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LIPID NANOEMULSION-BASED LIQUISOLID COMPACT TABLETS FOR ORAL DELIVERY OF CLOTRIMAZOLE: FABRICATION STRATEGIES, CHARACTERIZATIONS, ANTIMYCOTIC AND TOXICOLOGICAL EVALUATIONS

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This work aims to fabricate liquisolid compact tablets incorporating clotrimazole (CLOT)-loaded lipid nanoemulsions (LNE) for oral treatment of systemic fungal infections. Nanoemulsion was characterized for droplet size, rendered into free-flowing granules and compressed into liquisolid compact tablets, and evaluated using pharmacopoeial and non-pharmacopoeial methods. In-vitro and in vivo antifungal, stability and toxicological tests of the tablets were evaluated. LNE was nanosized (66.7 ± 5.7 – 121.6 ± 3.2 nm). Liquisolid tablets were stable, non-toxic, had uniform weight (341.4 ± 1.2 – 346.7 ± 0.8 mg), drug content uniformity (85.2 ± 0.1 – 99.8 ± 0.2 %), and had excellent disintegration (2.96 ± 0.8 – 5.88 ± 1.3 min), and controlled release property. In-vitro and in vivo antifungal evaluations revealed improved antimycotic activity of CLOT. The results highlight that CLOT-LNE liquisolid compact tablets is a promising carrier system with improved oral utility for the treatment of systemic fungal infections.

Keywords*: Clotrimazole; Lipid nanoemulsion; Liquisolid; Systemic mycoses; Candida albicans; Oral delivery*

INTRODUCTION

Fungal or mycotic infections are highly underrated but very important public health challenge in the underdeveloped and developing regions of the world with immense impacts on human morbidity and mortality. Studies have shown high similarities between fungal cells and their host cells, the propensity of fungi to invade and infect a variety of tissues in a single host as well as undergo morphogenic changes during host invasion to guarantee optimal survival.[1&2] Conventionally and based on the site of infection, fungal infections are classified into systemic, superficial, and subcutaneous infections. In the past decades, systemic mycoses have very low incidences compared with superficial and subcutaneous infections and were generally regarded as rare infections (GRARI). However, they are currently part of everyday infections owing to aging, malnutrition, autoimmune diseases, chronic kidney disease (CKD), diabetes mellitus (DM), HIV/AIDS, and certain medico-surgical manoeuvres that suppress human immune functions e.g. organ transplant and heart prosthesis.[3&4] Furthermore, there has been increased interest in systemic fungal infections (opportunistic and endemic) caused by *Candida albicans* because the fungi are ubiquitous and are implicated in deep mycotic infections affecting internal organs of the body especially in patients with severe morbidities such as chronic obstructive pulmonary disease

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(COPD), cancer, tuberculosis, and patients on antibiotics and immunosuppressant therapy.[5&6] Though the azoles are widely applied for oral treatment of systemic fungal infections, clotrimazole (1-[(2-chlorophenyl) diphenylmethyl]-1-H-imidazole) or CLOT, a wide spectrum imidazole antifungal drug that is active against *C. albicans*, is sparingly used.[7&8] Its mechanism of antifungal activity include inhibition of phospholipids and triglycerides biosynthesis in fungal cells, reduction of fungal oxidase and peroxidase enzymatic activities, and blockade of *C. albicans* transformation from spores to hyphae, resulting in the death of the fungi.[9] However, the low usage of CLOT in clinics for the treatment of systemic mycoses could be due to its poor aqueous solubility (0.49 µg/ml) or lipophilicity having a Log P of 6.1 and pKa 6.7, low oral bioavailability, short half-life (about 3 h), high frequency of dosing, rapid metabolism, and toxicity effects including dysuria, gastrointestinal problems, and depression.[7 – 10] Therefore, there is an urgent need to incorporate CLOT in a suitable carrier system like lipid-based nanoparticulate delivery system, which will guarantee enhanced oral solubility, bioavailability, efficacy, and safety of the drug for effective treatment of systemic mycoses.

According to extant literature, many workers have reported the use of novel drug delivery systems, including ufosomes[6], micro- and nano- emulsions[7,8], polymeric nanoparticles[9], nanogel[10], solid lipid nanoparticles[11], phospholipid vesicles[12], nanocapsules[13], nanoethosomal gel[14], nanomicelles[15], proliposomes[16], and lipospheres[17] to improve the biopharmaceutical dispositions of CLOT for enhanced patient compliance, and toxicity elimination. Lipid nanoemulsions (LNE) are transparent or translucent and stable submicron dispersions of immiscible liquids (oil and water) stabilized using surfactants with droplet size ranges of 20 - 200 nm. The utility of lipid nanoemulsions lies in their physical stability against creaming, sedimentation, and coalescence due to their nanometric droplet size generated using relatively low surfactant concentration, and excipients used (oil and surfactant) are generally regarded as safe (GRAS), biodegradable, and biocompatible[18]. Thus, it is envisaged that formulation of lipid nanoemulsions containing CLOT will facilitate increased solubility and bioavailability of the biopharmaceutics classification system (BCS) class II drug in which rate of dissolution is an important factor that influences drug absorption process due to enhanced intestinal absorption and distribution of the drug-bearing lipid droplets.

Liquisolid compact technique is an emerging technology for dosage form design which is based on rendering liquid medication into a freely compressible, non-adherent, and free-flowing powder suitable for direct compression with excellent potential to enhance the solubility and bioavailability of drugs. In this system, liquisolid tablets are prepared by physical mixture of the liquid drug (solutions, emulsions, and suspensions) with selected tablet excipients, carriers (lactose, cellulose, starch) and coating materials (colloidal silicon dioxide) to produce a homogenous flowable powder mixture by absorption and adsorption[19, 20]. Liquisolid compact technique is used to produce commercially viable tablets containing drugs with improved solubility, acceptable size, and weight to aid oral administration by swallowing. Formulation of liquisolid tablets is important due to the numerous advantages offered by tablet dosage form including cost-effective production, higher production rates, elimination of costly control steps involved in intravenous or vaginal dosage forms, possibility of administration of higher dose strength, ability to withstand handling, prolonged shelf-life, and possibility of sustained release of the API[21]. Pharmacoeconomically, oral administration of CLOT through liquisolid compact tablets implies a lower cost compared with intravenous or vaginal route. Patient considerations indicate that the oral route is the most convenient because tablets could be self-administered by the patient without professional skills or equipment, and tablets are generally portable. In our study, we compressed a physical mixture of CLOT lipid nanoemulsion (core liquid formulation) with appropriate carriers and coating excipients into liquisolid tablets. This is based on the hypothesis that LNE would enhance oral administration of CLOT by addressing the drawbacks of poor solubility, low bioavailability, and high frequency of dosing, while liquisolid compact tablets will resolve poor stability and produce better patient compliance for improved antifungal efficacy of the encapsulated drug.

MATERIALS AND METHODS

Chemicals

Clotrimazole was a donation from Nature and Nurture Pharmaceuticals, Nigeria. Soybean oil was bought from Aromachem, Essex, UK. Kolliphor® P188 was kindly donated by BASF SE, Ludwigshafen, Germany. Ethanol was purchased from JHD, Guangzhou, China. Polysorbate (Tween®) 80, polyethylene glycol (PEG) 400 and propylene glycol were obtained from Merck, Darmstadt, Germany. Labrasol was a donation from Gattefosse SAS, Saint-Priest, Cedex, France. Microcrystalline cellulose (Avicel® PH-102) was purchased from FMC Biopolymer, PA, USA. Amorphous colloidal silica (Aerosil® 300) was obtained from Evonik Industries AG, Hanau, Germany. Type 'A' sodium starch glycolate (Primojel®) was purchased from DFE Pharma, Goch, Germany. *Tetracarpidium conophorum* (Conophor or Walnut) oil was obtained from a batch processed in our laboratory.

Methods

Solubility assessment of CLOT in various excipients

Solubility evaluation of CLOT in various liquid lipids (soybean oil, coconut oil, conophor oil, and palm oil), surfactants (Kolliphor® P188, Labrasol, and Tween® 80), and co-surfactants (PEG 400, propylene glycol, and glycerol) was carried out by modified shake-flask method[22]. Briefly, an excess amount of CLOT was added to 2 ml of each test excipient in a small screw-capped plastic bottle and shaken mechanically for 72 h at 25 ± 1 °C to attain equilibrium solubility. The mixtures were centrifuged (Remi Equipments, Ltd, India) at 10,000 rpm for 15 min to exclude undissolved drug, and the supernatant was collected and filtered using a membrane filter (0.4 µm membrane). Appropriate dilutions of the aliquots of the filtrate were obtained using ethanol and drug concentration was determined in triplicate spectrophotometrically (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Malaysia) at 262 nm using ethanol as the blank solvent.

Construction of ternary phase diagrams

Ternary phase diagrams were constructed using the water titration (spontaneous emulsification) method. The diagrams were constructed by combining different weight ratios of the selected surfactant and co-surfactant, S_{mix} (1:1, 2:1, 1:2, 3:1 and 1:3) with the selected oil in ratios of 1:9 – 9:1 (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10). For any mixture, the total quantity of the surfactant, co-surfactant and oil concentrations always added to 100 %. The components were stirred using a magnetic stirrer (Ohaus Corporation, New Jersey, USA) for 5 min and the mixtures were titrated with distilled water followed by gentle agitation until equilibrium at 25 ± 2 °C. Thereafter, samples were visually examined for clarity and classified as clear nanoemulsion or emulsion. The phase behaviour of the disperse systems was represented on ternary phase diagrams with three apices: Conophor oil, water, and T80+PEG 400 (S_{mix}) using ProSim software[23].

Preparation of lipid nanoemulsions

Lipid nanoemulsions (LNE) were prepared by modified high pressure homogenization (HPH)[24]. LNE consists two phases (oil and aqueous) that were prepared separately. The oil phase, consisting of varying amounts of conophor oil (20 – 30 % w/w) and CLOT were completely dissolved with mild heat at 50 °C under slight stirring using a magnetic stirrer (Ohaus Corporation, New Jersey, USA), and the oil phase was allowed to cool down to ambient temperature (25 ± 2 °C). The aqueous phase was prepared by dissolving the hydrophilic surfactant, Tween® 80 (3.0 %w/w) and the co-surfactant, PEG 400 (1.5 %w/w) in double distilled water. The aqueous phase was added slowly in aliquots to the oil phase at room temperature and pre-homogenized with magnetic stirrer at 4,000 rpm for 5 min. The obtained primary coarse emulsion was homogenized with a high pressure homogenizer (Jinhu Ginhong Machinery Co., Ltd, Jiangsu, China) at 500 bars for 8 cycles for 10 min. The same protocol was applied in the formulation of drug-free LNE, and all batches of the LNE were filled in glass vials, sealed and stored at room temperature. Table 1 shows the compositions of the lipid nanoemulsions.

Determination of droplet size and size distribution of LNE

The LNE were diluted with double distilled water (1:200, v/v), filled in disposable polystyrene cuvettes, and inserted in Zetasizer nano ZS90 (Malvern Instruments Ltd, Worcestershire, UK) for determination of their

mean droplet size (intensity weighted droplet size), and size distribution (polydispersity index) in triplicate by dynamic light scattering (DLS) at 25 ± 2 °C followed by analysis of the intensity of the scattered light at an angle of 173°[22].

Calculation of amounts of coating and carrier materials

To prepare liquisolid compacts of CLOT-LNE, Avicel®PH-102 and Aerosil®300 were used as the carrier and coat systems respectively. According to the model prescribed by Spireas and Bolton[25], the ϕ-values of Avicel®PH-102 and Aerosil®300 were calculated for each batch of the CLOT-LNE, and the liquid load factor, L_f values (defined as the ratio of the weight of the liquid medication, W to the weight of the carrier material, Q) were determined and used to calculate the amount of Avicel®PH-102 needed, while the quantity of Aerosil®300 required was calculated from the assigned excipient ratio, R-value. Thus, in the study, Avicel®PH-102 and Aerosil®300 were used as the carrier and coating materials for the preparation of liquisolid compacts of CLOT-LNE with good flow properties and suitable for direct compression without the addition of any water miscible vehicle and the calculations were dependent on the weight of dry CLOT-LNE formulation used as the liquid medication.

According to the mathematical model, the flowable liquid retention potential of the Avicel®PH-102 and Aerosil®300 were determined using known weights of dry CLOT-LNE and each of the carrier and coating material, and the flowable liquid retention potential of each of the carrier and coating material is calculated using the relationship:

$$(\phi) = \frac{weight\ of\ CLOT - LNE}{weight\ of\ solid\ material} \qquad (1)$$

The liquid load factor, L_f for acceptable flowability was calculated based on the flowable liquid retention potential (ϕ) value of Avicel®PH-102 and Aerosil®300 and using excipient ratio, R value of 20 according to the relationship:

$$\text{Liquid load factor, } Lf = \phi_{Av} + \phi_{Ae}\left(\frac{1}{R}\right) \qquad (2)$$

Where ϕ_{Av} is the flowable liquid retention potential of the carrier material, Avicel® PH-102, and ϕ_{Ae} is the flowable liquid retention potential of the coating agent, Aerosil® 300.

The amount of the carrier material, Q was calculated from the relationship:

$$\text{Quantity of carrier material, } Q = \frac{Weight\ of\ CLOT - LNE}{Liquid\ load\ factor} \qquad (3)$$

The amount of the coating material, q was calculated from the relationship:

$$\text{Quantity of the coating material, } q = \frac{Quantity\ of\ the\ carrier\ material}{Excipient\ ratio} \qquad (4)$$

Preparation and micromeritics of powder mixtures

Porcelain mortar and pestle were used to manually mix the required quantity of carrier excipient (Avicel® PH-102) with an amount of the LNE containing CLOT equivalent to 20 mg until the nanoemulsion was completely used and efficiently absorbed. Aerosil® 300 was incorporated as the coating material with continuous mixing until excess fluid was adsorbed. This was followed by the addition of 5 % sodium starch glycolate (Primojel®) as disintegrant and 1 % magnesium stearate as the lubricant. CLOT-free granules were also prepared using similar method. The flow and compression properties of the liquisolid granules were evaluated by measuring the angle of repose (θ), Carr's compressibility index (C_i), and Hausner's ratio (H_r), in triplicate to guarantee data validity as previously described[26&27]. The study was done using 10 g of the granules and 200 taps respectively, and the parameters were calculated using the following formula:

$$\text{Angle of repose, } \tan\theta = \frac{2h}{d} \qquad (5)$$

$$\text{Carr's compressibility index, } C_i = \frac{d_2 - d_1}{d_2} X100 \qquad (6)$$

$$\text{Hausner's ratio, } H_r = \frac{d_2}{d_1} \qquad (7)$$

Where h is the height of the heap of the dry liquisolid mixture, d is the diameter of the base of the heap after fall from a pre-determined height, d_2 is the tapped or packed bulk density of the mixture, and d_1 is the poured or loose bulk density of the mixture.

