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# Research Article
# Development of Topical Antibacterial Gel Loaded with Cefadroxil Solid Lipid Nanoparticles: *In vivo* Wound Healing Activity and Epithelialization Study

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## Abstract

**Background and Objective:** Wound could be caused due to physical trauma or surgical incisions. The incidence of infection may extend the wound healing process. Thus, present study was aimed for the sustained drug release and site specificity in dermal region to eradicate the bacterial infection, thereby accelerate the wound healing process. Wound healing process was performed by the eradicating cutaneous infection with Cefadroxil loaded Solid Lipid Nanoparticles (SLN) based topical gels. **Materials and Methods:** Solid lipid nanoparticles of Cefadroxil (CEF) were prepared by solvent evaporation technique. Based on the particle and physicochemical characterizations one formulation was optimized. Selected formulation 1% w/w SLN4 was loaded in 1% w/v carbopol to develop topical antibacterial gel, which was evaluated for pH, spreadability, drug release and *in vivo* wound healing activity in male rats. **Results:** From the prepared five nanocarriers, SLN4 was selected that showed nanorange size, stable, monodispersed particles, with the optimum entrapment efficiency and drug loading. Fourier-transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD) presented that CEF was encapsulated and available in the non-crystalline form in SLN. Polymeric gel loaded with CEF-SLN4 has desired pH and spreadability with 86.76±0.12% of drug released in 8 h. Whereas *in vivo* wound healing activity was assessed as wound contraction and noted to be 99.73±0.93% within 3 weeks alongside complete epithelialization was achieved within 17 days by the application of CEF-SLN4 gel. **Conclusion:** Prepared CEF-SLN4 gel was stable, skin-compatible and spreadable with sustained drug release. Applied gel efficiently permeable deeper into skin layers, eradicate the bacterial growth and could be effective in treating the infectious wound in short-span of time. Therefore, CEF-SLN4 topical gel could be efficiently used for the faster wound healing treatment.

Key words: Cefadroxil, solid lipid nanoparticles, gel, topical, wound healing, bactericidal, bacterial infections

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Cefadroxil (CEF) is a semisynthetic first-generation cephalosporin antibiotic effective against both Gram-positive and negative organisms namely, Bacteroides fragilis, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Streptococcus pneumonia* and other species[1]. It acts by inhibiting the cell wall synthesis of microbe called as a bactericidal antibiotic. As per United States Food and Drug Administration (FDA or USFDA) Cefadroxil indicated for cutaneous infection caused by staphylococci and other systemic bacterial infections of urinary and respiratory tract infections[1]. The CEF should be cautiously used with the patients having hypersensitivity with penicillin due to its structural similarity. Physicochemical properties of CEF include, whitish-yellow crystalline powder, soluble in water and stable in acidic condition. The CEF is a slightly water-soluble drug with 28% protein binding and 1.5 h half-life[2]. Although it's well absorbed on oral administration but may cause nausea, antibiotic-induced diarrhea, vomiting and allergic rashes over the skin. The CEF reported to showed higher systemic half-life with prolonged efficacy compared to other drug moieties from the same category[3]. There are no pharmacokinetics modifications if CEF administered with food, many clinical studies postulate CEF is relatively more effective compared with cephradine or cephalexin, one dose could be equivalent to the 4 doses of other antibiotics in the treatment management of respiratory, urinary and skin infections[4]. The combined therapy of CEF oral and topical administration synergize the bactericidal effect in the skin region. Topical formulation of CEF is more effective to treat cutaneous staphylococcal infections[5]. Formulation scientists also explored the CEF for the accelerated wound healing treatment by inhibiting the possible accompanied bacterial infections[6]. Chitosan-based CEF nanoparticles showed enhanced wound healing process and maximum bacterial clearance by the topical application of the prepared CEF-gel[7]. After injury onset tissue lesion occurs, the regeneration and repair of tissue consist of proliferative extracellular matrix remodeling sequential events with allocation of blood and parenchymal cells[8]. Healing of wound takes place by contraction of myofibroblasts and reepithelialization[9]. The ancient era described the use of green paint containing copper over the wound by Egyptians since copper possesses bactericidal activity[10]. Wrapping of mummies also influences the wound bandages. The discovery of antiseptics in the 19th century helps to decrease the mortality rate by decreasing the infection[11]. By the end of the 20th century, there are about 5000 products are available or healing wounds[10]. Currently, nanocarriers are extensively explored for the dermal and cosmetics application due to its enhanced solubility, stability and site-specificity. Solid lipid nanoparticles attracted the attention of formulation technologists due to its scale-up production, less-toxicity, higher drug loading and controlled release features[12]. The SLN also exhibits enhanced drug penetration, cutaneous occlusions and skin hydrations[13]. Due to lipid usage in SLN it's physiologically accepted and known as nanosafe carrier[14].

The objective of the current investigation was to prepare CEF loaded SLN with stearic acid-lipid using pluronic F-127 as a stabilizer. Based on the particle and physicochemical characterization of one formula of SLN was optimized. The selected optimized CEF-SLN was further developed in topical CEF-SLN carbopol polymer-gel. Thereafter, further efficacy was evaluated by *in vivo* wound healing activity and epithelialization study in male rats.

## MATERIALS AND METHODS

**Materials:** Development and characterization part was done during August-November, 2019, whereas, *in vivo* wound healing and epithelialization study were performed in the period of December, 2019 to January, 2020 at the College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-kharj, Saudi Arabia.

Cefadroxil was obtained as a gift sample from Jazeera Pharmaceutical industries Riyadh, Saudi Arabia. Stearic acid, pluronic F-127 and dichloromethane were purchased from Sigma Aldrich, USA. Carbopol, propylene glycol and propylparaben, triethanolamine were procured Loba Chemie Pvt. Ltd., India. Milli-Q water used in the preparation and analysis was processed from the Milli-Q® Direct 8 water purification system. All other chemicals used were of analytical grades.

