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Novel *Capra hircus*-Based Miconazole Nitrate-Loaded Mucoadhesive Nanogels for Enhanced Treatment of Oral Candidiasis

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ABSTRACT

Natural products play a central role in organic chemistry and are good excipient sources for pharmaceutical formulations. The objective of this study was to formulate and evaluate *Capra hircus*-based miconazole nitrate (MN)-loaded mucoadhesive nanogels for improved delivery and enhanced antifungal activity in the treatment of oral candidiasis. The method of wet rendering was employed for the extraction of homolipid from *Capra hircus*. Lipid matrix consisting of 7:3 ratio of the *Capra hircus* homolipid and Phospholipon® 90G was then prepared by fusion method. MN-loaded nanoparticulate lipospheres were prepared at drug concentrations of 0, 0.25 and 0.5 %w/v by melt homogenization and thereafter characterized with respect to particle size, polydispersity index (PDI), entrapment efficiency (EE%) and drug loading (DL). The nanoparticulate formulations were transformed into nanogels by dispersion in Polycarbophil® and thereafter characterized in terms of drug content, rheology, mucoadhesion and compatibility by Fourier transform infra-red (FT-IR) spectroscopy. *In vitro* drug dissolution in simulated salivary fluid (SSF, pH 6.8) and antifungal activity were evaluated and compared with a marketed MN-loaded oral gel formulation. The nanoparticles with acceptable PDI values (0.08-0.7), EE% (48.69-65.21), DL (18.20-23.94) and average particle size (135.9-339.0 nm) were obtained. *Capra hircus*-based mucoadhesive nanogels exhibited good physicochemical properties with no strong chemical interaction between MN and the excipients used in the formulation, and gave significantly ($p<0.05$) greater drug release and anticandidal activity than marketed gel. *Carpra hircus*-based mucoadhesive nanogels can be used as an alternative sustained delivery system for MN in localized treatment of oral candidiasis.

Keywords: Nanoparticulate lipospheres, miconazole nitrate (MN), mucoadhesive nanogels, oral candidiasis, *Capra hircus*, antifungal activity

Introduction

Fungal infection has been on the increase due to an increase in the use of steroids, HIV infection coupled with resistant to current treatment. The need for developing novel drug delivery system for its management and treatment is necessary. The delivery of a required amount of drug to the target site in the body to achieve the desired therapeutic effect and maintain optimum drug plasma concentrations is the goal of every drug delivery system.[1,2] This means that for drug delivery system to be effective, the delivery rate of the drug is dictated by the body's need. In administrating a drug, different factors must be considered, and these factors include the properties of the drug, the disease involved and the time for desired therapeutic outcome.[3] Thus, there is need to develop new, convenient, and non-parenteral dosage forms with administrative routes that allow a drug to be rapidly dissolved and absorbed into the systemic circulation to achieve optimum plasma concentration. The oral mucosal lining has rich blood supply and drug through this route has rapid onset of action, enhanced bioavailability, ease of self-medication, increased patient compliance and avoidance of first pass and food effects. [4] These advantages make the oral mucosa route better than the enteral and parenteral routes of drug delivery.[4] Also, the mucoadhesive systems interact with dosage form and the absorptive mucosa, resulting in the absorbing tissue having high drug flux [5] which is applied in the treatment of fungi infections and other microbial infection. In humans, invasive fungal infections are mostly caused by candida species, producing mild mucocutaneous disorders to invasive disease involving different organs. People that have been on broad-spectrum antibiotics for a long time or people living with HIV infection, those that wear dentures, diabetes mellitus patients, or asthmatics that inhaled steroids are usually the patient with oral candidiasis.[6] The yeast from genus *Candida albicans* is the cause of the fungal infection called candidiasis.[6] Certain factors are considered in the treatments used to manage *Candida* infections which include; the anatomic site of the infection, the host immunity, the underlying disease, the virulence of the *Candida* strain causing the infection, and how susceptible the *Candida* species is to specific antifungal drugs.[6] The imidazole antifungal agents such as miconazole nitrate (MN) are used for the treatment of skin infections or superficial candidiasis (dermatophytosis and *Pityriasis versicolor*) vaginal, oropharyngeal, esophageal and mucocutaneous candidiasis, oral thrush, candiduria, histoplasmosis, blastomycosis, coccidioidomycosis, chromoblastomycosis, paracoccidioidomycosis, and the skin yeast infections.[7-9] The drug has also been used for the treatment of disseminated fungal infections when given intravenously by infusion.[10] Mucoadhesive formulation increases the residence time of the drug such as MN at the target site/organ/tissue thereby decreasing

