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(RESEARCH ARTICLE)

Preparation, physicochemical characterization, and *In vivo* evaluation of miconazole nitrate nanosponges for the treatment of systemic mycoses

Hilary Onwuchekwa [1], Emmanuel Uronnachi [1,*], Chukwuebuka Umeyor [1], Calistus Nwakile [1], Josephat Obasi [1], Ifeanyi Okeke [1] and Charles Esimone [2]

[1] *Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*
[2] *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*

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Abstract

Miconazole is a Biopharmaceutics Classification System (BCS) class II drug, thus limiting its oral bioavailability. Nanosponges can improve the oral bioavailability of poorly soluble drugs and sustain their release. The work was aimed at formulating miconazole nanosponges to enhance its solubility and consequently, oral bioavailability.

Batches of nanosponges incorporating different concentrations (1, 2 and 3 %) of miconazole nitrate were formed using ethyl cellulose (EC), polyvinyl alcohol (PVA), ethanol and Kolliphor® 188. They were characterized using percentage yield, encapsulation efficiency, Differential Scanning Calorimetry (DSC), Xray Diffraction (XRD), particle sizes, polydispersity index (PDI) and Scanning Electron Microscopy (SEM). *In vitro* antifungal evaluation against *Candida albicans* was assessed while *in vitro* release using simulated gastric fluid was done. Furthermore, *in vivo* release studies in rats were carried out using the bioassay method.

The percentage yield of the nanosponges were between 50 to 60 %. Encapsulation efficiencies ranged between 77 and 98 %. Particle sizes ranged between 266.5 and 781.1 nm. Particles were porous and spongy in appearance. Antifungal evaluation of the nanosponges against *Candida albicans* revealed activity like that of the pure drug. *In vitro* release studies showed a sustained release pattern for the nanosponges up till 12 hours whereas the unformulated drug peaked at 2 h. *In vivo* evaluation showed a concentration dependent increase in blood concentration of the drug with NS4 having the highest release.

Nanosponges containing miconazole nitrate were successfully formulated and evaluated and are a promising oral delivery system for miconazole nitrate.

Keywords: Miconazole nitrate; Bioavailability; Nanosponges; Bioassay; *In vivo* release

1. Introduction

Oral drug delivery systems have been the most convenient and acceptable method of drug delivery to patients. It is however fraught with challenges of drug solubility and permeability causing some chemical entities to be absorbed poorly from the GIT. It is postulated that about 40 % of new chemical entities have poor oral bioavailability [1].

* Corresponding author: Emmanuel Uronnachi
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Consequently, several approaches have been explored to improve the solubility of these poorly soluble drugs. Such approaches include solid dispersions, lipid and polymer based micro and nanoparticulate drug delivery systems, Self-emulsifying drug delivery systems (SEDDS), nanoemulsions, microemulsions, etc [2]. All of these have their advantages and limitations.

Miconazole nitrate (MN) is an effective antimycotic agent for the treatment of candida infections. Its poor aqueous solubility limits its use orally due to poor absorption from the gastrointestinal tract (GIT) [3]. Miconazole is a Biopharmaceutics Classification System (BCS) class II drug. It is weakly basic in nature and acts by inhibiting the enzyme, 14-α demethylase, which causes ergosterol synthesis [4-5], and inhibits fungal peroxidases causing cell death via peroxide accumulation [6].

Several researches have been conducted on formulations of miconazole. Maha *et al.* [7] formulated nanoemulsions and creams of miconazole nitrate and evaluated them for topical delivery. The formulations were stable over the period of evaluation with particle sizes ranging from 102.36 nm to 309.11 nm. Mady *et al*. [8] demonstrated an enhanced anti-candidal action of miconazole nitrate when combined with urea for treating buccal infections. Umeyor *et al*. [9] prepared miconazole nano-structured lipid carriers using admixtures of Softisan 154 and soybean oil and evaluated its antimalarial activity in vivo. The results showed similar clearance rates between the NLCs and positive control. Kenechukwu *et al*. [10] prepared dual-responsive microgels containing miconazole nitrate for vaginal drug delivery. Their results showed a better anticandidal activity of the microgels versus commercial gel preparations of miconazole. Kenechukwu *et al*. [11] prepared nanogels containing miconazole for treatment of oropharyngeal candidiasis. Their preparation showed better anticandidal activity than marketed formulations of miconazole at equivalent concentrations. Kenechukwu *et al*. [12] prepared molecularly PEGylated lipid matrices containing miconazole nitrate for treatment of vulvovaginal candidiasis. Their formulations exhibited better and significantly ($p < 0.05$) faster and more powerful fungicidal activity regarding killing rate constant values than commercial topical solution of MN (Fungusol) and pure MN sample. Uronnachi *et al*. [13-14] prepared Solidified reverse micellar microparticles of miconazole nitrate and evaluated its potential for improved oral bioavailability. *In vitro* release studies of the formulation showed an improved release of the formulations made from Softisan lipid matrices when compared with release from the pure miconazole nitrate drug. Gulati *et al*. [15] evaluated the use of miconazole microsponges for the treatment of diaper dermatitis. Vazquez and Sobel [16] prepared miconazole mucoadhesive tablets for treatment of oropharyngeal candidiasis. Their preparation was a convenient once-daily formulation for treatment of oropharyngeal candidiasis.

