]. The spectrum of PL-90G exhibited characteristic C–H stretching bands at 2924 cm⁻¹, a stretching band of ester carbonyl group at 1732.2 cm⁻¹ and an ester C–O stretching band at 1240 cm⁻¹. CTAB spectrum displayed C–H stretching vibrations at 2912 and 2850 cm⁻¹ for methyl and methylene groups, respectively. The bands in the region of 1400–1500 cm⁻¹ were due to C – H bending vibration[16]. The spectrum of physical mixture showed all the corresponding bands of the added excipients [16, 26]. Similarly, the spectrum of lyophilized NCD-LPX presented the distinctive corresponding bands to NCD, CTAB, and PL-90G at their respective wave numbers, indicating no chemical interactions among its constituents.

3.7. PXRD

Crystalline nature of NCD was detected by X-ray diffraction analysis which showed different peaks demonstrated in Fig. 3A. NCD diffractogram showed sharp peaks at 2θ of 9.5°, 14.8°, 17.3°, 19.4°, 20.8°, 23.8°, 25.5° and 29.8°. On the other hand, CTAB shows characteristic diffraction peaks at 2θ of 9.1°, 14.7°, 18.7°, 21.3°, 32.2°, 35.6° and 44.3° and several minor peaks and confirms its crystalline nature as reported in literature [16]. All these peaks of NCD and CTAB were absent in the NCD-LPX showing complete entrapment of NCD in the nanoparticle. These results complies with the literature as the drug crystalline nature was converted into amorphous [26].

3.8. DSC

The NCD thermogram showed endothermic peak at 230.12°C, complies to the drug melting point and high thermal stability [38](Fig. 3B). PL-90G endothermic peak was observed at 153.82°C in accordance with literature [39]. Similarly, CTAB presented a sharp endothermic peak at 106.99°C due to moisture loss, and another peak at 267.27°C which coincides with its melting range as given in the literature [16]. Endothermic peaks of physical mixture showed the corresponding NCD and PL90G peaks with slightly shifted to lower temperatures. However, thermogram of NCD-LPX revealed significant decrease in intensity of NCD, followed by the broadening and the significant shift in endothermic transition. This slight shift in peak position could be due to PL 90G melting before the NCD, which leads to partial or complete NCD dissolution in PL 90G lipid [16, 40].

3.9. Characterization of NCD-LPX-HPMC gel

3.9.1. Physical appearance

The formulated NCD-LPX-HPMC gel was inspected for color, transparency, and grittiness. NCD-LPX-HPMC gel was opaque, uniform and yellow in color, having smooth appearance. pH was found to be 6.12 ± 0.06, which was within the desired pH range. Table 2 shows the physical characteristics and pH of NCD-LPX-HPMC gel and HPMC gel.

Table 2
Physicochemical characterization of NCD-LPX-HPMC gel.

Parameters	HPMC gel	NCD-LPX-HPMC gel
Appearance	Transparent gel	Opaque gel
Colour	Colourless	Yellow
Homogeneity	Uniform	Uniform
pH	6.05 ± 0.02	6.12 ± 0.06
Note: NCD-LPX-HPMC gel: Niclosamide loaded Leciplex Hydroxypropyl methyl cellulose gel, HPMC gel: Hydroxypropyl methyl cellulose gel. Each value represents the mean ± Standard deviation n = 3		

3.9.2. Rheology

Rheological study was performed to check the viscosity of gel. Gel shows shear thinning rate which explains that gel viscosity decreases as the shear rate increases which shows non-Newtonian behavior which is beneficial in topical application to ensure high coverage (Supplementary Fig. SF3) [41, 42].

3.9.3. Spreadability

Spreadability was measured to evaluate the consistency of gel which was an important factor for the ease of application and eventually increase patient compliance [43]. It was calculated in % with a value of 211% ± 2.87 of NCD-LPX-HPMC gel. Results were in compliance with the reported acceptable range and also confirmed the smooth application of gel on the skin [44].

3.10. In vitro release study

NCD release from NCD-Dispersion, NCD-LPX and NCD-LPX-HPMC gel was evaluated through *in vitro* study by dialysis membrane method (Fig. 4A&B). Study was performed at physiological pH 5.5 and 7.4 which mimics the skin and blood pH respectively for 24 h. A significantly retarded drug release was observed from NCD-LPX-HPMC gel at both the pH, which was desired for this study to control the drug release over extended period of time. At pH 5.5, NCD-Dispersion, NCD-LPX and NCD-LPX-HPMC gel respectively exhibited 76%, 35% and 19% NCD release in 2 h, followed by 100%, 47% and 25% NCD released in 4 h, indicating a quick release from dispersion and LPX, when compared with LPX-HMPC gel Fig. 4A. NCD-LPX and NCD-LPX-HPMC gel respectively showed 82% and 64% NCD release in 24 h. At pH 7.4, almost all NCD (100%) was released from NCD-Dispersion in 2 h, whereas around 51% and 22% of NCD was released from NCD-LPX and NCD-LPX-HPMC gel in 2 h. Similarly, in 24, 98% NCD was released from NCD-LPX and 85% from NCD-LPX-HPMC gel, more sustained released of NCD from NCD-LPX-HPMC gel as shown in Fig. 4B. The results of the study revealed that all the NCD formulations had a pH dependent drug release. The NCD-Dispersion showed a burst release of NCD, whereas NCD-LPX and NCD-LPX-HPMC gel showed sustained release profiles, even if the former showed more control over the drug release.

3.10.1. Release kinetics

To identify the release pattern of NCD from NCD-Dispersion, NCD-LPX and NCD-LPX-HPMC gel different kinetic models including first order, zero order, Korsmeyer-Peppas, Hixon-Crowell, and Higuchi by using DD-solver were applied. Based on the highest R² value Korsmeyer-Peppas was chosen to be the best fit release kinetic model (Table 3), with R² values 0.9915 and 0.9934 at pH 5.5 and 7.4, respectively. Moreover, the diffusion exponent (n) was found 0.498 at pH 5.5. and 0.577 at pH 7.4, indicating non Fickian diffusion of NCD from NCD-LPX-HPMC gel.

