SOLIDIFIED REVERSE MICELLAR SOLUTION-BASED MUCOADHESIVE NANO-EMULGEL IMPROVES THE DISSOLUTION OF POORLY SOLUBLE IMIDAZOLE ANTIFUNGAL

FRANKLIN CHIMAIOBI KENECHUKWU; MUMUNI AUDU MOMOH, GOD'S POWER TOCHUKWU ISAAC, LILIAN CHINONSO NWEKWO, ANTHONY AMAECHI ATTAMA AND EMMANUEL CHINEDU IBEZIM

Drug Delivery and Nanomedicines Research Unit, Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Enugu, Nigeria.

ABSTRACT

Surface-modification using solidified reverse micellar solutions (SRMS) is a promising technique for improving the biopharmaceutical properties of active pharmaceutical ingredients. Here, we report on the formulation and characterization of colloidal nano-enabled emulsion impregnated gel delivery system based on SRMS containing mineral fat (beeswax) and surface-modifier [Phospholipon® 90H (P90H)] (3:7) - phospholipid-modified beeswax-based nano-emulgels - for prolonged duration of action and improved oromucosal bioavailability of miconazole nitrate (MN), a poorly water-soluble imidazole antifungal that is commonly associated with poor aqueous solubility and dissolution, low bioavailability and short half-life in the treatment of oropharyngeal candidiasis. Solid lipid nanoparticles (SLNs) prepared with P90H-modified beeswax-based lipid matrix (SRMS) by high-shear hot homogenization (melt-emulsification) had low polydispersity indices, with average particle size and encapsulation efficiency values in the range of 204.0 ± 2.9 to 263.0 ± 7.1 nm and 36.23 ± 2.01 to 58.87 ± 3.56%, respectively. SRMS-based nano-emulgels developed from the SLNs by dispersion in polycarbophil (a mucoadhesive polymer) were stable and had better prolonged drug release \( T_{100} = 36.25 - 63.67\% \) than marketed formulation (Daktarin® oral gel) which showed fast drug release \( T_{100} = 100\% \) in simulated salivary fluid (pH=6.8). This study has shown that phospholipid-modified beeswax-based lipid nano-emulgels could be employed as better alternative delivery system than Daktarin® oral gel to improve oropharyngeal dissolution and delivery of MN.

KEYWORDS: Solidified reverse micellar solution (SRMS); Miconazole nitrate (MN); Oropharyngeal drug delivery; Solid lipid nano-dispersions (SLN)-based nano-emulgel; Phospholipon® 90H; Beeswax.

INTRODUCTION

Oropharyngeal candidiasis (OPC) is one of the most common, treatable opportunistic fungal infections of the oral cavity, frequently observed in immunocompromised patients such as those suffering from acquired immune deficiency syndrome (AIDS), diabetes mellitus, metabolic disorders, poor nutrition, poor denture hygiene, infants and elderly, and heavy (chain) smokers and those undergoing cancer or antibiotic therapy [1-3]. It is caused primarily by Candida albicans and usually characterized by whitish thick patches (spots) or pseudomembranous plaques on tongue, palate more, inner cheek, and other oral cavity surfaces, sore throat and difficulty in swallowing (dysphagia), cracking at corners of the mouth where the lips meet, and redness or discomfort in the mouth area, sore and painful mouth, burning mouth or tongue, etc. [1, 4-9]. The infection can be treated with either topical antifungal agents (e.g., nystatin, clotrimazole, amphotericin B oral suspension) or systemic oral azoles (fluconazole, itraconazole, or posaconazole) [10, 11]. Even though several antifungal agents have been used for treating OPC, high concentration of these substances especially when administered systemically may pose ominous risk of drug...
resistance, several side effects, and drug–drug interactions [4-8].

