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Novel *Bos indicus* Fat-Based Nanoparticulate Lipospheres of Miconazole Nitrate as Enhanced Mucoadhesive Therapy for Oral Candidiasis

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Abstract: Nanoparticulate lipospheres of miconazole nitrate were formulated and incorporated into mucoadhesive gels for enhanced treatment of oral candidiasis. Extracted cow fat and Phospholipon® 90H (7:3) were used to prepare a lipid matrix by fusion. Nanoparticulate lipospheres with drug concentrations (0, 0.125, 0.25, 0.5 % w/w) were prepared using the lipid matrix (5.0 % w/w), polysorbate 80 (2.0 % w/w), sorbitol (4.0 % w/w) and distilled water by melt homogenization. The lipospheres characterized encapsulation efficiency (EE), drug loading capacity (DL), particle size, polydispersity index, morphology, and compatibility. Lipid-based gels were prepared using Polycarbophil® (1.0 % w/w), the nanoparticles and other excipients, by dispersion, then characterized regarding drug content, compatibility, rheology, ex-vivo mucoadhesive, in-vitro drug release, and anti-candidal activity. The EE, DL, and mucoadhesive strengths of the formulated gels were influenced by drug loads. The particles were smooth, nanometric, and spherical, with polydispersity indices in the range of 0.247 – 0.293. The gels were pseudoplastic. The gels ' extensive drug release profiles and anti-candidal activity were significantly superior to commercial Daktarin®. FT-IR confirmed the compatibility of the drug with excipients used in the formulations. The prospects of this study necessitate further optimization.

Keywords: oral candidiasis; miconazole nitrate; solid lipid nanoparticles; mucoadhesive nanogels; controlled drug release; anti-candidal activity.

1. Introduction

Among the various antifungal agents, miconazole nitrate, a broad-spectrum antifungal agent of the imidazole group, is widely used to treat oral candidiasis. However, the hydrophobic nature of this medication limits its absorption, leading to low bioavailability and shorter residence time in the oral mucosa. To resolve this, the dosing frequency is increased, which predisposes the patient to adverse effects, toxicity issues, and fungal resistance [1]. There is, therefore, a need to

develop improved delivery systems for miconazole antifungal drugs for enhanced treatment of oral candidiasis.

Solid lipid nanoparticles (SLNs) have caught the interest of researchers as these nano-scale lipids have shown great potential for attaining the goals of improved delivery as well as bioavailability of poorly water-soluble drugs like miconazole nitrate [2]. It is interesting to learn that a number of successful studies and reviews have been done regarding the incorporation of SLNs into pharmaceutical dosage forms and their use in drug delivery [3–7]. SLNs, as the name implies, are lipid-based nanoparticulate drug delivery systems. They consist of lipospheres made up of a hydrophobic fat core and stabilized by an outer monolayer of phospholipids. An outstanding advantage of lipospheres is that they are used to deliver both hydrophobic and hydrophilic drugs. Usually, poorly soluble drugs, like miconazole nitrate, are dispersed in the internal core of the solid fat matrix. Another major advantage of these nanoparticulate lipospheres over other delivery systems is the potential for extended-release of entrapped drugs due to their solid lipid matrix [8]. Some more qualities of interest include physical stability, high dispersibility in an aqueous medium, high entrapment efficiency, ease of preparation, and scale-up [9]. Following preparation, these lipid-based nanocarriers must be characterized to assure their suitability for *in vitro* and *in vivo* applications. To evaluate the physical transformation of lipospheres into nanoparticles, some analytical techniques are used. They include: ultraviolet-visible spectroscopy (UV-Vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), Dynamic light scattering (DLS), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM), etc. [10].

Over time, miconazole nitrate has been incorporated successfully into SLNs using various methods and formulated into mucoadhesive dosage forms for more convenient administration to mucosal sites [2,11]. Mucosal tissues in the body consist of mucous membranes and are densely vascularized, making them one of the most preferred sites for drug administration. These tissues offer advantages such as prolonged residence time of therapeutic agents, enhanced absorption, targeting and localization of the drug, and protection from degradative enzymes [12]. As mentioned earlier, the mucosal routes are highly vascularized; as a result of this, there is a considerably high flow of blood within these areas resulting in increased bioavailability upon drug absorption [12]. The most commonly explored mucosal sites for delivery of pharmacologically active agents include: the oral/buccal cavity, rectal route, conjunctiva/ocular, vaginal route, nasal cavity, and gastrointestinal tract (GIT) [13].

Most drugs' poor bioavailability and rapid degradation are conveniently circumvented when delivered via oral mucosal [14]. These drugs have been incorporated into mucoadhesive gel dosage forms for convenient delivery into the oral mucosa. Mucoadhesive gels offer additional advantages of biocompatibility, controlled release of the drugs, simplicity of preparation, and ease of administration [15].

Despite the modifications adopted in the formulation of mucoadhesive gels and other solid lipid delivery systems, there is insufficient literature on phospholipid-modified, fat-based mucoadhesive lipospheres of miconazole nitrate formulated in nanoparticulate scale. By way of contributing to knowledge in this aspect, the objectives of this study, therefore, are: (i) to adopt a combination of *Bos indicus* (cow) fat and Phospholipon®90 in the formulation of nanoparticulate

lipospheres encapsulating miconazole nitrate, (ii) to deliver miconazole nitrate from gel formulations of nanoparticulate lipospheres using polycarbophil as the mucoadhesive polymer, and (iii) to evaluate the gel formulations for the anti-candidal activity of miconazole nitrate vis-à-vis the commercial brand of miconazole nitrate oral gel (Daktarin®).

2. Materials and Methods

2.1. Materials.

The following materials were used in the study: miconazole nitrate USP (Gutic Biosciences Ltd., India), Daktarin® oral gel (Janssen Pharma, Beerse, Belgium), Phospholipon® 90H (P90H) (Phospholipid GmbH, Köln, Germany), Polycarbophil® (BASF, Germany), sorbitol (Gulshan Polyols Limited, India), sorbic acid (Apac Chemicals, USA), polysorbate 80 (Tween® 80) (Merck KGaA, Darmstadt, Germany), sodium sulfide (Krishna Chemicals, India), sodium chloride, potassium chloride, calcium chloride dehydrate, sodium hydroxide (BDH Chemicals Ltd., Pooles, England), methanol, ethanol (Sigma Aldrich, USA), urea BP (Anmol Chemicals, India), sodium dihydrogen phosphate (Shreeji Pharma International, India), Sabouraud dextrose agar (Titan Biotech Ltd., India), chloramphenicol (Sunways, India), glycerol (Wuppertal, Germany), bentonite (Opta Minerals, USA), activated charcoal (Taupo Carbon Producers Ltd., India), hydrochloric acid (GFS Chemicals, Inc., Columbus, OH). Clinical isolates of *Candida albicans* were obtained from the diagnostic laboratories of a secondary level hospital (Bishop Shanahan Hospital, Nsukka, Nigeria).