Table 3
R² values of kinetic release models for NCD release at pH 7.4 and 5.5.

Model	Model R ² value					
	pH 7.4			pH 5.5		
	NCD-Dispersion	NCD-LPX	LPX-NCD-HPMC gel	NCD-Dispersion	NCD-LPX	LPX-NCD-HPMC gel
Zero order	0.7550	0.7820	0.7966	0.4573	0.1865	0.7363
First order	0.9843	0.9273	0.9757	0.9475	0.7964	0.8761
Higuchi	0.9989	0.8206	0.9782	0.9638	0.8878	0.9875
Hixson-Crowell	0.9776	0.8131	0.9520	0.9086	0.7214	0.8390
Korsmeyer-Peppas	0.9991	0.9743	0.9934	0.9998	0.9857	0.9915
	n = 0.361	n = 0.309	n = 0.577	n = 0.377	n = 0.338	n = 0.498
Note: NCD-LPX-HPMC gel: Niclosamide loaded Leciplex Hydroxypropyl methyl cellulose gel, NCD-LPX: Niclosamide loaded Leciplex, NCD-Dispersion: Niclosamide Dispersion. Each value represents the mean ± Standard deviation n = 3						

3.11. Ex vivo permeation

Ex vivo permeation of NCD from NCD-Dispersion, NCD-LPX and NCD-LPX-HPMC gel was conducted at pH 7.4. As observed (Fig. 4C), a significantly enhanced NCD was permeated from NCD-LPX, followed by NCD-LPX-HPMC gel when compared with NCD dispersion. More specifically, 139 µg/cm² and 64 µg/cm² cumulative amount of NCD was permeated from NCD-LPX, and NCD-LPX-HPMC gel as compared to NCD dispersion which only permeate 26 µg/cm² in 24 h analysis.

3.12. Skin penetration by fluorescence microscopy

The skin penetration of Dye-LPX-HPMC gel was visualized under fluorescence microscope. The results showed that Rhodamine B loaded LPX -HPMC gel was deposited more in the dermal layer and resulted in high fluorescence marking in the dermis layer when compared with control group (Fig. 5A&B). These results indicated that NCD-LPX-HPMC gel was able to deliver high amount of NCD deep in the mice skin.

3.13. Deposition studies

Drug deposition studies were performed to check the deposition of drug in different layers of skin. The results showed that more NCD was deposited in the dermal layer then permeated through the skin as depicted in Fig. 5C. More specifically, the drug retained 35.17 ± 4.4% and 29.98 ± 4.17% on the dermis layer from NCD-LPX-HPMC gel and NCD-LPX respectively, whereas only 4.12% ± 0.62 was retained from NCD-Dispersion. Additionally, the permeated amount was less as compared to the drug deposition in the skin layers, thereby indicating the ability of the LPX system to retain drug at the intended site.

3.14. Antimicrobial assay

Microbroth dilution method was used to determine the antimicrobial efficacy of NCD-LPX-HPMC gel, NCD-LPX and NCD-Dispersion. The results showed that NCD-LPX-HPMC gel significantly reduced the microbial burden at the tested concentrations, however, maximum antimicrobial activity was observed at 100 µg/mL against both the *S. aureus* and MRSA as reported in Fig. 5D. When compared with NCD-LPX and NCD-Dispersion, the NCD-LPX-HPMC gel showed improved antimicrobial activity even a lower concentration against *S. aureus* and MRSA (Fig. 5E), **thereby indicating the ability of the prepared system to inhibit microbial growth when used in smaller quantity**. Similar results were observed in terms of the IC₅₀ values, which indicated that NCD-LPX-HPMC gel IC₅₀ values were the lowest against *S. aureus* (0.9639 ± 0.38) and MRSA (1.388 ± 0.40 µg/mL) as depicted in Table 4.

Table 4
Comparative IC₅₀ values of tested formulations against *S. aureus* and MRSA.

Formulation	IC ₅₀ Value (µg/mL)	
	<i>S. aureus</i>	MRSA
NCD-Dispersion	8.072 ± 0.89	14.28 ± 2.10
NCD-LPX	1.562 ± 0.59	4.849 ± 2.00
NCD-LPX-HPMC gel	0.9639 ± 0.38	1.388 ± 0.40
Note: NCD-LPX-HPMC gel: Niclosamide loaded Leciplex Hydroxypropyl methyl cellulose gel, NCD-LPX: Niclosamide loaded Leciplex, NCD-Dispersion: Niclosamide Dispersion, <i>S. aureus</i> : <i>Staphylococcus aureus</i> , MRSA: Methicillin-resistant <i>Staphylococcus aureus</i> . Each value represents the mean ± Standard deviation n = 3		

3.15. Wound contraction

Wound size contraction analysis results of NCD-LPX-HPMC gel are demonstrated in Fig. 6A, post induction of cellulitis and compared with NCD-Dispersion gel and untreated control group. The 4th day of animal studies was considered as day 0 of the treatment. A significantly enhanced wound size (8.53 ± 0.18 cm²) was observed in untreated group at day 5, whereas a lower wound size was reduced in NCD- dispersion treated group (2.49 ± 0.10 cm²). However, a significantly abridged wound size was observed in NCD-LPX-HPMC treated group (1.65 ± 0.02 cm²). Similarly on day 7, the wound sizes were respectively observed to be 9.20 ± 0.33 cm², 3.53 ± 0.12 cm² and 0.61 ± 0.02 cm², for untreated, NCD-Dispersion gel and NCD-LPX-HPMC gel treated groups. On day 10, a significantly reduced and near to wound closure size (0.53 ± 0.001 cm²) was observed in NCD-LPX-HPMC treated group, thereby indicating the treatment efficacy of the prepared formulation. On the other hand, the wound size was enlarged in NCD-Dispersion gel treated group (7.4 ± 0.22 cm²) and further enlarged in untreated group (10.1 ± 0.16 cm²), indicating the inefficiency of the NCD-dispersion group in the treatment of cellulitis.