Imidazole antifungals such as miconazole nitrate (MN) are administered mucosally for the treatment of oromucosal fungal infections such as OPC [12] owing to the following advantages of oromucosal drug delivery: high vascularization, the avoidance of systemic side effects and occurrence of first pass metabolism as well as relatively low enzymatic activity, which could improve drug bioavailability and, therefore, patient compliance [12]. Miconazole nitrate acts by means of a combination of two mechanisms: ergosterol biosynthesis inhibition, which causes lysis of fungal cell membranes because of the changes in both membrane integrity and fluidity and direct membrane damage of the fungal cells [13]. The drug is primarily used as an oral gel to treat OPC [14]; however, poor dissolution and lack of absorption make it a poor candidate for oral administration [2]. In addition, when used in localized therapy of OPC, resistance of the causative organism to the drug is a serious problem [11]. Furthermore, factors such as salivary secretion could lead to rapid elimination of the drug and hence increased dosing frequency [12, 15]. In fact, the oral gel is administered 3-4 times daily for OPC treatment [14]. Besides, for effective treatment, the drug must be delivered in sufficient concentration to the site of infection, which increases the toxicity concerns and associated side effects. The entrapment of MN in suitable vehicle would assist in its localized delivery and an improved solubility and availability at the targeted site (oral mucosa) coupled with a reduction in its associated undesirable side effects, which would culminate in administration of low doses and amelioration of the possibility of resistance.

In this study, solidified reverse micellar solution (SRMS) consisting of Phospholipon® 90H (a phospholipid) and beeswax (a hard fat) was prepared, characterized and employed to formulate and evaluate phospholipid-modified beeswax-based mucoadhesive lipid nano-emulgels for prolonged localized oromucosal delivery of MN. Phospholipids are attractive delivery vehicle forming a building block of some nanoparticles for some drug molecules to enhance the absorption across lipid-rich biological barriers and increase efficacy. As an amphipathic molecule, phospholipids possess a positively charged head group and two neutrally charged tail groups, a rare molecular characteristic that renders phospholipids miscible in both water and lipid and able to facilitate the crossing of the cell-membrane barrier [16, 17]. Therefore, the exploration of an effective phospholipid-modified nanoparticulate drug delivery system would prolong the delivery of MN and improve patient compliance to treatment which invariably would provide the following advantages: smaller drug doses with fewer and/or no side effects, longer residence time (less frequent dosing) with more target specificity and controlled release as well as improved compliance. Meanwhile, there is paucity of information on the use of phospholipid-modified beeswax-based (SRMS-based) mucoadhesive lipid nano-emulgels for improved controlled oropharyngeal delivery of antifungal drugs. Consequently, the aim of this study was to prepare and evaluate phospholipid-modified beeswax-based mucoadhesive lipid nano-emulgels for prolonged localized oromucosal delivery of MN.

MATERIALS AND METHODS

Materials

The pure sample of miconazole nitrate used was purchased from Gutic Biosciences Limited, India. Phospholipon® 90H (P90H) was kindly provided by Phospholipid GmbH (Köln, Germany). Other materials include methanol and ethanol (Sigma Aldrich, USA), sorbic acid (Foodchem Int. Co., China), Polysorbate 80 (Tween® 80) (Merck KGaA, Darmstadt, Germany), beeswax (white) (Ph. Eur. Carl Roth GmbH + Co. KG Karlsruhe, Germany), Polycarbophil (Noveon®) (Lubrizol Corporation, Ohio, USA) and distilled water (Lion water Ltd., University of Nigeria, Nsukka, Nigeria). The brand of commercially available miconazole nitrate oral gel used was Daktarin® oral gel (McNeil Products Ltd., Maidenhead, Berkshire, SL6 3UG, UK). All other chemicals and reagents used were analytical grade and obtained commercially.

Preparation of lipid matrix

The lipid matrix for solid lipid nanoparticles (SLNs) formulation was prepared by fusion [18] using Phospholipon® 90H and white beeswax (at 3:7 ratio). The lipids were weighed, melted together at 70 °C in a temperature-regulated oil bath and stirred until a homogenous, transparent melt of the solidified reverse micellar solution (SRMS) was obtained. The homogenous mixture of the SRMS was then stirred at room temperature until solidification. The SRMS was stored in airtight and moisture resistant glass bottles in the refrigerator until used.