**Preparation of cefadroxil loaded solid lipid nanoparticles:** Solid lipid nanoparticles were prepared by solvent emulsion evaporation technology by using a probe sonicator (Fisherbrand™ Q500 Sonicator with Probe-Fisher Scientific, New Hampshire, United States). Five formulations of SLN were prepared by dissolving 100 mg of Cefadroxil (CEF) in 3 mL dichloromethane and the specified amount of stearic acid as per Table 1 was melted at 70°C in 3 mL ethanol, both of this solution mixed and kept on sonication for 1 min for complete dissolution of this organic phase. The aqueous phase was prepared by solubilizing 250 mg of pluronic F-127 (surfactant) in 100 mL of Milli-Q water and cooled at 5°C in the refrigerator

Table 1 Composition of Cefadroxil loaded solid lipid nanoparticles

| Formulation code | Cefadroxil (mg) | Stearic acid (mg) | Pluronic F-127 (mg) |
|---|---|---|---|
| SLN1 | 100 | 250 | 250 |
| SLN2 | 100 | 300 | 250 |
| SLN3 | 100 | 350 | 250 |
| SLN4 | 100 | 400 | 250 |
| SLN5 | 100 | 450 | 250 |

overnight followed by homogenized (IKA t50 ultra-turrax heavy duty homogenizer, Germany)[15] at 1500 rpm for 15 min. Both organic and aqueous solvents emulsification was done in probe sonication (60 watts for 5 min) by adding organic phase dropwise in the aqueous phase. The dispersion medium was then stirred on a thermostatically controlled magnetic stirrer (WiseStir MSH-A, Wisd Laboratory Equipment, model: MSH-20D, Lindau Switzerland) at 1000 rpm at 50°C for 24 h to evaporate the organic solvents completely. Pre-washed (3-4 times with Milli-Q water) concentrated solid-lipid drug dispersion was lyophilized (Manifold freeze dryer, Millrock Technology, NY, United States) to get the dried SLN of Cefadroxil, which was preserved in moisture-resistant container (Desiccator) until further evaluations[16].

**Particle size, PDI and Zeta potential measurement CEF loaded SLNs:** Mean particle size and frequency were measured based on the Dynamic Light Scattering (DLS) principle involving the Brownian motion theory using zetasizer (Zetasizer Nano ZS90, Malvern Panalytical Ltd., United Kingdom)[17]. The sample under study was dispersed in demineralized water with (1:200) dilution factor, vortexed (IKA vortex 3 vortex mixer, Germany) for 5 min. The sample then filled in the specific sample cell as per the procedure (12 mm Glass cell (PCS1115)), Disposable folded capillary cell (DTS1070) for size and zeta-potential respectively. Measurement was carried out in triplicate for each sample at constant temperature 25°C and fixed laser beam angle of 90°C.

**Entrapment efficiency and drug loading calculation:** Entrapment efficiency and drug loading were analyzed by indirect method using UV-Spectroscopy (UV-VIS Spectrophotometer, V-630, Jasco, Pfungstadt, Germany) at λmax-224 nm. All formulae CEF-SLN dispersion before lyophilization was analyzed for Entrapment Efficiency (EE (%)) and Drug Loading (DL (%)) by cooled centrifugation of 2 mL of dispersion medium at 12000 rpm for 5 min (Hettich Universal 320, Tuttlingen, Germany). The supernatant solution was then passed through a membrane filter (Chromafil Xtra Syringe Filter, PTFE-20/25 0.25µm), the filtrate was then diluted with water-methanol solvent systems (75:25) and absorbance was measured for concentration calculation of unentrapped free CEF. The following equations were employed for EE (%) and DL (%) calculations[18,19].

$$\text{EE (\%)} = \frac{\text{CFL amount added} - \text{Free CFL}\left(\text{unentrapped}\right)}{\text{CFL amount added}} \times 100$$

$$\text{DL (\%)} = \frac{\text{Entrapped CFL}}{\text{Total theoretical weight of SLN}} \times 100$$

**Selection of optimized CEF loaded SLN:** Based on the particle characterization and drug analysis in the SLN, SLN4 formulation was optimized for further characterization.

**Scanning electron microscopy:** Test sample (SLN4) interacted with beam of electron and produces a tomographic micro image of nanoparticles that highlights the size and surface features. The sample under investigation was adhered on SEM stubs followed by gold plate coating. The selected area was then processed and magnified to extract the particle morphologic information (JEOL Model JSM-6490, Japan).

**Fourier-transform Infrared Spectroscopy (FTIR):** Chemical interaction between stearic acid, Pluronic F-127, CEF and CEF loaded SLN (SLN4) formulation was performed by FTIR spectroscopy (FTIR Spectrometers, FT/IR-4000, Jasco, Pfungstadt, Germany). Samples were triturated with anhydrous potassium bromide (KBr) a thin film was compressed in die and placed in the FTIR spectrometers followed by measuring the functional group peaks over the wave number region of 400-4000 $cm^{-1}$. The spectrum obtained were interpreted for possible chemical interactions[20].

**Differential Scanning Calorimetry (DSC) analysis:** Differential Scanning Calorimetry (DSC) analysis (DSC N-650, SCINCO, Seoul, Korea) was performed for CEF (pure drug) and optimized formulation SLN4. Accurately weigh 5 mg of CEF (pure drug) and lyophilized SLN4 were sealed separately in the aluminum pan. The samples were heated from 25-250°C at 10°C rate under the nitrogen condition (10 mL $min^{-1}$). Empty pan was placed as a reference, the obtained thermogram was processed for axis and measuring the melting point of the drug in the endothermic peak of the drug and tracing it in the formulation. The DSC analysis results could help the investigator to understand the recrystallization and melting behavior of the CEF (pure drug) and drug-loaded SLN4 formulation.

