A Novel Approach of Targeting Linezolid Nanoemulsion for the Management of Lymph Node Tuberculosis

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ABSTRACT: Tuberculosis (TB) represents a major public health problem, globally affecting children and adults. Lymphatic TB is the most common type of extrapulmonary tuberculosis, which affects the peripheral lymph nodes. This burgeoning disease requires a long-term treatment of multiple antibiotics to kill Mycobacterium tuberculosis, resulting in an increased rate of multidrug-resistant tuberculosis. To overcome drug resistance with the first-line antibiotics, linezolid W/O nanoemulsion was developed in this current work. W/O nanoemulsion was prepared by oil phase titration technique using sunflower oil, span 80 and tween 80, and optimized by pseudophase ternary diagrams. The particle size, polydispersity index, zeta potential, viscosity, and refractive index for the optimized formulation were found to be 92.32 nm, 0.066, $-21.9$ mV, 32.623 cP, and 1.453, respectively. Drug release from the developed nanoemulsion followed the zero-order kinetic. The antimicrobial efficacy study confirms the antibacterial potential of the developed nanoemulsion. In vivo studies conducted on Wistar rats confirms the lymphatic targeting with a high amount of drug at the target organ just after 8 h of dosing. As a result of the foregoing promising results, it may be inferred that the suggested nanoemulsion could be a viable therapy option for lymph node tuberculosis.

1. INTRODUCTION

Tuberculosis is the world’s most pernicious disease caused by Mycobacterium tuberculosis (MTB) and, mainly, affects the respiratory system.1 Although the bacteria may affect any system of the body, but normally it infect the lungs. Extrapulmonary TB occurs when tuberculosis develops outside of the lungs, such as lymph nodes, pleural membrane, and osteoarticular areas.2 Among the extrapulmonary TB, lymph node TB is the most common in the United States. In this type of TB, peripheral lymph nodes particularly anterior and posterior cervical chains are most commonly affected.3,4

For the management of different classes of TB, WHO has prescribed standard drug regimen. Anti-TB drugs are categorized into five classes; among them, the first line drugs are the most effective. But because of the development of bacterial resistance with the first class anti-TB drugs, there is a need to give attention to the newer classes of anti-TB drugs. Linezolid is one of newer class of antitubercular drugs, which is indicated for treatment of lymph node TB. Linezolid was selected as synthetic antibacterial agent of the oxazolidinone class of antibiotics. The drug is active against anaerobic, aerobic Gram-positive, and few Gram-negative bacteria. It comes under the newer class of antitubercular drug and has been explored by the researchers for effectiveness against multidrug resistant TB. But the indicated oral dose of linezolid for TB is 600 mg twice a day, which produces serious side effects and limits its use.5 Thus, there is a requirement of a suitable delivery system through which this dose can be minimized without compromising the drug effectiveness.

Various studies have been carried out by the researches to incorporate the anti-TB drugs into the drug delivery carriers, such as nanoparticles, microparticles, microemulsions and nanoemulsions, and solid lipid nanoparticles.6−10 Nowadays much attention is paid to lipid-based formulation with particular emphasis on self-emulsifying drug delivery system (SEDDS) and nanoemulsions which have broadly been researched as drug delivery systems. These are the colloidal scattering frameworks that are thermodynamically stable and are made up of two immiscible liquids blended along with the emulsifying agent (surfactants and cosurfactants) to shape a single phase.11

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In the current work, W/O nanoemulsion is explored for lymph node TB. It is a colloidal type of drug delivery system that interacts with the lipid membrane of the lymphatic system and results in enhanced vascular permeability due to passive targeting. When such a system is given orally it undergoes the intestinal uptake by the lymphoid follicles and Peyer’s patches of the GALT and transported to lymph by phagocytosis of macrophages and will release the anti-TB drug. Thereby incorporation of linezolid into the W/O type nano emulsion leads to dose reduction and hence support its use in lymph node TB.

2. MATERIALS AND METHODS

2.1. Materials. The drug Linezolid was obtained as a gift sample from Godavari drugs limited (Telangana, India). Span 80, Span 85, Tween 80, hydrochloride (HCl), and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used for the experiments were in analytical grades, obtained from SD Fine Chemical Limited (Mumbai, India).

