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Original Research Article

Formulation of Lecithin-Based Nanocapsules of Levofloxacin and *In Vitro* Evaluation of the Anti-Mycobacterium Potentials

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. Multidrug resistant TB (MDR-TB) remains a public health crisis. The aim of this study was to formulate and evaluate the anti-mycobacterium activity of levofloxacin (LVF) nano formulations against mycobacterium isolates. A 10 g quantity of the lecithin powder was placed in a beaker and 50 mL quantity of water added and heated on a water bath at 55ºC for 30 minutes. The oil and water phases were separated by centrifugation at 3000 rpm for 30 minutes. The gum/crude lecithin was dried in vacuum oven for 1 hour at 40 ºC. The solvent and lecithin were separated by decantation. The acetone was removed by heating at low temperature at 40 ºC and the powdered lecithin was packaged in screw-capped containers until further use. The percentage yield of the extracted lecithin ranged from 31.0±0.31% to 35.0±0.32%. The differential scanning calorimetry (DSC) thermograph of pure LVF showed two sharp endothermic peaks at 225.7ºC and 227.8ºC. The drug content of levofloxacin formulation using extracted lecithin (LEL) and levofloxacin formulation using reference lecithin (LRL) ranged from 96.9±0.17% to 98.6±0.12% respectively. The LVF nano capsules had activities against the mycobacterial isolates with minimum inhibitory concentrations (MICs) of 26.9µg/mL for LEL and 58.3µg/mL for LRL. The chitosan-fortified nano capsule formulation of LVF has potentials for further exploration and development for enhanced bioavailability and application against MDR-TB.

Keywords: Lecithin, Levofloxacin, Multi-drug resistance, Nano capsule, Tuberculosis.

Introduction

Tuberculosis (TB) is a well-known chronic infectious disease caused by the bacterium, *Mycobacterium tuberculosis*.[1] The human organ that is mostly affected is the lungs. It spreads through the air when people with TB cough, sneeze or spit.[2] According to World Health Organization (WHO), about a quarter of the human population is estimated to have been infected with this causative agent, *M. tuberculosis*.[3] An estimate of 5-10% of people who get infected with TB usually develop this disease. This chronic infection occurs when bacteria multiply in the body, which could also affect different organs.[4] Common symptoms associated with TB include: cough (which could persist for months), chest pain, weakness, fatigue, weight loss and night sweats.[4] According to a recent report from WHO, a total of 1.25 million people died from tuberculosis in 2023.[4] An estimated 10.8 million people fell ill with TB worldwide including 6.0 million men, 3.6 million women and 1.3 million children. Multidrug resistant TB (MDR-TB) remains a public health crisis and also a health security threat. One of the health targets of the United Nations Sustainability Development Goals (SDGs) is to end the TB epidemic by 2030.[4]

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MDR and extensive drug resistant (XDR-TB) regimens that are commonly used in the treatment of tuberculosis are generally toxic, expensive and also have longer duration of action.[5-9]

Levofloxacin (LVF) is a broad-spectrum antibiotic that belongs to the fluroquinolone group.[10] Its mechanism of action is based on its ability to inhibit topoisomerase II, topoisomerase IV and deoxyribonucleic acid (DNA) gyrase. These enzymes are required for DNA replication, transcription, repair and recombination.[11] Encapsulation of LVF in the proposed nano formulation is hoped to offer minimized dose and also help to reduce some of the adverse effects associated with the drug.[12] The emergence of multi-drug resistant TB (MDR-TB) which is resistance to at least levofloxacin is of great concern.[12] The issue of resistance is very common especially with the first-class and second-class anti TB-drugs. The increase in the occurrence of MDR-TB, necessitates the use of nano-technological approach, such as encapsulation of levofloxacin in lower doses than the existing form for effective drug delivery. The present study aimed to formulate and evaluate chitosan-fortified, lecithin-based nano capsules, containing lower doses of levofloxacin for enhanced bioavailability and therapeutic application in TB. The main specific objectives, were to develop low dose chitosan-fortified, lecithin-based nano capsule formulations containing 200 mg LVF, to characterize some physic-chemical properties of the LVF nano capsules and to evaluate the anti-mycobacterium activity of LVF nano formulations against mycobacterium isolates.

Materials and Methods

Materials

Soybean was obtained from Nsukka main market, Enugu State, Nigeria in January, 2019. Pure sample of LVF was obtained from Teva Pharmaceuticals, USA. Hexane (Fuji Chemical Industry, Co Ltd., Japan, 98% purity, 88.4 ppm), Acetone (Naphtha Pharmaceuticals USA, 99.9% purity, 750 ppm), Ethanol (Lucas Oil Products, Inc. USA, 95.6% purity, 2% concentration), Sodium Chloride (ProChem Inc, USA, 99.0% purity), Hydrogen Peroxide (ChemQuest International Inc. USA, 96% purity), Distilled Water (STC, UNN), Chloroform (Sigma-Aldrich), Lipoid® S 75 (reference lecithin) (70 % phosphatidylcholine), was obtained from (Lipoid GMBH, Germany), Neusilin® (magnesium alumino-metasilicate) (Fuji Chemical Industry, Co Ltd., Japan), Labrasol® (PEG-8 caprylic/capric glyceride), (Gattefosse, Saint-Priest Cedex, France). All other reagents and solvents used were analytical grade.

Equipment

Homogenizer (17879 Silverson Machines Ltd., England), pH meter (6305, Jenway, USA), UV-Vis Spectrophotometer (1800, Shimadzu, Germany), Fourier Transform Infra-Red (FTIR) spectrometer (Shimadzu, Japan), electronic weighing balance (20433, Ohaus, China), magnetic stirrer (Jenway magnetic stirrer plate 400), Optical microscope (Hund, Wettzlar, Germany), motic camera (Moticam 2.0 MP CMOS, China).

Method

Sourcing and preparation of Soy flour

Soybeans was purchased from Nsukka main market, 6.842942ºN latitude and 7.373266ºE longitude, Enugu State Nigeria in January, 2019. A herbarium Voucher Number of PCG/UNN/0313 *Glycine max* (L) was assigned. The soybean nuts were subsequently cracked, (PM and T Grinding Machine, Japan), followed by processing in a grain mill, and resulting flour sieved (Sieve no. 4).

Extraction of lecithin from soybeans

Lecithin extraction was carried out according to the aqueous degumming method reported by Eshratabadi, with slight modifications.[13] A 10.0 g quantity of the powder (flour) was placed in a beaker and 50 mL quantity of water added, and heated on a water bath at 55 ºC for 30 minutes. The oil and water phases were separated by centrifugation (SM800B-Uniscope, England), at 3000 rpm for 30 minutes. The gum/crude lecithin which was formed in the lower layer was then dried in vacuum oven (TF-P29, Thermo Fisher, Japan) for 1 hour at 40 ºC.[13] The process was repeated 5 times to get sufficient quantities of lecithin. The extract was subjected to purification in order to reduce neutral oil from crude lecithin. This was done by treating the oven-dried (TF-P29, Thermo Fisher, Japan) crude lecithin with acetone in the ratio of 1:6 (w/v), respectively and the mixture stirred for 1 hour. The solvent and lecithin were separated by decantation and the treatment was repeated until the solvent became colorless. The acetone was then removed by heating at low temperature using the magnetic stirrer (Jenway magnetic stirrer plate 400), at 40 ºC and the powdered lecithin obtained was packaged into sealed nylon bags in screw-capped containers until further use.

Characterization of the lecithin extract

The characterization of the soy lecithin extract was done by studying the physic-chemical characteristics such as colour, odour, taste and appearance. Other official tests such as acid value, saponification value, peroxide value, iodine value and free fatty acid content were carried out. The tests were done for both the extracted and the reference lecithin.[14]

Organoleptic evaluation

The colour was observed with the naked eye, while the odour was observed with the nose when the container was opened to detect the smell. The taste was observed by placing a little portion of the lecithin on the tongue, while the appearance was assessed by observing and feeling a little portion of the sample placed on the fingers.