**X-ray crystallography:** The XRD diffractograms of CEF (pure drug) and optimized formulation SLN4 were taken by Ni filtered Cu Kα radiation scattering at voltage 40 kV, current 40 mA, $2^{\theta}$ diffraction angel, continuous scan mode with a speed of 1.000 deg/min and at 25°C temperature by employing X-ray diffractometer (Ultima IV Multipurpose X-ray Diffraction System, Rigaku, Tokyo, Japan), goniometer (Ultima IV), counter (Scintillation).

**Development of CEF-SLN loaded carbopol gel:** Gels were prepared by dispersing carbopol (1 g) in distilled water (100 mL), soaking was continued overnight. The CEF-SLN (SLN4) formulation equivalent to 1% w/w was mixed, 0.1% w/v in were added to act as preservative and penetration enhancer, respectively. Homogenization of CLF-SLN (SLN4) loaded polymeric gel was done by impeller stirrer at 1000 rpm for 6 h (IKA® RW 20 digital dual-range mixer system, Germany) Skin pH and transparency was adjusted by adding 1-2 drops of triethanolamine opacifier[21].

**Characterization of CEF-SLN loaded carbopol gel**

**pH test:** Validated, pre-standardized pH meter (Bibby Scientific 3540 pH/Conductivity Meter, Keison Products, UK) was used to measure the hydrogen ion potential pH of the developed SLN4 CEF polymeric gel. Standard buffer solution of pH 7 and 10 was used as control. About 1 g of CEF loaded gel was dispersed in 10 mL of Milli-Q water, vortexed for 1 min, potentiometer electrode was then dipped in the sample, reading was measured at room temperature (25°C). The experiment was repeated thrice and the mean result was noted[22].

**Spreadability study:** Spreadability study was performed in the Multer and Muller suggested fabricated design. The instrument consists of 2 glass plates of 15×3.75 cm. The sample under test was sandwiched between 2 glass plates, 1 kg weight pressure was exerted on the top plate for 5 min for complete smearing of the gel between the glass plates. The lower glass plate was fixed on the wooden box engraved with scale while the upper plate was fixed with a thin metallic wire passing through the pulley, the other end of the wire was fitted with a weight holding hoke. Twenty grams weight disk was placed in the hoke[23]. Time take to roll the upper plate for 7.5 cm was measured by stopper watch, spreading property of the gel was determined (triplicate) by substituting the time (seconds) in the following equation:

$$S = \frac{M \times L}{T}$$

Where:

M = Weight tied to the upper glass plate

L = Length of glass plate

T = Time taken to travel 7.5 cm was measured in sec

***In vitro* drug release study and similarity factor calculation:** Drug release was performed by the Franz diffusion method by using modified Keshary-Chien cell. The CEF (1% w/w) equivalent of 10 mg, pure drug, SLN4 formulation, dissolved in phosphate buffer pH 6.8 and SLN4 loaded polymeric gel were added separately in the donor compartment, fixed with a pre-soaked dialysis membrane (cut off weight 12 kDa, 15.9×25 mm). The acceptor compartment filled with 100 mL of pH 6.8 phosphate buffer, the assembly was fixed and added with a magnetic bead maintained at 37±0.5°C. At pre-determined time intervals, a 1 mL sample was withdrawn with a replacement of phosphate buffer to provide a sink conditions. Samples of different time intervals (0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h) were analyzed by UV-Spectroscopy (UV-VIS Spectrophotometer, V-630, Jasco, Pfungstadt, Germany) at λmax-224 nm for all the 3 types (CEF, CEF-SLN4 and CEF-SLN4 loaded gel) in triplicates[24]. Graphs were drawn by plotting (%) drug released against time (h). The similarity factor was calculated by the equation suggested by Moore and Flanner. The drug release profiles were compared for CEF-SLN4 and SLN4 loaded gel at different time intervals using similarity factor $f_2$ endorsed by US-FDA (Food and Drug Administration) and with the help of an equation $f_2$ was calculated. If the value of $f_2$ found to be $\geq$50 then the samples understudy will be considered as similar[25]:

$$f_2 = 50 \log \left\{ \left[ 1 + \frac{1}{n} \sum_{t=1}^{n} w_t (R_t - T_t)^2 \right]^{(-0.5)} \right\} \times 100$$

where, $f_2$ is a similarity factor, n, $w_t$, $R_t$ and $T_t$ are number of observations, optional weight, reference-drug releases (%) and test-drug releases (%), respectively.

***In vivo* wound healing examination and epithelialization study**

**Grouping and dosing of animals:** Eighteen male rats weighing (200-250 g) were obtained from the animal house of the Department of Pharmacy, Prince Sattam Bin Abdulaziz University and divided into 3 groups (n = 5) for the experiments. They were acclimatized to the laboratory conditions for a week before the start of experiment and the rats were provided with standard food and water *ad libitum* in a controlled atmospheric condition. The animals were allowed free access to portable drinking water. Group I served

as control treated with the blank polymeric gel formulation. Group II was treated with the standard silver sulphadiazine (1% w/w) marketed formulation. Group III; animals were treated with CEF-SLN4 loaded polymeric gel.