From these research works, it can be gleaned that much of the work done on miconazole has been on its topical delivery enhancement. Hence the interest of this research is in delivering it orally for systemic use. Nanosponges have been proven by several researchers to improve the oral bioavailability of poorly soluble drugs, as well as sustain their drug release patterns [17-18].

In addition, there has been no published studies on the use of nanosponges for improving the oral bioavailability of miconazole nitrate, hence the decision to undertake this study. The work was, therefore, aimed at formulating and characterizing nanosponges containing miconazole nitrate as well as evaluating its antimycotic activity.

2. Material and methods

2.1. Materials

Kolliphor® P188 (BASF, Germany), Ethylcellulose, Miconazole Nitrate (Gutic Biosciences, India), Polyvinyl alcohol (Anmol chemicals, India), Ethanol (Qualichems, India). Other materials used were of analytic grade.

2.2. Methods

2.2.1. Preparation of nanosponges

Miconazole nanosponges were prepared using different proportions of ethyl cellulose and polyvinyl alcohol (PVA) by emulsion solvent diffusion technique [19].

The disperse phase consisting of a known weight of miconazole and specified quantity of ethylcellulose (the different quantities for the various batches are given in table 1) were dissolved in 100 ml of ethanol and slowly added to a definite amount of PVA and KP®188 in 100 ml of water as the continuous phase. The mixture was stirred at 5,000 rpm on a

magnetic stirrer for 5 h. The formed nanosponges were collected by filtration and dried in hot air oven at 40 ^{0}C for 24 h. Several batches were formulated including an unloaded batch according to Table 1.

Table 1 Composition of formulations (%w/w)

Formulation Code	MNZ (g)	Ethylcellulose (g)	Ethanol (ml)	PVA (g)	KP®188 (g)	Water (ml)
NS1	0.5	1.0	100	1.0	1.0	100
NS2	1.0	2.0	100	1.0	1.0	100
NS3	2.0	3.0	100	1.0	1.5	100
NS4	3.0	4.0	100	1.0	1.0	100
NS5	-	2.0	100	1.0	1.0	100

NS – Nanosponge, MNZ – miconazole, PVA – polyvinyl alcohol, KP®188 – Kolliphor®P188

2.2.2. Physicochemical characterization

Percentage yield determination

The percentage yield of the nanosponges was calculated using the formula

$$\text{Percentage yield} = \frac{\text{Mass of NS recovered}}{\text{Mass of solid excipients}} \text{X} \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Determination of encapsulation efficiency

The entrapped drug in the nanosponges was determined. Briefly, 0.25 g portions of the nanosponges were collected and dispersed in 25 ml of methanol for a period of about 12 h with intermittent shaking. At the end of this period the dispersion was filtered and diluted ten-fold with methanol before determining their absorbance using a spectrum Model 752 spectrophotometer (China) at a wavelength of 280 nm. Triplicate determinations were obtained per batch. Afterwards, the encapsulation efficiency (EE) of the entrapped drug was determined using the following formula:

$$\text{EE} = \frac{entrapped\ drug}{theoretical\ drug\ loaded} \quad \text{X} \quad \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots 4$$

Determination of Particle size

The average particle sizes of miconazole nanosponges were determined by photon correlation spectroscopy (PCS) using a Nano ZS-90 (Malvern Instruments limited, UK) at a fixed angle of 25°. Sample was diluted 10 times with distilled water and then it was analyzed for particle size.

Particle Morphology determination

The morphology of nanosponges was examined using Scanning Electron Microscopy (LEO 440I, UK). Sample was deposited on a glass slide and was kept under vacuum. The samples were coated with a thin gold/palladium layer using a sputter coater unit. The scanning electron microscope was operated at an acceleration voltage of 15 kV.

pH evaluation

To check the stability of the formulations, the pH was measured over a period of six months at regular intervals of 1 week, 1 month, 3 months and 6 months. Prior to use, the pH meter (Jenway 6505, UK) was calibrated using buffers 4 and 9.

Thermal analysis

This was carried out using Differential scanning calorimetry (DSC-60, Shimadzu Corporation, Japan) to check compatibility between drug and polymers. Indium and lead standards were used for calibration while 5 mg samples were heated (range 50-400 °C, 10 °C/min) in crimped aluminium pans under a nitrogen atmosphere. The enthalpy of fusion and melting point were automatically calculated.

X-ray Powder diffraction (XRD) analysis

XRD analyses were carried out on the nanosponges and excipients using a Rigaku miniflex 300 (Rigaku, Japan) diffractometer. Wide angle X-ray diffraction (WAXD) studies were done using Cu Kα (40 kV, 30 mA) at the rate of 1.2 °/min over a 2Θ range of 10 - 70 °. Intensities and d-spacings were obtained, while the three most intense peaks for each sample were observed.