Physico-chemical properties of extracted and reference lecithin

Determination of free fatty acid (FFA)

A (10.0 g) quantity of lecithin (extracted and reference) samples were respectively weighed into a 250 cm^3 flask followed by the addition of alcohol (ethanol, 20 mL). The mixture was boiled on a hot plate, until all the oil dissolved completely and phenolphthalein indicator (3 drops) was added. The solution was titrated with 0.1 M sodium hydroxide until a faint pink end point was observed and the titer value (T) recorded. The percentage FFA of the sample was calculated using the equation 1:[14]

$$\% \text{ FFA (as oleic acid)} = \frac{T \times M \times 28.2}{W} \quad (1)$$

T = volume of NaOH used (mL), M = molarity of sodium hydroxide used (40 M), W = weight of sample used (g), 28.2 = constant used for calculating oleic acid

Determination of acid value

The same procedure was repeated as that of the free fatty acid. But the equation 2 used was thus: $AV = \frac{56.1 \times M \times T}{W}$ (2)

AV = acid value, T = volume of sodium hydroxide used (mL), M = molarity of sodium hydroxide used (M), W= weight of sample used (g), 56.1 = molecular weight of potassium hydroxide.[14]

Determination of saponification value

The lecithin (extracted and reference) samples (4.0 g) were respectively weighed into a conical flask (702101, STEMart, Japan) and to this, was added 0.5 M KOH. The mixture was then heated using the magnetic stirrer (Jenway magnetic stirrer plate 400, China), to saponify the fat or oil. The unreacted KOH was then back titrated with 0.5 M HCl using phenolphthalein as indicator. A blank sample was also prepared and back titrated accordingly. The sample and blank titers (V_1 and V_2) were recorded. The experiment was repeated thrice. The saponification values of the samples were then calculated using equation 3.[14,15]

$$SV = \frac{[(V_2 - V_1) \times M \times 56.1]}{W} \quad (3)$$

Where SV = saponification value, V_1 = volume of HCl used for the sample (mL), V_2 = volume of HCl used for the blank (mL), M = molarity of HCl, W = weight of sample used (g), 56.1 = molecular weight of KOH.

Determination of peroxide value.

To a weighed sample (1.0 g) in a conical flask (702101, STEMart, Japan), was added powdered potassium iodide (1.0 g) and solvent mixture (2:1 glacial acetic acid: chloroform, v/v). The resulting solution was then placed on a water bath to dissolve properly and 5% potassium iodide (20 mL) was then added. The sample solution was then titrated with 0.002 N sodium thiosulphate solution using starch as indicator.[14,15] The experiment was repeated in triplicates. The peroxide values of the samples were calculated using the equation 4:

$$PV = 2 \times V \quad (4)$$

Where PV = peroxide value, V = volume of sodium thiosulphate used (ml), 2 = (N x 1000)/W, N = normality of sodium thiosulphate used (N), W = weight of samples used (g).

Determination of iodine value

A weighed quantity (0.1 g) of the sample was added to a 300 mL conical flask (702101, STEMart, Japan). A 20.0 mL carbon tetrachloride was added and sealed. A 25.0 mL Hanus solution (a mixture of iodine monobromide in glacial acetic acid) was added and sealed, then shook for 1 minute. It was sealed and left in a dark room for 30 minutes. 10.0 mL of 15% potassium iodide and 100 mL water was added and sealed, and then agitated for 30 seconds.[14,15] It was then titrated with 0.1 mol/L sodium thiosulphate and the titre used to calculate the iodine value using equation 5. The sample results (V_1) and that of the blank (V_2) were recorded. The experiment was repeated thrice:

$$IV = \frac{[(V_2 - V_1) \times M \times 12.7]}{W} \quad (5)$$

Where IV = iodine value, V_1 = volume of sodium thiosulphate used for the sample (mL), V_2 = volume of sodium thiosulphate used for the blank (mL), M = molarity of sodium thiosulphate used (M), W = weight of sample used (g), 12.7 = constant used to convert from milli-equivalent thiosulphate to gram (mol. weight of iodine, 126.9).

Determination of moisture content

The moisture content was determined using the Mettler Toledo moisturizer (MS 12001L, Bio Techno Lab, Mumbai). The moisturizer was set at 105 ºC for 30 minutes. To a tarred aluminum dish in the moisturizer, weighed quantity (5.0 g) was placed and the machine closed and started automatically.[16] After 30 minutes, the result was read off the print out screen as it appears in percentage. it was calculated using equation 6:

$$\text{Moisture content} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (6)$$

Determination of calibration curve of levofloxacin

Stock solution of LVF (1 mg/mL) was prepared by dissolving 100 mg of drug in 100 mL phosphate buffer pH 6.8 in 100 mL volumetric flask (to get 1000 µg/mL drug solution) with vigorous shaking and sonicated for about 10 minutes and this served as the first stock solution. 10 mL of first stock solution was further diluted to 100 mL with phosphate buffer pH 6.8 (to get a stock solution containing 100 µg/mL of drug) (stock solution 2). The stock solution was then filtered through Whatman® filter paper no 1. The respective samples in each test tube were added phosphate buffer (pH 6.8) to make total volume of 10 ml to produce (0.5, 1.0, 1.5, 2.0, 2.5 µg/mL) respectively. The absorbance of solutions of pure LVF was measured at 280 nm using the UV-spectrophotometer (Spectrumlab 752S, Hitachi, Japan) and a calibration curve was plotted between concentration of drug (µg/ml) on x-axis v/s absorbance on y-axis to get the linearity and regression equation.[17]

Formular development

Experimental design by the response surface, randomized, central composite design (CCD) using Design Expert® version 13 (Stat-Ease Inc., Minneapolis) was deployed in the development of the formular.[18] Nine (9) runs were performed at three different stirring rates of 10,000, 15,000 and 20,000 rpm, respectively (totaling 27 runs (1 axial point + 1 center point)) were performed for a face centered CCD ($\alpha = 1$) that employed 2 independent numeric factors, namely drug-lecithin combination mass ratio (X_1) and chitosan concentration (X_2), and 1 categoric factor (stirring rate, at levels 3). Two (2) dependent factors, namely particle size (Y_1) and entrapment efficiency (EE) (Y_2) were considered as the responses for the optimal formular selection.[19] The polynomial regression equations in the following form were used to express the influence of the independent variables (X_1 and X_2) on the selected responses in the design as shown in Table 1:

$$Y = ß_o + ß_1X_1 + ß_2X_2 + ß_3X_1X_2 + ß_4X_1^2 + ß_5X_2^2$$

where Y = response/dependent variable size or EE (%)

$ß_o$ = intercept representing the arithmetic mean of all quantitative outcomes of twenty-seven runs

$ß_1$ to $ß_5$ = Coefficients computed from the observed experimental values of Y

X_1 and X_2 = Coded levels of factors or independent variables

X_1X_2 = Factors interaction

X_1^2 and X_2^2 = Quadratic relationship terms.

The design for optimization of the nano capsule formulations at 10,000, 15,000 and 20,000 rpm are shown in Table 1. The estimations for the vesicle size and the EE were set at the ranges of 25 to 85 nm and 90 to 100%, respectively.

Preparation of nanoparticles of LVF

The nanoparticles of LVF were prepared by the mechanical dispersion method, (Table 2).[20] The selection was made based on the confirmation location on the response surface (Batch 5, Table 2) which corresponded to run 17 on the design. LVF, lecithin and cholesterol were mixed and dissolved in absolute ethanol (Lucas Oil Products, Inc. USA, 95.6% purity, 2% concentration), in a beaker. Chitosan was dispersed in 100 ml of acetic acid (Merck, 0.2 % concentration) solution in distilled water overnight. Sodium tripolyphosphate (STPP) was then dissolved in 10 mL of distilled water and added to the chitosan dispersion and stirred using magnetic stirrer (Jenway magnetic stirrer plate 400), at 100 rpm for 30 minutes. The chitosan/STPP solution was then added to the organic solution in drops using a syringe and stirred at 10,000 rpm for 45 minutes using Ultra-turax (IKA® T25 digital, Germany). Subsequently, the precipitate formed was collected after 2-3 hours by centrifugation at 4,000 rpm. The nanoparticles obtained were adsorbed by mixing with Neusilin® (Fuji Chemical Industry, Japan) to form powdered products which were packaged in screw-capped containers until further use. Table 3 shows the composition of the optimized formulations.

Characterization of nanocapsule formulation

Differential scanning calorimetry (DSC)

The compatibility of LVF, lecithin, chitosan, and other excipients was studied using a Differential Scanning Calorimeters (MicroCal PEAQ-DSC, Malvern Panalytical, Japan). Each sample or mixtures of samples (5.0 mg) was placed in sealed aluminum pans and scanned at heating rate of 10 ºC/minutes over temperature range of 30-300 ºC. A standard was placed in the reference pan. Baselines were determined using an empty pan and all the thermograms were baseline corrected.[20,21]

Fourier Transform Infrared spectroscopy (FTIR) test for optimized formulations.