Morphological evaluation

The microstructures of the optimized granules bearing clotrimazole were analyzed by microscopy a using scanning electron microscope, SEM (Phenom World, Eindhoven, Netherlands). Samples were digested in double distilled water and placed on the sample holder of the SEM at 50 °C to freeze-fracture the sample and allowed to dry overnight at ambient temperature. Following instrument stabilization, sample imaging was carried out at 15 kV, and obtained images were focused using a digital NavCam mode, and transferred to Phenom suite software for image analysis[28,29].

Thermal analysis

Differential scanning calorimeter (DSC, Mettler-Toledo, Beaumont Leys, Leicester, UK) bearing the rugged multiSTARe sensor with 56 thermocouples and STARe Software option 13.0 was used to study the thermal profiles of CLOT and the granules. An empty standard aluminium pan served as reference after baseline correction. DSC thermograms were obtained for the samples between 30 – 300 °C at a heating rate of 5 °C/min under a 20 ml/min nitrogen flux with a sample size of about 10 mg separately weighed and placed into a hermetically-sealed aluminium-plated crucible[28].

Fourier transform-infrared (FT-IR) spectroscopy

FT-IR spectroscopy to study the compatibility between the granules and clotrimazole was conducted using FT-IR M530 Spectrophotometer (Buck Scientific, Connecticut, USA). The spectra of the samples were recorded in the wavelength region of 3,500 - 1,000 cm^{-1} with threshold of 1.303, sensitivity of 50, and resolution of 2 cm^{-1} range. Baseline scanning was done using a potassium bromated plate cleaning with a tri-solvent mixture comprising acetone-toluene-methanol at 3:1:1 ratio. In each case, about 50 mg of each sample was dissolved in 0.1 ml nujul diluent, introduced into the potassium bromate plate, and rendered into transparent discs using a pressure of 5 tons of 5 min in a hydraulic press, and the spectrum of the pellets was recorded[28,29].

X-ray powder diffraction study

Wide angle X-ray powder diffraction (XRPD) of the granules and CLOT was performed using x-ray diffractometer (Empyrean® diffractometer, Malvern Ltd, Royston, UK) equipped with camera length of 480 mm. The granules were placed in a sample holder in the diffractometer and the samples were scanned over a range of 2 θ at 45 kV voltage, 40 mA current, scanning angle range of 10 – 70° and scan rate of 0.4°/s, and diffractographs were analyzed using NGRL® Flat Programme Software[28&29].

Production of liquisolid tablets of CLOT

The dry liquisolid granules were used to produce tablets by direct compression method. Granules were compacted for 60 s at force of 70 kgf in a 10-mm diameter die to produce 350 mg liquisolid compact tablets on a single punch machine (Proton® miniPress, Proton Engineering Ltd, Ahmedabad, India). Liquisolid tablets of CLOT were stored in an airtight container for 48 hrs in a desiccator using fused calcium chloride as desiccant to ensure recovery of tablet hardness and elasticity before evaluation[29&30].

Measurement of content uniformity of tablets

Uniformity of drug content was evaluated using twenty (20) tablets selected randomly from each batch. The tablets were weighed and crushed individually using porcelain mortar and pestle, and the powders were dissolved in ethanol for 30 min. The mixture was filtered using Whatman filter paper, and the absorbance of CLOT was measured spectrophotometrically (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Malaysia) at 262 nm using ethanol as the blank solvent. Drug content uniformity was determined based on standard calibration curve of CLOT in ethanol at 262 nm[31,32].

Determination of weight uniformity

Weight uniformity of the tablets was determined using twenty tablets (20) which were selected randomly from all batches and individual weight was evaluated using an electronic balance (Ohaus Adventurer, China).

The mean, standard deviation and percentage deviation of each batch was determined[33&34].

Evaluation of tablet friability

Friability of liquisolid compact tablets of CLOT was investigated using a friabilator (Henan, People's Republic of China). Ten (10) liquisolid compact tablets were selected randomly from each batch, dedusted, and weighed together using a sensitive electronic balance (Ohaus Adventurer, China), placed in the drum of the friabilitor, and tumbled under the constant speed of 25 rpm for 4 min. They were removed, dedusted again, and the final weight was determined. The loss in weight (friability) was recorded and used to calculate percentage friability of the tablets using the formula below[35&36]:

$$\text{Friability (\%)} = \frac{Loss\ of\ weight}{Initial\ weight} \times 100 \quad (8)$$

Measurement of tablet hardness (crushing strength)

The hardness or crushing strength test was carried out on the samples from each batch using a hardness tester (Monsanto Hardness tester, VinSyst, India). Briefly, a tablet was placed between the anvil and spindle of the hardness tester and the applied pressure at a constant speed of 0.1 mm/s was used to measure the force of fracture of the tablet. Each determination was done in triplicate for each batch and the mean force or hardness value was recorded[35&37].

Determination of disintegration time of tablets

Disintegration time study was performed using six (6) tablets from each batch which were selected randomly and placed separately into each of the six tubes of the rack of the disintegration unit (Erweka ZT-71, Germany). Double distilled water served as the study media, and the rack was raised and lowered at constant rate in 500 ml of the medium contained in a glass beaker maintained at 37 ± 1°C and the mean time taken for complete disintegration of the tablets was recorded[32&38].

***In-vitro* drug dissolution study**

Beer's curves for clotrimazole were obtained at 264.1 nm for SGF (pH 1.2) and 265.5 nm for SIF (pH 7.4) without enzymes respectively. *In-vitro* dissolution study was done using the USP drug dissolution apparatus II (paddle) (DIS 6000, Copley Scientific, UK) with SGF and SIF as the biorelevant media. All dissolution tests was done using freshly prepared 900 ml of SGF and SIF respectively, maintained at 37 ± 1 ^{0}C with paddle speed set at 50 rpm. In each case, one tablet from each batch of liquisolid compact tablets was immersed in the dissolution medium as the paddle was rotated. At pre-determined time intervals up to 12 hrs, 5 ml of the dissolution medium was withdrawn and analyzed spectrophotometrically (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Malaysia) at 264.1 nm for SGF and 265.5 nm for SIF respectively. For each 5 ml of sample withdrawn, an equivalent volume (5 ml) of the dissolution medium was added to the apparatus to maintain sink condition throughout the study period. The withdrawn samples were filtered and the cumulative percentage of drug released was determined with reference to the standard Beer's plot for clotrimazole in each of the dissolution medium used. Each determination in SGF and SIF was done in triplicate. Dissolution efficiency (DE) of the CLOT-LNE liquisolid compact tablets was evaluated as the area under the dissolution curve up to a given time and it is expressed as a percentage as follows: Dissolution efficiency (DE)=

$$\frac{\int_{t1}^{t2} ydt}{\int_{t1}^{t2} Y_{100}t} \times 100\% \quad (9)$$

Where y is the percentage of dissolved drug, DE is the area under the dissolution curve between the time point t_1 and t_2 expressed as a percentage of the curve at maximum dissolution, y_{100} over the same time period[39&40].

Study of mechanism of release and release kinetics of tablets

The drug dissolution data obtained from each batch were applied to different drug release mathematical models including zero order, first order, Higuchi, and Korsmeyer-Peppas models to study mechanism of release and release kinetics of the liquisolid compact tablets of CLOT. Zero-order release model refers to a system where the rate of drug release is constant and independent of its concentration, and the cumulative percentage of

drug release versus time is plotted. First-order model defines a system where rate of drug release is dependent on its concentration and logarithm percentage of drug remaining is plotted against time[41&42]. The Higuchi model describes where rate of drug release from an insoluble matrix is proportional to the square root of time and the plot of cumulative percentage of drug release against the square root of time is linear for controlled release[43]. Korsmeyer–Peppas model applies the 'n' value representing the drug release exponent or diffusional exponent, to study the mechanism of drug release. The kinetic model with the best fit based on linearity of the plots shown by the highest value of correlation coefficient, R^2 will be selected[29].

In-vitro antifungal sensitivity test

Clinical isolates of *C. albicans* and *Aspergillus niger* sensitive to clotrimazole were collected and tested *In-vitro* following protocols approved by the Clinical and Laboratory Standards Institute (CLSI). The organisms were suspended in normal saline and vortexed for 60 s, and the suspension was centrifuged at 5,000 rpm for 10 min and fungal cells were collected. The cells were washed with phosphate buffered saline (PBS) pH 7.4 thrice, suspended in PBS and counted to obtain 2×10^8 colony-forming units (CFU)/ml of fungal cells respectively. The antifungal activity of the CLOT liquisolid compact tablets and their placebo counterparts were assessed against *C. albicans* and *A. niger* by agar well diffusion method. Agar plates were inoculated with 0.5 MacFarland standard broth cultures of the test organisms. Then, 100 µl of each fungal suspension combined with 20 ml of Sabouraud dextrose agar (SDA) solution were poured into sterile Petri dishes and then allowed to cool and solidify at room temperature for 15 min. Reconstitution of the formulations was made by dissolving one tablet in 2 ml of 2 % Tween® 80. Thereafter, 8-mm diameter wells were punched into the solidified agar using a sterile cork borer and filled with 80 µl of the formulations, the positive (pure clotrimazole solution) and negative (placebo tablets) controls. Then, the culture plates were kept in sterile inoculation chambers for 2 h to facilitate diffusion of the test solutions and to allow sufficient time for the fungi to grow. Each Petri dish was incubated at 37 ± 0.1°C for 48 h. The diameter of the inhibition zone around each well for each fungus was measured at the end of the incubation time. Experiments were performed in triplicate for each fungus and each test sample and the antifungal activity was expressed as the average of inhibition zone diameters (in mm) produced by the formulations[8,9].

Animal use protocols

White albino rats (BALB/c strain) of both sexes weighing between 180 – 220 g were selected randomly from the animal facility in the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria and allowed to acclimatize in an environment maintained at 25 ± 2 °C, 12 hrs light/dark cycle. Before the study commenced, the animals were given free access to pellets (Guinea Feeds, Nigeria) and clean water *ad libitum*. Animal use complied with the ARRIVE guidelines and the study was performed according to the U.K. Animals (Scientific Procedures) Act, 1986 and the EU Directive 2010/63/ EU guidelines for animal experiments with the approval of the Animal Research Ethics Committee (AREC) of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

In vivo antifungal study

To determine the antifungal efficacy of the formulations, the study was carried out as previously described[14] with slight modifications. The rats were immunosuppressed 14 days before inoculation by controlled oral administration of dexamethasone (0.5 mg/l) and tetracycline (1 g/l) in their drinking water. After the initial 7 days of immunosuppression, dexamethasone and tetracycline were administered at the concentrations of 1 mg/l and 0.1 g/l respectively. The study was done using 2×10^8 colony-forming units (CFU)/ml of *C. albicans* cells. Inoculation of animals was done by intraperitoneal administration of 0.5 ml of the

yeast inoculum and immunosuppression was continued for 7 days after fungal challenge to allow for complete infection *in vivo*. Then, the animals were divided into 7 groups of 5 rats per group for treatment of infection from *C. albicans*. Groups 1 - 4 received 5 mg/kg of the CLOT-loaded liquisolid tablets in drinking water by gastric gavage. Groups 5 was administered with 5 mg/kg of CLOT solution (positive control), group 6 received the placebo liquisolid compact tablet as the negative control, while group 7 animals were untreated. At pre-determined time intervals up to 24 hrs, blood was withdrawn from the retro-orbital venous puncture of the animals using capillary tubes and collected into heparinized tubes and centrifuged at 5,000 rpm for 15 min and the yeast cells present in the collected plasma was counted by spreading each sample onto a Sabouraud dextrose agar (SDA) agar plate. The plates were incubated at 37 ± 0.1°C for 48 hrs and the number of viable colonies of the microorganism was counted for each sample. The antifungal activity of the CLOT-loaded liquisolid compact tablets was depicted by plotting the number of *C. albicans* (cfu/ml) that survived at each time point[14&44].

Toxicological assay

Portions of the blood samples collected through the retro-orbital venous punctures were used to assess haematological parameters [red blood cells (RBC), packed cell volume, haemoglobin, white blood cells (WBC) and its differentials] of the animals following standard procedures. Plasma obtained from centrifuged blood samples was assayed for evaluation of liver enzymes (aspartate transaminase – AST, alanine transaminase – ALT, alkaline phosphatase – ALP) using Randox kit and following manufacturer's protocols.

Storage stability study

Storage stability test was carried out on the liquisolid compact tablets of clotrimazole according to slightly modified guidelines of the International Conference on Harmonization (ICH) (Q1A, R2). Tablets from each batch were stored for 6 months in a humidity chamber at different conditions: 30 ± 2 °C/65 ± 5 %RH and 40 ± 2 °C/75 ± 5 %RH. After 6 months, samples were collected and subjected to drug content uniformity test as earlier described[28,45].