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the possibility of resistance to infectious agents. Lack of information on phospholipid-modified mucoadhesive nanoparticulate lipospheres of miconazole nitrate informs the decision for this research. The objectives of the study were to: formulate and characterize nanoparticulate lipospheres encapsulating MN; develop gel formulations of the nanoparticulate lipospheres using polycarbophil as bioadhesive polymer; and evaluate the gels for improved delivery and anticandidal activity of MN in relation to the commercially available MN oral gel.

Materials and Methods

Materials

The following materials were used in the study: miconazole nitrate USP pure powder (Gutic Biosciences limited, India), Daktarin® oral gel (Janssen Pharma, Beerse, Belgium), Phospholipon® 90G (P90G) (Phospholipid GmbH, Köln, Germany), Polycarbophil (Noveon®) (Lubrizol Corporation, Ohio, United States of America), sorbitol (HALAL &FSSAI, India), sorbic acid (Foodchem international corporation, China), polysorbate (Tween® 80) (Merck KGaA, Darmstadt, Germany), sodium sulfide dihydrate (Leap Labchem Co., Ltd, China), sodium chloride, potassium chloride, sodium hydroxide and calcium chloride dihydrate (BDH Chemicals Ltd, Pooles, England), methanol (Sigma Aldrich, USA), urea (Aarey Drugs & Pharmaceuticals Ltd Mumbia, India), sodium dihydrogen phosphate (Shreeji Pharma International, India), Sabouraud dextrose agar (SDA) (United Technology Trade Corp, USA), Fenicol® 250 mg (chloramphenicol, Nigeria), ethanol (Sigma Aldrich, USA) glycerol (P&G Chemicals, USA), bentonite (ETEC, Belgium), activated Charcoal (Res-Kem LLC, Pennslvania), concentrated hydrochloric acid (Qingdao Hot Chemicals Co. Ltd, China), and *Candida albicans* Bishop Shanaham Hospital, Nsukka, Nigeria).

Extraction and purification of Capra hircus (goat fat)

The method of wet rendering was employed in extracting and purification of the goat fat (*Capra hircus*) lipid as reported previously.[11] The goat fat was obtained from local slaughter house in Nsukka Coomodity Market, Nsukka, Enugu State, Nigeria.

Lipid matrix preparation for nanoparticulate lipospheres formulation

Fusion method was employed in preparing the lipid matrix [12] using *Capra hircus* (goat fat) and Phospholipon® 90G at 7:3 ratio. Solidified lipid matrix obtained was then stored in an airtight moisture resistant glass bottle until used.

Preparation of nanoparticulate lipospheres

The active ingredient (drug, MN), lipid matrix, Polysorbate 80 (Tween® 80) (mobile surfactant), sorbitol (cryoprotectant) and distilled water (vehicle) were used to prepare the MN-loaded nanoparticulate lipospheres by the high-shear melt homogenization method.[13, 14]

Determination of polydispersity indices and particle size

The particle size mean diameter (Z. Ave (nm) and polydispersity indices of nanoparticulate lipospheres formulations (PDI) were determined with a Zetasizer nano-ZS (Malvern Instrument, Worceshtire, UK) fortified with a 10mw He-NE laser using wavelength of 633 nm and a backscattering angle of 173^0 at 25^oC. Before photon correlation spectroscopic (PCS) analysis, deionized water was used to dilute all samples to get a suitable scattering intensity.[15]

Entrapment efficiency (EE%) and drug loading (DL)