3.16. Histopathological examination of skin tissue

H&E staining reports of the test samples are reported in Fig. 6B. As can be seen, skin sections in normal mice group revealed normal skin structure with intact epidermis and dermis and clearly visible skin appendages like hair follicles, sebaceous glands, dermal papillae, excretory ducts and secretory cells (Fig. 6BI). In contrast, the positive control group had obvious pathological alterations typical of cellulitis, including disruption of the skin architecture, vascular prominence, neutrophilic infiltration, and broadened interstitial spaces with some dermal edema as shown in (Fig. 6BII). Histological improvement was seen after the treatment with NCD-LPX-HPMC gel which included restoration of epidermal and dermal organization, reduction in inflammatory cell infiltration and decrease in edema (Fig. 6BIII). On the other hand, the NCD-Dispersion gel treated group although showed improved histopathology compared to the positive control group, yet the results were no convincing as reported with NCD-LPX-HPMC gel group, even if the inflammation was reduced and there was partial restoration of normal tissue structure (Fig. 6BIV). Overall, the NCD-LPX-HPMC gel had more protective and healing effects on skin damage caused by cellulitis; the group had a better histological recovery.

4. Discussion

Cellulitis, an acute inflammation or infection of dermis or subcutaneous tissue usually appears with symptoms of redness, pain, tenderness and elevated skin temperature of the respected area. It is generally caused by *Staphylococcus*, *streptococci* and Community acquired MRSA [45–47]. Traditional treatment of cellulitis involves antibiotic therapy, which is done orally in mild and moderate forms and intravenously in severe and complicated cases [5]. Although the use of antibiotics continues to be the main pillar of managing cellulitis, excessive use and wrong prescriptions of these drugs have also led to the emergence of antimicrobial resistance. Unjustified use of antibiotics causes development of resistance in organisms [48]. To overcome this issue NCD was an alternative new approach for the treatment of cellulitis as it is repurposed against Gram positive bacterial infection [10]. NCD acts through mechanism of inhibiting ATP production and causes cell death [49]. However, its low solubility and high permeability and low oral bioavailability (5.5%-10%) limits its therapeutic potential [12]. Many strategies can be employed to enhance the therapeutic efficacy of NCD for cellulitis. One of the best approaches is to make nanocarrier to facilitate delivery of NCD. LPX is a cationic surfactant and phospholipid based nanoparticle, these lipid based nanocarriers facilitate the delivery of hydrophobic as well as hydrophilic drugs [15]. LPX enhance the penetration of NCD to deep dermis layer [50]. Due to the low viscosity of NCD-LPX, it was suggested to incorporate it into HPMC gel to retain on skin application site. HPMC gel is selected as having good drug release and do not clog the skin pores and have cooling effect [22].

Thin film hydration method was used to fabricate NCD-LPX and after preoptimization studies, it was statistically optimized by using BBD. PS in an important parameter for topical drug delivery as it needs to penetrate up to dermal skin layer and give localize effects in the dermal layer to treat cellulitis. Increase in PL 90G concentration increases the PS, this may be due to an increased bilayer formation which eventually results to the development of multilamellar vesicles takes place [37, 51]. Moreover, it could be due increased viscosity of LPX, thereby forming larger vesicles [52, 53]. In the case of the cationic surfactant (CTAB) molar ratio the smaller vesicles were usually obtained at higher concentrations of the cationic surfactant, because the lipid was solubilized by the surfactant. However, the nature of the surfactant had a remarkable impact on the size of the particles of the liposomes prepared. Specifically, larger vesicles were generated in CTAB, suggesting that even though the solubilization of vesicles might be caused by the increase in cationic surfactant content [54]. Moreover, the combination of the bulky hydrophobic tail and the strong cationic head group that make up the entire molecule may increase the PS [55, 56]. ZP of the nano system indicate their stability. More specifically, a system with ZP value more than ± 30 mV are considered stable [57]. This surface charge of the vesicles plays a significant role in preventing the aggregation due to repulsion between the charged particles [58]. PL90G, CTAB, and NCD significantly influence ZP because each alters the surface charge environment of LPX. PL90G, a zwitterionic phospholipid, provides a baseline charge

through its polar head groups[59]. CTAB, a cationic surfactant, strongly increases positive surface charge, enhancing electrostatic repulsion and colloidal stability. Niclosamide, a weakly acidic drug, contributes negative charges at physiological pH, counterbalancing CTAB's effect. Overall positive ZP was due to CTAB. Similarly, %EE is another important feature of the nano system as it directly influences the bioavailability of drugs [60, 61]. A significantly higher %EE was observed with higher PL 90G. This could be explained by the structural integrity of LPX that was increased with a high PL 90G concentration, thereby increasing the %EE [16, 53]. Also, a higher value of lipid concentration increases the solubilization of NCD in the lipid phase, leading to enhanced %EE of NCD [61, 62]. Morphological analysis via TEM showed nanoparticles were within the range of required size i.e. $\approx$ 200 nm and validated the DLS results.

FTIR analysis indicated the presence of all functional groups of corresponding individual components including NCD, PL 90G, mannitol and in the physical mixture. Moreover, the respective characteristic peak were found in NCD-LPX, with slight shifts and decreased intensities or broadening, indicating successful incorporation of NCD in LPX. However, no covalent bonding or disappearance of the characteristic peaks of the principal species was observed, suggesting that the preparation of the formulation did not involve covalent bonding or chemical degradation.