2.2. Pseudoternary Phase Diagram Construction. To investigate concentration range of components for the existing boundary of W/O NEs, pseudoternary phase diagrams were constructed using oil titration method. The surfactant and cosurfactant used were Span 80 and Tween 80 as they attained the required HLB to emulsify the oil in these ratios. Three phase diagrams were prepared with the 1:1, 2:1, and 3:1 weight ratios of Span 80: Tween 80, respectively. Aqueous phase and the surfactant mixture were then mixed at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5 (w/w). These weight ratios of water and S: CoS were diluted dropwise with the oily phase (sunflower oil) under moderate agitation. After being equilibrated, the mixtures were assessed visually and determined as being W/O nanoemulsions by virtue of their transparency, nondispersibility, polarizing light, and flow ability.

2.3. Formulation of Nanoemulsion. Step 1: Primary W/O Nanoemulsion. A blend of distilled water and Smix were mixed with vortexing, followed by slow titration with oil phase. The visual observation of admixture was carried out for a clear transparent W/O nanoemulsions. The stable transparent primary W/O nanoemulsions were tested for nondispersibility test in water and observed under polarizing light for validation of continuous phase.

Step 2: Multiple W/O/W Nanoemulsion. For making W/O/W nanoemulsion, different composition of nanoemulsions were selected for incorporation of drug into the aqueous phase. 1% (w/w) of linezolid was dissolved in aqueous phase (distilled water) of all selected W/O nanoemulsion compositions. Then selected quantity of Smix and oil was added and stirred for 2 min to get W/O nanoemulsion. The obtained primary W/O nanoemulsions was dispersed with surfactant (tween 80) mixed water phase (3%−15% w/w), followed by homogenization at 10 000 rpm for 10 min. The obtained secondary nanoemulsion (W/O/W) was identified by water dispersion tests and the formulations that failed the water dispersibility tests were discarded.

2.4. Dispersion Stability Studies. 2.4.1. Heating Cooling Cycle. Six cycles between refrigerator temperature 4 and 45 °C with storage at each temperature of not less than 48 h was studied. Those formulations that were stable at these temperatures were subjected to centrifugation test. Temperature stability shelf life as a function of time and storage temperature was evaluated by visual inspection of the nanoemulsion system at different time period. Nanoemulsion was checked for the temperature stability and were kept at three different temperature range, that is, refrigerator, room temperature, and incubator then observed for any evidence of phase separation, flocculation, or precipitation.

2.4.2. Centrifugation. To estimate metastable systems, the optimized nanoemulsion formulation was centrifuged at 1000 rpm for 15 min at 5 °C and observed for any change in homogeneity of nanoemulsions.

2.4.3. Freeze Thaw Cycle. Nanoemulsion formulations were kept in refrigerator at 4 °C and then kept out to normal temperature (25 °C) at an interval of 2 h. Then formulations were observed for any change in homogeneity of nanoemulsions.

2.5. Droplet-Size, PDI, and Zeta Potential. Globule size of the W/O nanoemulsion was determined through photon correlation spectroscopy using Zetasizer Nano ZS90 (Malvern instruments) at 633 nm which is based on the principle of dynamic light scattering (DLS). To avoid multiple scattering effects, the multiple W/O/W emulsions were diluted in water (1:100). The polydispersity index (PDI) was a dimensionless measure of size distribution range derived using cumulant analysis ranged 0 to 1. A low PDI score indicates a monodispersed population.

Zeta potential (ζ) of the nano emulsions were determined after dispersing the test sample in distilled water (1:100). A potential difference across the dispersion medium and sample droplet serves the basis for zeta potential. Due to this difference, the charged droplets within the dispersion medium migrates toward the opposite charge electrode, which ultimately give rise to the positive or negative value of zeta potential.

2.6. Viscosity and Refractive Index. The viscosity of nanoemulsion was measured using rheometer (MCR101 Rheoplus of Anton Paar). It is done by using cone plate probe and procedure was carried out for 6 s. Refractive index of the prepared nanoemulsion formulation was determined using Abbes refractometer.

2.7. Fluorescence. Fluorescence microscopy was carried out to determine the size, shape and phases of the nanoemulsion droplets. Linezolid nanoemulsion was prepared by loading rhodamine dye in the aqueous phase. Further, the slides were prepared and subjected to the fluorescent microscopic examination.