Entrapment efficiency (EE%) and drug loading (DL) was carried out as reported earlier [16] with slight modification. A 5-mL volume of the undiluted formulated nanoparticulate lipospheres was placed in the upper chamber with sample recovery chamber fitted below the membrane in the lower compartment. Centrifuge (TDL-4 B. Bran Scientific and Instru. Co., England) was used to assemble the unit and rotated at 4,000 rpm for 2 h to extract into the recovery chamber 1 mL of the aqueous phase (1 mL). This volume was appropriately diluted with methanol and absorbance readings were obtained using UV-VIS spectrophotometer (6405 Jenway, UK) at 285 nm. The absorbance readings were obtained using UV-VIS spectrophotometry (6405Jenway, UK) at 285 nm after diluting the volume (1ml of the aqueous phase) with appropriate methanol. the drug content was estimated and the encapsulation efficiency of all batches of NLC was calculated with the formula below. [17].

$$\text{EE}\ (\%) = \frac{calculated\ drug\ content}{theoritical\ drug\ content}\ x\ 100$$

$$= \frac{total\ drug\ loded - dug\ in\ aqueous\ phase}{total\ drug\ loaded}\ x\ 100 \qquad eqn\ 1$$

$$\text{Drug loading capacity (DL)} = \frac{Wa}{W1}\ x\ 100 \qquad eqn\ 2$$

Where, W_l = weight of lipid added in the formulation and W_a = amount of miconazole nitrate entrapped in the lipid.

Preparation of lipid-based gels

Dispersion method was used in preparing the lipid-based gels (drug-loaded and unloaded). [18] Briefly, the preparation was done using the mucoadhesive agent (Polycarbophil, PCP) and other relevant ingredients (glycerol, sorbic acid and distilled water) coupled with nanoparticulate lipospheres formulations. The formulations were poured into an ointment jar and the pH raised to 6.8 by drop-wise addition of 0.5 M NaOH.

Determination of the drug content

To determine the drug content of the formulation, 5 ml volume of the gel formulations from each batch was measured into a 20 ml volumetric flask and10 ml of methanol added and mixed thoroughly for 30 min. The solvent was used to make the volume up to mark and then centrifuged at 4,000 rpm for 1 h. The content of drug in the solution (supernatant) was assayed using spectrophotometry at 285 nm.[17]

FT-IR spectroscopy

Fourier transform infra-red (FT-IR) spectroscopic analysis was conducted as reported previously [18]. A smart attenuated total reflection (SATR) accessory was used for data collection. A 0.1 ml volume of each batch of the nanogel formulations was mixed with 0.1 mL diluent. The solution was introduced into the potassium bromate (KBr) plate and compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum obtained.

Rheological and mucoadhesive studies

The viscosity of each gel formulation was measured using a digital viscometer (NDJ-5S Viscometer, Labscinces, England), with spindle number 04 at different speeds (6, 12, 30 and 60 rpm). The sample was placed in a 20-mL beaker and the spindle immersed in it and attached to the coupling nut such that the gel level was at the groove on the shaft. Ambient laboratory temperature (32 °C) was maintained while running the test and the viscosity (mPa.s) of each gel formulation was recorded.[19]

For mucoadhesive study, 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 inverted position to the ring of Du Nuoy tensiometer (ELMA tensiometer model 8600T, UK). 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 other membrane on the

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ring of Du Nuoy tensiometer for 5 min. The ring of the tensiometer was gradually raised until the two mucosal tissues got detached from each other. The procedure was repeated for other batches of the gel and new buccal membrane was used in each case. The mucoadhesive strength expressed as the detachment stress in dynes/cm^2 was determined from the minimal force that detached the exposed mucosal tissue from surface of each gel formulation.

$$\text{Mucoadhesive strength } \left(\frac{\text{dynes}}{Cm^2}\right) = \frac{F}{A} \qquad Eqn\ 3$$

Where, F is the force of detachment in dyne and A is exposed mucosa Area (1cm^2).

In vitro drug release studies

In vitro release study was carried out as reported previously.[19] A treated dialysis bag [Molecular weight cut off (MWCO) 8000 g/mol, CelluSep®, USA] was used and a 2 mL sample of each gel formulation was placed in it and both ends closed with a thread. Freshly prepared simulated salivary fluid (SSF, pH 6.8) was used as the release medium in which the formulation was suspended in from retort clamp. The release medium was stirred at a stirring rate of 100 rpm at temperature of 37 ± 0.5 ºC. A 5 mL samples was removed at regular interval of 15 min for 6 h, and the same volume of fresh release medium was added to maintain the same volume (i.e. sink condition). The filtrate, 5 mL obtained from the withdrawn sample using 0.21 mm filter device (Millipore®, Germany), was diluted with methanol and spectrophotometrically analyzed at 285 nm. The miconazole nitrate at each point in time was calculated from the absorbance reading and expressed as percentage drug released taking cognizance of the drug