Analysis of samples of the ingredients was carried out for qualitative compound identification using FTIR spectroscopy (IR Tracer-100, Jasco Corporation Luigi, Europe). The potassium bromide (KBr) pellet of approximately 1 mm diameter of the drug or drug-excipient mixture was prepared by grinding 3-5 mg of sample with 100 -150 mg of KBr in pressure compression machine. The drug-KBr/drug or excipient-KBr compact was then subjected to FTIR spectroscopy.[21]

Drug entrapment efficiency (EE)

The indirect method was adopted in calculating the entrapment efficiency of the nanoparticles.[22] A 100 mg quantity of the sample formulation (nanoparticles) was dissolved in 100 mL of phosphate buffer solution (PBS) (pH 6.8) and stirred at 100 rpm. The sample was further centrifuged at 10,000 rpm for 30 minutes. Subsequently, 1 mL of propan-2-ol was added to the supernatant liquid and shaken for 10 minutes. Then 1 mL was removed from the supernatant liquid and diluted to 10 mL using PBS (pH 6.8). The solution was then filtered using a 0.45 µm pore size (Advantech MFS, Germany) and analyzed spectrophotometrically at 280 nm for LVF and the EE calculated using the equation 7:

$$EE = \frac{\text{total amount of drug incorporated in formulation} - \text{free drug in supernatant}}{\text{total amount of drug}} \times 100 \quad (7)$$

Drug Loading Capacity (LC)

LC expresses the ratio between the entrapped active pharmaceutical ingredient (API) and the total weight of the lipids. LC was determined using the equation 8:

$$L\,C = \frac{\text{amount of drug in supernatant}}{\text{total amount of lipid matrix used in formulation}} \times 100 \quad (8)$$

Percentage yield

The nanoparticles from each batch were weighed to get the yield of nanoparticles formulated. The percentage yield was calculated using the equation 9:

$$\text{Percentage yield} = \frac{\text{weight or mass of product obtained}}{\text{total weight or mass of ingredients}} \times 100 \quad (9)$$

Table 1: Design for optimization of the nano capsule formulations at 10,000, 15,000 and 20,000 rpm

Run (Batch)	Factors (independent variables)		Responses (Dependent variables)	
	X_1 (mg)	X_2 (mg)	Vesicle size (nm)	EE (%)
1	0	0	85	100
2	-1	1	78	90
3	-1	1	74	98
4	-1	-1	73	99
5	0	-1	70	95
6	1	1	60	98
7	1	1	65	97
8	1	0	67	98
9	1	0	68	96
10	0	1	70	97
11	-1	0	64	98
12	-1	0	65	96
13	-1	1	55	95
14	1	0	50	95
15	-1	0	55	97
16	0	0	40	99
17	0	0	45	100
18	0	1	40	98
19	-1	-1	55	97
20	1	-1	50	98
21	1	1	55	99
22	0	-1	45	95
23	1	-1	50	97
24	1	-1	55	98
25	0	1	24	99
26	-1	-1	24	96
27	0	-1	28	95

X_1 = Drug-lecithin combination mass ratio, X_2 = Chitosan concentration, -1 = low, 0 = medium, 1 = high

Table 2: Composition for preformulation studies

Batch	Ingredients						
	Levofloxacin (mg)	**Lecithin® (g)**	**Cholesterol (%)**	**Ethanol (ml)**	**Chitosan (%)**	**STPP (%)**	**Acetic acid in distilled water qs (ml)**
1	200.0	0.05	0.1	10.0	0.1	0.1	100.0
2	200.0	1.0	0.1	10.0	0.1	0.1	100.0
3	200.0	1.5	0.1	10.0	0.1	0.1	100.0
4	200.0	0.05	0.1	10.0	0.2	0.1	100.0
5*	200.0	1.0	0.1	10.0	0.2	0.1	100.0
6	200.0	2.0	0.1	10.0	0.2	0.1	100.0
7	200.0	1.0	0.1	10.0	0.4	0.1	100.0
8	200.0	1.5	0.1	10.0	0.4	0.1	100.0
9	200.0	2.0	0.1	10.0	0.4	0.1	100.0

STPP = Sodium tripolyphosphate, * The selected formular.

Table 3: Composition for the preparation of optimized LVF nanoparticles

Batch	Ingredients						
	Levofloxacin (mg)	**Lecithin® (g)**	**Cholesterol (mg)**	**Ethanol (ml)**	**Chitosan (%)**	**STPP (%)**	**Acetic acid in DW qs (ml)**
LEL	200.0	1.0	0.1	10.0	0.1	0.1	100.0
LRL	200.0	1.0	0.1	10.0	0.1	0.1	100.0

LEL: Formulation of LVF using extracted lecithin, LRL: Formulation of LVF using reference lecithin, DW: Distilled water, STPP: Sodium tripolyphosphate.

Determination of surface morphology using scanning electron microscope (SEM)

Shape and surface morphology of LVF nanoparticles was studied using scanning electron microscopy (SEM) (Jeol USA, Model JSM-7900F). For shape and surface morphology, the nanoparticles were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument in order to neutralize the charging effects before scanning in SEM with an acceleration voltage of 20 KV.[22]

Particle size and polydispersity index (PDI)

The mean diameter and polydispersity index of LVF nanoparticles were also measured using a Zeta sizer (Nano-ZS, Hitachi, Japan). All the samples were diluted with a fixed amount of double distilled water to obtain a suitable scattering intensity, before photon correlation spectroscopic (PCS) analysis.[22]

Formulation of nano capsules

LVF nano capsules were prepared by using enteric coated capsules (entrinsic, Capsugel®, Germany). Each empty capsule shell was weighed using the electronic weighing balance (120-5DM, S. Mettler, Germany). A total of 60 capsules containing 200 mg of LVF were prepared. The formulated nanoparticles powder was filled into the capsule shell. After filling the capsule shell, the cap was used to close the shell. The weight of the capsule and powder were determined.[23] The weight of powder to be filled in the capsule was determined using the equation 10:

Weight of powder to be filled in the capsule = Tapped-Bulk density x Volume of the capsule. (10)

Physic-chemical properties of nano capsules

Uniformity of weight of nano capsules

Twenty (20) capsules were randomly selected from each batch. Using the analytical balance (120-5DM, S. Mettler, Germany), the 20 capsules were weighed together. The mean capsule weight was then calculated. Subsequently the capsules were weighed individually and the weights of the capsules recorded. The variations of individual capsule weights from the mean weight were determined, and the percentage deviations calculated.[24]

Drug content test for nano capsules

One (1) capsule from the optimized formulation was carefully opened, and the content poured into a beaker. The powder was dissolved in 50 mL of distilled water and filtered into a beaker. A 1 mL quantity of the filtrate was measured and transferred into a 100 mL volumetric flask which was made up to 100 mL mark with distilled water. 10 mL of the resulting solution was collected and put in a clean and dry test tube. The sample was analyzed using the spectrophotometer (Spectumlab 752S, Hitachi, Japan), at 280 nm for LVF.[25]

In vitro dissolution studies

The *in vitro* drug release studies were carried out using tablet dissolution test apparatus (Erweka DT-D, Heusens-tamm, Germany).[26] Initially, 900 mL of 0.1 N HCl at (pH1.2) was used as the dissolution medium for 2 hours at 50 rpm, maintained at 37±1.0 ºC. Samples were withdrawn at 5, 15, 30, 45, 60, 120 minutes in 0.1 N HCl. The dissolution medium was then changed to 900 mL of PBS (pH 6.8) and samples were withdrawn at 3, 4, 6, 7, 8, hours intervals. The samples were appropriately diluted with PBS (pH 6.8) and assayed spectrophotometrically at 280 nm for LVF.

In vitro drug release kinetics

Various kinetic models were used to describe the *in vitro* release kinetics and mechanisms of drug release from the nanoparticles.[27] The zero-order kinetics explains the systems where the drug release rate is independent of its concentration (eqn. 11). The first order kinetics is used to describe the release from systems where the release rate is dependent on concentration (eqn. 12). Higuchi model describes the release of drugs from the insoluble matrix as a square root of time (eqn. 13). Korsmeyer is used to describe the drug release from a polymeric system (eqn. 14):

$C_o - C_t = K_o t$

$C_t = C_o + K_o t$ (11)

C_t is the amount of drug released at time t,

C_o is the initial concentration of drug at time t = 0,

K_o is the zero-order rate constant.