**Excision wound model:** Excision wound was inflicted on the rats under i.v., ketamine (120 mg $kg^{-1}$ b.wt.) anesthesia. The posterior side of the rat was shaved and the wound was impressed on the sides of the central trunk using a scalpel and sharp scissors and sterilized with ethanol. The skin was excised from the marked area to obtain a wound measuring a maximum of 135 $mm^2$. After achieving complete hemostasis by blotting the wound with a cotton swab soaked in warm saline, the animals were placed individually in cages. After a day of wound formation, standard (Markated:1% w/w silver sulfadiazine) and test (1% CEF-SLN4 loaded gel) formulations were gently applied to cover the wounded area every for 24 h until complete healing will be reached. The wound diameter of every animal was measured at 1, 7, 14 and 21 days using a transparent ruler every week until epithelialization and complete wound closure was recorded. Wound healing activity was assessed by wound area, wound contraction rate (%) and epithelialization period[26]. Wound contraction (%) was calculated by using the following equation:

$$\text{Wound contraction (\%)} = \frac{\text{Initial wound size at day zero} - \text{Specific day wound size}}{\text{Initial wound size at day zero}} \times 100$$

The time needed for epithelialization was measured in days unit. The rate of epithelialization was indicated by the fall of scale leaving no raw wound behind. Epithelialization study: complete disappearance of the wound with regrowth of hair at the site of the wound after the process of scare formation and fall-off a new layer of epithelium will have formed, the whole of these events called epithelialization[27]. The time required for epithelialization in control, standard and test were calculated as mean epithelialization period (days).

**Statistical analysis:** Statistical analysis was performed by one-way analysis of variance (ANOVA) using Dunnett's multiple comparison tests. Each data value was expressed as Mean±SD. The $p<0.05$ was considered statistically significant (different).

## RESULTS

**Particle size, PDI and Zeta potential measurement CEF loaded SLNs:** The mean particle size of the SLN formulation ranged from 240.8±0.064 to 283.6±0.016 nm, reflecting all the particles within the nano-range, probe sonicator responsible to break the particles in the nanoscale. Particle size uniformity was assessed by PDI (polydispersity index), results showed that all the formulations were monodisperse as evident by PDI value from 0.25-0.45 (Fig. 1). The inter-particles repulsion due to anionic surface charge over the particles (-10.8 to -14.4) was the surface charge range (Fig. 2), the optimized SLN4 has -14 zeta potential that renders stability of prepared SLNs.

**Entrapment efficiency and drug loading calculation:** Drug entrapped inside the SLNs lipdic matrix was ranged from 35.42±0.041 to 75.71±0.061, whereas drug loading was 4.39±0.034 to 11.35±0.084 (Fig. 3). Entrapment efficiency was found to increase with increase in the lipid concentration, to encompassed the added drug sufficient lipid proportion required after saturation of the lipid core with drug EE was found to be slightly reduced. Optimized formulation SLN4 showed entrapment efficiency (75.71%) and drug loading (9.64%).

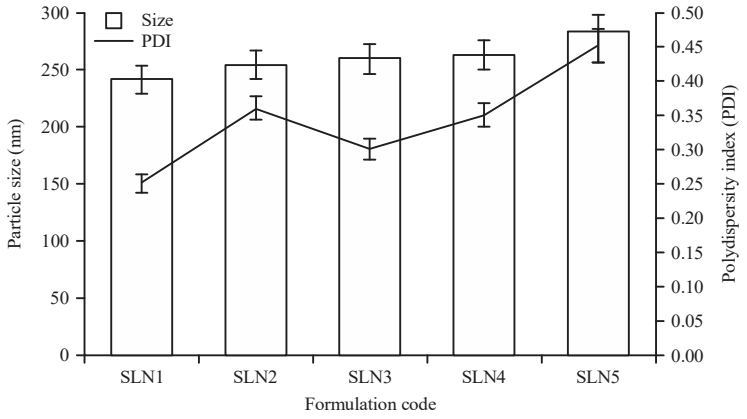


Fig. 1: Particle size and Polydispersity Index (PDI) measurement of Cefadroxil (CEF) loaded SLNs

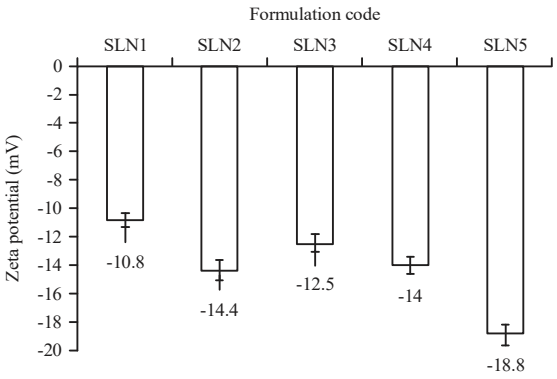


Fig. 2: Zeta potential measurement of Cefadroxil (CEF) loaded SLNs

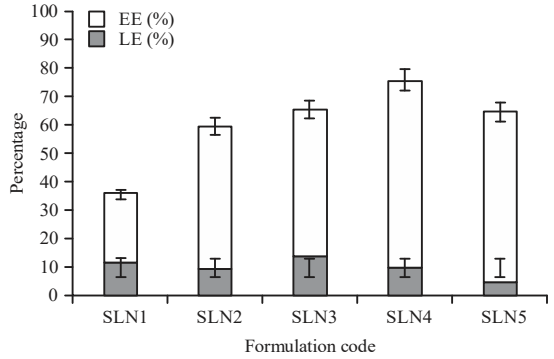


Fig. 3: Entrapment Efficiency (EE%) and drug loading efficiency (LE%) of CEF-SLNs

**Scanning electron microscopy:** The micrographs of optimized SLN4 nanocarrier were found to be spherical with smooth surface, white color scaled chips over the surface could be the adsorbed surfactant. The measured size of particles was in nanorange (Fig. 4a, b). Size plays a vital role for the permeation of drug through the stratum cornea, nano-size with less than 400 mol-daltons drug particles could be easily permeates deeper into the skin layer. The SLN4 loaded gel could be deeply permeated with occlusions and hydration by the developed topical formulations.