Statistical evaluation

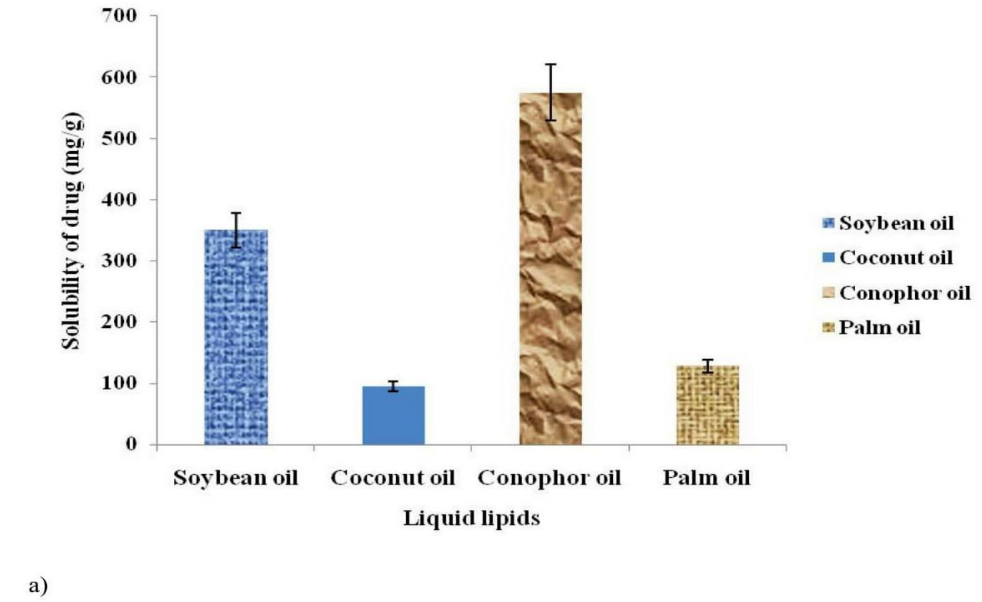
All data from the triplicate tests were expressed as mean ± standard deviation. Statistical significance of the differences in each study was determined at $p < 0.05$ by one-way analysis of variance (ANOVA) for grouped comparisons, and followed by student t-test using GraphPad Prism version 8.2.0 (Prisma, Graphpad Software, La Jolla, US) for analysis of data sets.

RESULTS AND DISCUSSION

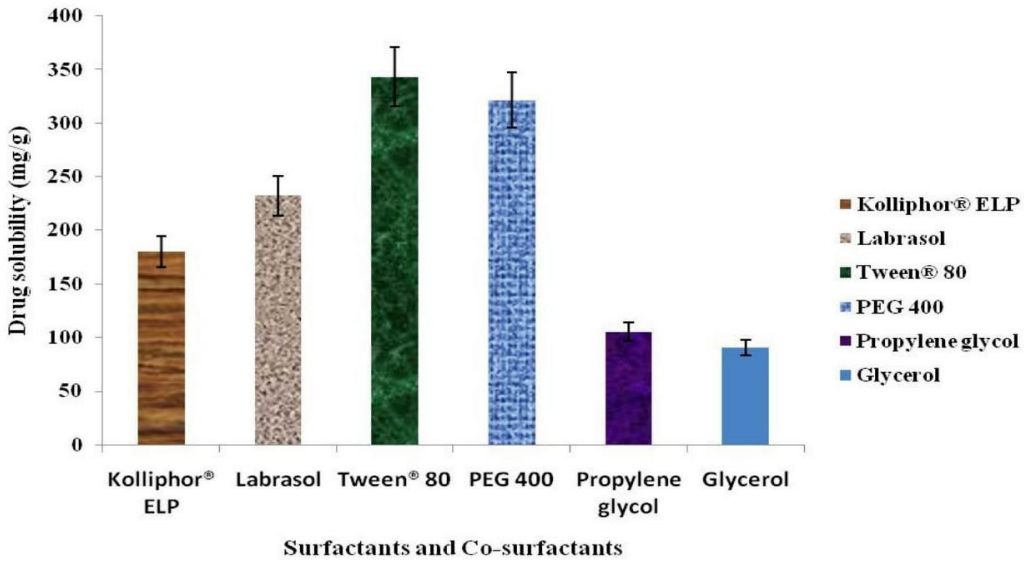
Results

Solubility screening of CLOT in excipients

This study was undertaken to identify the best inert oil and surfactants for the preparation of LNE of CLOT, and the solubility profiles of CLOT in oils, surfactants and co-surfactants are shown in Figure 1. The charts showed that CLOT was freely soluble in all the oils, surfactants, and co-surfactants tested because while an average amount of about 280 mg/g of CLOT solubilized in the oils as shown in Figure 1a, CLOT solubility in surfactants and co-surfactants averaged 250 and 170 mg/g respectively as seen in Figure 1b. Precisely, CLOT was most soluble (575.1 mg/g) in conophor oil, reasonably soluble in soybean and palm oils (350.45 and 128.3 mg/g), but least soluble in coconut oil (95.13 mg/g). Similarly, for the surfactants, the solubility of CLOT was highest (343.1 mg/g) in Tween® (polysorbate) 80 than Kolliphor® ELP (180.4 mg/g) and Labrasol (232.5 mg/g), while CLOT recorded the highest solubility in PEG 400 (321.6 mg/g) than in propylene glycol (105.4 mg/g) and glycerol (90.8 mg/g) for the co-surfactants.



a)



b)

Fig. 1: Solubility profiles of clotrimazole in various (a) oils (liquid lipid) (b) surfactants and co-surfactants. PEG 400 = Polyethylene glycol 400.

Ternary phase diagrams

Ternary phase diagrams were constructed based on data obtained through water titration of conophor oil, Tween®80 and PEG 400 as the surfactant and co-surfactant (S_{mix}) at different weight ratios. The phase diagrams were constructed to show the region of nanoemulsion for each diagram and to determine the optimal concentration of each of the components needed to formulate stable and clear nanoemulsions of CLOT. The phase diagrams are shown in Figure 2a-e and they revealed two distinct regions – the shaded region, which was characterized by visually homogenous and clear droplets, and the light (non-shaded) region, which was characterized by cloudy and unclear dispersions. From the diagrams, nanoemulsion region was about 20 % at S_{mix} 1:1 and increased to about 40 % at S_{mix} 2:1. The area of nanoemulsion was 30 % at S_{mix} 1:2 and remained unchanged at S_{mix} 1:3. However, there was further increase to about 35 % at S_{mix} 3:1.

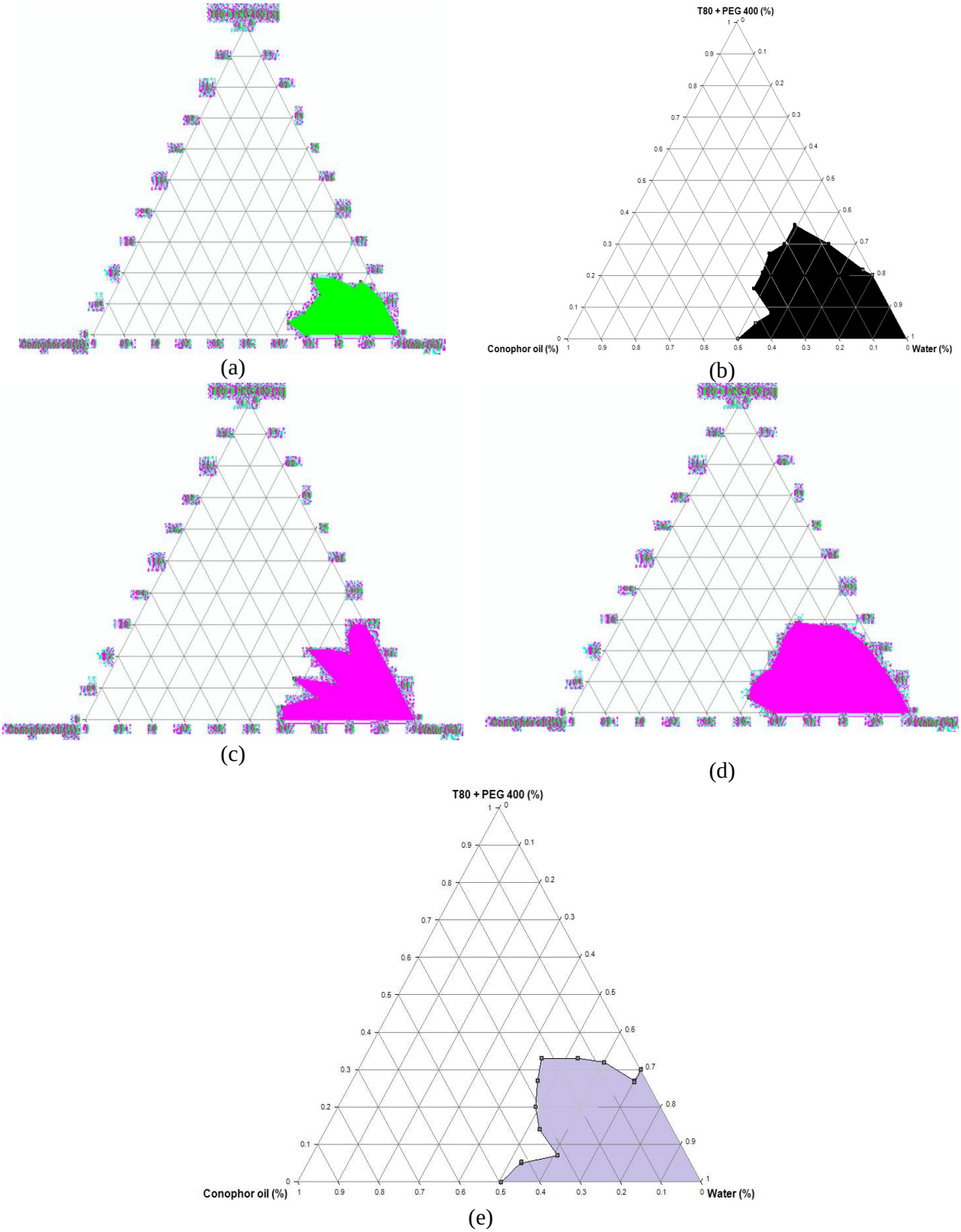


Fig. 2: Pseudo-ternary phase diagrams of the quaternary systems comprising Conophor oil/Tween®80/PEG 400/water at various S_{mix} ratios as follows (a) Tween® 80-PEG 400 1:1 (b) Tween® 80-PEG 400 2:1 (c) Tween® 80-PEG 400 1:2 (d) Tween® 80-PEG 400 1:3 (e) Tween® 80-PEG 400 3:1. T80 = Tween® 80, PEG = Polyethylene glycol.

Droplet size and polydispersity index of LNE

The results of the droplet sizes and polydispersity indices (PDI) of the various batches of LNE are shown in Table 1, while the intensity of the droplet size distribution is shown in Figure 3. As expected, the droplet sizes of the LNE were in the nanometer scale. Summarily, the average droplet sizes of batches A1 and A2 LNE containing CLOT ranged between 95.2 ± 5.3 and 121.6 ± 3.2 nm, the unloaded batch A3 recorded 66.7 ± 5.7 nm. Similarly, batches B1 and B2 LNE loaded with CLOT recorded average droplet sizes ranging between 88.4 ± 9.2 and 118.3 ± 1.1 nm, the unloaded batch B3 had droplet size of 72.1 ± 1.6 nm. In terms of polydispersity index, drug-loaded LNE could be said to be monodisperse in nature with PDI between 0.45 – 0.63 while the unloaded LNE are polydisperse with PDI of 0.71 and 0.79.

Table 1: Composition (%w/w) and physical properties of clotrimazole-loaded lipid nanoemulsion.

Batch	Conophor oil	CLOT	T80	PEG 400	DW	Droplet size (nm)	PDI
A1	30.0	0.5	3.0	1.5	100	95.2 ± 5.3	0.60 ± 0.01
A2	20.0	1.0	3.0	1.5	100	121.6 ± 3.2	0.45 ± 0.01
A3	20.0	ND	3.0	1.5	100	66.7 ± 5.7	0.57 ± 0.02
B1	20.0	0.5	3.0	1.5	100	88.4 ± 9.2	0.62 ± 0.01
B2	30.0	1.0	3.0	1.5	100	118.3 ± 1.1	0.59 ± 0.01
B3	20.0	ND	3.0	1.5	100	72.1 ± 1.6	0.63 ± 0.03

CLOT – Clotrimazole; T80 – Tween® 80; PEG 400 – Polyethylene glycol 400; DW – Distilled water; ND - No drug; A1, A2, B1, and B2 represent LNE batches which contain drug; A3 and B3 are LNE formulations which are drug-free or unloaded. PDI – polydispersity index.

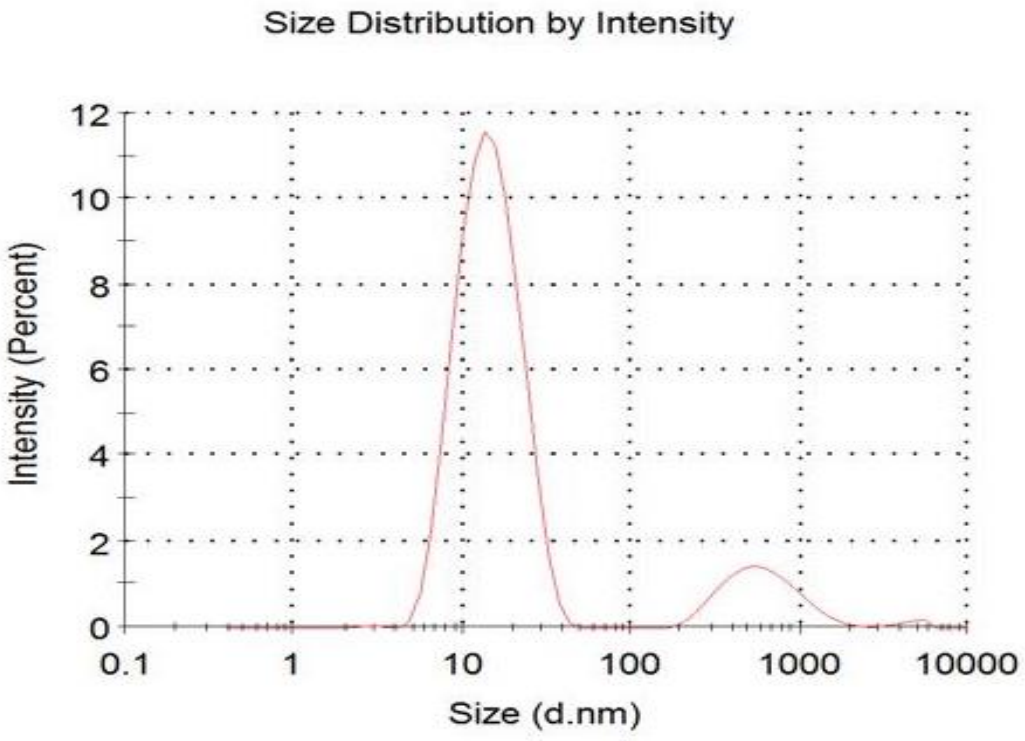


Fig. 3: Droplet size distribution curve for clotrimazole-loaded lipid nanoemulsion.