$\text{Log } C = \log C_o - K_1 t/2.303$ (12)

K_t is the first order rate equation expressed in time $^{-1}$ or per hour,

C_o is the initial concentration of the drug, C is the percent of drug remaining at time t

$$ft = Q = KH \cdot t_{1/2} \quad (13)$$

where, Q is the amount of drug released in time t per unit area, KH is the Higuchi dissolution constant

$$Mt / M\infty = K_{kp}t^n \quad (14)$$

where, Mt / M∞ is a fraction of drug released at time t, $K_{kp}$is the Korsmeyer release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices and the value of n characterizes the release mechanism of drug.

Antimycobacterial activity of the optimized formulations

The antimycobacterial activity test of the formulations was carried out using Tetrazolium (MTT) dye assay of micro broth dilution technique.[28, 29] Each formulated capsule was dissolved in a solution: 1 mL dimethyl sulfoxide (DMSO) (Invitrogen™, Chemical Company, Jamestown) in 9 mL sterile water (1:10 dilution), and further diluted 1:10 in 7H9 Middle brook broth to give the following final concentrations:

Sample A: LEL 1723 µg/mL;
Sample B: LRL 1867 µg/mL;
Sample C: Reference levofloxacin tablet 50 µg/mL;
Sample D: pure powder of levofloxacin 30 µg/mL (prepared by dissolving 300 mg of levofloxacin in 10 mL DMSO solution and diluted in 1:1000 by dispensing 30 µL of the levofloxacin solution in 30 ml 7H9 Middle-brook broth).

A 100 µL quantity of each sample was transferred to the first row of micro well plate (96 micro titer plate). A 50 µL of 7H9 Middle-brook broth supplemented with albumin dextrose complex (ADC) was transferred to the 2nd row of the micro wells. Then, 50 µL of test solution was transferred from the 1st well to the 2nd well, mixed thoroughly by pipetting up and down four times, and the process continued to well 11 from which 50 µL was withdrawn and discarded in order to maintain equal volumes and concentrations across the wells. A 50 µL of diluted culture of *Mycobacterium bovis* (BCG) and *Mycobacterium smegmatis*, was added to all the wells of the micro well plate, respectively, and incubated at 37 ºC for 7 days. Post incubation, 20 µL of tetrazolium salt dye was added to all the wells and allowed to incubate for 2 hours.

Data analysis

All the measurements were repeated in triplicates and the data obtained analyzed by Student *t*-test and One-Way Analysis of Variance (ANOVA). Statistical analysis was performed using Statistical Product and Services Solution software (SPSS, version 22.0 Inc., Chicago IL, USA) and Excel Microsoft Office version 2012. The results were presented as mean ± SD, and statistical differences between means considered significant at ($p < 0.05$).

Results and Discussion

Percentage yield of the extracted lecithin

The percentage yield of the extracted lecithin ranged from 31.0±0.31% to 35.0±0.32% without any significant difference ($p< 0.05$) as shown in Table 4. The lecithin was extracted up to 5 different times using the same methodology in order to get sufficient quantities of lecithin.

Organoleptic properties of extracted and reference lecithin

The organoleptic properties of both extracted and reference lecithin are displayed in Table 5. The extracted lecithin had a brownish-yellow colour and an appearance of fine to granular powder, while the reference lecithin was yellow in colour and sticky in nature. Both the extracted and reference lecithin were tasteless.

Physic-chemical and micromeritic properties of extracted and reference lecithin

Table 6 shows the physic-chemical properties of both the reference (Lipoid® S 75) and extracted lecithin. The moisture content determination of lecithin is very critical to its fluidity.[17,18] It is usually dried to a low moisture content of < 1 % in order to improve its 'keep-ability' and fluidity. From the results obtained, the reference lecithin had a moisture content of 0.05±0.01% and the extracted lecithin recorded 0.09±0.02%. Although the values obtained for the two batches were < 1 %, it showed that they were within the acceptable standard range for lecithin.[18] The extracted lecithin recorded a higher value of moisture content than the reference lecithin, without a significant difference ($p< 0.05$).

The free fatty acid (FFA), is used to determine the suitability of the oil for edible purposes.[18] A high percentage of FFA (above 1.5 %) is a determination of indication of unsuitability of the oil. The FFA of the extracted lecithin was 3.38±0.14%, while that of the reference was 4.12±0.21%. The extracted lecithin had a higher value of FFA than the reference lecithin without a significant difference ($p < 0.05$).

The saponification value is the number of milligrams of potassium hydroxide required to saponify completely 1 g of fat or oil.[19] It is used to indicate the presence of low or high proportion of lower fatty acids. The extracted lecithin had a higher value of saponification (43.20±6.80 mgKOH/g) compared to the reference (40.10±6.50 mgKOH/g) without a significant difference ($p< 0.05$).

The iodine value is used to determine the rancidity by oxidation of the oil.[19] The higher the iodine value, the greater is the liability of the oil or fat to become rancid by oxidation. The extracted lecithin had an iodine value of 102.40±0.65 mgI/g, while the reference had 104.50±1.07 mgI/g. The low iodine value of the extracted lecithin could be due to oxidation of polyunsaturated fatty acid during long isolation process.[19] The reference lecithin had a higher value of iodine than the extracted lecithin without a significant difference ($p< 0.05$).

The acid value is the measurement of free fatty acids present in the oil or fat.[19] The reference lecithin recorded 10.40±0.23 mgKOH/g, while the extracted recorded 10.50±0.30 mg/KOH/g. This showed that the extracted lecithin had a higher percentage of free fatty acid, compared to the reference. The acid value of food grade lecithin recommended by FAO/WHO are found to be up to 36 mg KOH/g of lecithin.[20] The extracted lecithin had a higher acid value than the reference, without a significant difference ($p < 0.05$).

Table 4: Percentage yield of the extracted lecithin (mean ± SD)

No. of times extracted	Yield (g)	Yield (%)
1	3.50	35.0 ± 0.32
2	3.30	33.0 ± 0.18
3	3.40	34.0 ± 0.36
4	3.10	31.0 ± 0.31
5	3.20	32.0 ± 0.30

Table 5: `Organoleptic properties of extracted and reference lecithin.

Organoleptic property	Extracted lecithin	Reference lecithin (Lipoid® S 75)
Colour	brownish-yellow	Yellow
Odour	Odourless	Odourless
Taste	Tasteless	Tasteless
Appearance	Fine to granular powder	`Sticky

Table 6: Physic-chemical properties of reference and extracted lecithin (mean ± SD).

Property	Reference Lecithin (Lipoid® S 75)	Extracted Lecithin
Moisture content	0.05 ± 0.01	0.09 ± 0.02
Free fatty acid	3.38 ± 0.14	4.12 ± 0.21
Saponification value	40.10 ± 6.50	43.20 ± 6.80
Acid value	10.40 ± 0.23	10.50 ± 0.30
Peroxide value	9.80 ± 0.31	10.30 ± 0.31
Iodine value	102.40 ± 0.65	104.50 ± 1.07

Preformulation studies
Formular development
The actual quantities of the variables for the CCD for each batch and the responses are shown in Table 7. The responses obtained ranged from 31.1 to 128.3 nm and 36.1 to 86.0 % for the vesicle size and EE, respectively. Table 8 represents the responses obtained by analysis of variance (ANOVA) at 95% confidence interval and fitted to statistical models using the Design Expert®. Table 9 represents relevant parameters, and confirmation of point parameters for vesicle size (nm) and EE (%). Fig. 1 represents the 3D surface plot for encapsulation efficiency. It was observed that the best-fitted was the quadratic model. The coded polynomial equation obtained for the vesicle size was:

$V = + 55.00 + 0.3190A + 3.25B + (-0.8148) C_1 + (-7.93) C_2 + (-3.50) AB + 1.53AC_1 + 1.34AC_2 + (-7.52) BC_1 + 4.22BC_2 + 4.62A^2 + (-3.71) B^2$

while that of the entrapment efficiency was:

$EE = 99.67 + 0.1738A + 0.4053B + 0.1111C_1 + 0.0000C_2 + 0.4167AB + 0.8047AC_1 + (-0.5791) AC_2 + 0.0000BC_1 + 0.4268BC_2 + (-1.33) A^2 + (-1.42) B^2$.