Preparation of solid lipid nanoparticles (SLNs)

Miconazole nitrate-loaded SLNs were prepared using the drug, lipid matrix, Polysorbate® 80
(Tween® 80) (mobile surfactant), sorbitol (cryoprotectant) and distilled water (vehicle) by the high shear hot emulsification-homogenization method [18]. Briefly, specified quantity of each lipid matrix (5 %w/w of the SLNs formulation) was placed in glass beaker and melted at 80 °C in a temperature-regulated oil bath on a hot-plate magnetic stirrer assembly and increasing amount of miconazole nitrate (0.25, 0.5 and 1.0 %w/w) was added to the melted lipid matrix. At the same time, an aqueous surfactant solution consisting of sorbitol (4 %w/w) and Polysorbate® 80 (2 %w/w) were prepared in a separate beaker and heated at the same temperature. The hot aqueous surfactant phase was then transferred into the hot lipid phase and thereafter homogenized using an Ultra-Turrax (T25 Basic IKA-Werke, Staufen, Germany) at 15,000 rpm for 20 min, and allowed to re-crystallize at room temperature. Unloaded SLN formulations were also prepared to serve as control. In summary, the SLNs contain lipid matrix (5.0 %w/w), Polysorbate® 80 (2.0 %w/w), sorbitol (4.0 %w/w), MN (0, 0.25, 0.5, 1.0 %w/w) and distilled water (q.s. to 100.0 %w/w).

**Estimation of entrapment efficiency (EE) and drug loading (DL)**

The encapsulation efficiency (EE) of MN-loaded SLNs was determined by ultrafiltration [16] using a microconcentrator (Vivaspin® 6, Vivascience, Hanover, Germany) consisting of filter membrane with molecular weight cut off (MWCO) of 10,000 Daltons. Undiluted SLN formulation (5 ml) was placed in the upper chamber and sample recovery chamber was fitted below the membrane in the lower compartment. The unit was assembled in a centrifuge (TDL-4 B, Bran Scientific and Instru. Co., England) and rotated at 4,000 rpm for 2 h to extract the aqueous phase (1 ml) into the recovery chamber. This volume was appropriately diluted with methanol and absorbance readings were obtained spectrophotometrically at a predetermined wavelength of maximum absorption of 285 nm. Drug content was estimated by reference to a standard Beer-Lambert’s plot, while the EE % was calculated using equation 1.

\[
EE(\%) = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad ... 1
\]

DL expresses the ratio between the entrapped active pharmaceutical ingredient (API) and total weight of the lipids [19]. It was determined using equation 2.

\[
DL(\%) = \frac{W_2}{W_1} \times 100 \quad ... 2
\]

Where, \(W_1\) is the weight of lipid added in the formulation and \(W_2\) is the amount of API entrapped by the lipid.

**Determination of surface charge, particle size and polydispersity indices**

The zeta potential of SLNs formulations was determined via electrophoretic mobility measurements using a Zetasizer Nano-ZS (Malvern Instruments, UK). The mean diameter and polydispersity index of both plain and drug-loaded SLNs were also measured using a Zetasizer Nano-ZS (Malvern Instruments, UK). All samples were diluted with a fixed amount of deionized water to obtain a suitable scattering intensity, before photon correlation spectroscopic (PCS) analysis.

**Preparation of mucoadhesive SRMS-based nano-emulges**

The mucoadhesive SRMS-based nano-emulges were prepared using the mucoadhesive agent (Polycarbophil®, PCP) and other relevant ingredients (glycerol, sorbic acid and distilled water) in addition to the SLN formulations by dispersion method [20]. Briefly, PCP (1.0 %w/w) was dispersed in 30 ml of distilled water in a glass beaker. After an overnight solubilization, glycerol (3.0 %w/w), sorbic acid (0.02 %w/w) and SLNs from each batch (20 ml) (to yield 0, 0.05, 0.10, 0.20 %w/w MN) were incorporated into the PCP aqueous dispersion, with continuous stirring after each addition. In each case, the system was made up to 100.0 %w/w with distilled water and stirred vigorously for 2 h until a homogenous consistent 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.