Granules flowability

Result of the micromeritic profiles of the granules is shown in Table 2. From the result, it was found that the angle of repose of the granules ranged between 22.15 ± 0.17 and 24.84 ± 0.88°, the Carr's compressibility index ranged between 7.11 ± 0.12 and 9.52 ± 0.75 %, and the recorded Hausner's ratio was in the range of 1.09 ± 0.04 and 1.11 ± 0.07. However, the differences recorded for each parameter are not significant (p> 0.05).

Morphology of granules

Visual inspection of the granules showed granular, consistent, free-flowing, and off-white to cream colour particles. The SEM surface morphology of the granules as shown in Figure 4 revealed that smooth, spherical, non-porous and irregularly-shaped microstructures (without surface drug crystals) that are homogenously packed were formed in the liquid-powder admixture.

Table 2: Some pre-compression and micromeritic properties of the LNE-based granules.

Batch	L_f	Q (mg)	q (mg)	Angle of repose (°)	Hausner's quotient	Compressibility index (%)
A1	0.23	217.4	10.87	22.15 ± 0.17	1.09 ± 0.04	7.11 ± 0.12
A2	0.23	282.6	14.13	22.07 ± 0.52	1.03 ± 0.01	7.85 ± 0.22
A3	0.23	208.7	10.44	23.11 ± 0.45	1.00 ± 0.07	9.52 ± 0.75
B1	0.23	226.1	11.31	23.72 ± 0.21	1.11 ± 0.07	8.01 ± 0.63
B2	0.23	269.6	13.48	22.55 ± 0.71	1.10 ± 0.09	8.76 ± 0.59
B3	0.23	195.7	9.79	24.84 ± 0.88	1.07 ± 0.08	7.97 ± 0.84

Data represents Mean ± standard deviation for triplicate determinations. A1, A2, B1, and B2 are LNE formulations which contain drug; A3 and B3 are LNE formulations which are drug-free or unloaded. L_f – Liquid load factor. Q – Quantity of carrier material. q – Quantity of coating material.

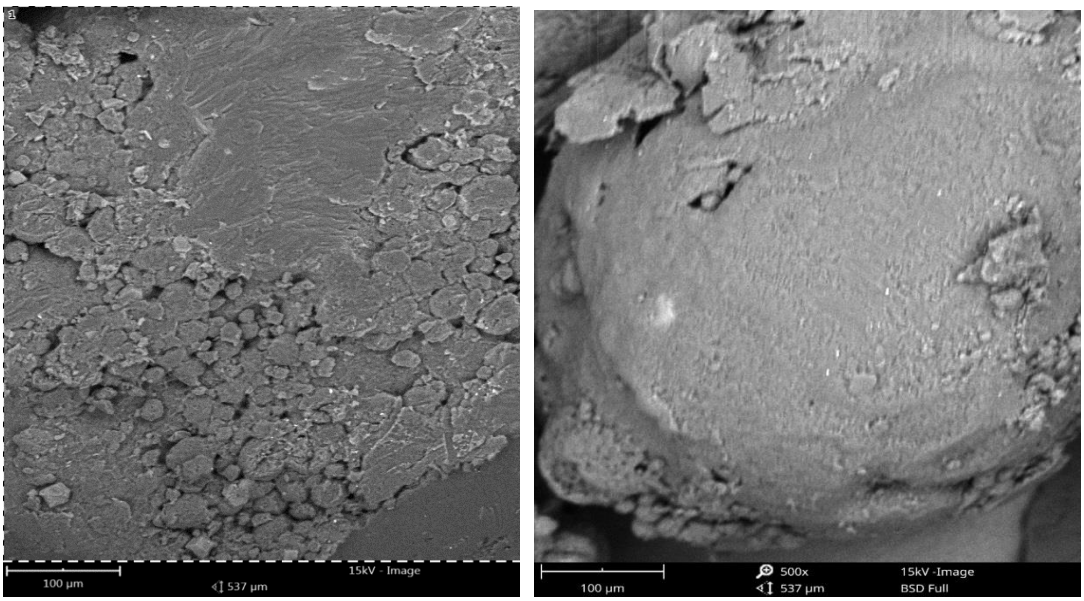


Fig. 4: Scanning electron micrographs (SEM) of selected clotrimazole-loaded lipid nanoemulsion (LNE)-based granules indicating smooth, spherical structures that are homogenously packed without surface drug crystals**.**

Thermal study by DSC

The result of the thermal behaviour of CLOT and the granules is shown in Figure 5. DSC thermogram of pure CLOT showed only one characteristic endothermic melting peak at 134.31 °C. The liquisolid granules yielded various characteristic single endothermic DSC curves with melting peaks lower than that of CLOT and ranging between 61.33 to 62.92 °C. It was observed that there was no peak due to CLOT in the granules.

Fourier-transform infrared (FT-IR) evaluation

Results of the FT-IR analysis are in Figure 6. The IR spectra of CLOT showed high wave bands at 3686.32 and 3455.59 cm^{-1} that correspond to −OH group and medium C=C-C stretching, mid-wave numbers at 2226.84 cm^{-1} ($-CH_2$ stretching), 1624.1 and 1349.57 cm^{-1} (benzene ring stretching), 1024.41 cm^{-1} (chlorobenzene and C= N stretching), and 667.85 and 705.44 cm^{-1} assigned to C-H stretching. These significant peaks observed in the IR spectra of the drug were retained in the CLOT-loaded granules without any untoward and significant shift in the positions of the peaks or the formation of novel peaks.

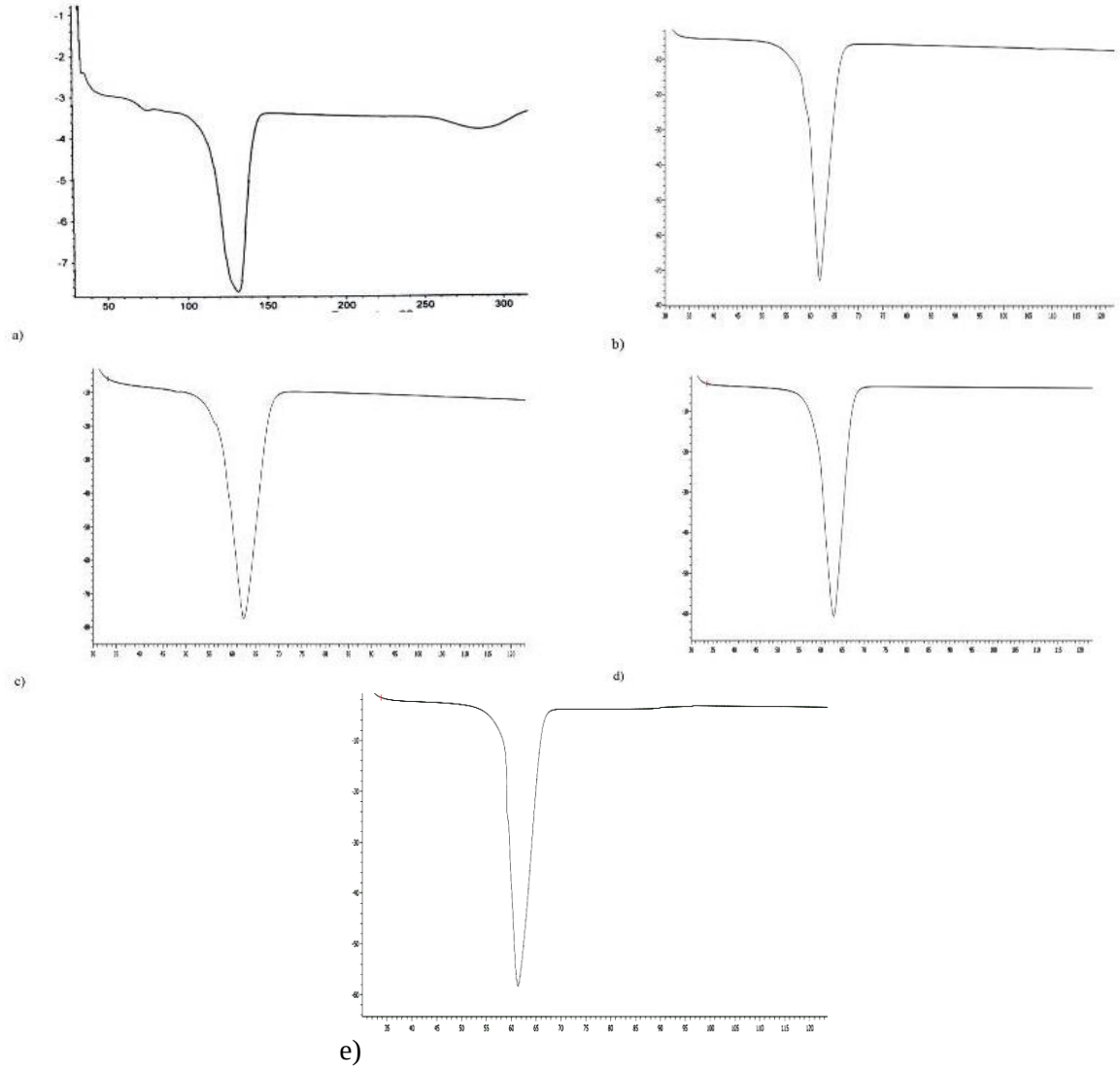


Fig. 5: Differential scanning calorimetry (DSC) thermograms of (a) Clotrimazole (CLOT), and granules prepared from (b) lipid nanoemulsion (LNE) A1 containing 0.5 %w/w CLOT (c) LNE A2 containing 1.0 %w/w CLOT (d) LNE B1 containing 0.5 %w/w CLOT (e) LNE B2 containing 1.0 %w/w CLOT.

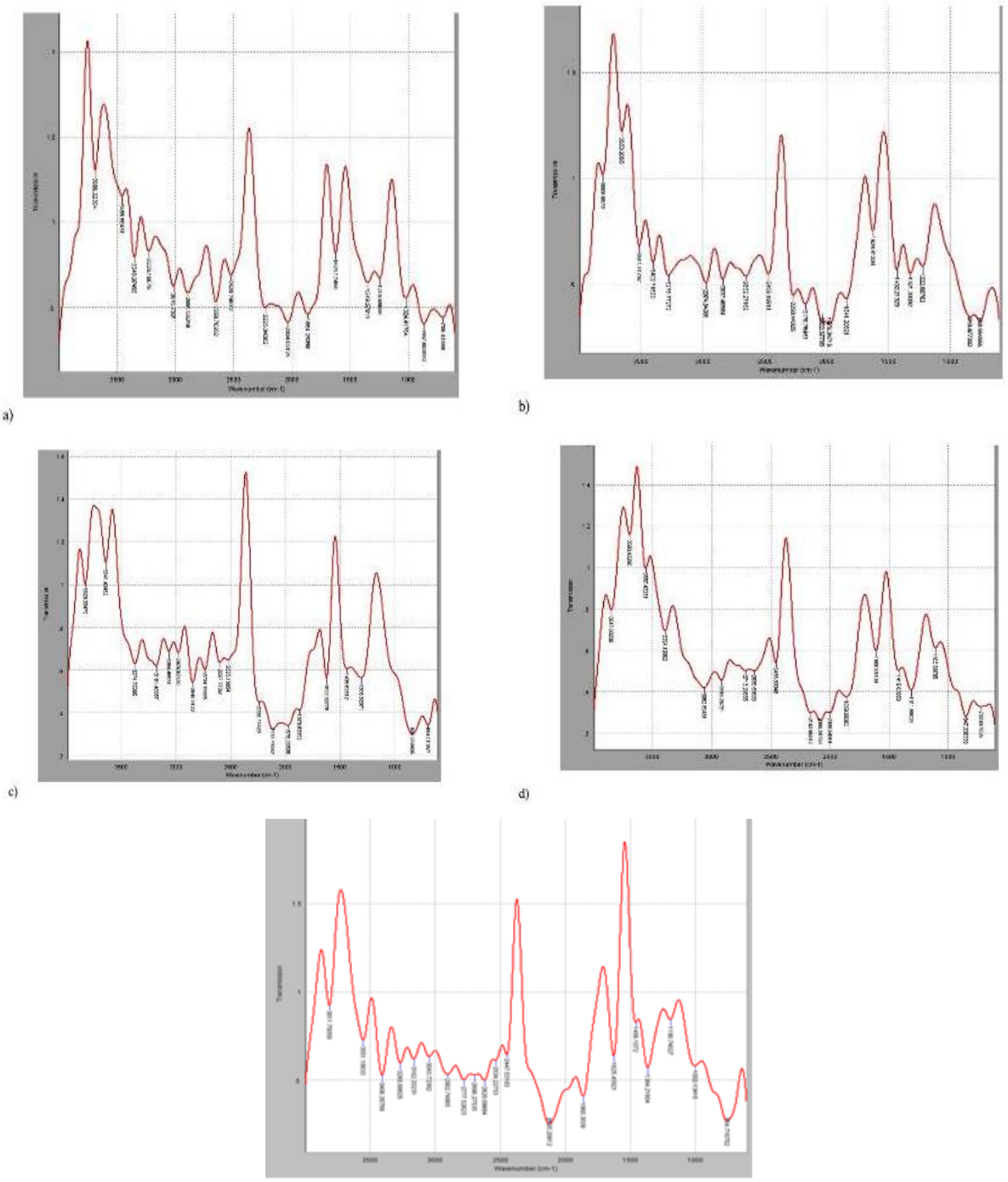


Fig. 6: FT-IR spectra of (a) Clotrimazole (CLOT), and granules prepared from (b) lipid nanoemulsion (LNE) A1 containing 0.5 %w/w CLOT (c) LNE A3 containing no drug (d) LNE B1 containing 0.5 %w/w CLOT (e) LNE B3 unloaded.