A = Drug-lecithin combination mass ratio, B = Chitosan concentration (mg), C = Stirring rate (rpm).

Table 7: Actual quantities for the optimization of the nano capsule formulations

Runs	Independent Factors				Responses	
	Actual quantities for X_1 (mg)	X_1	X_2 (mg)	Stirring rate (rpm)	Vesicle size (nm)	EE (%)
1	200:1000	1:5	0.2	level 3 of C	66.9	79.4
2	200:50	4:1	0.4	level 3 of C	47.8	73.4
3	200:50	4:1	0.4	level 2 of C	31.1	82.2
4	200:50	4:1	0.1	level 3 of C	62.7	59.8
5	200:1000	1:5	0.1	level 1 of C	34.2	58.7
6	200:2000	1:10	0.4	level 3 of C	114.8	55.2
7	200:2000	1:10	0.4	level 2 of C	128.3	46.4
8	200:2000	1:10	0.2	level 1 of C	79.5	55.9
9	200:2000	1:10	0.2	level 3 of C	125.1	56.4
10	200:1000	1:5	0.4	level 3 of C	124.9	52.5
11	200:50	4:1	0.2	level 2 of C	72.1	67.3
12	200:50	4:1	0.2	level 1 of C	50.4	70.2
13	200:50	4:1	0.4	level 1 of C	78.9	50.5
14	200:2000	1:10	0.2	level 2 of C	35.6	67.4
15	200:50	4:1	0.2	level 3 of C	87.0	65.8
16	200:1000	1:5	0.2	level 2 of C	48.0	67.7
17	200:1000	1:5	0.2	level 1 of C	70.3	50.3
18	200:1000	1:5	0.4	level 1 of C	67.1	61.5
19	200:50	4:1	0.1	level 1 of C	71.7	48.9
20	200:2000	1:10	0.1	level 2 of C	66.9	68.1
21	200:2000	1:10	0.4	level 1 of C	53.4	36.1
22	200:1000	1:5	0.1	level 3 of C	52.3	55.6
23	200:2000	1:10	0.1	level 3 of C	54.4	74.2
24	200:2000	1:10	0.1	level 1 of C	78.4	81.9
25	200:1000	1:5	0.4	level 2 of C	44.9	43.6
26	200:50	4:1	0.1	level 2 of C	47.5	86.0
27	200:1000	1:5	0.1	level 2 of C	42.6	57.7

X_1 = Drug-lecithin combination mass ratio (mg), X_2 = Chitosan concentration (mg). Stirring rates at level 1 = 10,000, level 2 = 15,000 and level 3 = 20,000 rpm

Table 8: Statistical parameters obtained for the ANOVA and CCD

Soure/parameter	Vesicle size	EE
Model	Quadratic	Quadratic
P-Value	0.1780	0.0174
R^2 value	0.5039	0.5761
Adjusted R^2	0.1401	0.2653
Predicted R^2	- 0.6023	- 0.3919
Adequate precision	4.9251	4.9346

Table 9: Confirmation of point optimization by face-centered CCD (X_1 and X_2).

Analyzed dependent variable (Response)	Predicted			Observed	Error (%)
	Mean ± SD	95 % PI low	95 % PI high	Mean ± SD	
Vesicle size (nm)	54.18 ± 14.67	17.086	91.2839	70.3 ± 0.82	17.4054
EE (%)	99.78 ± 1.37	96.307	103.248	50.3 ± 0.47	1.6283

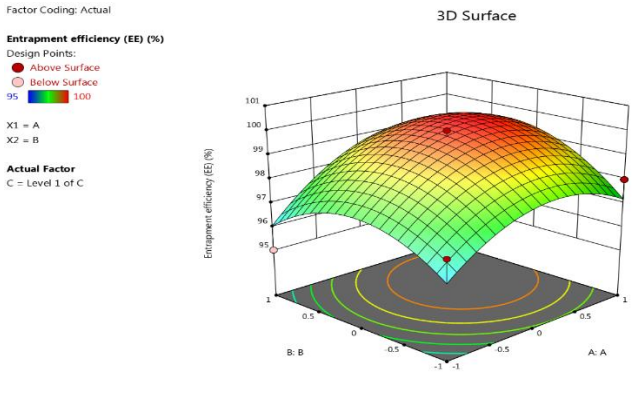
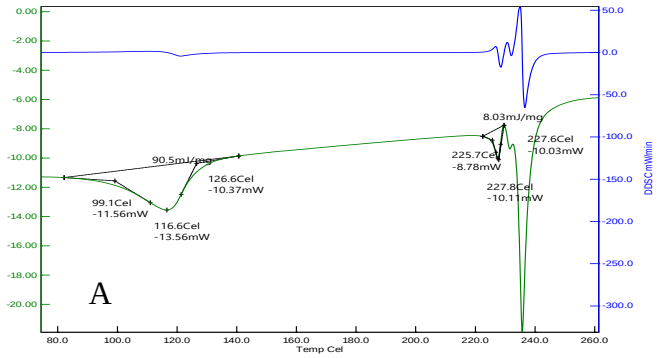


Figure 1: 3D surface plot for encapsulation efficiency

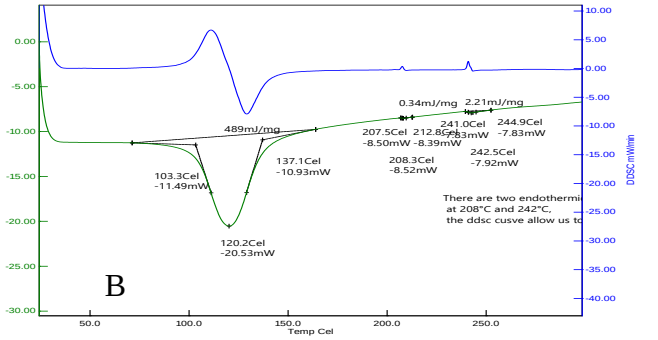
Characterization of optimized formulations.
Differential scanning calorimetry (DSC)
DSC is a thermal technique that is used in measuring the changes that occur in a material when subjected to increased temperature (heat) at the same rate to a reference. It is employed in studying some physic-chemical properties of materials involving heat changes, including interaction of substances combined in formulations.[21,22] Figure 2 and Table 10 show the results of the DSC of LVF formulations.
The thermograph of pure LVF (Fig. 2a) shows two sharp endothermic peaks at 225.7 ºC and 227.8 ºC. The peaks were within the melting range of LVF (225 – 227 ºC).[22] This observation confirms the purity of the LVF sample used. The thermograph also shows a broad endothermic peak at 116.6 ºC, which might be attributed to the dehydration of the hydrated LVF.[22]
The thermograph of the LEL formulation (Fig. 2b) also shows two endothermic peaks at 208 ºC and 242 ºC. These might represent the melting peaks of the LVF moieties shifted as a result of the presence of excipients in the formulation.[22] Shifting of melting peaks in DSC thermographs have been attributed to amorphous distribution of the drug.[21,22] The observation of the melting peaks attributable to LVF, confirms the presence and compatibility of the drug compound with the excipients used in the formulation.
Figure (2c) shows the thermograph obtained for the LVF nano capsules using the reference lecithin® (LRL), while the broad endothermic peak at 99.0 ºC might be attributed to dehydration, the rest of the thermograph did not show distinct thermal transition peaks. This might imply that the LVF particles could have been molecularly dispersed in the carriers in amorphous forms.[22]

Table 10: DSC studies of the levofloxacin formulations.

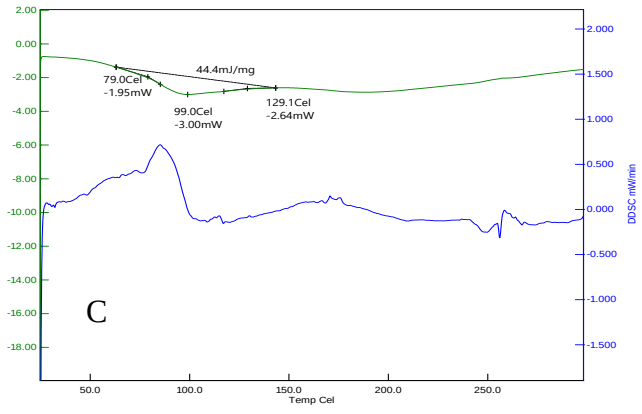
Sample	Thermal change (ºC)					
	Type	Onset	Endset	Mid	Interpretation	Heat change (Enthalpy) (mJ/mg)
Levofloxacin (LVF)	endothermic	99.1	126.6	116.6	dehydration	90.50
	endothermic			225.3	melting peak	8.03
	endothermic	227.6	227.8	227.7	melting peak	
LEL						
	endothermic	103.3	137.1	120.2	dehydration	489
	endothermic	207.5	212.8	208.0	melting peak	0.34
	endothermic	241.0	244.9	242.5	melting peak	2.21
LRL	endothermic	79.0	129.1	99.0	dehydration	44.4



Levofloxacin.