NCD crystalline peaks at its characteristic angle as reported in the literature [26] were observed. Also, crystalline nature of CTAB was also found. However, conversion of crystalline nature of NCD into amorphous in the NCD-LPX was depicted in the XRD spectra, which could be attributed to the optimum formulation strategy and the formation of nanosized LPX. The conversion of crystalline NCD into amorphous will enhance the drug solubility, dissolution and bioavailability [63].

DSC thermogram showed the distinct melting points of each excipient according to the given literature. NCD melting point 230.12°C was in accordance with the literature reported [64]. Slight shift of peak was observed in physical mixture, the reason for which could be that PL 90G melted before giving lipid environment to the drug and hence which makes the NCD to solubilize [40]. However, no endothermic peaks were observed in NCD-LPX, thereby indicating the thermal stability of the formulation and conversion of crystalline drug into amorphous.

NCD-LPX was further incorporated into HPMC gel in order to retain them at the site application for topical delivery. Physiochemical characterization of the developed formulation were investigated including uniformity, color and pH. Gels with more alkaline and acidic pH respectively cause scaly skin, and skin irritation [65]. The pH of the formulation was within range 4.2–6.5 that is believed to be suitable for topical application. Similarly, NCD-LPX-HPMC gel spreadability depicted that gel covers maximum area of skin and give maximum therapeutic effect, which means that all the prepared gels had distended as a result of a slight amount of shear [66]. Additionally, rheological examination of the NCD-LPX-HPMC gel showed its optimum viscosity, and suitable flow properties required to obtain therapeutic efficacy of the loaded drug [67, 68].

Diffusion rate of the drug out of the nano carrier system is of paramount importance since it indicates the concentration of drug at the site of action and duration of action. [69]. The pH of the release medium affected the release of the drug. Increase in the release of NCD was noted at pH 7.4 as compared to pH 5.5, which can be attributed to pH-dependent ionization of the drug. NCD is a weakly acidic compound having a pKa of $\sim$ 6.5. The pKa is lower than the pH of the media, which is 7.4, and under the pH conditions the maximum concentration of NCD was in the ionized form, leading to increased aqueous solubility, and decreased lipid affinity. Drug was easily released from the vesicular system into the release medium and hence the drug release is enhanced [70]. Delayed release of NCD from NCD-LPX can be due to encapsulation of drug into the liposome bilayer structure, which restricts diffusion of the drug into the external medium [16, 71]. Also the LPX has smaller PS and increased surface area which is why high distance drug travel to release from matrix that was in accordance with the literature reported [15, 27]. After incorporating NCD-LPX into HPMC gel further retard its diffusion. When hydrated, HPMC swells up and become a hydrated three-dimensional network of polymer which envelops the formulation. This thickened gel layer provides a diffusional barrier and increases the path tortuosity and decreases the mobility of the drug in this region, resulting in a prolonged drug release. The drug release from HPMC matrices is controlled mostly by diffusion through the hydrated gel layer, plus progressive relaxation and erosion of the polymer [22].

Their permeation in the skin depends in a large measure is influenced by such factors as the elasticity of the membrane, its hydrophilicity. and the stability of the vesicle structure [72]. A significantly enhanced cumulative permeation of NCD from NCD-LPX and NCD-LPX-HPMC gel could be due to the structure of LPX which includes phospholipid and CTAB (cationic surfactant) which increases interaction with the negative charge of the skin, thereby increasing the partitioning of the drug into the stratum corneum, and facilitating transport of the drug across the skin [50]. Additionally, the small particle size provides a large surface area of contact with the skin, increasing the adhesion and penetration of the drug through the intercellular lipid pathway. Other LPX formulations have demonstrated an improved absorption of drugs across the biologic membrane [16].

Antimicrobial assay indicated a significantly improved efficacy of NCD-LPX-HPMC gel against S.A and MRSA, via maximum inhibition at all the tested concentrations. Moreover, the prepared formulation was effective even at a low concentration of 1.6 μ g/ml. This could be possibly due to sustained drug release from NCD-LPX-HPMC gel which allows NCD to act more efficiently and inhibits bacteria, this could be due to high permeation and deposition of drug [16]. High fluorescence imaging and increased dermal deposition of NCD-LPX-HPMC gel depicted that maximum drug was penetrated into the dermal layer, and the possible reason could be explained by the combined effect of LPX nanoparticles and HPMC gel. HPMC increases the viscosity and skin adhesion, which results in longer contact of NCD and allows them to be released slowly [73], whereas LPX penetrate deeper into the dermis [16]. Conversely, NCD-Dispersion had low viscosity and solubility, which led to low levels of dermal retention [74].

The therapeutic efficacy of NCD-LPX-HPMC gel in the *S. aureus*-induced cellulitis model can be attributed to the synergistic roles of its components. Niclosamide, a broad-spectrum antimicrobial agent, demonstrated potent activity against *S. aureus*, while liposomal encapsulation enhanced drug entrapment, stability, and penetration into infected dermal tissue[50]. The positive or balanced zeta potential imparted by CTAB and PL90G facilitated stronger electrostatic interactions with bacterial membranes, thereby improving drug delivery at the infection site. Incorporation into the HPMC gel matrix provided viscosity, spreadability, and sustained release, ensuring prolonged contact with the wound surface and reducing dosing frequency. *In vivo*, treated mice exhibited faster resolution of erythema, edema, and purulent discharge compared to controls, with histopathology confirming reduced neutrophilic infiltration, improved collagen deposition, and accelerated re-epithelialization. The formulation also minimized systemic exposure, lowering the risk of toxicity

while maintaining local bioavailability. Collectively, these findings highlight the translational potential of NCD-LPX-HPMC gel as a patient-friendly, topical nanogel therapy for bacterial skin infections, offering improved efficacy, safety, and compliance compared to conventional treatments.