XRPD analysis

XRPD diffractograms of the granules and CLOT are shown in Figure 7. The result showed that CLOT registered characteristic diffraction peaks at (2θ) 8.8°, 10.9°, 11.5°, 13.5°, 15.1°, 18.8°, 19.2°, 20.1°, 21.7°, 24.3°, 25.6°, and 26.4° respectively. Interestingly, the diffractograms of the liquid-solid granules did not show the entire major peaks of the drug as could be seen from Figure 6. The observed differences between the XRPD peaks of CLOT and the liquisolid granules were very significant ($p < 0.05$).

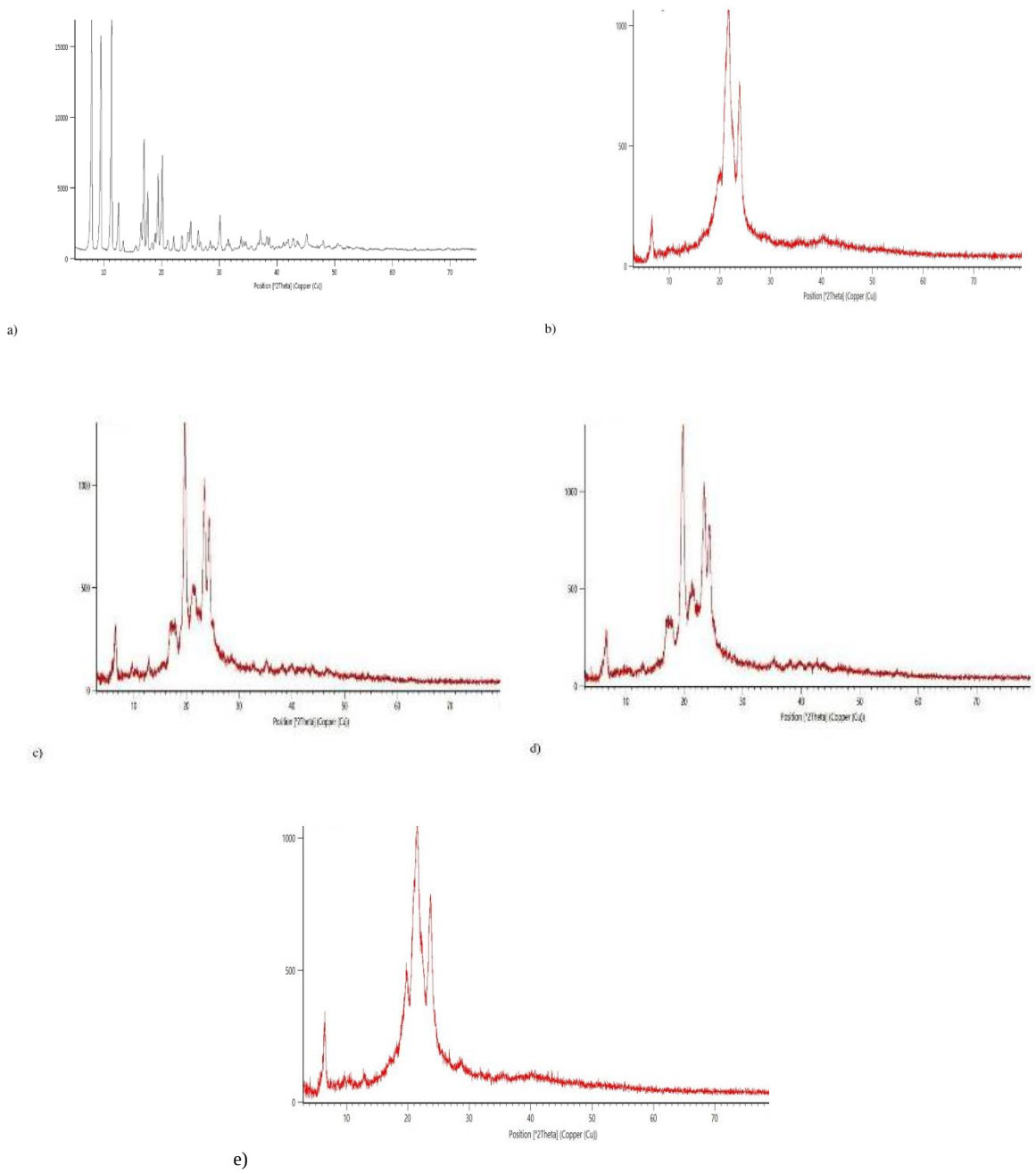


Fig. 7: X-ray diffractograms of (a) Clotrimazole (CLOT), and granules prepared from (b) lipid nanoemulsion (LNE) A1 containing 0.5 %w/w CLOT (c) LNE A2 containing 1.0 %w/w CLOT (d) LNE B1 containing 0.5 %w/w CLOT (e) LNE B2 containing 1.0 %w/w CLOT.

Drug content uniformity

Result of the drug content uniformity assessment of the liquisolid tablets shown in Table 3 indicates varying and significant ($p<0.05$) content of CLOT ranging between 85.2 ± 0.1 and 99.8 ± 0.2 %. Precisely, batch B tablets are more uniform in drug content than batch A liquisolid tablets because TB1 and TB2 tablets recorded 95.8 ± 1.5 and 99.8 ± 0.2 % drug content respectively, whereas for batch A tablets, while TA1 tablets recorded 97.6 ± 0.5 % of drug content, TA2 liquisolid tablets had 85.2 ± 0.1 % CLOT content. However, inter-batch comparisons indicate no significant ($p>0.05$) difference. In contrast, TA3 and TB3 are placebo liquisolid tablets because they contain no clotrimazole.

Table 3: Physicotechnical properties of clotrimazole-lipid nanoemulsion liquisolid compact tablets.

Batch	Drug content uniformity (%)	Drug content uniformity (%) (6 months)	Weight uniformity (mg)	Friability (%)	Hardness (KgF)	Disintegration time (min)
TA1	97.6 ± 0.5	90.4 ± 1.1	341.4 ± 1.2	0.31 ± 0.01	4.52 ± 0.12	2.96 ± 0.8
TA2	85.2 ± 0.1	82.7 ± 2.4	344.3 ± 0.4	0.27 ± 0.01	5.35 ± 0.32	4.15 ± 1.1
TA3	ND	ND	342.1 ± 0.7	0.53 ± 0.03	4.35 ± 0.56	3.55 ± 0.5
TB1	95.8 ± 1.5	89.3 ± 1.7	345.5 ± 0.5	0.44 ± 0.02	5.67 ± 0.22	4.50 ± 2.1
TB2	99.8 ± 0.2	92.6 ± 0.8	346.7 ± 0.8	0.25 ± 0.01	6.55 ± 1.22	5.88 ± 1.3
TB3	ND	ND	343.2 ± 0.7	0.28 ± 0.04	6.75 ± 0.31	3.78 ± 2.2

Data represents Mean ± standard deviation for triplicate determinations. TA1, TA2, TB1, and TB2 are liquisolid compact tablets containing clotrimazole; TA3 and TB3 are liquisolid compact tablets that are drug-free or unloaded. ND – No drug.

Weight uniformity test

Result of weight uniformity test of the CLOT-LNE liquisolid compact tablets is shown in Table 3. It could be seen that the tablets had weights ranging between 341.4 ± 1.2 and 346.7 ± 0.8 mg. It was observed that drug loading influenced tablet weight uniformity as increased drug loading resulted in increased weight. For instance, the data showed that in batch A liquisolid tablets, TA1 and TA2 had weights 341.4 ± 1.2 and 344.3 ± 0.4 mg respectively, with drug loading of 0.5 and 1.0 %w/w of CLOT. Similar observation was made for batch B tablets. However, placebo tablets TA3 and TB3 had low weights of 342.1 ± 0.7 and 343.2 ± 0.7 mg respectively.

Tablet friability

Result of the friability test is shown in Table 3 because the CLOT-LNE liquisolid tablets had significant ($p< 0.05$) friability ranging from 0.25 ± 0.01 to 0.53 ± 0.03 %. Specifically, batch A liquisolid tablets had friability values between 0.27 ± 0.01 and 0.53 ± 0.03 % while batch B tablets recorded friability values ranging between 0.25 ± 0.01 and 0.44 ± 0.02 %.

Hardness of tablets

The result of the hardness test is shown in Table 3. It could be seen from the data that all tablet batches had significant ($p<0.05$) hardness values ranging from 4.35 ± 0.56 to 6.75 ± 0.31 kgF. There was no significant ($p> 0.05$) difference in the hardness data for batches TA1 – TA3 with values ranging from 4.35 ± 0.56 to 5.35 ± 0.32 kgF and that of batches TB1 – TB3 had hardness values ranging between 5.67 ± 0.22 and 6.75 ± 0.31 kgF.

Disintegration time test

Result of the disintegration time study is shown in Table 3. The data showed that the liquisolid compact tablets of CLOT disintegrated completely between 2.96 ± 0.8 and 5.88 ± 1.3 min. Inter-batch comparison of disintegration times revealed for batch A formulations, batch TA1 had disintegration time of 2.96 ± 0.8 min while batch TA3 disintegrated within 3.55 ± 0.5 min. It was observed that liquisolid tablets formulated from LNE with high liquid lipid (oil) content disintegrated faster than formulations which parent LNE had low content of conophor oil. Similar scenario was observed with batch B liquisolid tablets, though there is no huge difference ($p> 0.05$) in the disintegration times of batches A and B liquisolid compact tablets.

***In-vitro* dissolution study**

The dissolution profiles of the CLOT-LNE liquisolid tablets in SGF (pH 1.2) and SIF (pH 7.4) at 37 ± 1 °C are shown in Figures 8 and 9 respectively. Within the study period of 12 hrs and as shown in Figure 8, the liquisolid compact tablets batches TA1 and TB1 produced significant ($p< 0.05$) drug release maxima of 55.13 ± 4.5 and 50.10 ± 6.8 % respectively in 2 h in SGF while 92.45 ± 9.3 and 88.10 ± 7.5 % of CLOT respectively were the significant ($p< 0.05$) amounts released in SIF after 12 h. Similarly in Figure 9, batches TA2 and TB2 liquisolid tablets significantly ($p<0.05$) released 58.13 ± 5.2 and 53.10 ± 2.2 % of CLOT respectively in SGF within 2 hrs,

and significantly ($p<0.05$) released 94.75 ± 3.2 and 91.10 ± 1.0 % of drug respectively in SIF. Further assessment of the dissolution features of the liquisolid tablets led to the evaluation of their dissolution efficiency (DE%) in order to ascertain their chance of remaining dissolved and in a prolonged contact with the physiologic milieu of the gastrointestinal system with potential high bioavailability. $DE\%_{2\ h}$ of TA1 and TB1 recorded statistically significant ($p<0.05$) values of 54.92 ± 4.4 and 49.81 ± 6.6 % respectively in SGF and this increased significantly ($p<0.05$) in SIF to 92.05 ± 9.0 and 87.65 ± 7.3 % respectively for $DE\%_{12\ h}$. Similarly, TA2 and TB2 had significant ($p<0.05$) $DE\%_{2\ h}$ values at 58.01 ± 5.1 and 52.85 ± 2.0 % respectively in SGF which significantly ($p< 0.05$) increased in SIF to 94.00 ± 2.7 and 90.73 ± 1.3 % respectively for $DE\%_{12\ h}$.

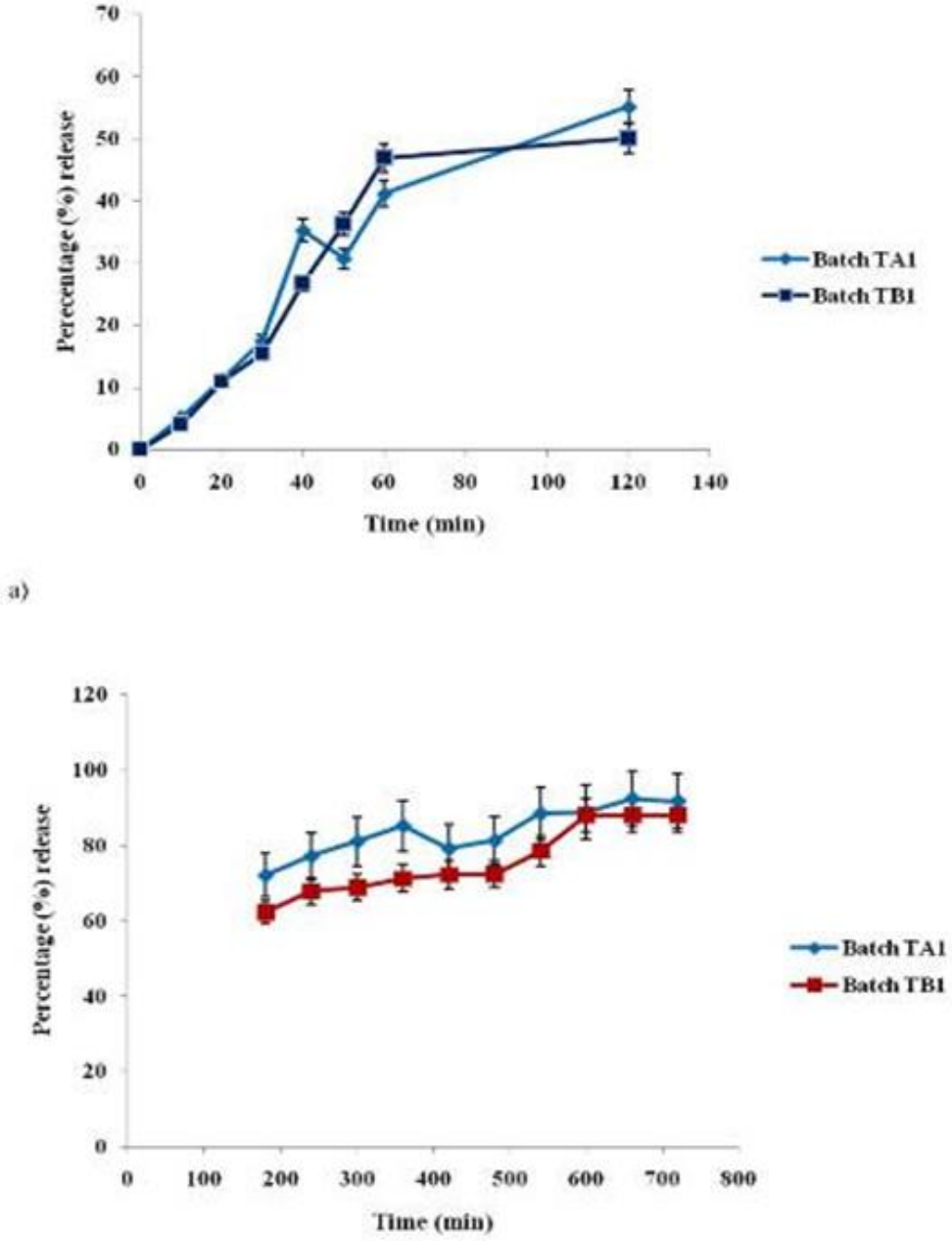


Fig. 8: *In-vitro* dissolution profiles of clotrimazole (CLOT) from (a) liquisolid compact tablets, TA1 and TB1 containing 0.5 %w/w of CLOT in simulated gastric fluid (SGF), and (b) liquisolid compact tablets, TA1 and TB1 containing 0.5 %w/w of CLOT in simulated intestinal fluid (SIF).