Inhibition zone diameters (IZDs)

The *Candida* strain was obtained from oral swab of an immunosuppressed female HIV-positive patient suffering from oral thrush. The isolate was identified, purified and kept on SDA agar slant as bench culture at 4 ^{o}C as reported previously.[20] Sterile water was used to suspend the overnight culture of *Candida albicans.* The isolates were standardize using 0.5 MacFarland turbidity Standard as reported previously.[18] To determine the inhibition zone diameter, 2 ml each of the gel formulations as well as the commercial sample was prepared and used to carry out sensitivity test using the cup-plate agar well diffusion method.[21] Thereafter, the inhibition zone diameters (IZDs) were measured.

Statistical analysis

All experimental set up was in triplicates to ensure validity of statistical analysis. Mean ± SD were calculated. One-way Analysis of variance (ANOVA) and Student's t-test were obtained from the pooled results using Statistical Package for Social Sciences (SPSS) version 16.0. Differences were considered significant for p-values ≤ 0.05.

Results and Discussion

Physicochemical properties of the nanoparticulate lipospheres

Figure 1 (A, B, C and D) shows the nanoparticle size distribution by intensity for all batches which is captured in Table 1. The particle sizes (nm) as shown in Figure 1 1(A-D) for the different concentrations (S_0-S_3) are within the range of 135-339 nm which agrees with nanoparticles diameter as reported previously. [21] Nanoparticle represents one billionth of a meter or 10^{-9} m and the length is a metric unit. [22] The PdI of the formulations; S_0, S_1, S_2 and S_3 are shown in Table 1. Values obtained were within the mid-range values of PdI between 0.08-0.7, a range over which the distribution algorithm best operates.[23] Values of PdI greater than 0.7 indicates very broad size distribution and may contain large particles or aggregates that could lead to sedimentation.[24] The EE (%) and DL increased with increase in drug concentration up to batch S_2 and then decreased with further increase. This is expected since a saturation threshold can be reached beyond which a decreased is seen due to excess drug concentration as shown in Table 1 for S_2 and S_3. This could be due to displacement of some active pharmaceutical ingredient (MN) from the active sites of the lipid matrix.

Table 1: Physicochemical properties of the nanoparticulatelipospheres

Sample	Z-Av. (nm)	Pdi	EE (%)	DL (%)
S_0	135.9	0.503	-	-
S_1	155.3	0.363	48.69	18.50
S_2	289.8	0.308	65.21	23.94
S_3	339.0	0.424	61.45	18.20

Fourier Transform Infrared (FT-IR) spectroscopy of the formulations

Table 2 shows the vibration bonds of the pure MN and that of the prepared gel formulations. Compatibility between the drug and the excipients of the formulation S_1 Gel, S_2 Gel and S_3 Gel at different concentrations (0.125, 0.25 and 0.5mg/ml) were compared. It was shown that, there were no disappearances or marked shift in the peak position of MN in the drug spectra of the formulations from that of the pure MN as shown in Table 2. The absence of interfering peaks shows there were no unwanted reactions between MN and other excipients used in the formulation which agrees with.[20] The aromatic CH stretch, aromatic C=C, aliphatic CH stretch, CH2 stretch, CH bending and imidazole C-N stretch of the different functional groups in MN was present and comparable with that of the pure sample, hence the drug can be used with the excipient without causing instability or incompatibility problems.

Drug content and mucoadhesive properties of the gel formulations

Figure 2 is a double axis graph showing the drug content and the mucoadhesive properties of the gel formulations. The formulation with highest drug content was S_2 Gel. The percentage drug content of the formulation was within the acceptable range 85 to 115%, which is consistent with earlier report.[25] Results of the mucoadhesive studies represented by the graph in Figure 2 shows that the formulated S_2 Gel had the highest mucoadhesive strength while the least mucoadhesive strength was recorded by S_1 Gel. The mucoadhesive strength tends to be optimum at optimum concentration, the decrease in mucoadhesive strength as recorded by S_3 Gel could be as a result of saturation which led to decreased adhesion due to low diffusion of water into the polymer network, resulting in the polymer network not swelling sufficiently and decreasing the rate of interpenetration between mucin and the polymer, in agreement with a study reported elsewhere.[25] The mucoadhesive property exhibited by the formulated gels allows the slow clearance of the formulation from the mucosa leading to extended release of the drug.