Conclusion

The NCD-LPX-HPMC gel was designed to deliver niclosamide (NCD) effectively into deeper layers of the skin and to provide meaningful relief in cellulitis. The optimized liposomal system (LPX) showed favorable particle size, zeta potential, and high entrapment efficiency, with nanoscale dimensions confirmed by TEM. Compatibility of excipients was validated by FTIR, while XRD and DSC analyses demonstrated successful transformation of NCD into an amorphous encapsulated form. Incorporation into the HPMC gel produced a formulation with uniform consistency, skin-friendly pH, smooth spreadability, and desirable rheological properties for topical use. *In vitro* studies revealed sustained release profile of the NCD-LPX-HPMC gel, while *ex vivo* permeation confirmed its deeper dermal penetration and retention. *In vivo* and histopathological evaluations further highlighted the gel's safety and therapeutic potency, showing significant inhibition of *S.A*-induced cellulitis. Taken together, these findings suggest that NCD-LPX-HPMC gel offers a promising, patient-friendly approach for topical niclosamide delivery, combining efficacy, safety, and sustained action to address bacterial skin infections.

Declarations

CRedit authorship contribution statement

A.N and A.B: Writing – original draft, Investigation, Methodology, Data curation.

F.Z and A.A: Formal analysis, Software, Resources.

A.A.F and A.A: Validation; Software, Visualization,

A.A.L: Writing and Review, Formal analysis.

F.U.D: Supervision, Resources, Project Conceptualization, Funding acquisition.

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Data availability:

Data will be made available on request.

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Figures

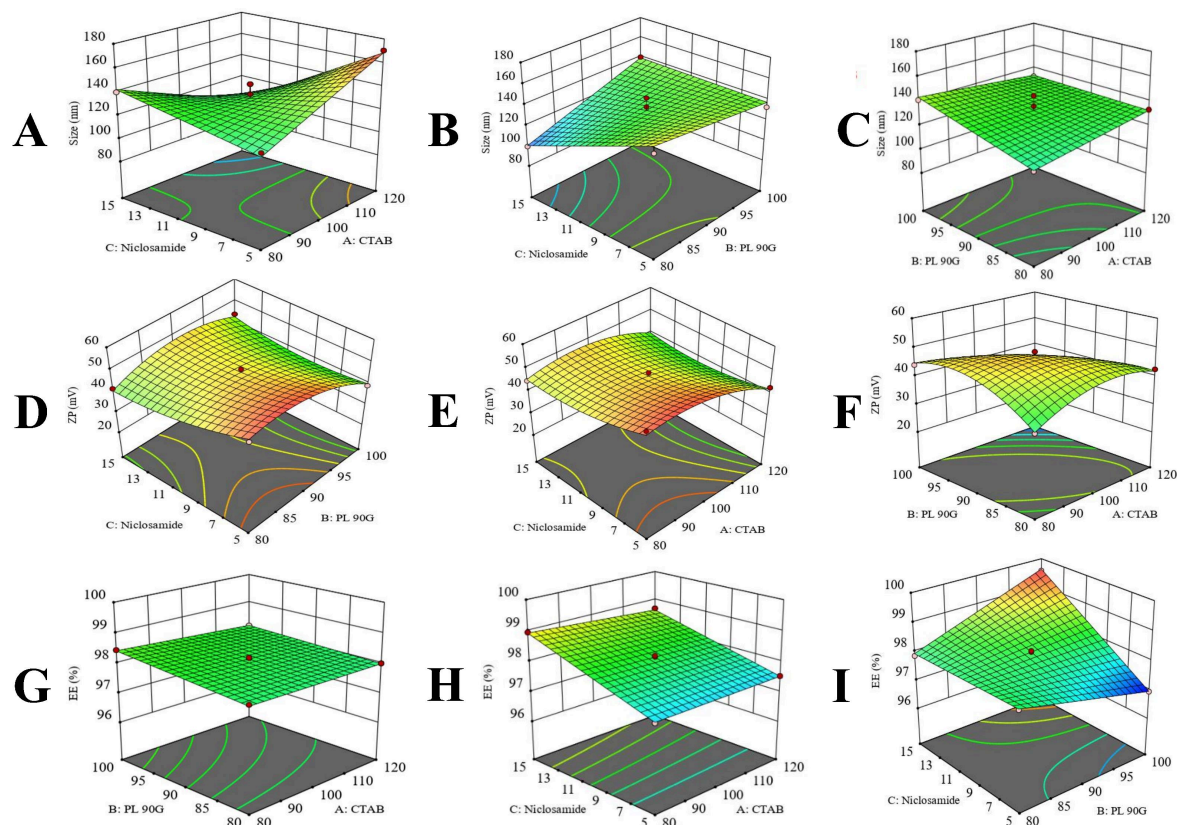


Fig.1

Figure 1

3D Graphs depicting effect of independent variables on PS (A-C), ZP (D-F), %EE (G-I). Note: Effect of NCD and CTAB (A), NCD and PL 90G (B), and PL 90G and CTAB (C) on PS. Effect of NCD and PL 90G (D), NCD and CTAB (E) and PL 90G and CTAB (F) on ZP. Effect of PL 90G and CTAB (G), NCD and CTAB (H), and PL 90G and NCD (I) on %EE. Abbreviations. Particle size, ZP: Zeta potential, EE: Entrapment efficiency, CTAB: Hexadecyltrimethylammonium bromide, PL 90G: Phospholipon® 90G, NCD: Niclosamide.