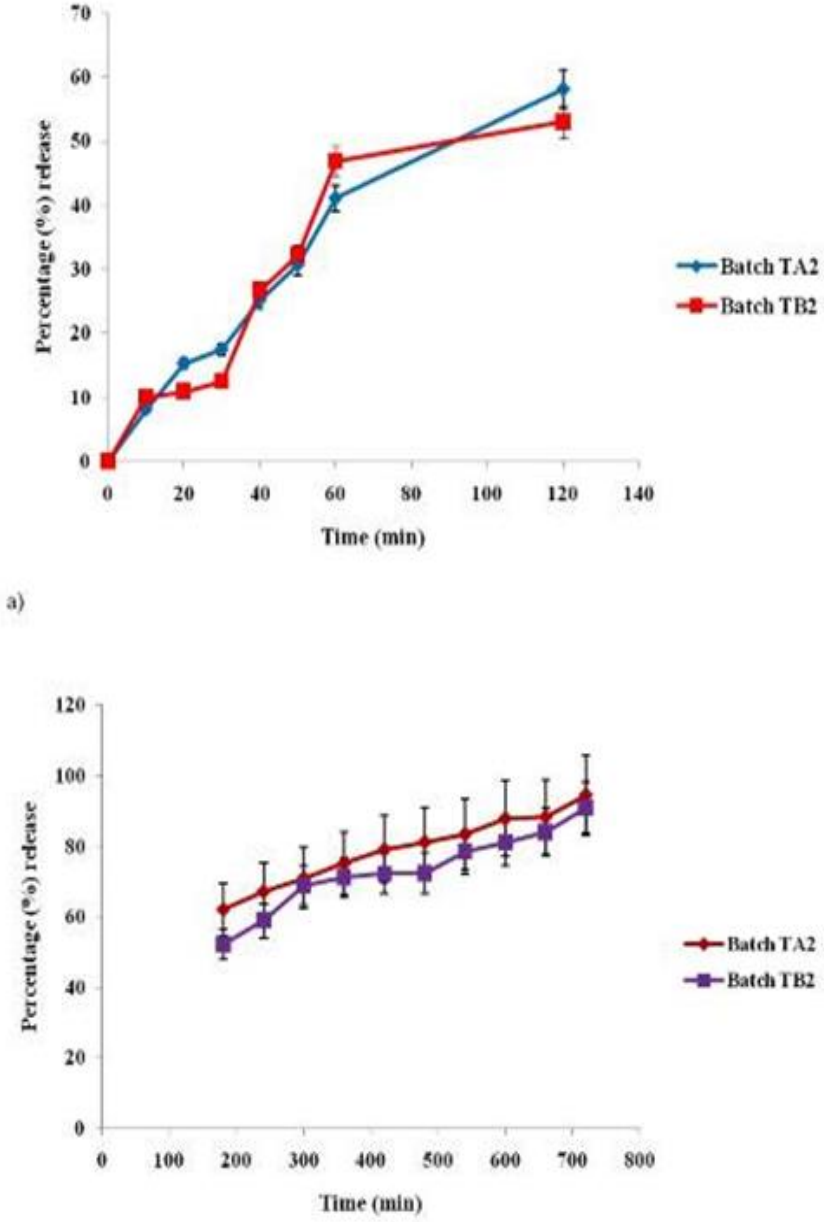


Fig. 9: *In-vitro* dissolution profiles of clotrimazole (CLOT) from (a) liquisolid compact tablets, TA2 and TB2 containing 1.0 %w/w of CLOT in simulated gastric fluid (SGF), and (b) liquisolid compact tablets, TA2 and TB2 containing 1.0 %w/w of CLOT in simulated intestinal fluid (SIF).

Mechanism of release and release kinetics of tablets

Result of the drug release kinetics and release mechanism of the liquisolid compact tablets is shown in Table 4. From the data in SGF, batches TA1 and TB1 with correlation coefficient > 0.99 fitted into the Higuchi square root of time kinetic model of drug release. First order and Higuchi models best describe the kinetic of release of CLOT from batches TA2 and TB2 which recorded correlation coefficient greater than 0.9 for both models. The release exponent (n) for batches TA1 and TB1 is less than 0.89. Batch TA2 had release exponent of 0.101 while batch TB2 had release exponent of 0.854. In SIF, first order and Higuchi square root of time models best describe the drug release kinetics of batches TA1, TB1 and TB2 because their correlation coefficient was higher than 0.9. Batch TA2 fitted well into the Higuchi model of drug release with the highest correlation coefficient of 0.997. The release exponents (n) for all the batches are less than 0.89.

Table 4: Release kinetics of drug from CLOT-LNE liquisolid compact tablets.

Batch	Media	Zero order	First order	Higuchi	Korsmeyer-Peppas	
		r^2	r^2	r^2	r^2	n
TA1	SGF	0.476	0.889	0.998	0.995	0.756
TB1		0.948	0.875	0.997	0.245	0.857
TA2		0.964	0.997	0.996	0.947	0.101
TB2		0.898	0.983	0.985	0.756	0.936
TA1	SIF	0.856	0.991	0.994	0.315	0.657
TB1		0.931	0.986	0.984	0.928	0.367
TA2		0.976	0.859	0.997	0.648	0.689
TB2		0.789	0.986	0.989	0.746	0.787

TA1, TA2, TB1, and TB2 are liquisolid compact tablets containing clotrimazole at various amounts; r^2 = squared correlation coefficient; 'n' represents the release exponent in Korsmeyer-Peppas drug release model. SGF = simulated gastric fluid; SIF = simulated intestinal fluid. CLOT = clotrimazole; LNE = lipid nanoemulsion.

***In-vitro* antifungal screening**

The results of the *In-vitro* antifungal screening of the CLOT-LNE liquisolid compact tablets, placebo tablets, and CLOT solution against *C. albicans* and *A. niger* are indicated in Figure 10. The data showed significant ($p< 0.05$) zones of inhibition produced against the test fungi but the antimycotic effect was proportional to drug loading. This is because batches TA2 and TB2 loaded with 1.0 %w/w of CLOT gave the highest ($p< 0.05$) inhibitions against *C. albicans* (22.67 ± 0.7 and 23.33 ± 1.4 mm) and *A. niger* (24.62 ± 1.5 and 20.67 ± 1.2 mm) respectively, than TA1 and TB1 loaded with 0.5 %w/w of CLOT and which produced their highest ($p< 0.05$) inhibitions against *C. albicans* (20.67 ± 0.1 and 22.33 ± 2.1 mm) and *A. niger* (19.05 ± 1.8 and 16.67 ± 0.9 mm) respectively.

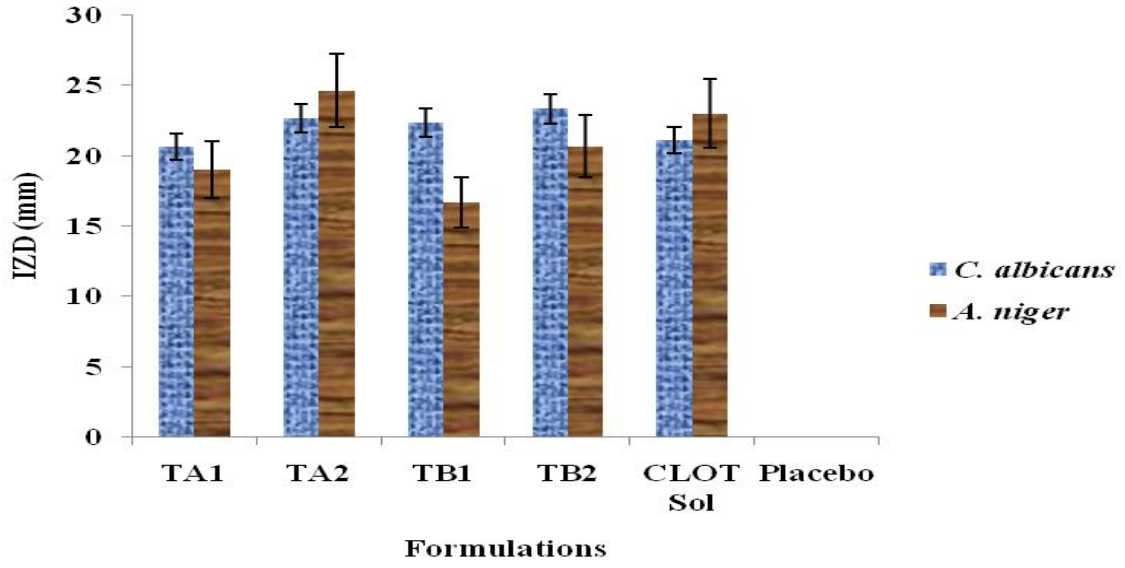


Fig. 10: *In-vitro* antifungal activity profiles of clotrimazole-lipid nanoemulsion (CLOT-LNE) liquisolid compact tablets against *Candida albicans* and *Aspergillus niger*. TA1 and TB1 containing 0.5 %w/w of CLOT, TA2 and TB2 containing 1.0 %w/w of CLOT. IZD = inhibition zone diameter.

***In-vivo* antifungal study**

The *in vivo* antifungal activity of the CLOT-LNE liquisolid compact tablets, CLOT solution and the placebo liquisolid tablets was tested in *C. albicans*-inoculated rats, and the result in illustrated in Figure 11. From the obtained data, it could be seen that *C. albicans* colonies in the experimental rats treated with liquisolid compact tablets of CLOT and the CLOT solution decreased after 1 h in comparison with the placebo and untreated groups, but the reduction of yeast colonies produced by CLOT-LNE liquisolid tablets was higher ($p < 0.05$) than that recorded by CLOT solution. The highest or most significant ($p < 0.05$) reduction of *C. albicans* colonies in the treated animals by the liquisolid tablets was obtained at the 12th hr followed by a sustained reduction in yeast load of the study animals up till 24 hrs when there was total fungi clearance. In contrast, the highest antifungal activity for CLOT solution was obtained after 2 hrs but this trend was not sustained, while the placebo tablets and the untreated groups maintained very high fungal loads *in vivo*.

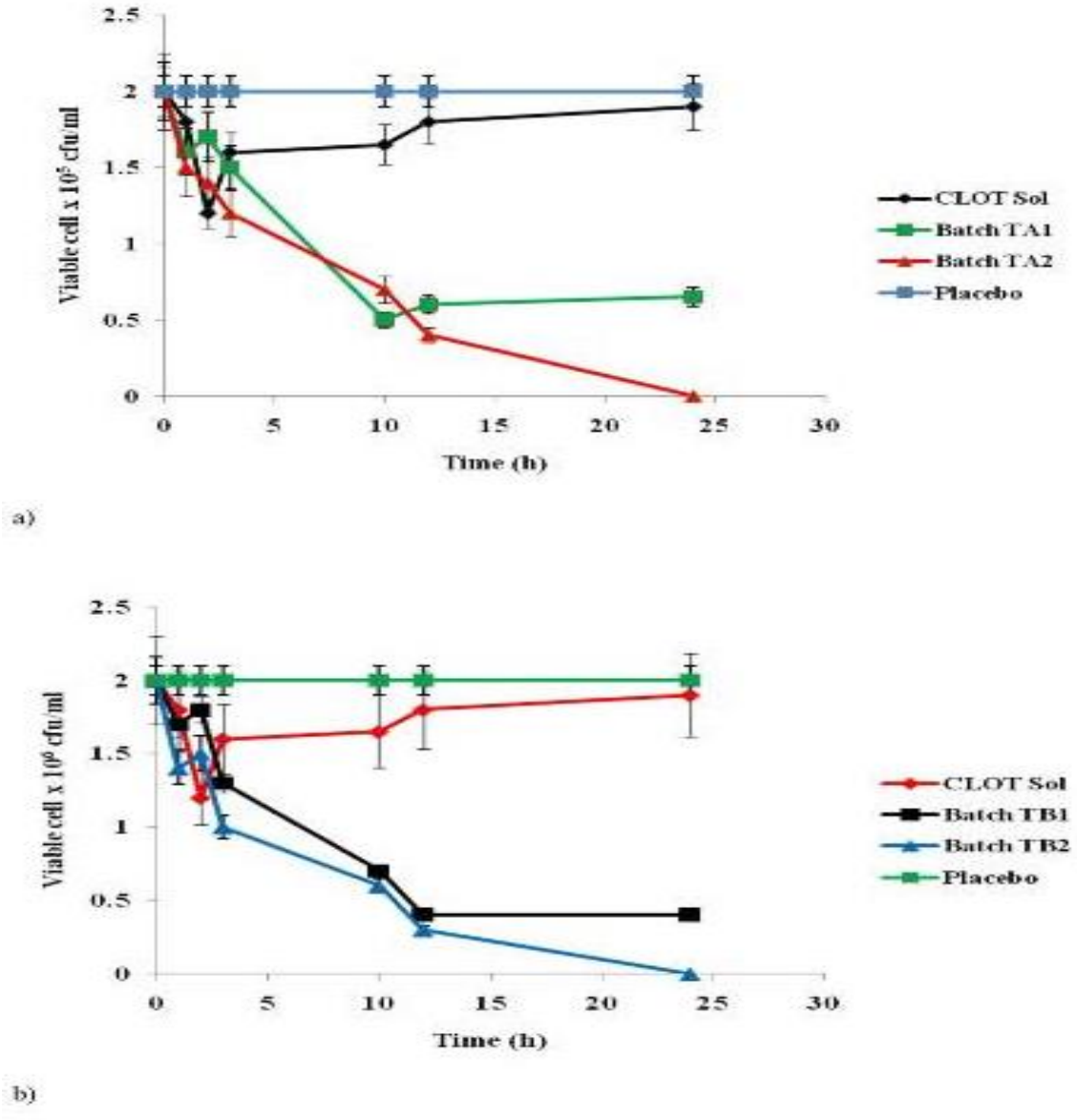


Fig. 11*: In-vivo* antifungal activity profiles of (a) clotrimazole-lipid nanoemulsion (CLOT-LNE) liquisolid compact tablets (TA1 and TA2 containing 0.5 and 1.0 %w/w of CLOT) and (b) clotrimazole-lipid nanoemulsion (CLOT-LNE) liquisolid compact tablets (TB1 and TB2 containing 0.5 and 1.0 %w/w of CLOT) against *Candida albicans*.