**Fourier-transform Infrared Spectroscopy (FTIR):** Identical peaks of CEF (pure drug) were observed at 1122.37, 1562.06, 1758.76, 2582.22 and 3513.67 $cm^{-1}$ (C-N: stretching, N-H: bending, -C=O stretching, =C-H: bending and O-H: stretching), respectively, in the fingerprint region of the compound. All these major identical peaks of CEF were absent in the formulation of SLN4 indicating the shielding effects by polymer and drug-polymer compatibility (Fig. 5).

**Differential Scanning Calorimetry (DSC) analysis:** The thermogram of CEF (pure drug) showed a sharp endothermic peak at 220.95°C. The absence of a sharp melting point peak in the SLN4 formulation suggested that the CEF drug was entrapped in the solid stearic acid particles and it's available in the amorphous nature (Fig. 6). Non-crystalline availability of the drug inside the nano-cage increases the stability of the formulation.

**X-ray crystallography:** The XRD diffractogram of CEF (pure drug) showed 2 θ characteristic peaks at 14.500, 18.800, 23.300, 26.800, 28.800, 32.900, 40.800 and 47.900, indicating crystalline nature of the Cefadroxil-drug (Fig. 7). All these crystalline peaks were absent in SLN4 formulation, but the presence of stearic acid causes few spiked peaks in the formulation. Reduced peaks in the SLN4 diffractogram confirm the decreased crystallinity, amorphous nature of the CEF.

**pH test:** The pH of the optimized formulation SLN4 loaded polymeric gel formulation was 6.56±0.012, which was comparable with skin pH. Therefore, the developed antibiotic gel was considered to be compatible with skin and meant for dermal application.

**Spreadability:** The spreadability of the SLN4 loaded gel was found to be 102 g/cm/sec, indicated easy spreadability of the prepared antibiotic topical gel formulation. Spreadability of gel was inversely proportional to the spreading time and consistency of the prepared gel. Good spreadability desired to cover the infected area with the antibiotic gel and for patient compliance.

***In vitro* drug release study and similarity factor calculation:** The results of CEF (pure drug), CEF-SLN4 and SLN4 loaded polymeric gel *in vitro* release were found to be 100% at 4th h and 90.67±0.34, 86.76±0.12% at up to 8th h, respectively. The CEF-SLN4 showed slightly higher drug release, compared to SLN4 loaded gel the reason could be the viscous nature of the polymeric gel, carbopol used in the preparation of gel may retard the release of the drug, but the difference was insignificant. To measure the influence of polymer on the drug release from the SLN shell similarity factor ($f_2$) was calculated by considering CEF-SLN4 as test and SLN4 loaded gel as reference. The diffusion drug profiles of both CEF-SLN4 and SLN4 loaded gel can be considered as similar based on the $f_2$ value: 51.129, which was more than 50.

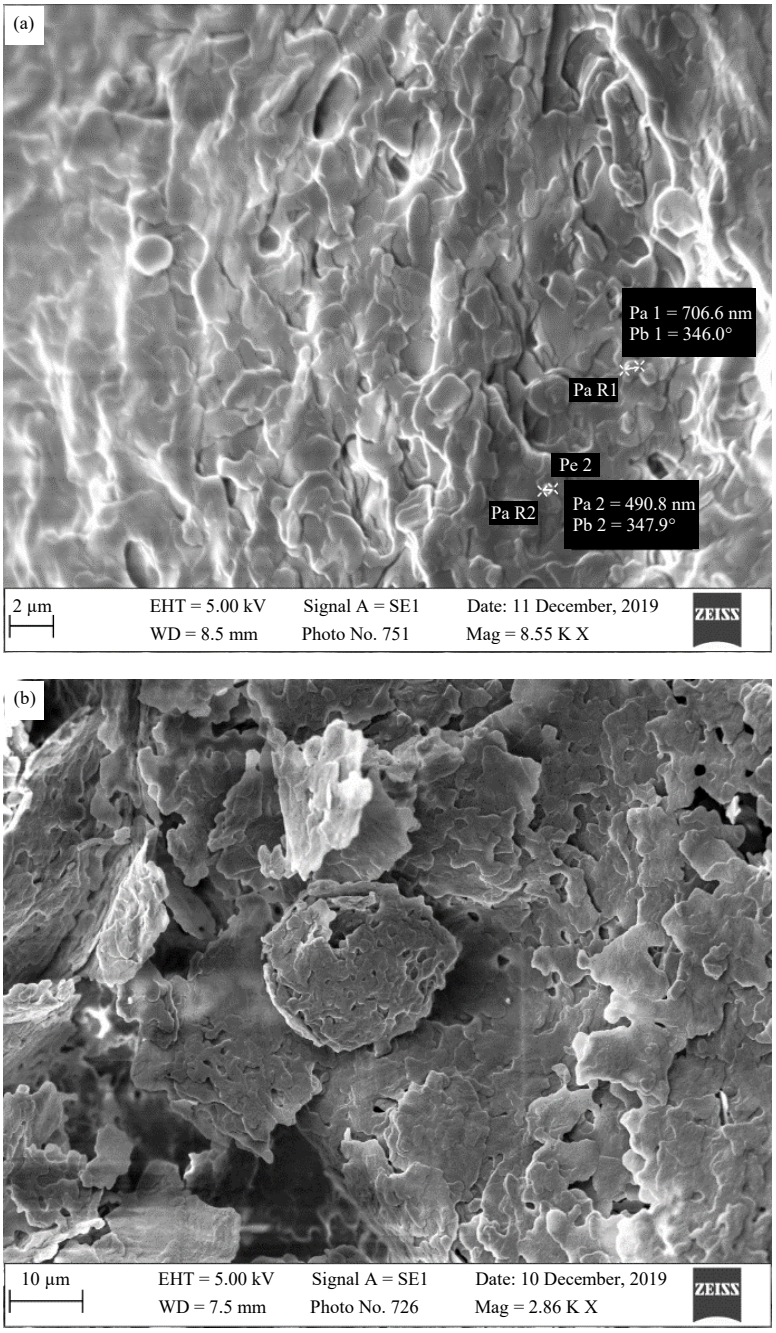


Fig. 4(a-b): Scanning electron microscopy of SLN4
EHT: Electron high tension, WD: Working distance, Mag: Magnitude, Pa: Particle