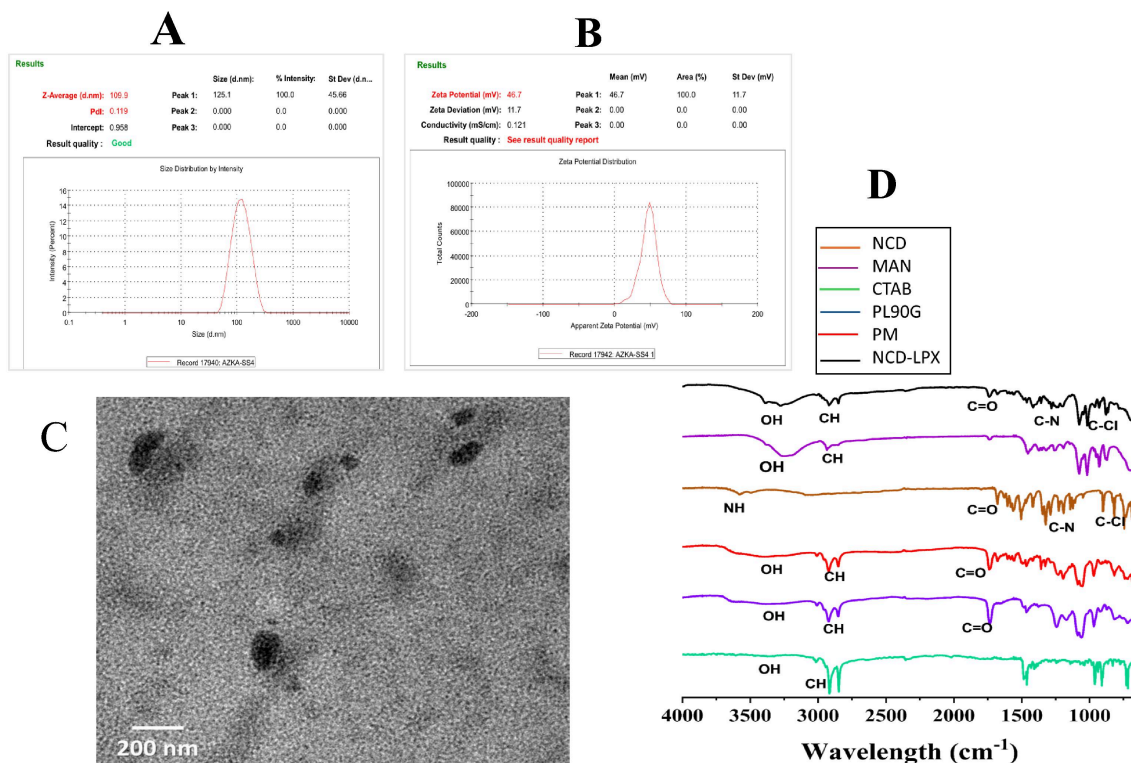


Fig.2

Figure 2

DLS characterization of NCD-LPX, (A) Particle size; (B) Zeta potential, and TEM analysis. *Note: NCD-LPX: Niclosamide loaded leciplex.* (C) Fourier transform infrared (FTIR) spectroscopy of NCD-LPX and its components. *Note: NCD-LPX: Niclosamide loaded leciplex, MAN: Mannitol, CTAB, PL90G, PM: Physical mixture and NCD: Niclosamide*

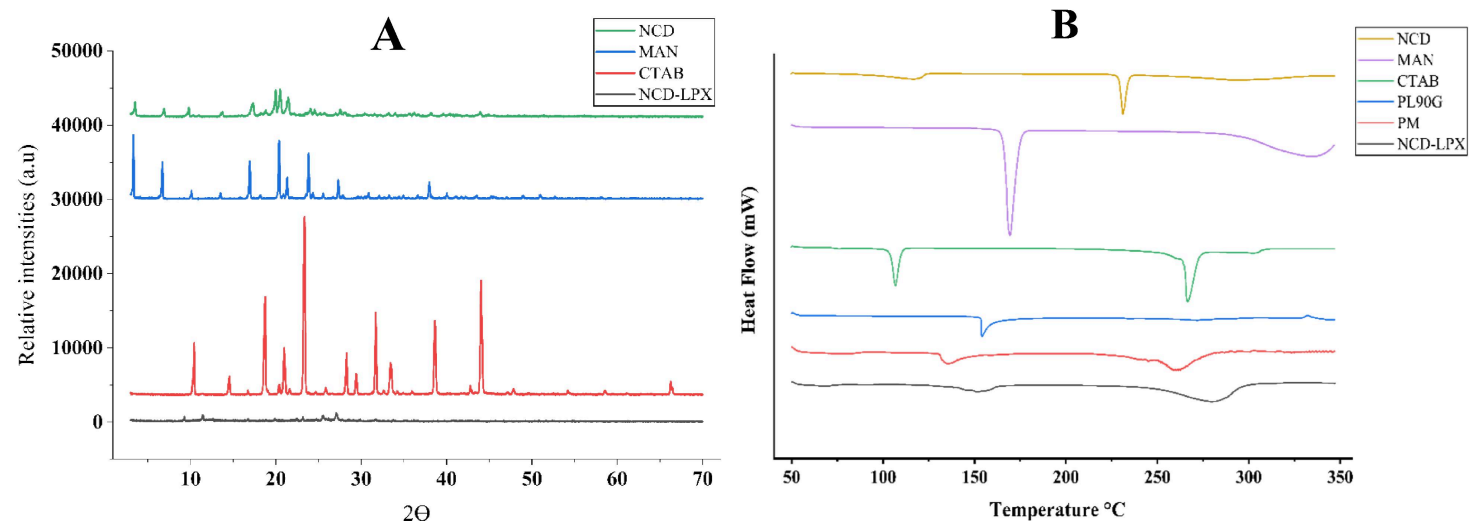


Fig.3

Figure 3

(A) Powder X-ray Diffraction (PXRD) of NCD-LPX and its components. *Note: NCD-LPX: Niclosamide loaded leciplex, MAN: Mannitol, CTAB, and NCD: Niclosamide;* (B), DSC analysis of NCD-LPX and its components. *Note: NCD-LPX: Niclosamide loaded leciplex, MAN: Mannitol, CTAB, PL90G, PM: Physical mixture and NCD: Niclosamide.*