Table 2: FT-IR spectra data of Miconazole Nitrate (MN) and the formulation

Group	MN	S_1GEL	S_2GEL	S_3GEL
Aromatic				
C-H stretch	3070	3045	3045.3	3053.02
C=C	1525	1536.04	1575.64	1539.9
Aliphatic				
C-H stretch	2920	2917.92	2887.38	2890.9
CH_2 stretch	2995	2987.4	2968.1	2983.54
C-H bending	1385	1323.74	1377.78	1350.76
Imidazole C-N stretch	3140	3168.82	3122.5	3145.66

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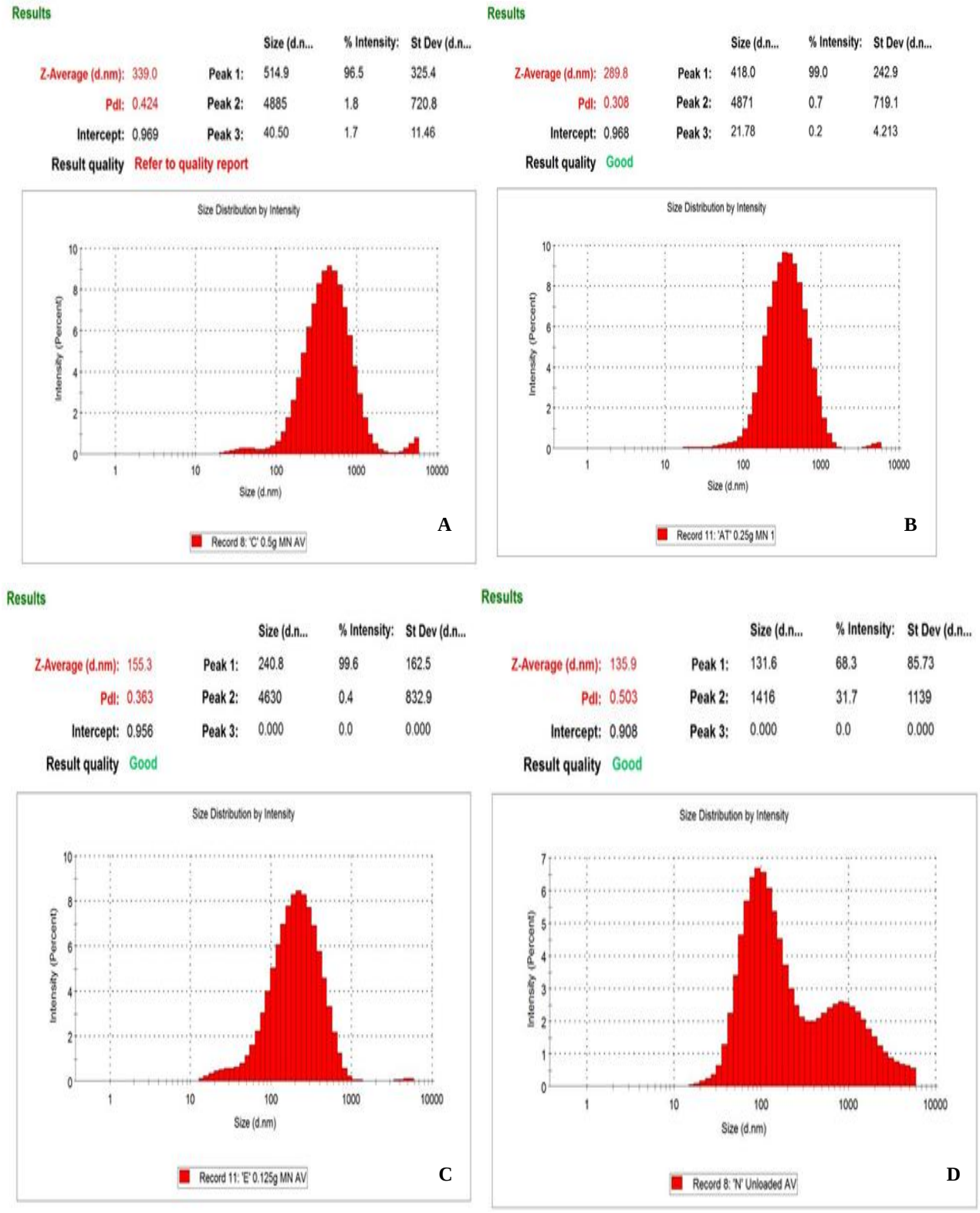