LEL Formulation

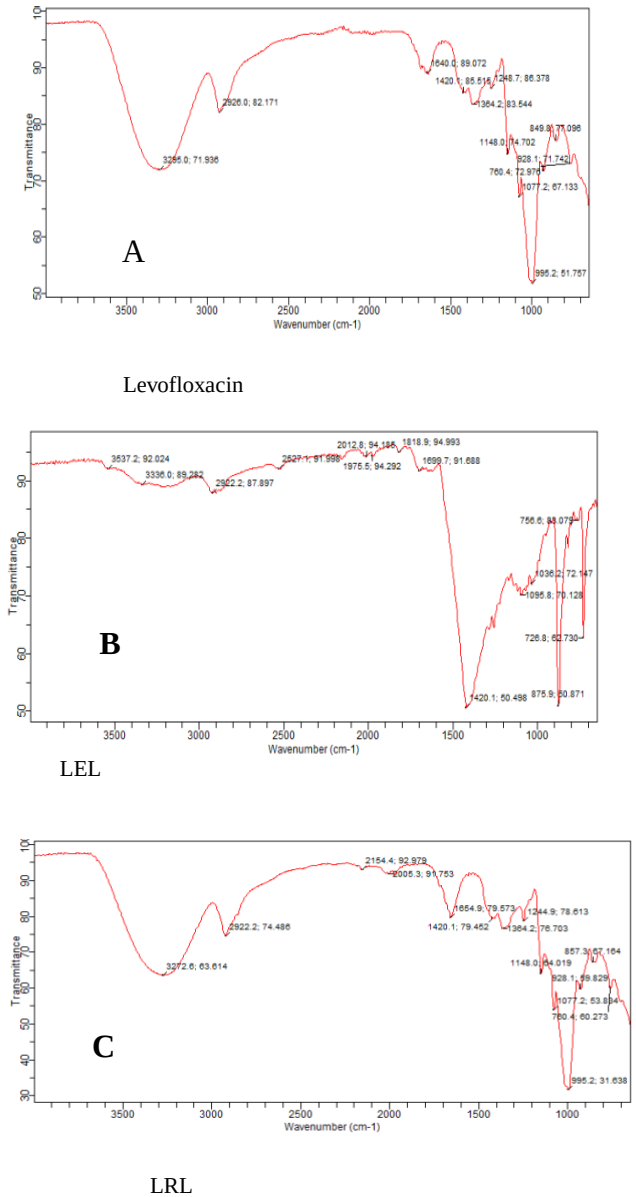


LRL Formulation

Figure 2: DSC thermograms of (a) levofloxacin, (b) levofloxacin formulated with extracted lecithin (LEL), (c) levofloxacin formulated with reference lecithin (LRL)

Fourier transform infra-red spectroscopy of optimized formulations (FTIR).
The results of the FTIR spectra are presented in Figure 3. LVF (Fig. 3a) had characteristic peaks at 3268.9 cm^{-1} due to carboxylic group, 2926.0 cm^{-1} due to alkanes group stretching, 1636.3 cm^{-1} due to stretching of the carbonyl group, 1144.3 and 991.5 cm^{-1} due to presence of halogen groups.[23] According to Beny*et al*., the LVF had aromatic, C-methyl, carbonyl, C-N and fluoro-group characteristic bonds at 3266,

2933, 1722, 129 and 1087 cm^{-1} respectively.[30] Also according to Gaurav *et al*., the FTIR of the LVF had characteristic peaks at 1725.0 cm^{-1} (carbonyl group), 1892.1 cm^{-1} (carbonyl group of quinolone moiety and 293.8 cm^{-1} aromatic C-H stretching).[25] The FTIR characteristic peaks of LEL (Fig. 3b) were found at 3265.4, 1979.2, 1558.0, 1418.4, 1349.3 and 884.0 cm^{-1} representing the -OH group, C=O, C=C stretching vibration, C-O and C-C stretching vibrations and C-N stretching vibration, respectively.[31] The prominent peaks of LVF were retained in spectra of LEL indicating the drugs and polymers does not exhibit any interaction and are suitable to be formulated as nano capsules. The FTIR characteristic peaks of LRL formulation (Fig. 3c) were found at 3406.6, 2113.4, 1036.3, 1371.7 and 976.6 representing the –OH group, nitriles stretching vibration, C=O, C=C stretching vibration and C-O, C-C stretching vibrations, respectively. The prominent peaks of LVF were retained in spectra of LRL indicating the drugs and polymers does not exhibit any interaction and are suitable to be formulated as nanocapsules.[25]



Levofloxacin

LEL

LRL

Figure 3: FTIR spectra of (a) levofloxacin, (b) levofloxacin formulated with extracted lecithin (LEL), (c) levofloxacin formulated with reference lecithin (LRL)

Encapsulation efficiency, Loading Capacity, percentage yield, drug content and uniformity of weight of optimized formulations.

The indirect method was used to determine the EE.[26] This was done by determining the actual amount of drugs entrapped within the nanoparticles by measuring the amount of free drug in the supernatant recovered after centrifugation and washing of the nanoparticles. EE can be used to judge the suitability of any drug carrier. The mechanical dispersion method and solvent evaporation technique were considered as an efficient method for preparation of the LVF loaded nanoparticles, since they could avoid high temperatures. Similar results were obtained by Rojanarat*et al*., who found that as the concentration of polymer increases, the EE and drug content increased, with more encapsulation of the drug particles.[32] As shown in Table 11, the EE of the optimized formulations were above 90%.

The percentage yield or recovery rate of the formulation has a direct relationship to the methodology.[32] Table 11 shows that maximum yield was obtained in the loaded formulations. High values (> 70 %) of the percentage of the nanoparticles recovered from the formulation are a strong indication that the formulation technique adopted was reliable. The role of any drug delivery system (DDS) is to deliver the drug incorporated to the target tissues intact with little or no toxic effect on the organ.

The overall drug loading capacity was low, although the LEL formulation had a lower value than the LRL formulation without a significant difference ($p < 0.05$). This suggests that LVF is a hydrophilic drug and the method used in the formulation could reduce the drug loading. Despite the optimization of process variables, LVF loading was low, which indicated that modification of the formulation approaches might be necessary in order to improve drug loading. The ability of the nanoparticles to accommodate active molecules is an important property that is achieved by the EE and LC. While EE % defines the ratio between the weight of entrapped drug and the total weight of API added to the formulation, LC expresses the ratio between the entrapped drug and the total weight of the carrier polymer(s). The two parameters are dependent on the formulation method adopted and the hydrophilicity of the drug.[28]

As shown in Table 11, the result of the drug content of the optimized formulations passed the BP specifications.[24] The drug content was studied in order to determine whether they complied with BP standards, and also to know if the drug was lost during the preparation process. They were within the ranges of 90 -110% of the average value. The drug content of the LEL formulation was higher than the LRL formulation without a significant difference ($p < 0.05$). The optimal formular was selected based on the initially set criteria obtained from the pre-formulations on EE and percentage yield.