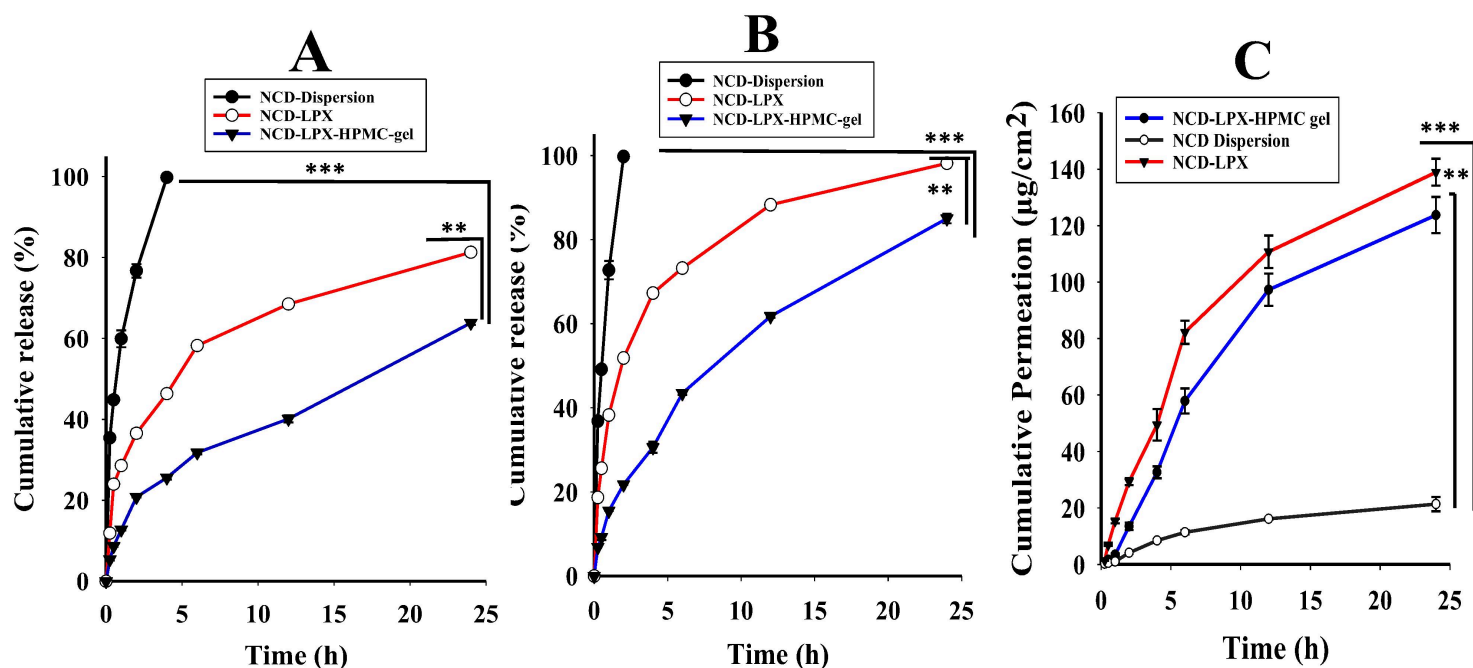


Fig.4

Figure 4

Cumulative release in vitro release at pH 5.5 ((A)), and pH 7.4(B). Cumulative permeation (C) of NCD from NCD-LPX-HPMC gel, NCD-Dispersion and NCD-LPX. Note: Statistical significance measured by Two-way Anova followed by Tukey's test, between NCD-LPX-HPMC and NCD-Dispersion and NCD-LPX and NCD-Dispersion. Where $p < 0.01 = **$ and $p < 0.001 = ***$. NCD-LPX-HPMC gel: Niclosamide loaded leciplex in Hydroxypropyl methylcellulose gel, NCD-dispersion: Niclosamide dispersion, NCD-LPX: Niclosamide loaded leciplex, n = Diffusion exponent, R^2 : Co-efficient of determination. Each value represents the mean $\pm$ Standard deviation ($n=3$).