**Preparation of simulated salivary fluid (SSF)**

Simulated salivary fluid (SSF, pH 6.8) was used as a dissolution medium to mimic salivary fluid. It was prepared following established procedure [21] using the following ingredients (g/l): sodium chloride (0.40), calcium chloride dehydrate (0.795), potassium chloride (0.40), sodium sulfide dehydrate (0.005), sodium dihydrogen phosphate (0.78), urea (1.00) and distilled water (q.s. to 1000 ml). The pH was adjusted to 6.8 by drop wise addition of dilute hydrochloric acid solution.
Determination of drug content of the mucoadhesive SRMS-based nano-emulgels
A 5 ml volume of each batch of the gel formulations was measured and placed in a 20 ml volumetric flask. A 10 ml volume of methanol was added and mixed thoroughly for 30 min. The volume was made up to the mark with the solvent and then centrifuged at 4,000 rpm for 1 h. The drug content of the solution (supernatant) was spectrophotometrically assayed at wavelength of 285 nm.

In vitro release of the mucoadhesive SRMS-based nano-emulgels
In vitro release studies were performed in 250 ml of SSF (pH 6.8) as a release medium using dialysis membrane and thermo-regulated hot plate magnetic stirring method [11]. The dialysis membrane (test dialysis bag) was cut open at both ends and soaked in release medium prior to analysis. Approximately 2 ml sample of each gel formulation was placed in the treated dialysis bag and both ends were closed. The membrane was then tied to the retort stand and suspended in the dissolution medium (SSF, pH 6.8) (200 ml) maintained at 37 °C and stirred at 100 rpm. At various time intervals (0, 1, 2, 4, 6, 8, 10 and 12 h), 5 ml of dissolution fluid was collected and replaced with freshly prepared SSF to maintain a sink condition while the experiment was carried out in triplicates. Withdrawn samples were centrifuged, filtered and diluted, and various amounts of MN released at various time intervals were quantified spectrophotometrically with reference to standard Beer-Lambert’s plot of MN at predetermined wavelength against the blank (SSF). The experiment was repeated using equivalent commercially available miconazole oral gel (Daktarin® oral gel). The percentages of MN released at various time intervals were then plotted against time.

Stability studies
The drug contents of the mucoadhesive SRMS-based nano-emulgels were re-checked after three months of storage at ambient temperature.

Data and statistical analysis
All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± SD. Student’s t-test were performed on the data sets generated using Statistical Package for Social Sciences (SPSS) software, version 12 (Chicago, IL). Differences were considered significant at \( p < 0.05 \).

RESULTS
Encapsulation efficiency and loading capacity of the SLNs
Table 1 shows the EE % and DL of the developed SLNs. In the present study, the lipid content of SLN dispersions was set at 5 % w/w for beeswax-based formulations and MN concentration was varied from 0.25 to 1.0 % w/w to estimate the effect of this increasing drug concentration on encapsulation and loading efficiency. The encapsulation efficiency (EE %) and drug loading (DL) of the SLNs are presented in Table 1. The results showed that the EE % were 44.50 ± 1.99, 58.87 ± 3.56 and 36.23 ± 2.01 % for beeswax-based SLNs containing respectively, 0.25, 0.5 and 1.0 % w/w of MN. Drug EE % increased with increase in the concentration of MN up till 0.5 % w/w for all batches, yielding maximum EE % of 58.87 %. Thereafter, the drug EE % decreased with increasing drug loading. So, the SLNs loaded with 0.5 % w/w MN resulted in higher EE %, while those loaded with 0.25 % w/w MN gave the least. Similarly, the DL values were 12.49 ± 3.05, 18.24 ± 2.99 and 9.82 ± 0.67 % for SLNs containing, respectively, 0.25, 0.5 and 1.0 % w/w of MN. The results of the DL (Table 1) equally indicate that drug loading increased with increase in the concentration of MN up till 0.5 % w/w for all batches, yielding maximum DL of 18.24 %. Thereafter, the DL decreased with further addition of MN. So, the SLNs loaded with 0.5 % w/w MN resulted in higher DL, while those loaded with 0.25 % w/w MN gave the least.