2.2. Methods.

2.2.1. Extraction and purification of *Bos indicus* (cow) fat.

The method of wet rendering was employed in the extraction of the lipid from cow fat [16]. Extraneous materials were physically removed from the adipose tissue, cut into smaller pieces, and subjected to moist heat in a water bath for 45 min. The molten fat was separated from the aqueous phase after filtering using a muslin cloth. The rancid odor of the extracted fat was removed using a mixture of 1 g activated charcoal and 9 g bentonite. The activated charcoal-bentonite-fat blend was placed in the oven at 90 ºC for 2 h. The molten fat was pressed through filter paper sheets to obtain an odorless product.

2.2.2. Preparation of lipid matrix for nanoparticulate lipospheres formulation.

The lipid matrix was prepared by fusion method [17] using the purified cow fat and Phospholipon® 90H ratio of 7:3. Thus, 49 g of cow fat and 21 g of P90H were transferred into a glass beaker in a liquid paraffin bath. The contents were melted together at a regulated temperature of 70 ºC. The melt was stirred continuously until a homogenous, transparent white mixture was obtained. The lipid matrix was stirred further until it attained room temperature, whereby it solidified. It was protected in an airtight, moisture-resistant glass bottle and preserved in the refrigerator until needed.

2.2.3. Preparation of nanoparticulate lipospheres.

Nanoparticulate lipospheres of miconazole nitrate were prepared using the hot homogenization method [18]. A specified quantity of the lipid matrix (5 % w/w of the lipospheres formulation) was placed in a glass beaker and melted at 80 ºC in the temperature-regulated heater (IKA Instruments, India). Miconazole nitrate (0.25 % w/w) was added to the melted lipid matrix. An aqueous surfactant solution consisting of sorbitol (4 %w/w) and polysorbate 80 (2 %w/w) was prepared in a separate beaker and heated at the same temperature. The hot aqueous surfactant phase was then dispersed in the hot lipid phase using an Ultra-Turrax T25 (IKA-Werke, Staufen, Germany) homogenizer at 1000 rpm for 5 min. The obtained pre-emulsion was homogenized at 12,000 rpm for 15 min and allowed to cool/re-crystallize at room temperature. The procedure was repeated with 0.5 % w/w and 1.0 % w/w concentrations of the miconazole nitrate. An unloaded (zero-drug) nanoparticulate lipospheres formulation was also prepared to serve as the control.

2.2.4. Encapsulation efficiency (EE) and drug loading (DL).

Vivaspin filter tubes (microconcentrators) (Vivaspin, Germany) consisting of filter membrane with a molecular weight cut-off (MWCO) of 10,000 at the base of the sample donor chamber were used. From the undiluted nanoparticulate lipospheres formulation, a volume of 5 ml was placed in the upper chamber, followed by a recovery chamber underneath. After that, the sample was centrifuged at 4,000 rpm for 2 h in a centrifuge (TDL-4B. Bran Scientific and Instru. Co., England), and the aqueous phase (extract) was collected into the recovery chamber. The extract was appropriately diluted with methanol, and absorbance readings were obtained using a UV-Vis spectrophotometer (6405 Jenway, UK) at 285 nm. Drug content was estimated by reference to a standard Beer-Lambert plot. The encapsulation efficiency and drug loading capacity were calculated using equations 1 and 2.

$$Encapsualtion\ efficiency = \frac{actual\ drug\ content}{theoritical\ drug\ content} \text{ x } 100\ \% \qquad \text{Eqn. 1}$$

$$Drug\ loading\ capacity = \frac{W_a}{W_1} \text{ x } 100\ \% \qquad \text{Eqn. 2}$$

where W_a is the amount of miconazole nitrate entrapped by the lipid, and W_l is the weight of lipid in the formulation.

2.2.5. Particle size and polydispersity indices.

Mean diameter, Z_{av} (nm), and polydispersity index (PDI) of the nanoparticulate lipospheres formulations were measured using a Zeta sizer nano-ZS (Malvern Instrument, Worcestershire, UK). The photon correlation spectroscopic (PCS) analysis was performed at a wavelength of 633 nm and 25 ºC using a backscattering angle of 173 degrees after suitable dilution with double-distilled water.

2.2.6. Canning electron microscopy.

The morphological characteristics of the nanoparticulate lipospheres formulations were determined by a scanning electron microscope (JEOL-JSM-6 360, Japan) at different magnifications. One drop of the sample was placed on a slide, and excess water was allowed to

evaporate at room temperature. The slide was attached to the specimen holder using double-coated adhesive tape. The gold coating was performed under vacuum using a sputter coater (model JFC-1100, JEOL, Japan) for 10 min, and then investigated 20 kV.

2.2.7. Compatibility study.

Fourier transform-infrared (FT-IR) spectroscopic analysis was conducted in the National Centre for Energy Research, the University of Nigeria, using a Shimadzu FT-IR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan), and the spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1} with a threshold of 1.303, the sensitivity of 50 and resolution of 2 cm^{-1} range. A smart attenuated total reflection (SATR) accessory was used for data collection. The potassium bromate (KBr) plate used for the study was cleaned with a tri-solvent (acetone-toluene-methanol at 3:1:1 ratio) mixture for baseline scanning. For each batch of the nanoparticulate lipospheres formulations, 0.1 ml was measured and mixed with 0.1 ml nujol oil, after which the mixture was compressed into discs employing KBr plate and a hydraulic press. Thereafter, the discs' spectrum was obtained using Gram A1 spectroscopy software and TQ Analyzer 1.