2.8. In Vitro Drug Release Study. For nanoemulsion, in vitro drug release study was performed by the dialysis bag method and the dialysis bag was made up of cellulose membrane (Sigma, USA, molecular weight 12 000 g/mol). Three ml of formulation was filled in the dialysis bag. Drug release media comprises of 100 mL of phosphate buffer (pH 6.8) with thermoregulation maintained at temperature of 37 ± 2 °C with 100 rpm. An aliquot of release media was withdrawn at different time interval for 4 h and sink conditions were maintained throughout the study. Withdrawn samples were analyzed by UV-spectroscopy at λmax 251.5 nm. All the studies were performed in triplicate (n = 3). Further, to elucidate the release mechanism, in vitro release data was fitted to zero-order, first-order, Higuchi and Korsemeyer-Peppas model.

In vitro release of nanoemulsion from capsule and linezolid tablet Linox (Unichem, Mumbai, India), USP dissolution apparatus type 1 (basket type) was used separately. Capsules and tablets were kept in the basket. Phosphate buffer (900 mL, pH 6.8) was used as a dissolution media. The basket was
positioned in media vessel made up of glass. The temperature of the media inside the vessel was maintained at 37 ± 2°C and rotation speed was set to 100 rpm. Aliquots of dissolution media were withdrawn at different time interval for 4 h and analyzed by UV-spectroscopy at 251.5 nm. Sink conditions were maintained throughout the study and sampling were performed in triplicate (n = 3).

2.9. Antimicrobial Efficacy Study. Colorimetric method was used to study the antimicrobial activity of the optimized formulation against *Mycobacterium smegmatis*. *Mycobacterium smegmatis* ATCC 607 was cultured at 37°C in test tube containing 3 mL of the KIRCHNER’S medium to maintain turbidity equal to that of a no. 1 McFarland standard (approx 3 × 10^7 CFU/mL) and diluting the culture 1:5 in broth.21 After the incubation time of 7 days, alamar blue (indicator) was added into all the four cultured test tubes containing control, pure linezolid (2 μg/mL), placebo nanoemulsion (11.11 μL), and linezolid nanoemulsion (11.11 μL). Color change was observed in all the test tubes.22 Further, the percent cells survival was measured by the counting the colony forming units (CFU mL^-1^).

2.10. In Vivo Studies. The in vivo study was approved by the Institutional Ethical Committee of Animal Research, Jamia Hamdard, New Delhi, India (protocol approval no. 1428), and all the “principles of laboratory animal care” were followed during the study. Wistar rats (120–140 g) were used for the study of orally administered nanoemulsion formulations. Before treatment, the animals were fasted overnight. In this study design, 24 Wistar rats were divided into four groups. Group 1 was treated as control and administered with normal saline (4 mL) and group 2 is administered with the linezolid nanoemulsion (0.64 mg in 4 mL) by oral gavage.9 At regular intervals of 0.5, 2, 4, and 24 h, the animals were sacrificed and lymph nodes, thymus, intestine, and spleen were weighed separately and placed in 3 mL of normal saline solution. Then the normal saline solution (3 mL) containing tissues were homogenized for 1 min. Further, 0.5 mL of cold methanol was added into the mixture and the whole mixture was centrifuged (120 000 rpm, 10 min). The supernatant solution was taken out and filtered through 0.22 μm membrane filter. The solutions were then transferred into RP-HPLC vials and analyzed by the already developed and validated RP-HPLC method for the % drug release of drug in above-mentioned tissues with respect to time.

A developed and validated RP-HPLC method was used for analysis of drug in tissue.23 Lachro CART C-18 chromatographic column with dimensions of 250 × 4 mm and 5 μm particle size was used. Isocratic mode of analysis was performed with the mobile phase consisting of methanol:water (50:50 v/v) with a flow rate maintained at 1 mL/min. Wavelength was selected 251.5 nm, and the total run time of 10 min was set for the drug estimation.