Particles properties of the SLNs
Fig. 1 with Table 1 shows the particle properties of the SLNs. Photon correlation spectroscopy (PCS) data showed that the mean particle size of MN-free SLNs was 263.0 ± 7.1 nm, while MN-loaded SLNs containing 0.25, 0.5 and 1.0 % w/w of MN had particle sizes of 262.4 ± 13.0, 243.5 ± 6.8 and 204.0 ± 2.9 nm (for beeswax-based SLNs), respectively. Similarly, the polydispersity index of MN-free SLNs was 0.274 ± 0.04, while MN-loaded SLNs containing 0.25, 0.5 and 1.0 % w/w of MN had polydispersity indices of 0.239 ± 0.01, 0.248 ± 0.06 and 0.238 ± 0.02, respectively. Meanwhile all particles were within nanometer size ranges as expected for well-formed SLNs (Fig. 1a-1d). The zeta potential (ZP) values obtained for the formulations were -32.9 ± 1.7 mV for drug-free SLNs and -36.7 ± 2.5, -39.8 ± 0.9 and -40.1 ± 1.3
### Table 1: Some physicochemical properties of the solid lipid nanoparticles (SLNs)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Z-Av. (nm)</th>
<th>PDI</th>
<th>ZP (mV)</th>
<th>EE (%)</th>
<th>DL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>263.0 ± 7.1</td>
<td>0.274 ± 0.04</td>
<td>-32.9 ± 1.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F1</td>
<td>262.4 ± 13.0</td>
<td>0.239 ± 0.01</td>
<td>-36.7 ± 2.5</td>
<td>44.50 ± 1.99</td>
<td>12.49 ± 3.05</td>
</tr>
<tr>
<td>F2</td>
<td>243.5 ± 6.8</td>
<td>0.248 ± 0.06</td>
<td>-39.8 ± 0.9</td>
<td>58.87 ± 3.56</td>
<td>18.24 ± 2.99</td>
</tr>
<tr>
<td>F3</td>
<td>204.0 ± 2.9</td>
<td>0.238 ± 0.02</td>
<td>-40.1 ± 1.3</td>
<td>36.23 ± 2.01</td>
<td>9.82 ± 0.67</td>
</tr>
</tbody>
</table>

Key: Z-Av means average particle size; PDI means polydispersity indices; ZP means zeta potential; EE means encapsulation efficiency; DL means drug loading; F0, F1, F2 and F3 are beeswax-based SLNs; F1, F2 and F3 contain increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of miconazole nitrate, while F0 is plain or unloaded SLNs.

![Size distribution of SRMS-based SLNs](image)

**Figure 1**: Size distribution of SRMS-based SLNs (a) unloaded SLN based on beeswax and P90H (F0) (b) 0.25 %w/w MN-loaded SLN based on beeswax and P90H (F1) (c) 0.5 %w/w MN-loaded SLN based on beeswax and P90H (F2) and (d) 1.0 %w/w MN-loaded SLN based on beeswax and P90H (F3). Key: F1, F2 and F3 are SRMS-based SLNs containing increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of miconazole nitrate while S0 is plain or unloaded SRMS-based SLNs.
Figure 2: Time-resolved stability of the mucoadhesive SRMS-based nano-emulgels after three months of storage at room temperature. Key: F₁ Gel, F₂ Gel and F₃ Gel are mucoadhesive SRMS-based nano-emulgels containing increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of miconazole nitrate.