2.2.8. Preparation of gels.

Lipid-based gels consisting of the nanoparticulate lipospheres, Polycarbophil® as the bioadhesive polymer, glycerol, sorbic acid and distilled water were formulated employing the dispersion method [19]. To do this, 1.0 % w/w of the bioadhesive polymer was dispersed in 30 ml of distilled water in a glass beaker and allowed to solubilize overnight. Thereafter, glycerol (3.0 % w/w), sorbic acid (0.1 % w/w), and nanoparticulate lipospheres from each batch (20 ml) were incorporated into the PCP aqueous dispersion, with continuous stirring. Then the system was made up to volume and stirred vigorously for 2 h until a consistent homogenous gel was formed. The formulation was then poured into an ointment jar, and the pH was raised to 6.8 by drop-wise addition of 0.5 M NaOH.

2.2.9. Determination of the drug content.

For each batch of the hydrogel preparation, 5 ml was added to 10 ml of methanol in a 20 ml volumetric flask. The volume was made up to the 20 ml mark with methanol and mixed thoroughly for 30 min. The solution was then centrifuged at 4000 rpm for 1 h. The drug content of each supernatant was assayed spectrophotometrically at 285 nm.

2.2.10. Rheological evaluation.

The viscosity of each gel formulation was measured using a digital viscometer (NDJ-5S Viscometer, Lab. Sciences, England), with spindle number 04 at different speeds (6, 12, 30, and 60 rpm). The spindle was immersed in the sample in a 20 ml beaker and attached to the coupling nut such that the gel level was at the groove on the shaft. The test was run at ambient laboratory temperature (32 °C), and each hydrogel formulation's viscosity (mPa.s) was recorded.

2.2.11. *Ex-vivo* mucoadhesive studies.

Buccal mucosal tissue was carefully removed from the buccal tissue of cow, kept frozen, and thawed in saline at ambient temperature just before use. The mucin was scraped from the buccal mucosal tissue leaving a thin membrane that was used as the model membrane. The membrane measuring (1 cm x 1 cm) was mounted and held in place on a smooth plastic surface using a pin while another mucosal section of the same area was fixed in an inverted position to the ring of a Du Nuoy tensiometer (ELMA, model 8600T, UK). Afterward, 100 µl of the gel formulation was placed on the mucosal surface. The plastic surface mounted with mucosal membrane and gel formulation was attached firmly to the membrane on the ring of Du Nuoy tensiometer for 5 min. The ring of the tensiometer was gradually raised until the two mucosal tissues were detached from each other. The procedure was repeated for other batches of the gel, and a new buccal membrane was used in each case. The mucoadhesive strength (dynes/cm^2) was determined from the minimal force required to detach the mucosal tissue from gel formulation, using equation 3.

$$Mucoadhersive\ strength\ \left(\frac{F\ (dynes)}{A\ (cm^2)}\right) \quad \text{Eqn. 3}$$

where, F = Detachment force (dynes),

A = Area of mucosa exposed (cm^2).

2.2.12. Preparation of synthetic salivary fluid (SSF, pH = 6.8).

Simulated salivary fluid (pH 6.8) was prepared using established protocols [20]. The SSF served as a medium for the release studies and to mimic the physiological pH. About 0.40 g of sodium chloride, 0.795 g of calcium chloride dehydrate, 0.40 g potassium chloride, 0.005 g of sodium sulfide, 0.78 g of sodium dihydrogen phosphate, and 1.0 g of urea were dissolved in a sufficient quantity of distilled water to produce 100 ml. The pH was adjusted to 6.8 by drop-wise addition of 0.1 M hydrochloric acid solution.

2.2.13. In vitro release studies.

In vitro release studies were performed using 250 ml of SSF (pH 6.8) as the release medium. The dialysis membrane (dialysis bag) was cut open at both ends and soaked in the release medium before analysis. Approximately 2 ml of each gel formulation was placed in the treated dialysis bag [molecular weight cut off (MWCO) 8000 g/mol, CelluSep®, USA] and both ends were tied with a thread and suspended in the release medium with continuous stirring at a rate of 100 rpm at 37 ± 0.5 ºC. Equal volumes, 5 ml, were withdrawn regularly at 15-min intervals for 6 h. The same volume (5 ml) of fresh release medium was added to replace the withdrawn quantity to maintain the sink condition. Each withdrawn sample was appropriately diluted with methanol, filtered using a 210 nm filter device (Millipore®, Germany), and the filtrate was analyzed spectrophotometrically at 285 nm. The released miconazole nitrate was calculated from the absorbance readings and expressed as a percentage of drug content in the 2 ml gel formulation. For comparison, this procedure was repeated using an equivalent quantity of commercially available miconazole nitrate topical cream (Daktarin® oral gel).

2.2.14. Isolation of *Candida albicans*.

Specimens collected with an oral swab from a female HIV-positive patient exhibiting thrush were streaked directly on sterile Sabouraud dextrose agar fortified with 0.05 % chloramphenicol. The plates were incubated aerobically at 35 °C for 48 h. To ensure purity, representative colonies were picked from the plate and sub-cultured on sterile SDA agar plates. The isolates of *Candida albicans* were characterized based on standard phenotypic tools (butyrous, opaque, irregular). The isolates were preserved on SDA agar slants at 4 °C. For use, overnight cultures of the *Candida albicans* were suspended in sterile distilled water to achieve turbidity comparable to 0.5 MacFarland's standards.

2.2.16. Anti-candidal activities of the miconazole nitrate-loaded nanoparticulate lipospheres.

Approximately 0.1 ml of the standardized microbial suspension was mixed with 20 ml of sterile modified SDA at 40 °C and poured into a sterile Petri dish. The seeded agar was caused to solidify. Exactly 2 ml of each batch of the gel formulations as well as the commercial sample (Daktarin® oral gel) were prepared by respectively dissolving 30 mg of batch F_1, 26.7 mg of F_2, 20 mg of batch F_3, as well as 15 mg of Daktarin® oral gel in 2.0 ml of sterile water. The solutions were used to determine inhibition zone diameters using the agar well diffusion method [21].

2.2.17. Statistical analysis.

All experiments were performed in at least three replicates for the validity of the statistical analysis. Results were expressed as mean ± SD. Differences were considered significant for p-values < 0.05, using the Student's t-test model.

3. Results and Discussion.

3.1. Results.

3.1.1. Size of nanoparticulate lipospheres.

The Z_{av} of the formulations ranged from 200.4 to 236.2 nm (Table 1). These values are within the standard nanometer range of 10 − 1000 nm for solid lipid nanoparticles [22].