Table 11 shows the results of the weight uniformity test carried out on the LVF and INH nano capsules. The result showed that the mean weight of the LVF ranged from 222.0± 0.01 to 220.0±0.03 mg for LEL and LRL respectively. The weight uniformity test was performed on the capsules to determine its compliance with USP specifications. All the nano capsules passed the weight uniformity test as the percentage of weight deviation was within the USP limits of ± 5% of average weight. The BP stipulates that tablet with an average weight of 300 mg or more should have percentage deviation not greater than 15.0%.[24]

Morphology using scanning electron microscope (SEM)

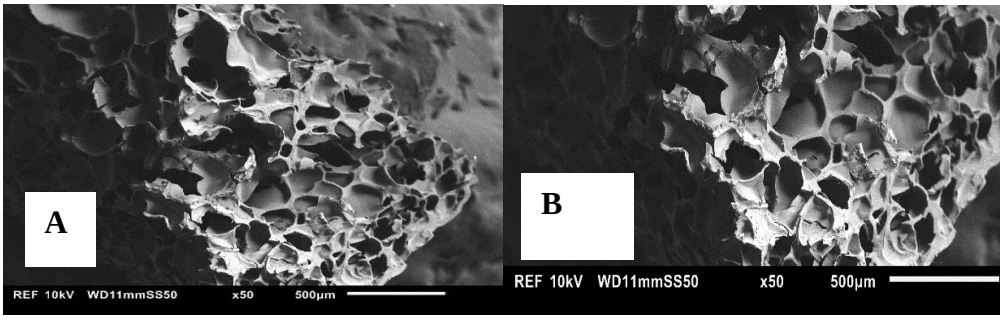
Figure 4 shows the detailed morphological features of the nanoparticles based on optimized parameters as obtained from the scanning electron microscope. The micrographs showed that the nanoparticles were spherically shaped, although Ramados*set al*., reported that the formulated nanoparticles had spherical shape with size ranging from 25 to 55 nm.[33] Overall, the LVF nanoparticles were spherically shaped with a smooth surface and spherical vesicles were present.

Particle size of optimized formulations.

The particle sizes of orally administered LVF significantly affect their oral absorption and bio-distribution, which ultimately determine the therapeutic efficacy. Figure 5 represents the particle sizes of LEL and LRL formulations. It is important to mention that although this is a nanoparticle formulation, we cannot rule out the presence of microparticles.[22] Particle size may be a function of either one or more of the following: formulation excipients, degree of homogenization,

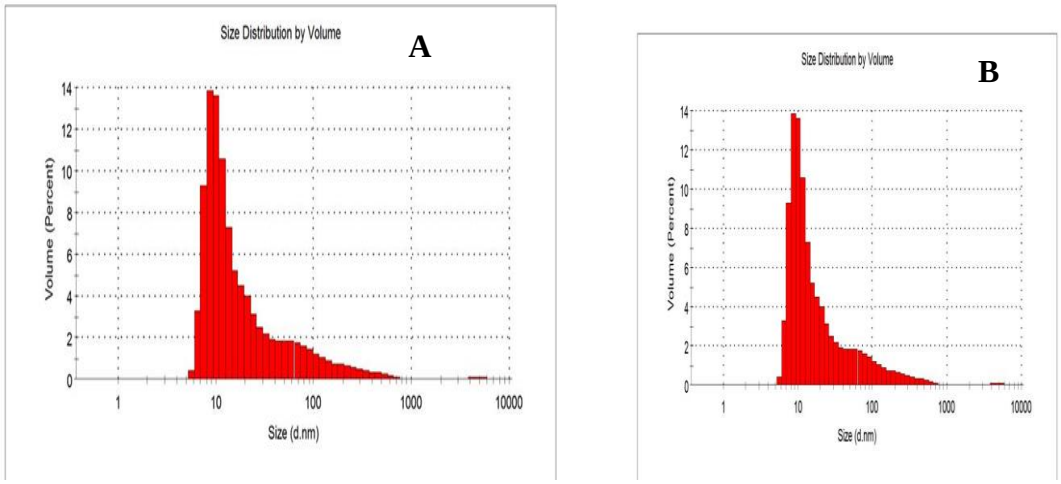
homogenization pressure, rate of particle size growth and crystal habit of the particle.[22] Presence of active drug in the formulation and the need to achieve thermodynamic stability in the absence of electrostatic repulsion are usually motivating factors to particle size change.

Table 12 shows that the levofloxacin formulation with reference lecithin (LRL) had a smaller particle size when compared to the extracted lecithin (LEL). Therefore, in terms of increase in particle size, LEL > LRL. Additionally, the polymer concentration and the amount of lecithin added to the formulation may have affected the particle size distribution.[22]



LEL LRL

Figure 4: SEM photomicrographs of (a) LEL and (b) LRL



LEL LRL

Figure 5: Particle size graphs of (a) LEL and (b) LRL

Polydispersity index (PDI)

Table 13 shows the PDI of the optimized batches. PDI is a representation of the distribution of size population within a given sample.[34] PDI describes the degree of non-uniformity of any size distribution. PDI > 0.7 indicates a broad particle size distribution, while a PDI of 0.0 indicates perfectly uniform sample. A PDI of 1.0 is a highly polydispersity sample that has multiple particle size populations. In polymer-based nanoparticles, values of 0.2 and below are acceptable in practice.[34] This is usually an indication of the uniformity of the particle size. The LEL formulation recorded a higher value of PDI than the LRL formulation without a significant difference ($p < 0.05$).

Table 11: Encapsulation efficiency (%), loading capacity (%), percentage yield (%), drug content (%) and uniformity of weight (mg) of the optimized nanocapsule formulations.

Batch	EE	LC	Yield	Drug content	Uniformity of weight
LEL	94.0 ± 0.28	48.8 ± 0.19	85.38 ± 0.54	98.6 ± 0.12	222.0 ± 0.01
LRL	94.4 ± 0.22	53.2 ± 0.09	92.11 ± 0.95	96.9 ± 0.17	220.0 ± 0.03

LEL = Formulation of LVF using extracted lecithin, LRL = Formulation of LVF using reference lecithin

Table 12: Particle size of the optimized formulations

Batch	Particle size (nm)
LEL	96.64 ± 0.04
LRL	94.60 ± 0.81

LEL = Formulation of LVF using extracted lecithin, LRL = Formulation of LVF using reference lecithin

Table 13: The PDI values of optimized batches

Batch	PDI
LEL	0.284 ± 0.08
LRL	0.270 ± 0.05

LEL = Formulation of LVF using extracted lecithin, LRL = Formulation of LVF using reference lecithin

Dissolution studies of the tablets

The results of the drug release profile are presented in Figures (6a-d). It shows that the amount of drug released, increased steadily with time up to the 8-hour period for Figures (6a-d). The result indicated that the chitosan-fortified formulations in both LEL and LRL retarded the release of LVF from the nanoparticles (Fig. 6c). This was found to be dependent upon the concentrations of the polymer and the cross-linking agents in accordance with a previous report by Zhou *et al*.[35] The cumulative release behavior of the LEL formulation showed that the LVF-loaded nanoparticles released 45.67% of the drug over the 8 hours period. The release profile of the LRL formulation at pH 1.2 and 6.8 shows that the amount of drug released, increased steadily with time. At 6 hours, 26.41% of the drug was released (Fig. 6b). This implied that the formulation was affected by the concentrations of the polymer and the cross-linking agent in accordance with a previous report.[35] Fig.6c shows the drug release profile of LEL and LR. The amount of drug released, increased steadily with time. The release profile of LRL, LEL and CL (Fig. 6d), shows that LRL recorded a maximum of drug release at 45.76% over the 8 hours period in a controlled manner. CL recorded the highest drug release within the 8 hours period. Independent variables like the polymer concentration and concentration of cross-linking agent affected the release of LVF from the nanoparticles.[32] Report by Beny*et al*., showed that the cross-linking agent affected the release of drug from the formulations resulting in increase in the polymer density and reduction of the macromolecular chain mobility which resulted in the decrease in drug release due to formulation of more stable and rigid spheres.[30] The release profile was characterized by a good sustained release properties with no burst effect. Being a sustained release dosage form, it will improve patient compliance, maintain the therapeutic action of the drug, reduce the incidence and severity of systemic side effect and the total amount of drug administered over the period of drug treatment.

In vitro drug release kinetics of the nano capsules

Table 14 represents the different mathematical models used to describe the drug release kinetics. For the LVF tablet formulations (LEL and LRL), the Korsmeyer model had the highest degree of correlation coefficient (0.9963). According to Korsmeyer-Peppa's law, when the release exponent (n) is between $0.45 < n = 0.89$, it indicates that the drug release from the system was non-Fickian transport.[26]

Results of the antimycobacterial test of the optimized formulations.