Figure 3: *In vitro* release profiles of MN-loaded mucoadhesive SRMS-based nano-emulgels in SSF (pH 6.8). Key: F₁ Gel, F₂ Gel and F₃ Gel are mucoadhesive SRMS-based nano-emulgels containing increasing concentrations (0.25, 0.5 and 1.0 %w/w, respectively) of miconazole nitrate while Daktarin® oral gel is a commercially available gel containing 2.0 %w/w miconazole nitrate.
Drug content of the mucoadhesive SRMS-based nano-emulgel

The drug contents of the mucoadhesive SRMS-based nano-emulgel are depicted in Fig. 2. The percentage drug content of MN-loaded mucoadhesive SRMS-based nano-emulgel was 81.54 ± 2.07 % for batch F1 Gel (with 0.25 %w/w of MN), and 89.38 ± 0.54 % for batch F2 Gel (with 0.5 %w/w of MN). However, the latter had the greatest drug content because further increase in the amount of MN led to a decrease in the percentage drug content. Batch F3 Gel (with 1.0 %w/w of MN) had drug content of 86.91 ± 0.39 %. So, mucoadhesive SRMS-based nano-emulgel formulation batch F1 Gel containing the least amount (0.25 %w/w) of MN had the lowest percentage drug content while that having 0.5 %w/w of MN (batch F2 Gel) gave the highest.

In vitro drug dissolution from the mucoadhesive SRMS-based nano-emulgel

Fig. 3 shows the in vitro dissolution profiles of MN-containing gel formulations, and the release of MN from the mucoadhesive SRMS-based nano-emulgel over a period of 12 h was compared with the MN release from the commercial oral gel of MN (Daktarin®). Fig. 3 shows that the commercial oral gel formulation demonstrated a faster release of MN than the mucoadhesive SRMS-based nano-emulgel formulations, with nearly 100 % of the drug released after 6 h. The release profiles of MN-loaded mucoadhesive SRMS-based nano-emulgel formulations (Fig. 6a) revealed that mucoadhesive SRMS-based nano-emulgel containing 0.25, 0.5 and 1.0 %w/w of MN (i.e. batches F1 Gel, F2 Gel and F3 Gel) showed an initial release of 16.10, 15.60 and 15.00 %, respectively, within the first hour. The mucoadhesive SRMS-based nano-emulgel formulations prolonged the release of MN for up to 12 h, and the cumulative amounts of MN released from the mucoadhesive SRMS-based nano-emulgel containing 0.25, 0.5 and 1.0 %w/w of MN (i.e. batches F1 Gel, F2 Gel and F3 Gel) were 63.67, 48.50 and 55.00 %, respectively. Thus, mucoadhesive SRMS-based nano-emulgel containing 0.5 %w/w of MN (i.e. batch F2 Gel) showed the least cumulative amount of MN released and hence the best controlled drug release, while mucoadhesive SRMS-based nano-emulgel containing 0.25 %w/w of MN (i.e. batch F1 Gel) showed the greatest cumulative amount of MN released and thus the worst controlled drug release.

Stability of the developed mucoadhesive SRMS-based nano-emulgel

The stability of the mucoadhesive SRMS-based nano-emulgel was based on drug content analysis immediately after preparation and after 90 days of storage at ambient temperature, and the result is presented in Fig. 2. After three months of storage, the percentage drug contents were 81.22 ± 3.16, 88.13 ± 1.98 and 85.59 ± 0.97 % for mucoadhesive SRMS-based nano-emulgel batches F1 Gel, F2 Gel and F3 Gel, respectively.

DISCUSSION

The results of EE% and DL suggest that MN attained saturation solubility in the P90H-structured beeswax matrix at 0.5 %w/w loading, and so more of the drug could not be solubilized and encapsulated with further MN loading, which is in perfect agreement with earlier reports on lipid particulate carrier systems [19, 22, 23]. The varied EE % may be as a result of API (active pharmaceutical ingredient) and matrix physicochemical and material characteristics. The lipid contents improved the EE % of MN in the SLNs. The reason could be the enhanced amorphicity of binary mixture of beeswax and P90H.