2.11. Lymphatic Targeting Efficiency. Targeting efficiencies of linezolid to the lymphatic system and plasma were calculated as the ratio of the concentration of linezolid in lymph nodes to plasma at different sampling time. All data are expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1. Pseudoternary Phase Diagram. Pseudoternary phase diagrams were constructed to study the existence of nanoemulsion formation zone. Pseudoternary phase diagrams were constructed using sunflower oil and Span 80 and Tween 80 as the surfactant and cosurfactant respectively for primary W/O nanoemulsion. For the optimization of the nanoemulsion, varied ratio of surfactant and cosurfactant were used and their effect on nanoemulsion formation was assessed. A large W/O nanoemulsion region was found in the phase diagram when the Smix ratio was 1:1. With the Smix ratio of 2:1, nanoemulsion region gets decreases in the phase diagram. Further as shown in Figure 1 the region decreases with Smix ratio of 3:1. The obtained primary W/O nanoemulsion was dispersed in tween 80-water mixture (3%–15% w/w), followed by high pressure homogenization, to get W/O/W

Figure 1. Pseudoternary phase diagram of the quaternary system containing sunflower oil, water, Span 80, and Tween 80 with span 80: Tween 80 ratio fixed at 3:1, 2:1, and 1:1.
nanoemulsions. The obtained formulation was authenticated by dispersibility tests in distilled water.

3.2. Formulation of Nanoemulsion. Oil phase titration method was used for the development of all the nanoemulsion formulations and Table 1 shows the recipe of various selected formulations compositions. The selection criteria was kept at minimum 3% aqueous phase to dissolve the drug under unsaturated condition and to avoid precipitation.

3.3. Dispersion Stability Study. The formulations were tested for different dispersion stability tests (Table 2). Only those formulations that showed no phase separation, creaming, cracking, coalescence, or phase inversion upon these stress tests were selected for further studies. The thermodynamic stability studies indicated that formulations containing more than 4% internal phase showed instability. Some formulations with less than 4% internal phase also failed the thermodynamic stability studies. This might be due to the insufficient amount of Smix or inappropriate ratio of emulsifiers and coemulsifiers. The resultant of Smix composition is responsible for providing a flexible water−oil interface and easy emulsification. The dispersibility tests showed that the formulation homogenized with more than 9% w/w tween 80−water mixture gets quickly dispersed. The dispersibility results confirmed the existence of water as an external phase.

3.4. Characterization of Nanoemulsion. The average globule size of the selected formulations was found to be in the range of 92.32−169.7 nm as shown in the Table 3. Among these, formulation F-1 had the minimum size with a polydispersity index (PDI) of 0.066. The narrow PDI shows droplet homogeneity with 98.4% droplet was found to be 69.85 nm size range (Figure 2). The optimized formulation (F-1) showed 1.6% droplet with polydisperded size range, which is very insignificant.

Zeta potential of nanoemulsion formulation (F-1) was found to be −21.9 mV, which confers formulation stability as the formulation resist aggregation. The zeta potential data showed droplet size has inverse relationship (Table 3). Here, nanoemulsion formulation (F-1) is an optimized formulation.

Table 1. Compositions of Various W/O Linezolid Nanoemulsion Formulations

| code | % w/w of component | water | Smix | oil | Smix ratio | Smix:water ratio |
|------|---------------------|-------|------|-----|------------|-----------------|
| F1   | 3.2                 | 28.8  | 68   |     | 1:1        | 9:1             |
| F2   | 3.6                 | 28.8  | 67.6 |     | 8:2        |                 |
| F3   | 4.11                | 28.8  | 67.09|     | 7:3        |                 |
| F4   | 4.86                | 28.8  | 66.4 |     | 6:4        |                 |
| F5   | 5.76                | 28.8  | 65.44|     | 5:5        |                 |
| F6   | 3.2                 | 28.8  | 68   |     | 2:1        | 9:1             |
| F7   | 3.6                 | 28.8  | 67.6 |     | 8:2        |                 |
| F8   | 3.2                 | 28.8  | 67.09|     | 7:3        |                 |
| F9   | 4.86                | 28.8  | 66.4 |     | 6:4        |                 |
| F10  | 3.2                 | 28.8  | 68   |     | 2:1        | 9:1             |
| F11  | 3.6                 | 28.8  | 67.6 |     | 8:2        |                 |
| F12  | 4.11                | 28.8  | 67.09|     | 7:3        |                 |

“Selected formulations for further studies.

| batch no. | centrifugation | heating cooling | freeze thaw cycle |
|-----------|----------------|-----------------|-------------------|
| F1        | ×              | ×               | ×                 |
| F2        | ×              | ×               | ×                 |
| F3        | √              | ×               | √                 |
| F4        | √              | ×               | ×                 |
| F5        | √              | √               | √                 |
| F6        | ×              | ×               | ×                 |
| F7        | ×              | ×               | ×                 |
| F8        | √              | ×               | ×                 |
| F9        | √              | √               | √                 |
| F10       | ×              | ×               | ×                 |
| F11       | √              | ×               | ×                 |
| F12       | √              | √               | √                 |

“Selected formulations for further studies.