3.1.2. Polydispersity indices.

The PDI for F_o, F_1, F_2 and F_3 were 0.247, 0.284, 0.284 and 0.283 respectively. These values fall within the acceptable range of 0.0 to 1.0 [23]. It can also be inferred that the nanoparticles were almost perfectly uniform in size distribution.

3.1.3. Encapsulation efficiency EE % and drug loading capacity DL. %

As shown in Table 1, the EE % and DL % were in the range of 35.32 – 56.78 % and 8.29 – 10.94 %, and respectively, this shows the ability of the SLNs to entrap a significant amount of the drug in each formulation. It was also observed that F_o had no recorded values because the formulation was unloaded with miconazole nitrate. The EE % and DL % increased with drug

concentration from 42.30 % and 10.94 % in F_1 to 56.78 % and 17.42 % in F_2. However, a decline was observed in F_3 (35.32 % and 8.29 %). This could be attributed to possible saturation attained due to an increase in drug concentration.

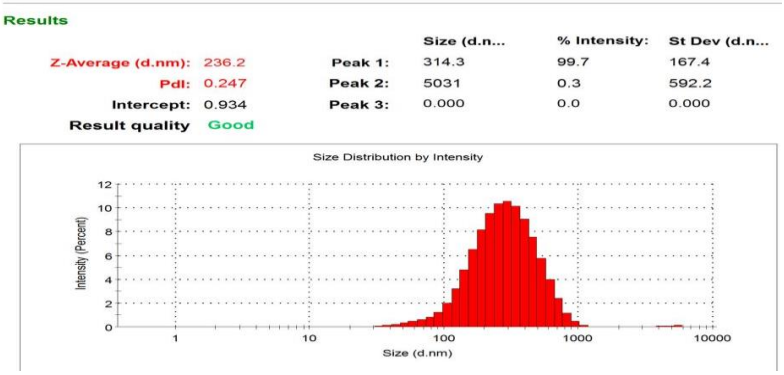


Figure 1. Size distribution report for (F_o) unloaded.

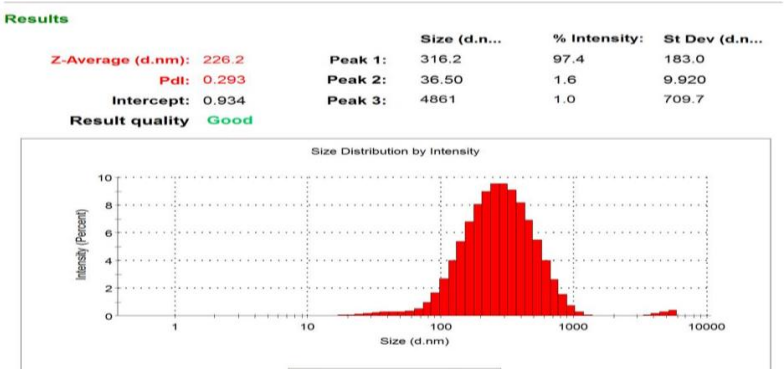


Figure 2. Size distribution report for (F_1) 0.25 mg MN.

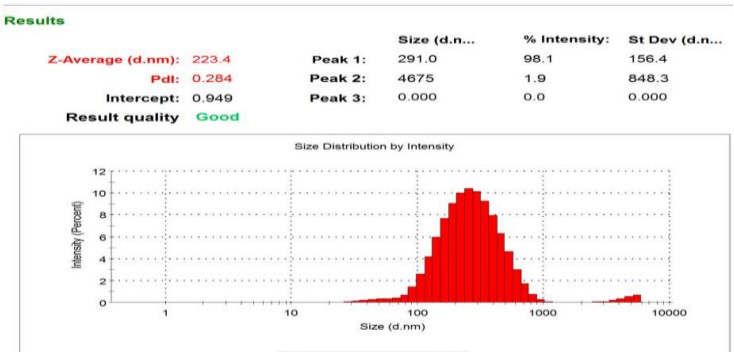


Figure 3. Size distribution report for (F_2) 0.5 mg MN.

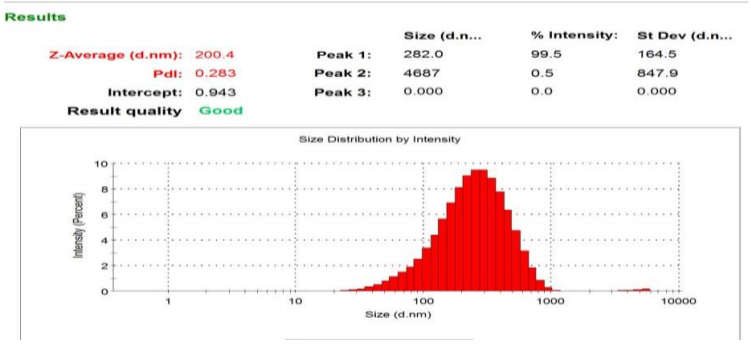


Figure 4. Size distribution report for (F_3) 1.0 mg MN

Table 1. Physicochemical properties of the lipospheres (Batches F_o, F_1, F_2 and F_3).

Batch	Z_{av} (nm)	PDI	EE (%)	DL (%)
F_o	236.2	0.247	-	-
F_1	223.4	0.284	42.30	10.94
F_2	233.4	0.284	56.78	17.42
F_3	200.4	0.283	35.32	8.29

Key: F_o is unloaded, F_1, F_2 and F_3 contain increasing concentrations (0.25, 0.5 and 1.0 w/w respectively) of miconazole nitrate.