Particle characterization of SLNs is essential to ensure the production of stable product of suitable quality. Physical stability and cellular uptake of nanoparticles are affected by particle size [23, 24]. Size distribution is affected by stirring rate, temperature and type and amounts of polymers and/or lipids as well as viscosity of the continuous phase [25, 26]. Drug loading resulted to an insignificant (p > 0.05) decrease in both the mean particle size and polydispersity indices of the SLNs. The reason for this effect is unknown but may be related to increased solubilization of the lipophilic drug in the core of the lipid matrix as well as formation of other structures such as mixed nanomicelles within the SLN dispersions [24, 27, 28]. The polydispersity indices of MN-loaded SLNs indicated low PDI and narrow distribution that would result in physical stability of the formulations, with little or no potential for particle growth [24]. This is especially true as the particle size analysis was carried out after more than one month of preparation of the SLNs and storage at ambient temperature (28.0 ± 3.0 °C). The stability of the formulations was also confirmed by the zeta potential or surface charge measurement, which showed that the values were above [30 mV], as
those recommended for aqueous nanoparticle dispersions to be considered stable [23, 25]. This suggests that the surface properties of the particles in all formulations were not altered during the 1-month storage period (before the analysis). The reason for the decrease in the percentage drug content with further increase in drug loading is uncertain, but may be related to saturation of the matrix core of the mucoadhesive SRMS-based nano-emulgel formulations with increase in drug concentration that resulted in difficulty in entrapment of MN, while the initial increase in percentage drug content with increase in drug loading is in perfect agreement with earlier reports that hydrogel-lipids could favour drug encapsulation in lipidic systems such as mucoadhesive SRMS-based nano-emulgel [29-31], which ultimately would culminate in enhanced pharmacokinetics and pharmacodynamics of encapsulated drugs and bioactives [32, 33].

The release profiles of MN from the mucoadhesive SRMS-based nano-emulgel formulations was investigated in SSF to simulate the oromucosal environment to aid in the prediction of the release profile of the various formulations in the oral mucosa [11]. In addition, initial drug strength was unified among all formulations screened to nullify the effect of concentration gradient [21]. The mucoadhesive SRMS-based nano-emulgel formulations had the tendency to fully sustain the release of MN, as had been demonstrated for gel formulations encapsulating MN in previous studies [30, 35]. The prolonged release of MN from the mucoadhesive SRMS-based nano-emulgel formulations in SSF may be a result of the slow rate of hydration and swelling of the mucoadhesive SRMS-based nanogel formulations in SSF, which, in turn, could be attributable to the properties of the excipients used in preparing the mucoadhesive SRMS-based nano-emulgel gels. The slow release could also be a consequence of the decreasing residual amount of MN in the mucoadhesive SRMS-based nano-emulgel formulations and the build-up of drug concentration in the dissolution medium in the course of time [36, 37]. Furthermore, PCP possesses bioadhesive property [20], which is an added advantage since the residence time of MN would be prolonged in the oral mucosa for maximum antifungal activity of the API; this would culminate in effective localized treatment of OPC. Meanwhile, SLNs would prevent rapid drug release due to their solid lipid matrix, which in turn would reduce drug mobility [26]. The higher prolonged MN release may be ascribed to the lipidic composition of the SLNs as well as their small size [2], providing the deposit effect for MN in the oral mucosa, leading to an effective treatment of oromucosal fungal infections such as OPC. The SLNs were prepared using a homolipid (beeswax) and a phospholipid (P90H). The phospholipid not only modified the encapsulation efficiency of the SLN but also modified the release properties. This was because of the complex structure of the phospholipid formed at the particle surface. Surface modification improves both the drug pay-load capacity and sustained release potential [16, 17]. Incorporation of SLNs into the gel formulations is expected to impart controlled release property on the mucoadhesive SRMS-based nano-emulgel [2]. In addition, the incorporation of SLNs into the PCP hydrogel should delay the release of MN since the three-dimensional network structure of the hydrogel provides an additional diffusion barrier [20, 32]. On the other hand, MN in the commercial oral gel formulation (Daktarin®) was not encapsulated and could be released more rapidly. In other words, a controlled release of MN was observed from the mucoadhesive SRMS-based nano-emulgel formulations, in contrast to the commercial Daktarin® oral gel, which demonstrated a faster release of MN. The in vitro drug release profiles equally revealed that the hydrogel-lipid complex structure considerably slowed the release of the entrapped drug, extending up to 12 h. These findings were expected, and can be attributed to an increase in the number of barriers to the passive diffusion of the drug. When MN is incorporated into the mucoadhesive SRMS-based nano-emulgels, the SLNs act as a drug-reservoir surrounded by a protective layer of gelling system which enables drug release over a prolonged time, that is, up to 12 h. These findings are similar to a recent report [29]. Overall results of the in vitro drug release indicate the mucoadhesive SRMS-based nano-emulgel formulations as a promising oromucosal delivery system for controlled release of MN for effective localized treatment of OPC.