Table 2. Phase Separation and Cracking Behavior of Different Formulation Batches against Centrifugation, Heating Cooling Cycle, and Freeze Thaw Cycle

Table 3. Mean Droplet Size, PDI, and Zeta Potential of the Selected Nanoemulsion Formulations

| formulation code | globule size (nm) (mean ± SD, n = 3) | polydispersity index (PDI) | zeta potential (mV) (mean ± SD, n = 3) |
|------------------|--------------------------------------|---------------------------|----------------------------------------|
| F1               | 92.32 ± 7.85                         | 0.066                     | −21.9 ± 3.58                           |
| F2               | 99.79 ± 8.93                         | 0.091                     | −20.33 ± 6.22                          |
| F6               | 112.98 ± 10.12                       | 0.124                     | −19.73 ± 4.25                          |
| F7               | 123.30 ± 12.67                       | 0.045                     | −19.06 ± 8.24                          |
| F10              | 169.70 ± 13.98                       | 0.242                     | −17.52 ± 4.27                          |

Figure 2. Particle size distribution (a) and zeta potential (b) graph of the optimized formulation.
having stable zeta potential in the nanoscale (+30 mV to −30 mV).

Refractive index of nanoemulsion formulations (F-1), was determined using Abbes refractometer and it was found to be 1.453. This indicates the isotropic nature of the formulation and signifies absence of drug and excipient chemical interaction.

The viscosity of nanoemulsion formulation was found to be 32.623 cP. On increasing the shear rate, there is no considerable increase in the viscosity of the formulation.

Fluorescence microscopy of the F-1 formulation was carried out, and Figure 3 depicts the size and shape of the particles with water as an internal phase. This further confirmed the existence of W/O/W emulsion.

3.5. In Vitro Drug Release Study. For nanoemulsion in vitro release study was performed in triplicate manner and the samples were analyzed by UV spectroscopy at 251.5 nm. Release data was fitted into kinetic models. Nanoemulsion followed zero-order kinetic model ($R^2 = 0.9958$), which describes that the drug release is independent of time.

Capsules filled with nanoemulsion were subjected for in vitro drug release using USP-dissolution apparatus type 1 (Basket type). The samples were analyzed by UV spectroscopy at wavelength of 251.5 nm. The dissolution data was fitted into kinetic models. Nanoemulsion filled in capsules followed the Higuchi model ($R^2 = 0.9834$), where the drug release is dependent on the square root of time because of the diffusion of the drug from the nanoemulsion and then from the capsule. Whereas the drug release from the tablet followed the first order kinetics ($R^2 = 0.964$), which is the characteristic of immediate release pharmaceutical dosage forms.\(^\text{24}\) A comparative drug release from the nanoemulsion, marketed tablet and capsule is depicted in the Figure 4. The dissolution graph showed that almost 100% of linezolid was released from tablet and capsule dosage form in 4 h dissolution time whereas less than 60% of linezolid was released from W/O/W nanoemulsion. This also authenticate the existence of multiple diffusion layers in case of W/O/W nanomulsion formulations.

3.6. Antimicrobial Efficacy Study. Colorimetric method was adopted to assess the antimicrobial activity of the formulation against *Mycobacterium smegmatis*. Change in color from blue to pink was observed in the control test tube and placebo nanoemulsion containing test tube, whereas light pink color was observed in pure linezolid containing test tube and linezolid nanoemulsion containing test tube. This indicates that drug and drug containing nanoemulsion were able to kill the colonies of *Mycobacterium smegmatis*. The percent cell survival is depicted in the Figure 5. It was noteworthy that the percentage cell survival was approximately 25% and 8% for pure linezolid and linezolid loaded nanoemulsions as compared to control; thus, the efficacy of developed nanoemulsions was found as approximately 3 times more than pure linezolid.