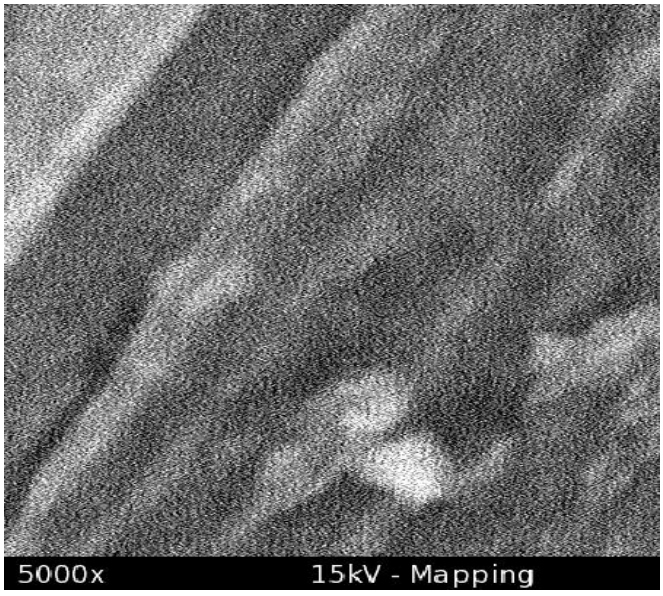
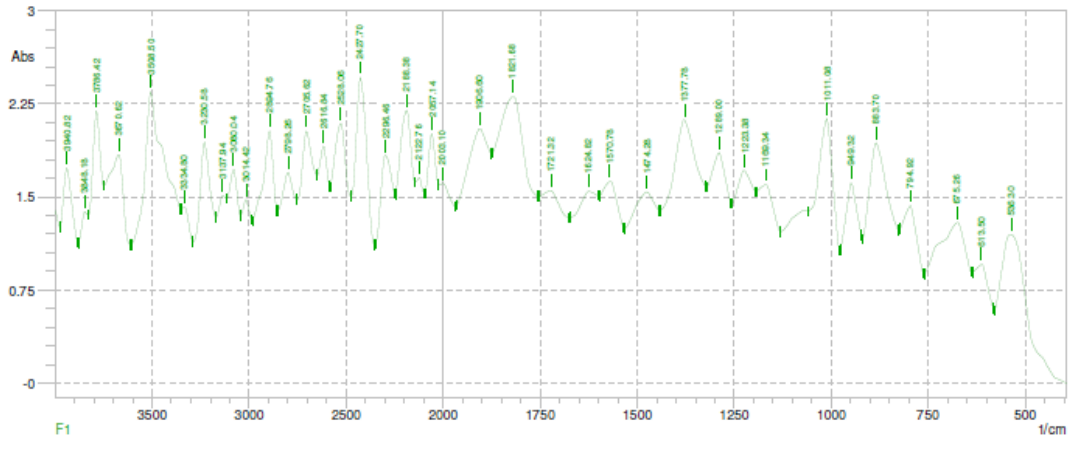


Figure 5. Scanning electron micrograph (SEM) of miconazole nitrate-loaded nanoparticulate lipospheres.

3.1.4. Morphology of the SLNs.

From the scanning electron micrograph (Figure 5), it can be inferred that the nanoparticles were morphologically spherical and smooth with no agglomerates or precipitates, which could interfere with the stability of the nanoparticles. They were also seen to be well dispersed and of uniform size.



(**a**)

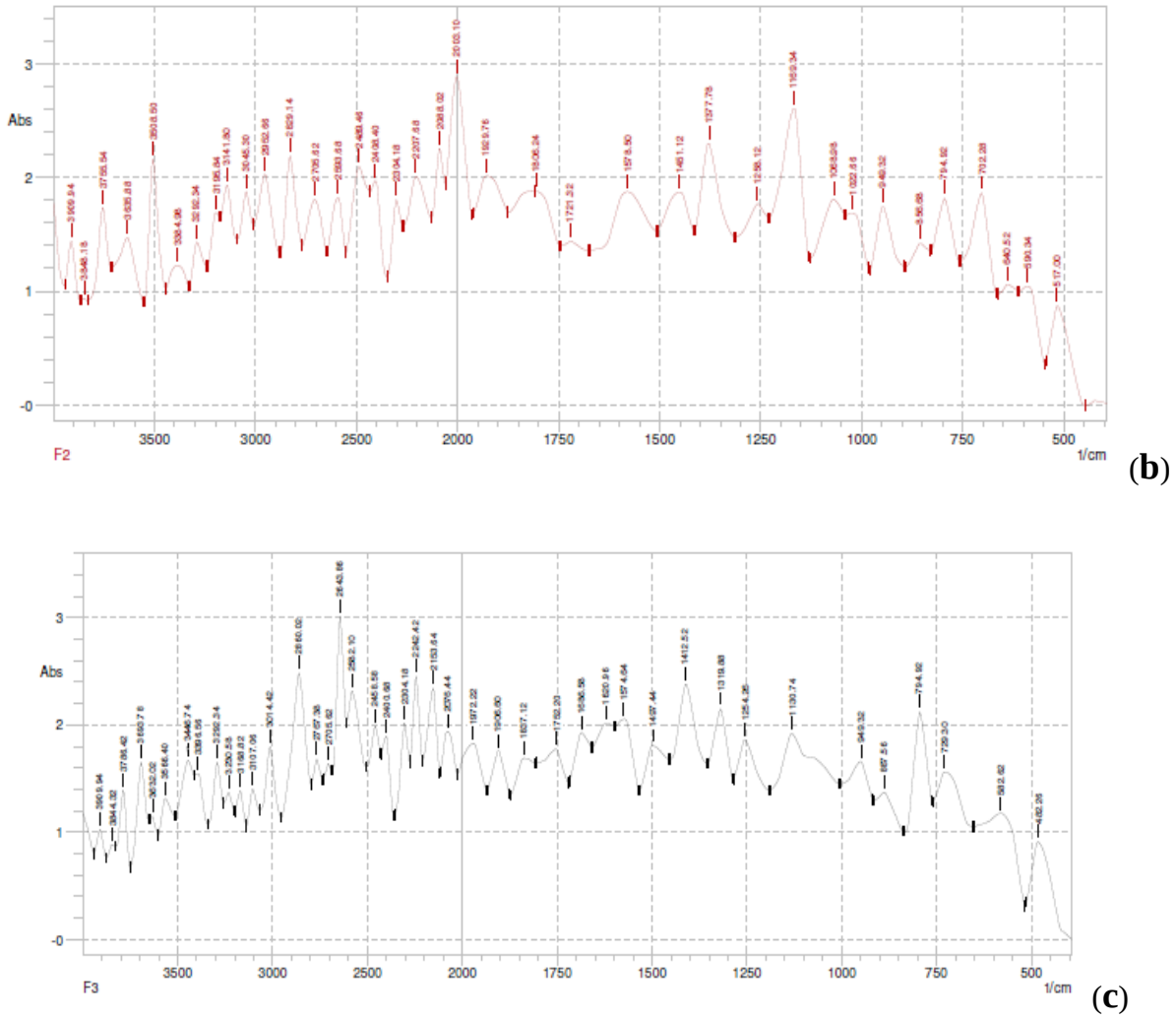


Figure 6. This figure represents: (**a**) FT-IR spectra of miconazole nitrate-loaded nanoparticulate lipospheres for batch F_1; (**b**) FT-IR spectra of miconazole nitrate-loaded nanoparticulate lipospheres for batch F_2; (**c**) FT-IR spectra of miconazole nitrate-loaded nanoparticulate lipospheres for batch F_3.