Toxicity assessment

Result of the toxicity study of the tablets is shown in Table 5. The result indicate no significant ($p > 0.05$) differences in the mean haematological factors measured from the blood samples of animal groups that received the CLOT-LNE liquisolid compact tablets relative to the control groups and the basal. However, the haematologic parameters recorded by the CLOT solution were higher ($p<0.05$) than that mobilized in the placebo group. The result also revealed that the hepatic factors from the CLOT-LNE liquisolid tablet groups were slightly ($p>0.05$) lower compared with the controls and the basal, but the placebo produced slight higher levels of AST and ALT relative to the CLOT solution.

Storage stability evaluation

The result of the storage stability assessment is indicated in Table 3 and the data revealed that there was no significant ($p > 0.05$) changes in the evaluated drug content uniformity of the CLOT-LNE liquisolid compact tablets after 6 months of storage and sampling according to ICH (Q1A, R2) guidelines because the tablets retained acceptable contents of CLOT.

Table 5: Effects of CLOT-LNE liquisolid compact tablets on the haematologic and hepatic factors.

Biomarker	Basal values	TA1	TA2	TB1	TB2	CLOT Solution	Placebo
Hb (g/dl)	13.8 ± 1.5	13.6 ± 0.1	12.9 ± 1.1	13.0 ± 0.5	13.2 ± 0.8	12.4 ± 0.2	11.6 ± 0.3
PCV (%)	39.6 ± 2.1	35.5 ± 0.5	37.2 ± 0.8	36.8 ± 0.4	39.1 ± 0.2	33.7 ± 0.1	31.8 ± 0.1
RBC ($\times 10^6/cm^3$)	6.5 ± 1.0	6.1 ± 0.3	5.8 ± 0.1	6.3 ± 0.9	6.0 ± 1.1	5.5 ± 0.5	5.0 ± 0.8
WBC ($\times 10^3/cm^3$)	9.1 ± 1.7	8.6 ± 0.7	8.8 ± 0.5	8.9 ± 0.1	8.7 ± 0.8	8.1 ± 0.2	7.5 ± 0.1
Lym (%)	71.5 ± 1.2	68.7 ± 1.0	69.3 ± 0.8	66.8 ± 0.5	70.4 ± 1.1	63.3 ± 0.3	60.0 ± 0.2
Neut (%)	30.3 ± 0.8	26.2 ± 0.2	28.8 ± 0.7	27.7 ± 0.2	29.1 ± 0.7	23.5 ± 0.1	21.6 ± 0.1
Mono (%)	1.2 ± 0.1	1.6 ± 0.3	1.4 ± 0.1	1.1 ± 0.7	1.2 ± 0.2	1.0 ± 0.2	0.7 ± 0.0
Baso (%)	2.2 ± 0.5	2.5 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	1.9 ± 0.6	1.7 ± 0.1	1.5 ± 0.2
Oes (%)	3.2 ± 0.7	2.6 ± 0.3	2.9 ± 0.6	2.7 ± 0.4	3.0 ± 0.7	2.2 ± 0.1	1.8 ± 0.4
PLT ($\times 10^3/cm^3$)	3.5 ± 1.1	3.6 ± 0.7	3.0 ± 0.4	3.1 ± 0.8	3.4 ± 0.3	2.6 ± 0.2	2.3 ± 0.1
AST (U/L)	80.2 ± 7.3	78.7 ± 9.2	75.2 ± 5.7	74.1 ± 7.7	77.3 ± 5.7	79.1 ± 1.5	80.3 ± 2.7
ALP (U/L)	77.4 ± 8.1	66.2 ± 5.2	70.5 ± 9.1	71.5 ± 4.1	75.1 ± 6.6	76.5 ± 4.1	75.8 ± 1.4
ALT (U/L)	88.7 ± 6.5	80.5 ± 7.1	85.1 ± 8.0	82.1 ± 9.5	84.1 ± 5.5	86.1 ± 1.5	87.1 ± 2.5

TA1, TA2, TB1, and TB2 are liquisolid compact tablets containing clotrimazole at various amounts. Hb – haemoglobin; PCV – packed cell volume; RBC – red blood cell; WBC – white blood cell; Lym – lymphocytes; Neut – neutrophils; Mono – monocytes; Baso – basophils; Oes – Oesinophils; PLT – platelets; AST - aspartate transaminase;ALP - alkaline phosphatise; ALT - alanine transaminase.

Discussion

Solubility screening of CLOT in various oils, surfactants and co-surfactants is important because using the appropriate formulation excipients will guarantee a clear, monophasic, and stable disperse system. Furthermore, selecting the most suitable excipients will facilitate drug solubilization and entrapment[22&23]. The shake-flask technique applied in the test has high adaptability and has been utilized for investigating the solubility of various biomolecules[22&30&46]. High drug solubility could be due to the non-formation of liquid crystalline mesophases or avoidance of chance crystallization in the oil and surfactants[22]. Thus, conophor (walnut) oil, Tween® 80 and PEG 400 which are generally regarded as safe (GRAS) due to their non-toxic and non-irritant properties, were selected for further investigation. Our previous study showed that conophor oil possesses three major fatty acids namely *n*-hexadecanoic acid, 9(Z)-octadecenoic acid, and *cis*-13-octadecenoic acid, which have been reportedly used as important adjuvants in lipid-based formulations for oral and topical applications, and in the manufacture of cosmetics. Perhaps, these fatty acids produced high CLOT fluidity in conophor oil resulting in enhanced solubility[23]. Selecting the right surfactant and co-surfactant for the preparation of LNE depends on their compatibility with the oil phase, safety, and ability to ensure the reduction of the surface free energy that exists at the oil-water interface[47]. Tween®80 (surfactant) and PEG 400 (co-surfactant) were selected because they produced the highest solubility of CLOT. They are non-ionic surfactants that will emulsify systems at very low concentration, and are also non-toxic. Hence, it is expected that they will not produce gastrointestinal irritation which favours oral administration. Furthermore, combining Tween®80 and PEG 400 with HLB values of 15 and 9.7 respectively, will favour production of stable LNE because the co-surfactant molecules will be distributed within the surfactant resulting in decreased interaction between the polar heads of the surfactants with improved interfacial film fluidity which effectively shields the oil phase with increased hydrocarbon tail penetration and formation of stable film[30].

The phase diagrams revealed changes in areas of nanoemulsion formation with changes in S_{mix} ratio. This is in agreement with previous report[30]. Maximum area of nanoemulsion (about 40 %) was obtained at S_{mix} 2:1 while nanoemulsion region was significantly reduced yielding a minimum area that was recorded at S_{mix} 1:1. This indicates that increasing the surfactant (Tween® 80) concentration relative to the co-surfactant (PEG 400) concentration yielded significant increase in area covered by the nanoemulsion formation. Within the nanoemulsion region, very fine oil-in-water droplets are formed with gentle agitation. This is facilitated by the ability of the surfactant molecules localized at the surface of the emulsion droplets to reduce interfacial free energy and provide a mechanical barrier to coalescence, while molecules of the co-surfactant increases surfactant interfacial fluidity by creating tangible voids among surfactant molecules. These phenomena would favour the formation of thermodynamically stable and spontaneous nanoemulsion as well as lead to improved solubilization of candidate drug[28&48]. Since, the nanoemulsion region also indicates emulsification efficiency of each S_{mix} ratio; it means that S_{mix} ratio 2:1 with the maximum nanoemulsion area had the best emulsification efficiency than the other S_{mix} ratios, and would result in reduced interfacial tension to the minimal level and enhanced surfactant/co-surfactant packing with the formation of a highly flexible coherent film at the oil/water interface[30]. Thus, S_{mix} ratio 2:1 was selected for the preparation of nanoemulsion.

Droplet size and size distribution evaluation are very important methods in the characterization of LNE. The result revealed that drug incorporation significantly ($p< 0.05$) affected droplet sizes with increases compared to the unloaded batches but with narrow deviation values. The variations in the droplet sizes could be due to the dynamic properties of LNE which maintained all the droplets in a steady state of flux with considerable degree of flocculation[8]. The increase in droplet sizes of the CLOT-loaded LNE confirms the molecular solubilization of CLOT in the oil-water dispersion, and lowering the oil-water interfacial tension by Tween®80 and PEG 400 might have nullified any chance formation of coagulum and secondary nucleation resulting in improved drug solubilization[28]. Considering the report that monodispersed samples have PDI values between 0.1 – 0.7 and polydisperse systems have PDI> 0.7, it could be noted that

the CLOT-loaded LNE are monodispersed while the unloaded LNE were polydispersed[49]. In addition, there was no drug precipitation or recrystallization in the loaded LNE throughout the study because the non-ionic surfactants lowered droplet-droplet interaction and coalescence due to their double layer repulsive effect giving a stable dispersion[28].

The study of flow characteristics of granules is an important aspect of particle engineering in the production of tablets because granules with excellent flow properties promote homogenous mixing of tablet ingredients (drug and excipients), give optimum die fill, yield tablets with uniform weight and diameter, produce tablets with uniform content of active pharmaceutical ingredient (API), and the desired therapeutic outcome[30&50]. Study of the flowability of the LNE-based granules for the direct compression of liquisolid tablets was done by measuring the angle of repose, compressibility index, and Hausner's quotient which show the degree of compressibility and interparticulate interactions between the granules particles[26]. The data obtained are in agreement with pharmacopoeial requirements for excellent flowability and compressibility of granules[35]. The excellent flow characteristic of the granules might be due to very low interparticulate friction that existed between the granular particles[51], and this property will boost direct compression of the dry granules owing to their cohesive nature, good particulate intimacy, and deformation tendency[52]. Results of the study infer that the adapted liquisolid technique has great potential for improving the flow and compression properties of granules for tablet production, and this could be ideal when commercial manufacturing of tablets and optimum quality control of produced tablets are considered.

Morphological evaluation is an important characterization step in powder technology. From the SEM micrograph, it could be inferred that the liquisolid admixtures were adequately blended and that the LNE formulations were compatible with the solid excipients used in producing the granules. The observation indicates that the formulation technique was reliable because it did not produce any untoward powder packing in the liquisolid admixture, and confirms the theory that liquid vehicles attenuate crystallinity of granules resulting in particles with smooth topology[53].

DSC was employed to study the material purity, thermal properties (heat changes – melting and recrystallization), and compatibility attributes of the drug and granules. The slight variation between this DSC scan and other reports on CLOT[6,54,55] could be due to differences in instrument scan rate settings or the actual state of CLOT used in these studies. Nonetheless, an endothermic melting peak for CLOT was designated. The disappearance of the endothermic melting peak of CLOT in the granules infers that the drug was entirely solubilized in the LNE and entrapped in the liquid-solid admixture perhaps due to internal molecular rearrangement of the nanoemulsion droplets and then, the powder particles. It also showed that CLOT completely transformed from crystallinity to amorphicity within the LNE and the powder mix and was compatible with the powder ingredients[6&54&55]. The crystalline-amorphous transition could be an advantage in the sense that it might favour sustained release of CLOT from the liquisolid tablets following oral administration.

To demonstrate the possibility of incorporation of CLOT in liquisolid compact tablets and study any strong interactions among the formulation components, FT-IR spectra of CLOT and the liquid solid granules were obtained by measuring differences in energy distribution between the components. FT-IR spectra of the granules confirmed that the drug was compatible with the formulation excipients. It also indicates that the drug was successfully incorporated and solubilized in the LNE and evenly distributed in the granules. However, the unloaded granules did not reveal the presence of these bands owing to the absence of CLOT in these batches[44].

XRPD is a method applied in the study of polymorphic and molecular changes of drug molecules, and also investigates possible interactions between drug molecules and excipients used in formulations. The XRPD fingerprints of CLOT highlights its crystalline nature. Absence of the CLOT bands in the diffractograms of the granules confirms the solubilization of CLOT in the LNE and its even distribution in the granules, as well as its transition from crystalline to amorphous state. Amorphization of CLOT molecules is important to ensure improved drug loading and entrapment. This scenario is in agreement with findings from similar studies[28,56,57]. The few peaks of the drug molecules seen in the

diffractograms of the granules are necessary to promote stability of the granules. It was observed that novel diffraction peaks were not found in the diffractograms of the granules and perhaps, this confirms the FT-IR observation that no strong or untoward interaction took place between the drug molecules and the formulation components[58].

Attaining drug content uniformity in the CLOT-LNE liquisolid compact tablets is an official quality control requirement because it signifies that the production of a batch followed measurable and reproducible protocols, underscores the capacity of the formulation to accommodate the loaded drug molecules, and indicates the possibility of the formulation to produce the desired therapeutic effect upon administration. The obtained result showed that the tablets passed the test because they complied with pharmacopoeial limits of acceptance or rejection that the drug content of each individual tablet must fall between 85 and 115 % of the average content[32,38]. Therefore, this result attests to the high quality of the formulations owing to acceptable content consistency of the API, homogenous distribution of CLOT in the granules, possibility of good weight uniformity, and the reproducibility of the manufacturing technique. Considering the primary LNE formulation, it was observed that batches with high content of conophor oil were more uniform in content of API than those with lesser oil content. This correlates easily with high solubility of CLOT in conophor oil and highlights the importance of drug solubility in the design of the dosage form.