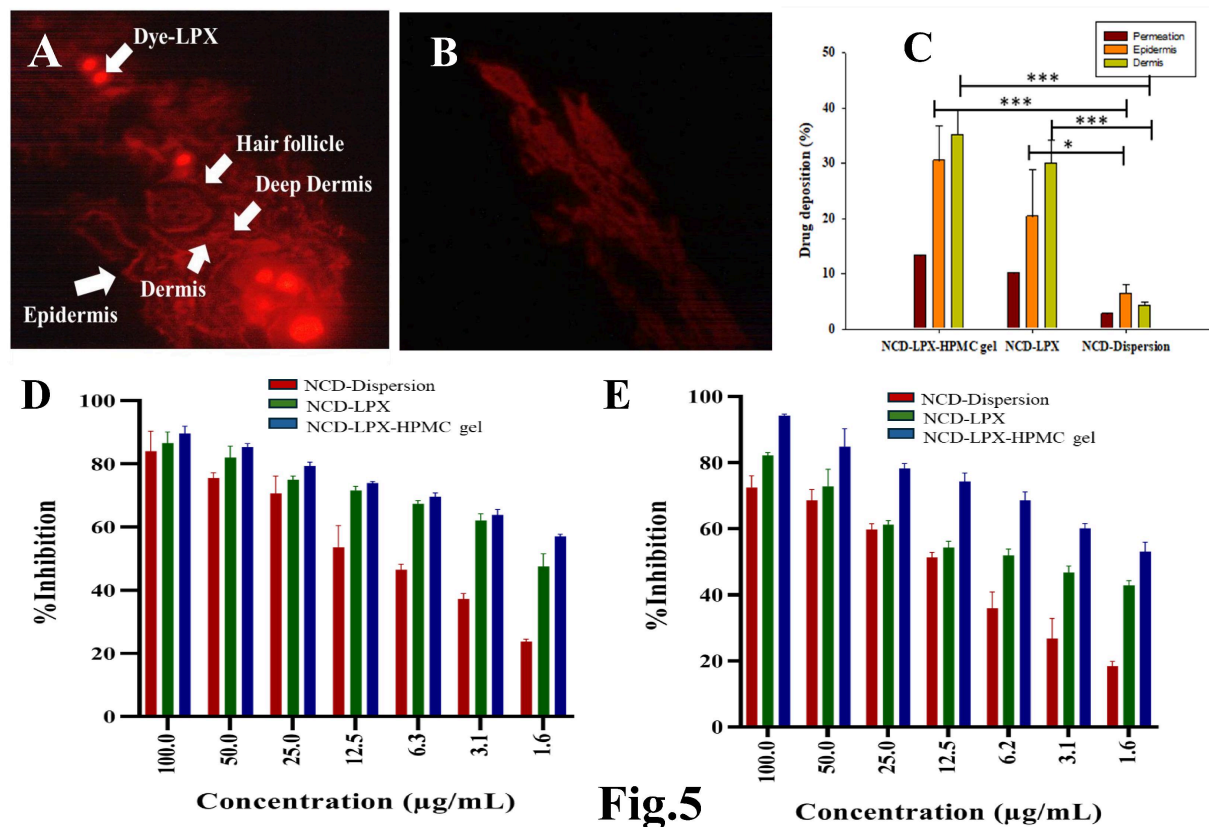


Fig.5

Figure 5

Fluorescence imaging of Rhodamine-loaded LPX (A) and Blank skin (B). Deposition of NCD from NCD-LPX-HPMC gel, NCD-Dispersion and NCD-LPX in epidermis and dermis layers of the skin (C). Note: Rhodamine-loaded LPX: Rhodamine-loaded lecplex. Statistical significance measured by Two-way Anova followed by Tukey's test, between NCD-LPX-HPMC and NCD-Dispersion and NCD-LPX and NCD-Dispersion * where $p < 0.05 = *$; $p < 0.01 = **$ and $p < 0.001 = ***$. NCD-LPX-HPMC gel: Niclosamide loaded lecplex hydroxypropyl methyl cellulose gel, NCD-LPX: Niclosamide loaded lecplex, NCD-Dispersion: Niclosamide Dispersion. Analysis was performed in triplicate ($n=3$). Minimum inhibitory concentration (MIC) of tested formulations against *S. aureus* (D), and (E) MRSA.

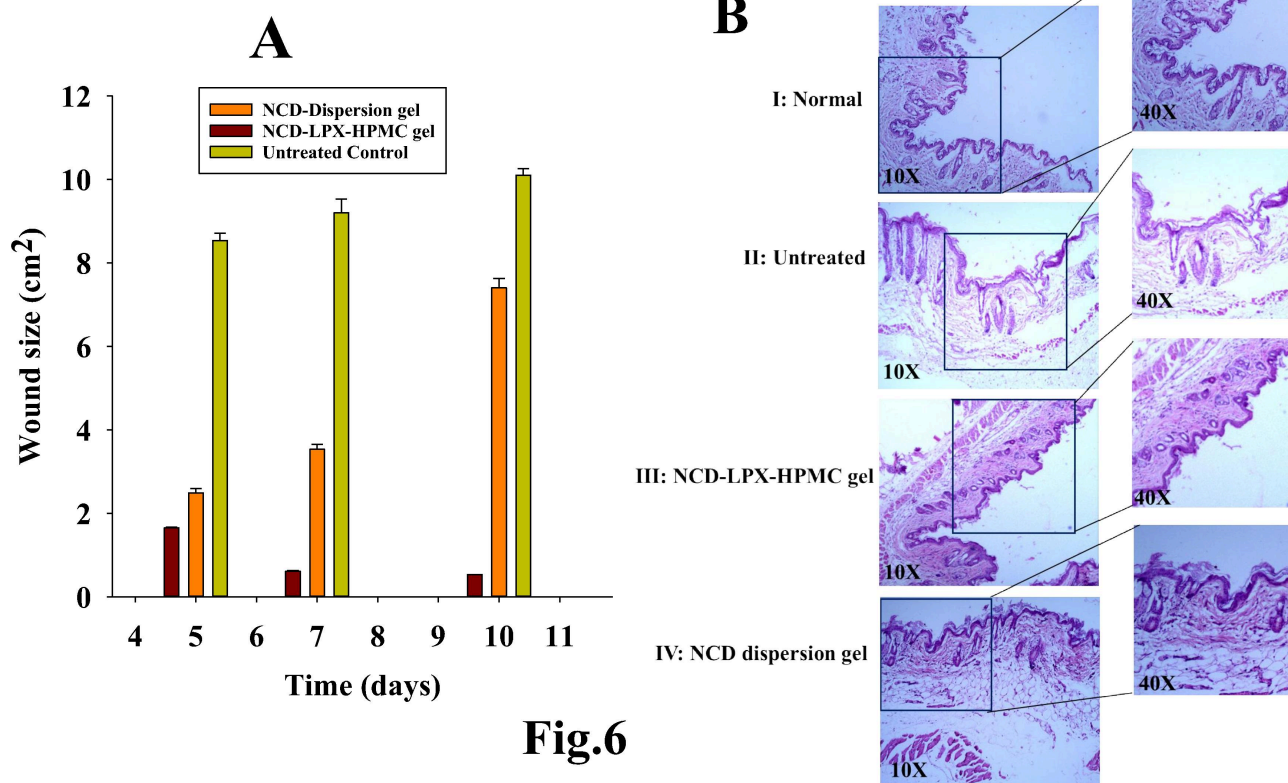


Fig.6

Figure 6

Wound healing effect at day 5,7 and 10 (A), and H&E examination of skin tissue of mice (B) at day 10. (I). Normal group; (II). Untreated group; (III) NCD-LPX-HPMC gel; and (IV) NCD-Dispersion gel in mice model. Note: *NCD-LPX-HPMC gel*: Niclosamide loaded lecithin hydroxypropyl methyl cellulose gel, *NCD-Dispersion*: Niclosamide Dispersion. Each value represents the mean $\pm$ Standard deviation $n=3$

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