Table 2. Fourier transform infra-red [FT-IR] spectrum of miconazole nitrate-loaded nanoparticulate lipospheres batches F_1, F_2, and F_3.

Absorption band of miconazole nitrate (cm^{-1})	**Absorption band of F_1(cm^{-1})**	**Functional groups for F_1**	**Absorption band of F_2(cm^{-1})**	**Functional groups for F_2**	**Absorption band of F_3(cm^{-1})**	**Functional groups for F_3**
3140	3137.94	Imidazole C-N stretch	3141.80	Imidazole C-N stretch	3168.82	Imidazole C-N stretch
3070	3080.04	Aliphatic CH stretch	3045.30	Aliphatic CH stretch	3014.42	Aliphatic CH stretch
2995			2952.66	Aliphatic CH_2 stretch		
2920	2894.76	Aliphatic CH stretch			2860.02	Aliphatic CH stretch
1566	1570.78	C=C aromatic	1578.50	C=C aromatic	1574.64	C=C aromatic
1525						
1445	1474.28	-CH_2- bending	1451.12	-CH_2- bending	1497.44	-CH_2- bending
1385	1377.78	C-H bending (aliphatic)	1377.78	C-H bending (aliphatic)	1389.88	C-H bending (aliphatic)

Absorption band of miconazole nitrate (cm^{-1})	Absorption band of F_1(cm^{-1})	Functional groups for F_1	Absorption band of F_2(cm^{-1})	Functional groups for F_2	Absorption band of F_3(cm^{-1})	Functional groups for F_3
1310	1289.00	C-N stretch			1319.88	C-N stretch
1070	1011.08	C-C stretch	1068.98	C-C stretch	1130.74	C-C stretch
710	794.92	C-H bonding	702.28	C-H bonding	729.30	C-H bonding

3.1.5. Compatibility study.

The IR frequencies of pure miconazole nitrate and MN-SLN formulations were represented in Figure 6 and Table 2. The FT-IR spectrum of the formulations revealed peaks that correspond to the absorption band of pure miconazole nitrate. The slight differences in peaks can be attributed to functional groups of excipients used in the formulation and reactions/impurities encountered during the laboratory work.

3.1.6. *Ex-vivo* mucoadhesive study.

From the results presented in Figure 7, it was observed that the formulations had good mucoadhesive properties. This can be attributed to incorporating the mucoadhesive agent PCP [24]. The F_2 gel, however, had the highest mucoadhesive strength at 34.6 dynes/cm^2, which indicates that this formulation will have extended residence time in the oromucosa, prolonged release of the drug, and therefore enhanced efficacy.

3.1.7. Drug content.

From Figure 7, it was also observed that the percentage drug content of each gel formulation increased (F_1 Gel = 89.32 %, F_2 Gel = 91.46 % and F_3 Gel=93.50 %) with increase in drug concentrations (0.25, 0.5 and 1.0 w/w for F_1, F_2 and F_3 respectively). There was no recorded value for the unloaded batch, F_o..

3.1.8. Viscosity/shear rate.

Results from Figure 8 showed no significant difference in the viscosities of the unloaded and MN-loaded formulations. However, the viscosities of each formulation were seen to decrease with an increase in shear rate (6 rpm, 12 rpm, 30 rpm, 60 rpm).

3.1.9. In vitro drug release in SSF (pH 6.8).

Figure 9 compares the release profiles of the drug-loaded mucoadhesive gels with the commercial Daktarin® oral gel for a period of 6 h. Initial drug release of about 1, 3, 5, and 7% w/w were observed within the first few minutes for the gel formulations and the Daktarin® oral gel. The F_1 and F_2 gels had the most release at 75 % and 90 %, respectively for 6 hours compared to the Daktarin® oral gel [74 %].

3.1.10. Antimicrobial studies.

This study was done to determine the sensitivity of *C. albicans* to the formulated gels. The F_1, F_2, and F_3 batches had significant inhibition zone diameters (IZDs) against the test organism

(Figure 10). However, in comparison to Daktarin® oral gel with IZD of about 18 mm, the F_1 and F_2 gels had higher IZDs of approximately 21 mm and 23 mm, respectively.

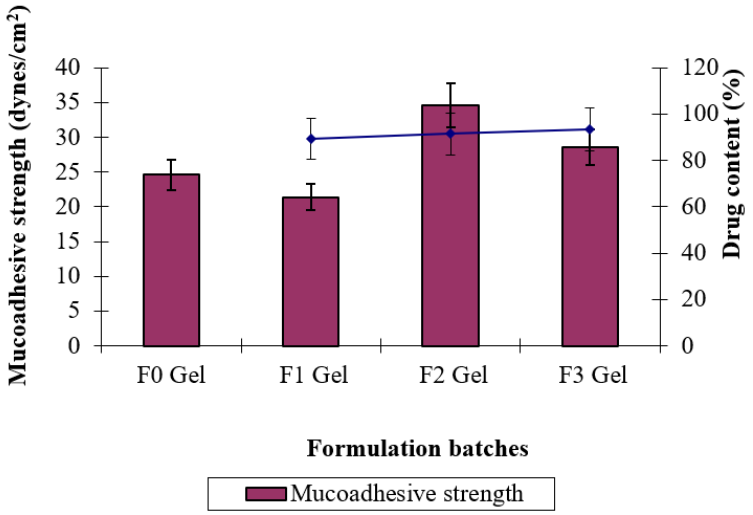


Figure 7. Drug content and mucoadhesive properties of the gel formulations.

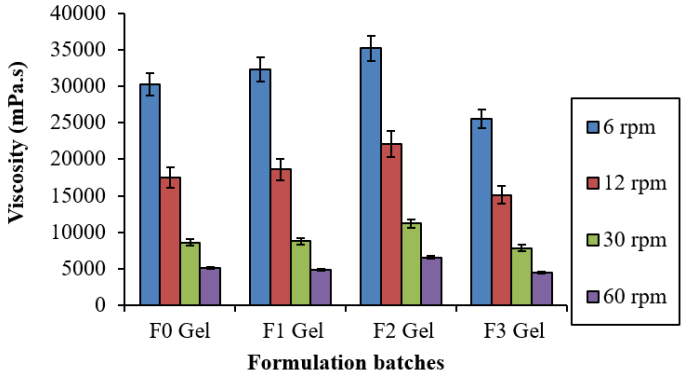


Figure 8. Viscosity profiles of the gel formulations.