Figure 1: Size distribution by intensity for**: [A]** batch S_3, [B]batch S_2, **[C]** batch S_1 and **[D]** batch S_0.

Viscosity of the formulated gel

Figure 3 depicts the viscosity profile of the gel formulations. The result shows the rheological property of S_0 Gel, S_1 Gel, S_2 Gel and S_3 Gel. The graph shows decreased viscosity with increasing shear rate (shear thinning behaviour). Batches S_1 Gel and S_3 Gel showed the same progression as S_0 Gel. The variation displayed by S_2 Gel at 6, 12, 30 and 60 rpm could be as a result of structural rearrangement due to dilatant behavior especially between 12 and 30 rpm, although S_2 Gel generally exhibited pseudoplastic behavior at high shear rates (i.e. between 6 and 12 rpm and between 30 and 60 rpm). This could be as a result of small changes within the fluid structure, such that the fluid rearranges the microscale geometries to ease shearing.[26] Viscous materials that show a decrease in viscosity with increasing shear rate are said to be pseudoplastic.

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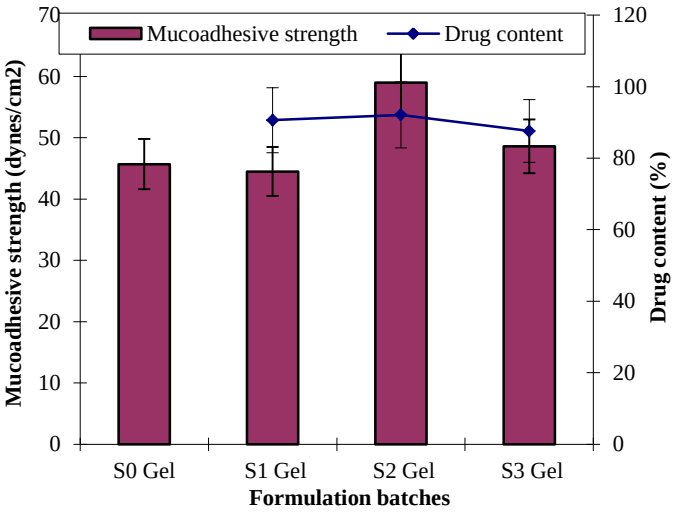

Figure 2: Drug content and mucoadhesive properties of the gel formulations

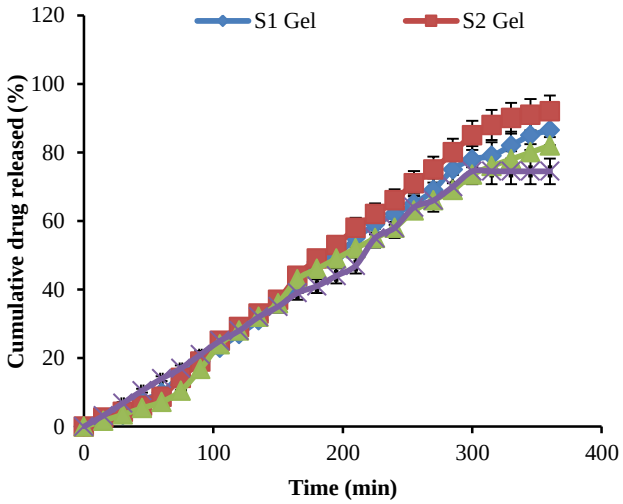

Figure 4: *In vitro* dissolution profiles of the drug-loaded gel formulation in SSF (pH 6.8).