Figure 8 revealed biphasic release pattern within the first 3 h about 60% drug was released in both CEF-SLN4 and SLN4 followed by a sustained release mechanism. The initial burst release could be due to the diffusion of unentrapped surface adsorbed drug, the sustained release mechanism may be due to the increased diffusion path length of drug and hindering effect of the stearic acid-lipid shell.

***In vivo* wound healing examination and epithelialization study:** The order of wound healing was found to control>standard>test (SLN4 gel), complete healing was observed at 21 days for the wound applied with topical SLN4 gel in comparison to marketed antibiotic topical gel. The wound contraction at first (0) day was considered as 0% with an average wound size of 125.33 $mm^2$ in all the

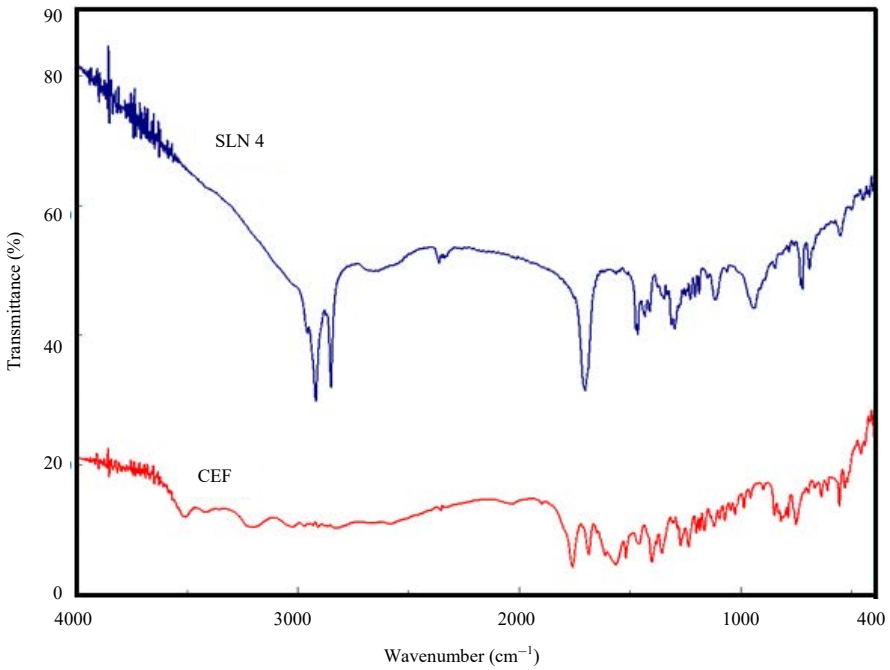


Fig. 5: Fourier-transform Infrared Spectroscopy (FTIR) of CEF-SLN4

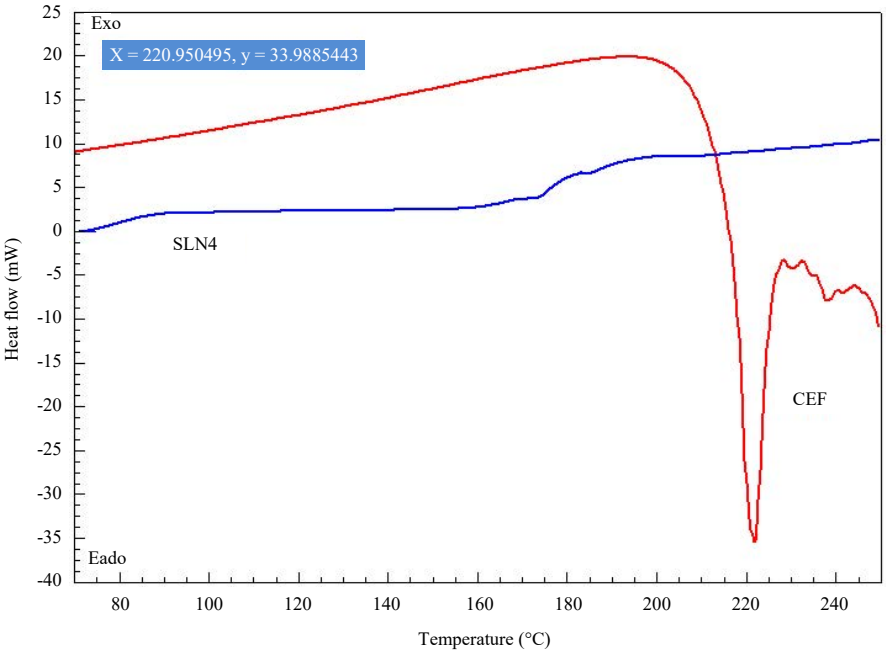


Fig. 6: Differential Scanning Calorimetry (DSC) analysis of CEF-SLN4

3 groups of control (blank), standard (marketed product silver sulphadiazine 1% w/w) and test (SLN4 loaded gel). The wound area (%) contraction was found to be 15±1.3, 63±2.3, 91.77±2.4% at 7, 14, 21 days, respectively in the control group. The application of standard (marketed product silver sulphadiazine 1% w/w) led to the healing of wound with contraction of 46.23±1.2, 82.27±2.1 and 99.20±2.5% at 7, 14 and 21 days, respectively. *In vivo* wound healing of SLN4 gel (test group) showed 52.20±2.1, 84.77±2.4 and 99.73±1.6% of wound contraction at 7, 14, 21 days. After 3rd week (21 days) all-male rats of test group completely healed by the topical application of SLN4 gel, the results were on par with the standard marketed product (Fig. 9).