Assessment of the stability of novel formulations is always very important in drug product design and development. Stability could be viewed from the degradation of the active ingredients or physical property of the formulation [19]. So, in order to determine the change in drug content on storage, stability study was carried out. The stability test results showed insignificant difference in drug content of MN before and after storage for three months, an indication that the mucoadhesive SRMS-based nano-emulgel formulations were stable at the experimental storage conditions [24].
CONCLUSIONS
The amphiphilic nature of phospholipids renders them miscible in both water and lipid and able to facilitate the crossing of the cell-membrane barrier; thus, they can solubilize hydrophobic drugs such as MN and facilitate its entry across oromucosal layers. In this investigation, beeswax-based mucoadhesive SRMS-based nano-emulgels were formulated and evaluated for prolonged localized delivery of MN for effective treatment of OPC. The SLNs had low polydispersity indices, within nanometer size range. The developed mucoadhesive SRMS-based nano-emulgel possessed better prolonged drug release properties than marketed formulation (Daktarin® oral gel). Thus, development of oropharyngeal MN mucoadhesive SRM possesses better prolonged drug release properties than marketed formulation (Daktarin® oral gel). This work makes part of the doctoral activities of Franklin Chimaobi Kenechukwu. We thank Phospholipid GmbH, Köln, Germany for providing Phospholipon® 90H (P90H) used in this study. We also acknowledge Lubrizol Corporation, Ohio, United States of America for the kind gift of Noveon® (Polycarbophil). This research received financial support from Tertiary Education Trust Fund (TETFund) (Grant no. TETFUND/DESS/NRF/STI/13/) by Government of Nigeria. Dr. Kenechukwu also acknowledges the support received from the African-German Network of Excellence in Science (AGNES), the German Federal Ministry of Education and Research (BMBF) and the Alexander von Humboldt Foundation (AvH).

ACKNOWLEDGEMENTS
This work makes part of the doctoral activities of Franklin Chimaobi Kenechukwu. We thank Phospholipid GmbH, Köln, Germany for providing Phospholipon® 90H (P90H) used in this study. We also acknowledge Lubrizol Corporation, Ohio, United States of America for the kind gift of Noveon® (Polycarbophil). This research received financial support from Tertiary Education Trust Fund (TETFund) (Grant no. TETFUND/DESS/NRF/STI/13/) by Government of Nigeria. Dr. Kenechukwu also acknowledges the support received from the African-German Network of Excellence in Science (AGNES), the German Federal Ministry of Education and Research (BMBF) and the Alexander von Humboldt Foundation (AvH).

REFERENCES
13. van den Bossche H. Biochemical effects of miconazole on fungi – I: effects on the uptake and/or utilization of purines, pyrimidines,


34. Nnamani PO, Kenechukwu FC, Dibua EU, Ogbonna CC, Monemeh UL, Attama AA. Transdermal microgels of gentamicin. European Journal of Pharmaceutics and Biopharmaceutics. 84, 2013:345–354.