The narrow values of the calculated percentage deviations (data not shown) indicate that the tablets had significant ($p< 0.05$) uniformity of weight which falls within acceptable pharmacopoeial ranges[29,38]. Therefore, the CLOT-LNE liquisolid tablets passed the screening because all the batches met standardized limits of pharmacopoeial specifications which prescribes that for tablets with average weight greater than 250 mg, not more than 2 tablets should deviate from the average weight by more than ± 5%, and none by ± 10%[38], and confirms that the tablets were produced following a robust manufacturing technique.

All the tablets passed the friability test because the CLOT-LNE liquisolid tablets had values lower than the pharmacopoeial specified upper limit of acceptance for friability of tablets set at ≤ 1[38]. It was observed that none of the liquisolid tablets from all of the batches was defaced with any major cracks or fractures. The low friability rating (below 1 %) of the tablets indicates that the tablets had acceptable mechanical strength, toughness, and can withstand abrasive effects due to handling. Furthermore, the data confirms the robustness of the formulation technique, which is vital for potential production on a commercial scale.

Ideal hardness property of the CLOT-LNE liquisolid tablets implies that the tablets should maintain acceptable disintegration and drug dissolution profiles[32]. It could be seen from the data that all liquisolid tablet batches passed the test. Since the acceptable standard for hardness or crushing strength for normal uncoated tablets is ≥ 4 kgF[53], it could be deduced that the tablets will withstand the risk of damage owing to poor handling during manufacture, packaging, commercial distribution, storage, and patient use.

Though the liquisolid tablets of CLOT were sufficiently hard to withstand physicomechanical abrasion, they were adequately soft to produce optimum disintegration following oral administration[59]. The significant ($p< 0.05$) time regimen obtained from the study is ideal for tablets intended to disintegrate in the gastrointestinal tract (GIT) after swallowing because longer disintegration time may result in delayed release of the drug from the formulation and impeded bioavailability for initiation of any significant therapeutic activity. The acceptable disintegration times recorded by the tablets imply that adequate amount of hydro-channels were created in the tablets which facilitated unhindered fluid flux into the tablets followed by absorption, swelling, and disentanglement of the tablet particles resulting in disintegration. This is reasonable considering that the liquisolid tablets were produced using Type 'A' sodium starch glycolate (Primojel®), a superdisintegrant, which not only enhanced the physicotechnical properties of the tablets but also created a positive impact on their disintegration, in tandem with earlier report[21].

The dissolution profiles of the liquisolid tablets showed significant ($p< 0.05$) release of CLOT from the tablets in a slow and sustained manner representing a biphasic release pattern. Data from the study highlighted the significant ($p< 0.05$) dissolution of CLOT in the

biorelevant media used signaling that liquisolid compact tablets could be applied to improve the gastrointestinal stability and solubility of CLOT, in concord with previous report[60]. This is important since it might enhance the applicability of the drug for the oral treatment of systemic fungal infections caused by fungi susceptible to clotrimazole. The high dissolution of CLOT from the formulations suggests that the drug has a good chance of reaching excellent bioavailability *in vivo* after swallowing for the clinical treatment of systemic mycoses, and it is expected that the controlled release property of the liquisolid tablets would result in the administration of decreased dosage regimen of CLOT, improved patient compliance, and reduced untoward effects[28]. Result from this study clearly showed that loading the LNE of CLOT onto a liquid-solid system encouraged an increase in the rate and amount of CLOT released in the test media. This is true considering that though CLOT was prepared in a liquid system, it was entrapped and molecularly distributed in the liquid-solid powder system with enhanced solubilization due to large surface area available for the powder mix and the level of dispersion of CLOT-bearing oil droplets endowed with enhanced wetting properties[27]. This is highly advantageous since it promotes accelerated absorption of drug to produce therapeutic concentrations in addition to the overall increase in drug absorption which occurs in the gastric and upper duodenal segments[61]. In addition, all the tablet batches conformed to acceptable pharmacopoeial specification of attaining at least 75 – 80 % of drug release[35]. The dissolution profile of the liquisolid tablets was strongly reinforced by high DE% values for the formulations indicating a promising possibility of the liquisolid tablets to remain dissolved and be in prolonged contact with the physiologic milieu of the gastrointestinal system with potential high bioavailability, considering that drug bioavailability is greatly affected by its dissolubility in the enteric system[39]. The evaluation showed that the DE% values did not show any significant ($p > 0.05$) difference between the dissolution profiles of the formulations. The high DE% values could be attributed to the high wettability and increased surface area of CLOT available for dissolution due to its molecular disposition in the dissolution medium, as described by the Noyes-Whitney relationship[59]. Furthermore, the increased dissolution of CLOT could serve as a confirmation of its reduced crystallinity and extant amorphicity as revealed by its DSC and XRD analyses. These pieces of evidences position the liquisolid compact tablets as important vehicle for enhanced bioavailability of CLOT following oral administration.

Data obtained from the *In-vitro* dissolution study were applied to evaluate the drug release kinetics and release mechanism of the liquisolid compact tablets using different kinetic models, and selection of the best fit was based on the graphically-determined square of the correlation coefficient (r^2) for each model as shown in Table 4. The Higuchi square root of time kinetic model of drug release from batches TA1 and TB1 imply that the batches experienced diffusion-controlled release of CLOT. The first order and Higuchi models of release of CLOT from batches TA2 and TB2 indicate interplay of concentration-dependent and diffusion-dependent drug release, and this is reasonable because release of CLOT from the tablets would diminish over time with decrease in drug concentration. Since the release exponent (n) for batches TA1 and TB1 is less than 0.89 but greater than 0.45, it suggests that their release mechanism is non-Fickian or anomalous, batch TA2 with release exponent of 0.101 which is greater than 0.89 underwent super case II transport, while batch TB2 that had a release exponent of 0.854 experienced case II transport. This suggests that swelling, erosion and diffusion of the drug from the matrix system of the formulations control mechanism of release[37,62]. However in SIF, the first order and Higuchi square root of time models and the release exponents (n) of TA1, TB1, TA2, and TB2 imply that the drug transport mechanism of TA1, TB1, TA2, and TB2 is non-Fickian, indicating that these batches experienced diffusion and erosion-controlled drug release.

The *In-vitro* antifungal activity of the CLOT-LNE liquisolid compact tablets, placebo tablets, and CLOT solution against *C. albicans* and *A. niger* might be attributed to the ability of CLOT to diffuse from the test formulations or solution after 48 h of incubation corresponding with CLOT release profile as observed in the *In-vitro* dissolution test. The result suggests that formulating CLOT as liquisolid compact tablets did not alter or diminish or eliminate its innate susceptibility against *C. albicans* and *A.*

niger probably on account of the excellent stability of the formulation and internalization of the drug dispersion by the yeast cells[63]. The result revealed that whereas batch B formulations recorded highest inhibitions against *C. albicans*, batch A formulations recorded the highest inhibitions against *A. niger*. Thus, the order of susceptibility of the tablets could be represented as *A. niger* > *C. albicans* for batch A liquisolid tablets, while the reverse holds true for the batch B tablets. In comparison with the controls, all batches of the liquisolid compact tablets recorded higher antifungal activity than the CLOT solution positive control. However, the CLOT solution had antifungal activity than the negative control which did not inhibit the proliferation of *C. albicans* and *A. niger* owing to the absence of drug in the batches (TA3 and TB3). The varying zones of inhibition recorded by the CLOT-LNE liquisolid compact tablets as well as the CLOT solution reflects the capacity of CLOT to disrupt and permeate the yeast cell membrane with possible alteration or degradation of the yeast cell proteins and lipids[23,64]. The result from this screening supports our hypothesis that the formulation of CLOT as liquisolid compact tablets will enhance its oral antifungal activity, and demonstrates the potential applicability of the liquisolid tablets of CLOT for the oral treatment of systemic fungal infections[9].

Prior to treatment, there was effective inoculation and proliferation of yeast in the animals as confirmed from the similarity in the fungal colony count of all animal groups. The result clearly indicates that developing liquisolid tablets encapsulating CLOT endowed the drug with significant ($p< 0.05$) antimycotic effectiveness against *C. albicans* over a period of 24 hrs compared to the control agents. The rapid reduction of the yeast colony and antifungal activity of the tablets could be due to the immediate release of CLOT from the tablets; thus, validating the excellent release of the drug recorded in the *In-vitro* dissolution study and *In-vitro* antifungal evaluation. This is in addition to the high possibility of increased dissolution, improved absorption and bioavailability of CLOT from the formulations in the systemic circulation of the animals. The highest or most significant ($p< 0.05$) reduction of *C. albicans* colonies in the treated animals by the liquisolid tablets implies that there was no significant ($p> 0.05$) difference in the colony reduction potential produced by all batches of the liquisolid tablets. Despite the controlled release effect of the CLOT-LNE liquisolid tablets, batches TA2 and TB2 loaded with 1.5 %w/w of CLOT produced the best yeast clearance with sustained release property and could be nominated as the most suitable formulations for potential industrial production and oral applicability in the clinical treatment of systemic mycoses. Furthermore, the controlled release effect will improve compliance and decrease the development of resistance to the oral dosage form due to *C. albicans*, in addition to decreased or outright elimination of the appearance of untoward effects after swallowing[28].

Toxicological evaluation was necessary so as to rule out implicating CLOT-LNE liquisolid compact tablets in any potential haematologic or hepatotoxic effects due to oral administration of the formulation. As the hepatic biomarkers (AST, ALT, ALP) are vital indicators of the state of health of the liver and the tissue is prominently involved in metabolism of orally administered drugs, it was evident from the results that the CLOT-LNE liquisolid compact tablets were safe and non-toxic because the liver enzymes in the animals that received the drug-loaded formulations were significantly lower relative to the controls and the basal, and this submission was consolidated by the levels of measured haematologic parameters[65]. Therefore, the data suggest that oral administration of CLOT-LNE liquisolid compact tablets would not cause hepatic and haematologic damages *in vivo* at the administered dose.

Assessment of the storage stability of a formulation is an important pharmaceutical quality management system (QMS) protocol which describes the capacity of the formulation to maintain its original desired quality attributes throughout its shelf-life[28,45]. Thus, the result demonstrates the ability of the liquisolid tablets to retain their acceptable physicotechnical properties when stored at ambient and elevated temperature conditions. Perhaps, the importance of this result is that the liquisolid tablets would probably meet the prescription of current international pharmaceutical law that a formulation should retain a minimum of 90 – 95 % active pharmaceutical ingredient (API) integrity throughout its life span[66]. In addition, physical examination of the tablets by visual inspection revealed that they were smooth and

had a regular shape without any sign of cracking or discolouration throughout the study period.

Conclusion

For the first time, we demonstrated the fabrication of lipid nanoemulsion-based liquisolid compact tablets incorporating clotrimazole and evaluated their physicotechnical properties for oral treatment of systemic mycoses. The free-flowing powders prepared from LNE formulations had excellent micromeritic and compressibility properties. They were stable, consistent in shape, and less friable. They had uniform weight, acceptable hardness, drug content uniformity, and had disintegration and dissolution properties within pharmacopoeial specifications. Furthermore, the liquisolid tablets were non-toxic and enhanced the ability of CLOT to produce excellent *In-vitro* and *in vivo* activity against *C. albicans* and *A. niger*, and this outcome underscored the potential effectiveness in the use of CLOT-LNE liquisolid tablets for oral treatment of systemic fungal infections. Thus, these findings satisfied the hypothesis of this study. The results from this study provided incontrovertible evidences that CLOT-LNE liquisolid tablets is a reliable carrier system with excellent flowability and compressibility profiles for oral delivery of CLOT with improved drug solubility, absorption, and bioavailability for the treatment of systemic fungal infections. Further investigations will be required to evaluate the toxicity effect of the CLOT-LNE liquisolid tablets on renal tissues following oral administration, as well as extrapolate essential pharmacokinetic factors of the tablets *in vivo* before optimization for clinical application is considered.

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Competing interests

The authors declare that there are no conflicts of interests regarding the publication of this manuscript.

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نشرة العلوم الصيدليـــــة
جامعة أسيوط

أقراص مضغوطة سائلة قائمة على مستحلب النانو الدهني للتوصيل الفمى لعقار كلوتريمازول: استراتيجيات التصنيع ، والتوصيف، وتقييم السمية و الفاعلية المضادة للفطريات

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تهدف هذه الدراسة إلى تصنيع أقراص مضغوطة سائلة تحتوي على مستحلبات نانوية دهنيـــة محملة بالكلوتريمازول (LNE) لعلاج الالتهابات الفطرية عن طريق الفم. تم تقييم مستحلب النانو مـــن حيث حجم القطرة ، وتم تحويله إلى حبيبات حرة التدفق وضغطه في أقراص مضغوطة سائلة ، وتقييمه باستخدام الطرق الفارماكوبية وغير الفارماكوبية. تم تقييم اختبارات الثبات والســـمية للأقـــراص فـــي المختبر وفي الجسم الحي. كان قياس قرة المستحلبات النانوية الدهنية (LNE) فى نطاق (٦٦,٧ ± ٥,٧ – ١٢١,٦ ± ٣,٢ نانومتر). و كانت الأقراص مستقرة ، وغير سامة ، ولهـــا نطـــاق وزن متقـــارب (٣٤١,٤ ± ١,٢ – ٣٤٦,٧ ± ٠,٨ مجم) ، وكذلك محتوى دوائى متقارب (٨٥,٢ ± ٠,١ – ٩٩,٨ ± ٠,٢٪) ، و كان لها تفكك ممتاز (٢,٩٦ ± ٠,٨ – ٥,٨٨ ± ١,٣ دقيقة) ، وخواص انطلاق منضـــبط للعقار . أظهرت تقييمات الفاعلية المضادة للفطريات في المختبر وفي الجسم الحي تحسنًا في الفاعليـــة لعقار الكلوتريمازول. تسلط هذه النتائج الضوء على أن الأقراص المصاغة هي نظام حامل واعد مـــع فائدة محسنة عن طريق الفم لعلاج العدوى الفطرية.