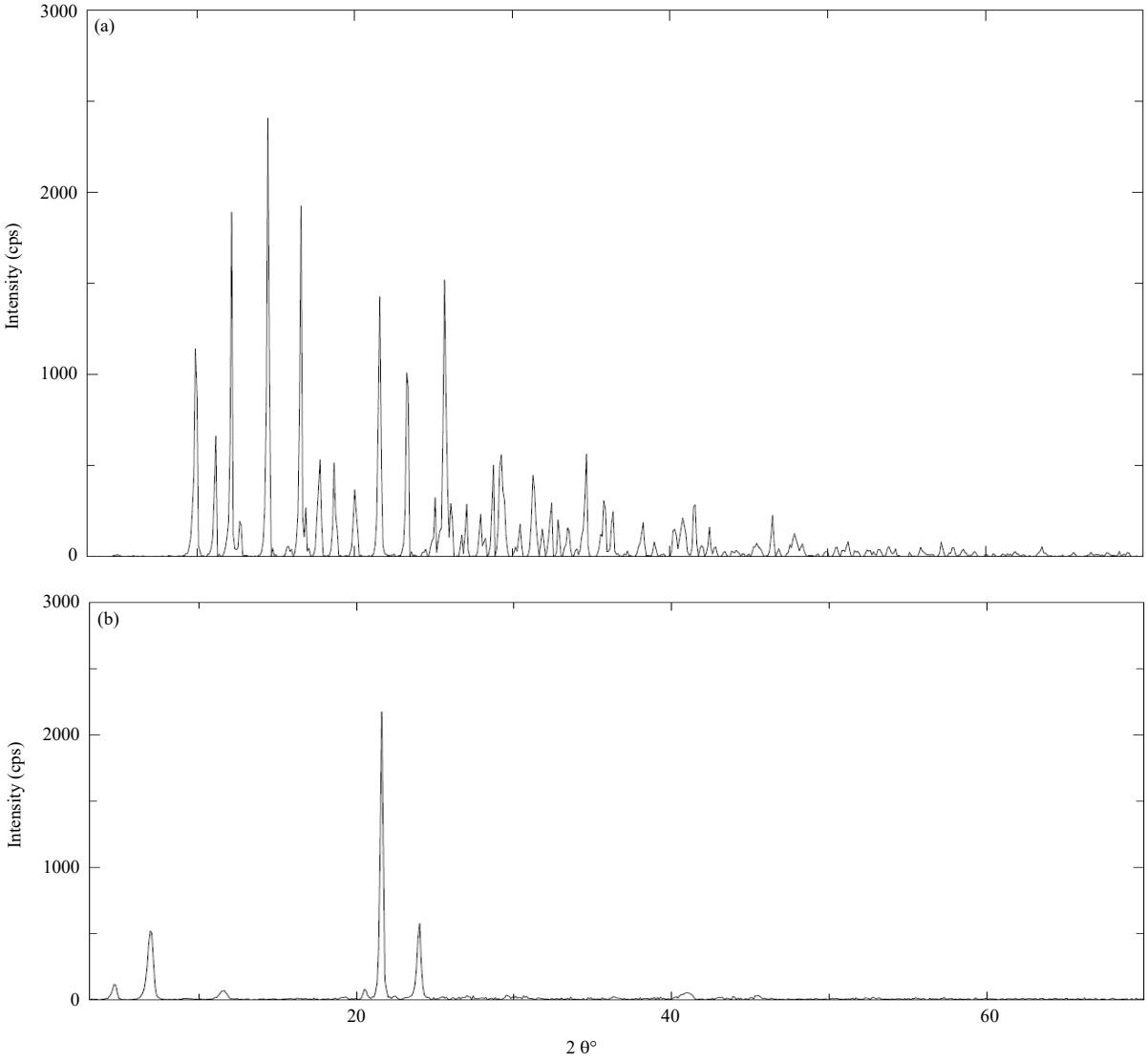


Fig. 7(a-b): X-Ray crystallography of (a) CEF and (b) SLN4

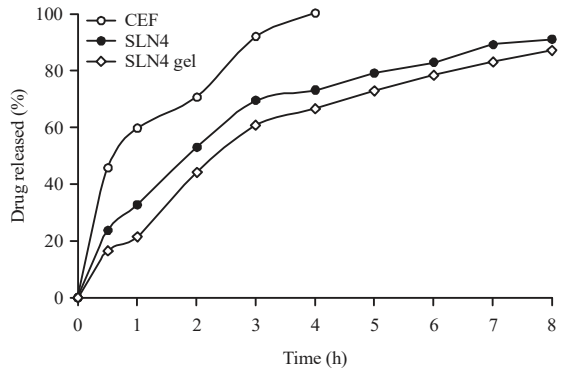


Fig. 8: *In vitro* drug release of CEF, SLN4 and SLN4-loaded gel

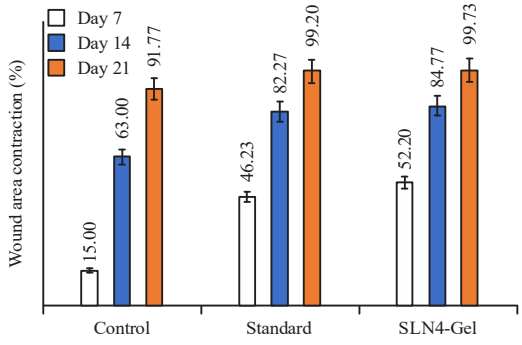


Fig. 9: *In vivo* wound contraction examination in male rats SLN4 loaded gel


Fig. 10: *In vivo* wound healing and epithelialization study of SLN4 loaded gel

Epithelialization was completed in 27±0.7, 21±0.4 and 17±0.4 days for control, standard and test group (Fig. 10), relatively short time was observed in the test animals applied with the SLN4 gel in comparison to standard (marketed product silver sulphadiazine 1% w/w) and control group of animals (un-treated). The reason for complete and fast healing could be the permeation of the CEF antibiotic deeper into the skin layer followed by complete eradication of the microbial strains. Stearic acid a lipid enhances the permeation of drugs in the lipoidal skin layer that further synergized by the nanosize of SLN drug carriers.

## DISCUSSION

Optimized formulation SLN4 showed particle size (263±0.048 nm PDI and 0.35, -14 zeta potential, entrapment efficiency was 75.71 with 9.64% drug loading capacity. Particle size was found to increase with the increase in the stearic acid, monodisperse nature of dispersion could be due to the process and pluronic F-127 stabilizer. Polydispersity index less than ≤0.5 considered as a particle with uniform size-frequency distribution[19]. FTIR, DSC, XRD and SEM revealing drug was entrapped in the spherical lipid matrix[20]. The CEF-SLN4 loaded gel exhibits skin compatibility pH (6.56±0.012) and desired spreadability (102 g/cm/sec). *In vitro* drug release of SLN4 was 90.67±0.34% and for CEF SLN4 loaded gel was found to be 86.76±0.12% in 8th h diffusion study, slight decrease release of SLN4 in the polymeric gel was acceptable as per similarity factor ($f_2$: 51.12). *In vivo* wound healing activity in male rats displayed 99.73% of wound contraction within 3 weeks and complete epithelialization within 17 days by CEF-SLN4 gel.

All the five nanocarriers (SLN1-5) were in nano-range, colloidal particles within 10-1000 nm range, called as nanoparticles. SLNs considered as the latest generation of the lipid emulsion with globules in nano-size range. There was progressive growth in the particle size was observed with increase in the lipid content (SA)[28]. Due to its small size SLNs offers increased surface area, higher loading efficiency and penetration property[29]. Promising SLN4 composed of CEF, stearic acid and pluronic F-127 in the ratio of 2:8:5 was considered as an optimum that produces the desired size and surface potential[30]. Polydispersity index indicated the frequency of particles in the dispersion, if PDI ≤0.3 then it can be considered as monodisperse nanoparticles. All the prepared SLNs showed increased colloidal stability due to the existence of inter-particle repulsive forces over the surface caused by pluronic F-127. Gyulai *et al.*[31] reported that pluronic F-127 induces more stable dispersion as compared to pluronic F-108 due to difference in chemical and polymeric chain ratio, responsible for particles surface adsorption. Entrapment efficiency results also showed increase in EE (%) with increase in the lipid concentration, if lipid content is high in SLN less chances of drug escape in the external phase, the results are with the agreement of montelukast loaded SLNs as reported