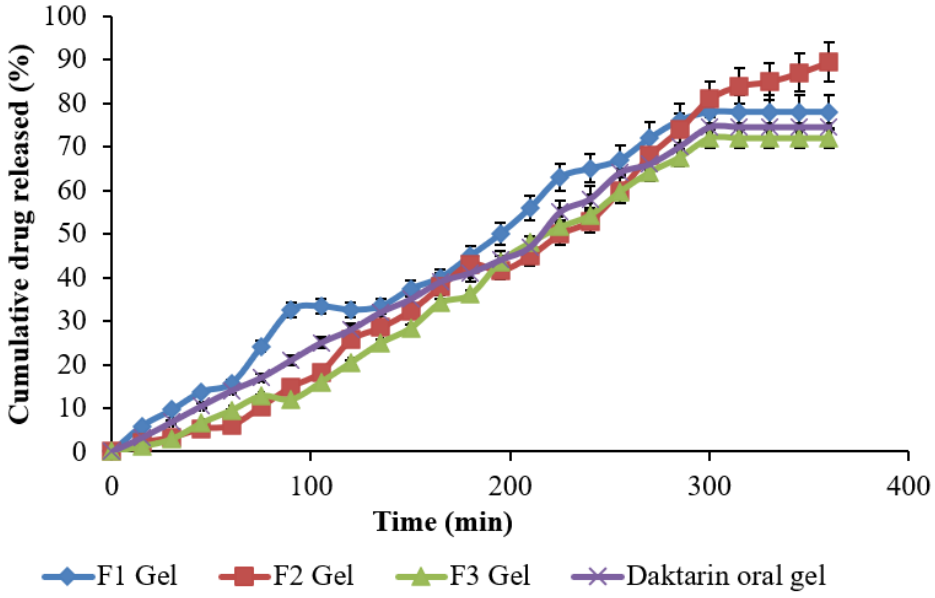


Figure 9. *In vitro* release studies of the drug-loaded gel formulations in SSF (pH 6.8).

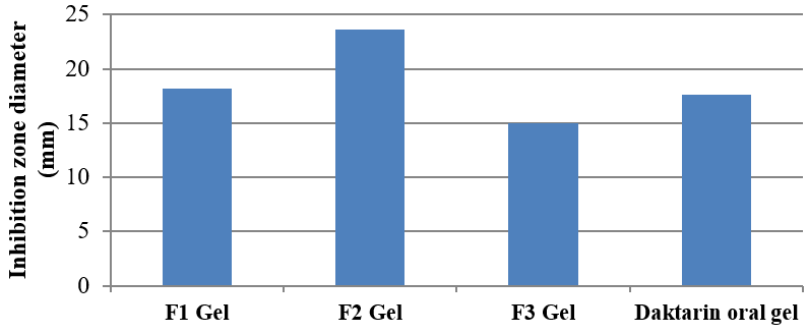


Figure 10. *In vitro* anti-candidal activity of drug-loaded gel formulations.

3.2. Discussion.

The hot homogenization method is ideal for the formulation of nanoparticulate lipospheres of hydrophobic drugs such as miconazole nitrate [25]. This method enables the drug to be encapsulated into the lipid matrix and not in the outer coating of the nanoparticles. The homogenization cycle runs from 200 – 20,000 rpm for nanoparticle preparations for about 5 – 10 min. In this study, the pre-emulsion (melted lipid matrix + aqueous surfactant) was stirred for 5 min and homogenized at 12,000 rpm for 15 min to obtain lipospheres of nano-sizes. Also, the nano-range particle sizes were achieved through increased processing temperature (80 ºC) [24]. At this temperature, it is believed that nanosized particles are formed upon a decrease in viscosity of the lipid phase [26].

Particle size and size distribution significantly affect the stability of lipospheres. Smaller particles with uniform size distribution tend to exhibit better stability. This is because they have fewer propensities to form aggregates during storage. Also, the wider surface areas of these particles enable the incorporation of a significantly high amount of therapeutic agent. The diameter of nanoparticulate lipospheres generally ranges from 10 – 1000 nm [27]. As seen in Table 1, the average sizes of the formulated lipospheres were 236.2 nm, 223.4 nm, 233.4 nm, and 200.4 nm for F_o to F_3, respectively. These formulations, therefore, can be said to yield optimal nano sizes. It is interesting to note that formulation parameters, such as quantities and types of lipids, surfactants, and even the drugs used, can significantly affect the particle size and distribution of the nanoparticles [28]. It has been observed that an increase in the concentration of the surfactant led to smaller particle sizes [29]. However, in this study, a single concentration of surfactant (polysorbate 80 at 2 % w/w) was used, resulting in the statistically insignificant variations in Z_{av} values recorded.

The size range, size distribution, and average diameter of nanoparticulate formulations, in other words, the degree of homogeneity, is measured with a parameter known as Polydispersity index (PDI). The numerical value for measuring PDI ranges from 0.0 (for perfectly uniform) – 1.0 (for highly heterogeneous particles) [30]. In other words, lower PDI values of nanoparticles in a formulation indicate homogeneity and vice versa [31]. In this study, the highest PDI value of the formulated lipospheres was 0.284 (for F_1 and F_2). These values indicate that the particle sizes were homogenous and monodispersed.

The main objectives for formulating liposphere delivery systems are enhanced bioavailability, targeted delivery, and sustained drug release. The actualization of these objectives

is greatly dependent on the successful incorporation of therapeutic molecules into the formulated lipospheres. This can be expressed using parameters such as encapsulation efficiency (EE) and drug loading capacity (DL) [32]. The EE is expressed as a ratio between the weight of entrapped drug molecules and a total weight of drugs incorporated. On the other hand, the DL is expressed as a ratio between the entrapped drug and the total weight of the lipids used in the formulation [24]. The type of phospholipids, the solubility of the drug, type of surfactant, and formulation methods are considered the rate-determining factors for establishing EE and DL. The lipophilic nature of drug molecules largely influences the efficiency with which these molecules are encapsulated into the hydrophobic core of lipospheres [33,34]. Also, it is known that the use of unsaturated phospholipids increases membrane fluidity which leads to leakage of the entrapped molecules [35]. The presence of Phospholipon®90 led to the enhanced EE observed in F_2. However, from Table 1, the decline in EE from F_2 to F_3, despite the increase in drug concentration, can be explained as an increase in the ratio of drug to lipid matrix from F_1 to F_2, leading to saturation of the matrix.