3.7. In Vivo Studies. When RP-HPLC of the drug was performed in the tissue samples, retention time was observed at 2.50 min, and linearity was found in the range of 0.3–25 μg/mL ($R^2 = 0.991$). The concentrations of the linezolid in the tissues were estimated with the help of standard plot. As shown in the Table 4, maximum drug release in the lymph nodes, spleen, and thymus was found after 24 h of dosing whereas in the thymus maximum drug release was achieved after 8 h of dosing. This suggests the possible transport of drug in the tissues.

3.8. Lymphatic Targeting Efficiency. By quantifying the ratio of lymph node concentration to plasma concentration, we were able to compare lymphatic targeting efficiency. The formulations were shown to be more concentrated in plasma in the first 4 h of sampling, but accumulated inside the lymphatic
system following the second 8-h sampling. Overall drug concentration and targeting efficiency were considerably higher (p < 0.05) in lymph nodes, spleen, and thymus compared to plasma, but negligible in lymph nodes (Figure 6). Linezolid lymphatic targeting efficiency was increased by more than 35 times. The results showed that nanoemulsion more effectively transported drugs to the lymphatic tissue.15

Table 4. Percent Drug Release in Lymph Nodes, Spleen, Thymus, and Intestine of Rat with Respect to Time

| sampling time (h) | lymph nodes (%DR ± SD) | spleen (%DR ± SD) | thymus (%DR ± SD) | intestine (%DR ± SD) |
|-------------------|------------------------|------------------|------------------|---------------------|
| 4                 | 0.8 ± 0.02             | 0.12 ± 0.01      | 0.35 ± 0.098     | 89.72 ± 1.23        |
| 8                 | 14.10 ± 0.82           | 8.96 ± 2.32      | 66.98 ± 10.35    | 09 ± 2.34           |
| 24                | 75.45 ± 4.39           | 8.3 ± 2.56       | 10.21 ± 1.35     | 02 ± 0.23           |

Figure 6. Comparative lymphatic targeting efficiency of Linezolid to lymph node, spleen, and thymus from the encapsulated W/O nanoemulsion formulation.

4. CONCLUSION

The combined results suggest that the developed W/O nanoemulsion of linezolid is capable of reaching to the lymph nodes through the lymphatic transport after oral administration. Characterization of the formulation indicates that the nanoemulsion was successfully developed with desirable attributes suitable for lymphatic targeting. Further, antimicrobial efficacy study supports the potential of nanoemulsion to kill *Mycobacterium smegmatis*. Finally, it can be concluded that the optimized linezolid W/O nanoemulsion can be a promising approach for lymphatic targeting and a better management option for lymphatic tuberculosis.

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References
(1) Mundayoor, S.; Shinnick, T. M. Identification of genes involved in the resistance of mycobacteria to killing by macrophages. *Ann. N.Y. Acad. Sci.* 1994, 730, 26–36.

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A.C. and P.J. have contributed equally in this research and are first authors. Conceptualization, Methodology, Investigation, Data curation, A.C. and P.J.; Software, Validation, Writing—original draft, A.C. and P.J.; Writing—review and editing, A.C. and P.J.; Formal analysis, S.M.; Resources, G.M., M.J.; Supervision, Z.I.; Project administration, A.M. and Z.I. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

Author Contributions
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(2) Brisson-Noël, A.; Lecossier, D.; Nassif, X.; Gicquel, B.; Lévy-Frebault, V.; Hance, A. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. *The Lancet* 1989, 334 (8671), 1069–1071.

(3) Getahun, H.; Matteelli, A.; Chaisson, R. E.; Raviglione, M. Latent Mycobacterium tuberculosis infection. *NEJM*. 2015, 372 (22), 2127–2135.

(4) Lerner, T. R.; de Souza Carvalho-Wodarcz; Repnik, U.; Russell, M. R.; Borel, S.; Diedrich, C. R.; Rohde, M.; Wainwright, H.; Collinson, L. M.; Wilkinson, R. J.; Griffiths, G.; et al. Lymphatic endothelial cells are a replicative niche for *Mycobacterium tuberculosis*. *J. Clin. Investig.* 2016, 126 (3), 1093–1108.

(5) Livermore, D. M. Linezolid in vitro, mechanism and antibacterial spectrum. *J. Antimicrob. Chemother.* 2003, 51, 9ii–16.