by Priyanka and Hasan[32]. The FTIR, DSC and XRD study revealed that there was no physicochemical interaction between polymers used and drug, drug was encapsulated in the lipid core and available in the non-crystalline form[20]. Selected SLN4 was loaded in carbopol to develop topical gel. pH test showed its skin compatibility, the viscosity of the formulation was optimum that gives suitable spreadability. Drug diffusion of developed SLNs gel was slightly decreased from the SLN4, but similarity index factor revealed drug release was significantly similar[33]. Drug released from the gel was in sustained manner and the presence of lipid in formulation could help for deeper permeation of drug through the skin layers[27,34].

Optimized SLN4 loaded Cefadroxil topical gels showed sustained drug release with dermal permeation of drug that supports effective bactericidal action and significantly accelerates the wound healing activity. Basha *et al.*[8] reported Cefadroxil could be used in wound dressing material as a potential antibacterial agent.

Therefore, it could be suggested that prepared antibacterial gel of nanoparticle size, lipid nature and broad spectrum of CEF may eradicate the bacterial load at the wound site thereby accelerate wound healing process. The ease of preparation will enable mass production of the formulation and its feasibility for clinical implications. Involvement of nanotechnology may hinder the cost of the product and patient compliance.

## CONCLUSION

Cefadroxil is topically effective bactericidal and more effective against the large spectrum of bacteria. Solid lipid nanoparticles considered to be efficient nanocarrier with higher drug loading capacity and penetration enhancement property. The use of lipid (SA) in the preparation of SLNs concomitant with nanoparticle size increases the drug penetration deeper into the skin layer, thereby eradicating infection from lower skin layers. Drug releases from the SLN lipid matrix were sustained as desired for effective infectious treatment. *In vivo* wound healing activity showed a higher contraction percentage for the developed formulation with enhanced epithelialization rate. Therefore, it could be concluded that the developed formulation is an alternative topical formulation for safe and fast wound healing treatment.

## SIGNIFICANCE STATEMENT

This study discovers the application of nanocarriers composed of solid lipid that enhances the permeation of drug with sustained drug release that helps the Cefadroxil to kill the bacteria and eradicate the infection. The gel could be easily spreadable over the applied infected area and hydrates the skin with the occlusiveness of the topical application. The study uncovers the beneficial antibacterial effectiveness of Cefadroxil on topical application that many researchers were not able to explore. Thus, a new theory on the development of SLNs and preparation of antibacterial topical gel for wound healing and possibly other combinations, may be arrived at.

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