The two most commonly used techniques in determining the morphology of lipid nanoparticles are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These techniques are used to establish the morphology, surface characteristics, size, and stability of materials in the nanometer (nm) or micrometer (µm) scale. The SEM works by focusing electron beams over a sample. These electrons interact directly with the atoms in the samples providing information about shapes and sizes as well as the presence/absence of aggregation [36]. SEM can magnify up to 1,000,000 times, thereby revealing minute details of the given sample [37]. These morphological details are carefully analyzed as the EE, physical stability, bioavailability, and targeted release of the therapeutic molecules are highly dependent on their morphologies. In the present study, morphological analysis shows spherical lipospheres with a large surface area to volume ratio. Therefore, the formulated lipospheres can be said to possess characteristics that would enhance the bioavailability and penetrability of the drug in the oral mucosa.

The functional groups and corresponding chemical bonds of nanosized particle formulations were analyzed using FT-IR. This technique is highly sensitive regarding the identification of atomic/molecular groups. The FT-IR identifies chemical bonds and functional groups by producing an infrared absorption spectrum like molecular fingerprints [24]. Functional groups of miconazole nitrate were identified from the IR spectra obtained in this study. There were no interactions between the drug and formulation excipients. At frequency intervals of 4000 to 400 $cm^{-}1$, F_1, F_2, and F_3 showed absorption bands between 3140 cm^{-1} and 710 cm^{-1}. These bands correspond to stretching vibrations of CH, C=C, CH_2, C-N, and C-C functional groups. The absorption bands corresponding to 3140 and 1310 cm^{-1} gave the imidazole C-N stretching, which is typical of the antifungal class to which miconazole belongs. At 3070 to 2920 cm^{-1}, all formulations showed aliphatic CH_2 and CH stretching, and at 1445, 1385, and 710 cm^{-1}, the aliphatic -CH_2- and C-H bonds were obtained. The preponderance of miconazole's -CH- moiety accounts for its hydrophobicity.

The formulations from this study are designed for adherence to the oral mucosa. It is also intended that interaction with the mucosal surface would enable prolonged drug retention at the application site. In order to achieve these, some factors were considered, viz: structure of the

mucosal membranes, physicochemical properties of the mucoadhesive polymer, and other mucoadhesive formulations [38]. Mucoadhesive gels have been formulated using various methods and used as delivery systems to mucosal sites such as the buccal, oral, nasal, ocular, rectal, and vaginal routes [39]. In this study, the nanoparticulate lipospheres of miconazole nitrate were successfully formulated into mucoadhesive gels. This drug delivery system is convenient for administering miconazole nitrate into the oral mucosa. The product was characterized by higher bioavailability and mucoadhesive as well as the prolonged release of the antifungal agent. Obviously, the mucoadhesive properties of polycarbophil (PCP) contributed to the high levels of entanglement within the viscous mucus layer [40].

This study's gel formulations were pseudoplastic in nature as they exhibited stable forms at rest but became less viscous when agitated [41]. Pseudo plasticity results from a colloidal network structure that aligns in the shear direction, thereby decreasing the viscosity as the shear rate increases [41]. It was observed that all the gel formulations had maximum viscosities at 6 rpm.

The *in vitro* drug release is usually done using the dialysis method [42]. Alternatively, the Franz diffusion cell can be used. In either technique, the principle of sink condition applies, and suitable temperatures are required [43]. The essence of *in vitro* release studies is to ascertain the sustained release of therapeutic molecules incorporated into lipospheres [44]. Generally, the high surface area of lipospheres facilitates contact with the mucous membrane, resulting in the release of therapeutic molecules. Usually, drug release follows these processes: firstly, water penetrates through the outer coat into the lipid core, the entrapped drug is dissolved, then migrates from the core to the external environment. For hydrophobic drugs such as miconazole nitrate, dissolution of the drug is a major challenge as it is the rate-limiting step in the release process. Therefore, the migration of drugs of this kind could be through diffusion from the core to the mucus membrane [45]. As shown in Figure 9, the study results are consistent with the mechanism of drug release in the oromucosal site, i.e., in SSF at pH 6.8. No burst release was observed, indicating that the drug was homogenously dispersed in the matrix of the gel formulations [42]. The controlled release observed in the F_1 and F_2 gels was attributed to the slow discharging of the drug from the lipid matrices of the lipospheres through dissolution and diffusion mechanisms [9]. The controlled release could also result from a concurrent decrease in the amount of drug in the gel formulations and build-up of drug concentration in the dissolution medium over time, which indicates that the drug release was diffusion-controlled [46].

The enhanced antifungal property of miconazole nitrate resulted from improved penetration of the drug-loaded SLNs into the fungal cell walls, thereby effectively inhibiting the synthesis of ergosterol, which plays a major role in the preservation of cell integrity and viability. Ergosterol also aids in cell function and regeneration [47]. The high IZDs recorded against *C. albicans* indicated the antifungal activity of miconazole with the SLNs [43]. The F_2 gel, having the most optimal physicochemical properties, equally had the highest IZD. Compared to the conventionally used Daktarin® oral gel, the F_1 and F_2 mucoadhesive gels had higher IZDs against C. *albicans.*

4. Conclusions

This study led to the successful incorporation of a lipophilic drug (miconazole nitrate) into nanoparticulate lipospheres, thereby enhancing the drug's *in vitro* solubility, bioavailability, and anti-candidal action. Controlled release of the drug in the simulated oromucosal solution was also achieved with the SLNs. The formulation may be incorporated into a mucoadhesive gel for the convenient treatment of oral candidiasis. The encapsulation efficiency results showed that the drugs were successfully entrapped in the lipospheres. The formulations were able to release miconazole nitrate over a period of 360 min continuously. The F_2 had the most optimal physicochemical and antifungal properties compared to F_1, F_2, and Daktarin®. There is an indication that miconazole nitrate solid lipid nanoparticles could be a promising alternative in treating oral candidiasis.

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Conflicts of Interest

The authors declare no conflict of interest.

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