The optimized formulations were tested against two (2) clinical isolates (*Mycobacterium bovis* and *Mycobacterium smegmatis*). The optimized formulations, commercial drug of LVF, and pure sample of levofloxacin was used in the evaluation of anti-mycobacterial activity as shown in Tables 15. A colour change from blue to pink indicated mycobacterial growth and the minimum inhibitory concentration (MIC) was interpreted as the lowest concentration that prevented the colour change. According to Rastogi *et al*.,[36] the MIC values of LVF against *M. intracellulare*, *M. avium* and *M. kansaii* were 8.0 µg/mL, 0.5 µg/mL and 0.25 µg/mL, respectively. LVF has also been shown to exhibit *in vitro* minimum inhibitory concentration (MIC) of 2 µg/mL or less against most (≥ 90%) strains of the following microorganisms; *Staphylococcus haemolyticus*, *Streptococcus agalactiae* and *Streptococcus milleri*.[36]

The LVF formulated with the extracted and reference lecithin samples had MICs of 26.9 µg/mL and 58.3 µg/mL, respectively. The mean MIC values of the reference LVF tablet showed that it had a lower MIC than the formulated LVF tablets without a significant difference ($p< 0.05$). Sample A (LEL) formulation had an MIC value of 26.9 µg/mL, while sample B (LRL) had an MIC value of 58.3 µg/mL (Tables 15 and 16), thus the formulation of LVF with the extracted lecithin had a lower MIC value compared to that of the reference lecithin without a significant difference ($p< 0.05$). The pure sample of LVF had an MIC of 0.025 µg/mL for both organisms (Table 16). Well 12, served as a control for organism activity. *In vivo* studies of *Mycobacterium tuberculosis* suggested that LVF activity was comparable with that produced by two-folds greater dosage of ofloxacin, although the minimum inhibitory concentration required to inhibit the growth of 90% of organisms (MIC_{90}) values for both drugs were similar at 1 µg/mL.Sparfloxacin had better activity with MIC_{90} of 0.5 µg per mL.[29] Several workers have found similar findings *in vitro*.[37,38] The result obtained shows that LEL and LRL, had higher potentials of activity against the Mycobacterial isolates than the conventional capsules. The higher activity may be attributed to increased permeation of the bacterial cell wall.[37]

Table 14: The zero, first order, Higuchi, Korsmeyer-Peppas, model for drug release determination in optimized formulation tablets and commercial brands

	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
Batch	r^2	r^2	r^2	r^2	N
LEL	0.8229	0.9093	0.9912	0.9963	0.558
LRL	0.9755	0.8697	0.9724	0.9756	0.547
CL	0.5161	0.6305	0.6747	0.9968	0.057

r^2 = Coefficient of correlation, n = release exponent.

LEL= Formulation of LVF using extracted lecithin, LRL= Formulation of LVF using reference lecithin, CL = Commercial levofloxacin

Table 15: Antituberculosis activities of the formulations against *Mycobacterium bovis* (BCG).

Sample	**Antibacterial activity against *M. bovis***												**MIC (µg/mL)**
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
A	-	-	-	-	-	-	+	+	+	+	+	+	26.9
B	-	-	-	-	-	-	+	+	+	+	+	+	58.3
C	-	-	-	-	-	-	-	-	-	-	+	+	0.04
D	-	-	-	-	-	-	-	-	+	+	+	+	0.05

Sample A (LEL) = Formulation of LVF using extracted lecithin

Sample B (LRL) = Formulation of LVF using reference lecithin

Sample C: Reference LVF, Sample D = Pure powder sample of LVF

(-) = inhibition of test organism (activity), (+) = growth of test organism (no activity), Well 12 is control for organism viability.

Table 16: Antituberculosis activities of the formulations against *Mycobacterium smegmatis*

Sample	Antibacterial activity against *M. smegmatis*												MIC (µg/mL)
Well	1	2	3	4	5	6	7	8	9	10	11	12	
A	-	-	-	-	-	-	+	+	+	+	+	+	26.9
B	-	-	-	-	-	-	+	+	+	+	+	+	58.3
C	-	-	-	-	-	-	-	-	-	-	+	+	0.04
D	-	-	-	-	-	-	-	-	-	+	+	+	0.025

Sample A (LEL) = Formulation of LVF using extracted lecithin
Sample B (LRL) = Formulation of LVF using reference lecithin
Sample C = Reference LVF (Levotil®, 500 mg, M & B)
Sample D = Pure powder sample of LVF, (-) = inhibition of test organism (activity), (+) = growth of test organism (no activity), Well 12 is control for organism viability

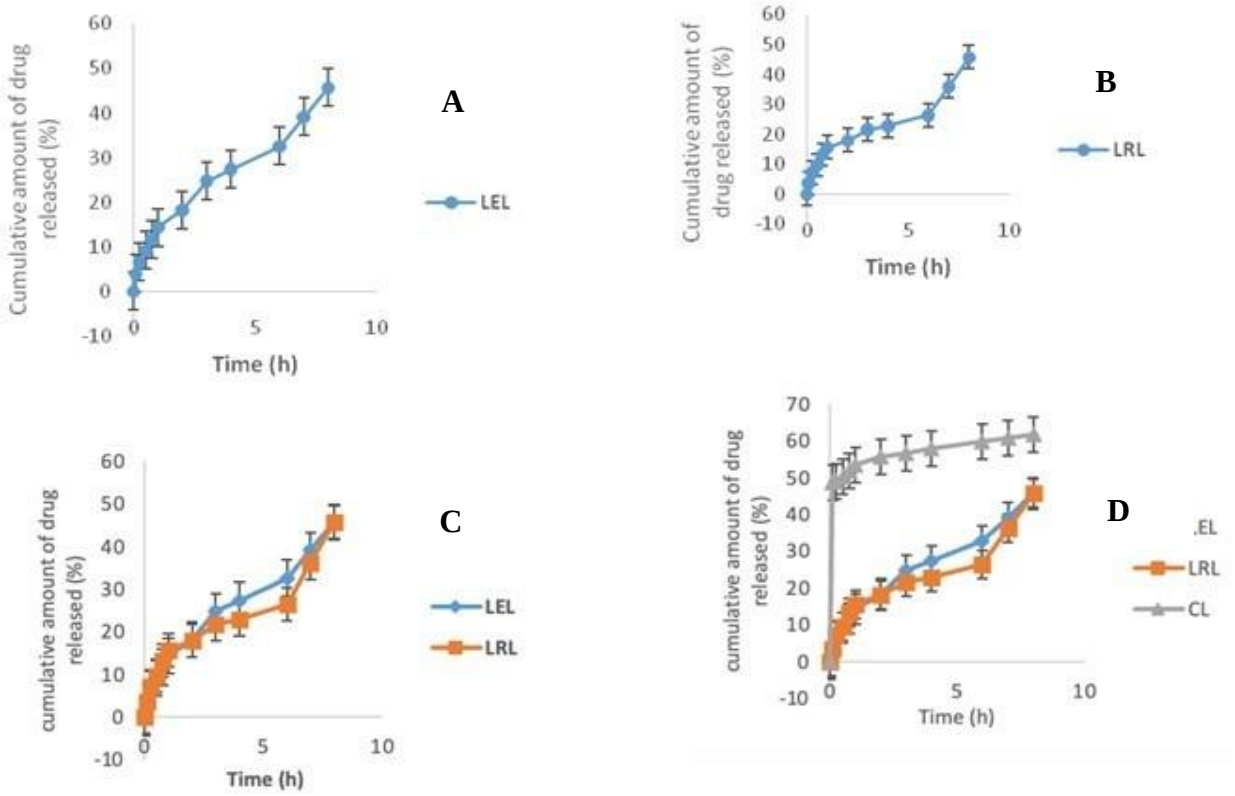


Figure 6: Drug release profiles of (a) LEL, (b) LRL (c) LEL and LRL (d) LEL, LRL and commercial levofloxacin (CL).

Conclusion

The extracted lecithin showed some physic-chemical properties comparable to those of the reference (Lecithin ®, S 75, Lipoid, Germany). Nano capsules containing LVF were formulated using the lecithin-samples fortified with chitosan for enhanced permeation. Although the LVF nano capsules had activities against the Mycobacterial isolates, the MICs of (LEL = 26.9 µg/ml; LRL = 58.3 µg/ml), were significantly ($p< 0.05$) higher than that of the reference commercial tablet (0.04 µg/mL). The optimized nano formulations showed controlled release of the active constituents over the period of 8 hours, unlike the reference conventional capsule formulations. The results present the chitosan-fortified nanocapsule formulations of LVF with potentials for further exploration and development for enhanced bioavailability and application against MDR-TB. This field requires further exploration, so as to effectively scale up all its aspects and subsequently produce commercially available chitosan-fortified, lecithin-based nanocapsule formulations of LVF and INH. Further research is therefore recommended for validation and to possibly undertake *in vivo* studies and industrial development.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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