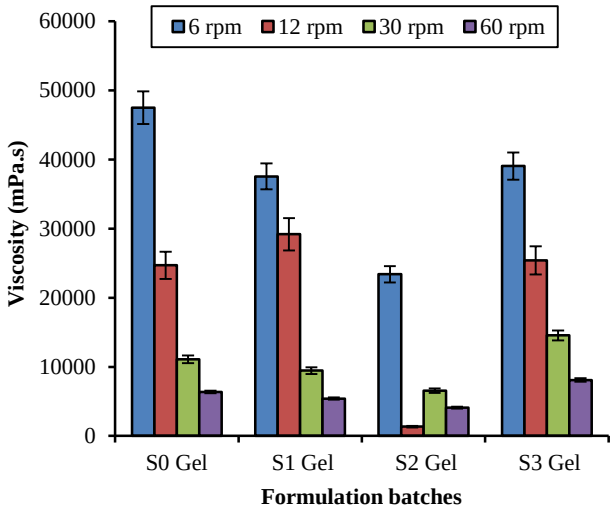

Figure 3: Viscosity profiles of the gel formulations

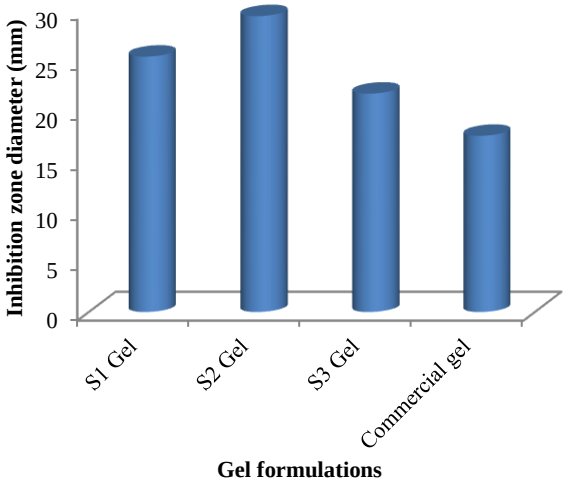

Figure 5: *In vitro* anti-candida activity of drug-loaded gel formulations.

In vitro dissolution profiles of the drug-loaded gel formulation using SSF (pH 6.8)

Figure 4 shows the i*n vitro* dissolution profiles of the gel formulation (drug-loaded) in simulated salivary fluid, SSF (pH 6.8) compared with equivalent commercially available gel (Daktarin® oral gel). Batch S_2 Gel had the highest release which was 92 %, followed by batch S_1 Gel with release of 83 % and batch S_3 Gel with release of 78 %. Commercially available oral gel had the lowest release of 69 %. The cumulative release of the formulations compared to that of commercially available oral gel was significant ($p < 0.05$). Although commercially available oral gel had a faster release, its cumulative release rate was lower than those of MN-loaded nanogels, which had a slower release and higher cumulative release rate indicating the drug can be applied less frequently with sustain optimum released concentration. The nature of the formulated gel could be the cause of the slower and sustained release of the API which it's believed to be protected by several passive barriers leading to slower but sustained release of the API (MN).[19, 28]

In vitro anti-candidal activity of drug-loaded gel formulations

Figure 5 shows the anticandidal activity of the gel formulations compared with commercially available MN oral gel. There was significant ($p < 0.05$) difference in the anti-candidal activities of the formulated nanogels and Daktarin® oral gel. The diameters of the zone of inhibition of the formulations (S_1 Gel, S_2 Gel, S_3 Gel and the commercially available oral gel) were 26, 30, 22 and 17 mm, respectively. This shows that sustain optimum release of the gel formulations gave a better inhibition of C*andida albicans* when compared to the commercial gel formulation which agrees with earlier report [19]. The increased IZD as observed in the formulated gel could be due to its lipid nature, high drug loading ability, sustained release and better permeation of the nano drug particle into the organism which results in the inhibition of the fungal enzyme 14α-sterol demethylase hence reducing the production of ergosterol and subsequently death. [29]

Conclusion

Capra-hircus based miconazole nitrate-loaded nanoparticulate lipospheres were formulated and evaluated for enhanced treatment of oral candidiasis. The *in vitro* release and anticandidal studies show that optimized formulation was more effective than the commercially available miconazole nitrate oral gel. The formulated *Capra hircus*-based mucoadhesive nanogel of MN can sustain the delivery of the drug, has the potential to be used for extended delivery of the MN and enhanced antifungal activity with improved patient compliance.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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