(6) Bansal, T.; Mustafa, G.; Khan, Z. I.; Ahmad, F. J.; Khar, R. K.; Talegaonkar, S. Solid Self-noemulsifyingdiverity system. a platform technology for formulation of poorly soluble drug. *Crit Rev. Tier Drug CarSyst.* 2008, 25 (1), 63–116.

(7) Rani, N. P.; Suriyaparaksh, T.; Senthumarai, R. Formulation and evaluation of rifampicin and gatifloxacin niosomes on logarithmic-phase cultures of *Mycobacterium tuberculosis*. *Int. J. Pharma Bi Sci.* 2010, 1 (4), 379–387.

(8) Saifulullah, B.; Arulselvan, P.; El Zowalaty, M. E.; Fukurazi, S.; Webster, T. J.; Gellich, B.; Hussein, M. Z. Development of a highly biocompatible antituberculosis nanodelivery formulation based on para-aminosalicylic acid—zinc layered hydroxide nanocomposites. *Sci. World J.* 2014, 2014, 401460.

(9) Zhou, A.; Lu, T.; Wang, L.; Lu, C.; Wang, L.; Wan, M.; Wu, H. Lymphatic transport of puerarin occurs after oral administration of different lipid-based formulations to unconscious lymph duct-cannulated rats. *Pharm. Dev Technol.* 2014, 19 (6), 743–747.

(10) Wais, M.; Azil, M.; Goswami, P.; Agnihotri, J.; Nadeem, S. Nanoemulsion-based transdermal drug delivery system for the treatment of tuberculosis. *Recent Pat Antinfact Drug Discovery* 2018, 12 (2), 107–119.

(11) Patel, P. A.; Chaulang, G. M.; Akolkotkar, A.; Mutha, S. S.; Hardikar, S. R.; Bhosle, A. V. Self emulsifying drug delivery system, A review. *Res. J. Pharm. Technol.* 2008, 1 (4), 313–323.

(12) Shakeel, F.; Ramadan, W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. *Colloids Surf., B* 2010, 75 (1), 356–362.

(13) Mustafa, G.; Baboota, S.; Ali, J.; Kumar, N.; Singh, T.; Bhattacharyya, A.; Ahuja, A. Effect of homogenization on the fate of true nanoemulsion in brain translocation, A gamma scintigraphic evaluation. *Sci. Adv. Material.* 2012, 4 (7), 739–748.

(14) Sigward, E.; Mignet, N.; Rat, P.; Dutot, M.; Muhamed, S.; Guigner, J. M.; Scherman, D.; Brossard, D.; Crauste-Manciet, S. Formulation and cytotoxicity evaluation of new self-emulsifying multiple W/O/W nanoemulsions. *Int. J. Nanomed* 2013, 8, 611–625.

(15) Jang, J. H.; Jeong, S. H.; Lee, Y. B. Enhanced Lymphatic Delivery of Methotrexate Using W/O/W Nanoemulsion, In Vitro Characterization and Pharmacokinetic Study. *Pharmaceutics* 2020, 12, 978.

(16) Shafiq, S.; Shakeel, F.; Talegaonkar, S.; Ahmad, F. J.; Khan, Z. I.; Ali, M. Development and bioavailability assessment of ramipril nanoemulsion formulation. *Eur. J. Pharm. Biopharm* 2007, 66 (2), 227–243.

(17) Shafiq, S.; Shakeel, F.; Talegaonkar, S.; Ahmad, F. J.; Khan, Z. I.; Ali, M. Design and development of oral oil in water ramipril nanoemulsion formulation, in vitro and in vivo assessment. *J. Biomed. Nanotechnol.* 2007, 3 (1), 28–44.

(18) Talegaonkar, S.; Khan, Z. I.; Ahmad, F. J. Formulation and development of nanoemulsion bearing atorvastatin calcium, in vitro evaluation. *J. Diapers Sci. Technol.* 2009, 30, 1–12.

(19) Shafiq-un-Nabi, S.; Shakeel, F.; Talegaonkar, S.; Ali, J.; Baboota, S.; Ahuja, A.; Khan, R. K.; Ali, M. Formulation development and optimization using nanoemulsion technique, a technical note. *AAPS pharmaceutich.* 2007, 8 (2), E12–7.