2.2.3. Antimycotic evaluation of formulations

Agar well diffusion assay

Briefly, the media [Sabouraud Dextrose Agar (Titan, biotech, India)] was prepared and treated according to the manufacturer's specifications. A 65 g of the media was dispersed in 1 L of sterile distilled water and sterilized at 121 ˚C for 15 min. The media was allowed to cool to 50 ˚C and later dispensed into 90 mm sterile agar plates and left to set. The sterile SDA plates were inoculated with the test culture (*C. albicans)* previously standardized to MacFarland suspension by surface spreading method using sterile cotton swab which was dipped into the inoculum and excess was removed by rotating the swab several times against the inside wall of the tube above the level of the fluid. This was done to obtain uniformity of the inoculum. A sterile cork borer was used to make eight wells (8 mm in diameter) on each of the SDA plates. Aliquots of 80 µl of each formulation dilutions, reconstituted in DMSO at concentrations of 5, 2.5, 1.25, 0.63 and 0.31 mg/mL for the N1, N2, N3, N4 N5 and MZ respectively were applied in each of the wells in the culture plates. DMSO served as the negative control. The cultures were incubated at 25 ± 2 ˚C for 48 h. The antimicrobial potential of the formulations was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each formulation, three replicates were conducted against each organism.

Determination of minimum inhibitory concentrations (MICs) of formulation by broth dilution

The method described by *European Committee for Antimicrobial Susceptibility Testing* (EUCAST) was adopted with slight modifications. A stock concentration of 5 mg/mL of each test formulation was made in sterile test tubes. Dilutions were prepared at twice the desired final concentration. Then, two-fold serial dilution of the stock was done using sterile Sabouraud Dextrose broth. A volume (1 ml) of fungal test suspension (*C. albicans*) previously standardized to MacFarland standard was added into each tube containing the diluted formulation, the tubes were capped then incubated at 25 ˚C for 48 h. After incubation, Results were read when sufficient growth of the test organism (i.e. obvious turbidity in the positive growth control), no growth in the uninoculated or negative growth control (where present) and when a purity plate showed the test organism to be pure. Then 20 µl of each mixture (formulation/organism combination) in the different test tubes was spread (plated) over the surface of Sabouraud dextrose agar plate that had been dried properly before incubating appropriately. The minimum inhibitory concentration of each formulation against the test organism was determined by checking for visible colonies on the surface of the agar plate after incubation. Then the plate (concentration) having no growth was taken as the MIC.

Minimum biocidal concentration (MBC)

For the MBC, MIC plates having no visible colony were further incubated at 25 ˚C for 24 h. then the MBC was determined by checking for visible colonies on the surface of the agar plate after incubation. Afterwards, the plate (concentration) having no growth was taken as the MBC.

Antimycotic standard calibration curve based on Inhibition Zone Dimeter (IZD)

Agar-well diffusion method was adapted for this study. Serial dilutions of pure miconazole were prepared as follows in animal serum: 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.1562 mg/ml. Sabouraud dextrose agar (SDA) was prepared and treated according to the manufacturer's specification by dispersing 65 g in 1L of sterile distilled water and sterilized at 121 °C for 15 min. The media was allowed to cool to 50 °C and later dispensed into 90 mm sterile agar plates and left to set. The sterile SDA plates were inoculated with the test culture *C. albicans* previously standardized to McFarland suspension. A sterile cork borer was used to make six wells (8 mm in diameter) on each of the SDA plates and 3 drops of each dilution were introduced into the wells. The plates were allowed to stand at room temperature (25 ± 2 °C) for 5 min followed by incubation at 25 °C for 48 h. The IZDs were measured in triplicate for each dilution. The mean IZDs were used to construct a standard calibration curve against the known concentrations of the drug.

2.2.4. Beer's plot of miconazole nitrate in 0.1N HCl

Different concentrations of miconazole nitrate were prepared using 0.1 N HCl. The absorbances of these concentrations were determined using a UV-Visible spectrophotometer at a wavelength of 272 nm Subsequently, the results obtained were used to plot a graph of absorbance against concentration (Beer's plot).

2.2.5. In vitro release

This was done using Simulated gastric fluid (0.1 N HCl). 100 mg quantities of the nanosponges were placed in dialysis membranes (MWCO 12,000 Da) and put in 900 mL of the dissolution medium. At regular time intervals, 5 ml portions were withdrawn from the dissolution medium and replaced with fresh fluid. The withdrawn samples will be filtered and analyzed for drug content using a Spectrophotometer at a wavelength of 272 nm. The *in vitro* release study was carried out for 8 hours.

2.2.6. Bioassay-guided in vivo antimycotic study of miconazole in rat serum

Healthy white albino rats (n = 30) of both sexes weighing 190 – 250 g were randomly selected for this study. They were kept in separate cages and allowed free access to food and water before the study. The rats were grouped into six (A to F) of five rats each. They were administered orally with 3, 4 and 5 mg/kg of miconazole-loaded nanosponges for batches NS1, NS2, NS3, and NS4 respectively. Standard drug (MNZ) was administered orally at the dose of 5 mg/kg as the positive control and unloaded formulation (NS5) were also administered orally at the dose of 5 mg/kg as negative control. Prior to administration, blood samples were drawn from the retroorbital venous plexus of the rats to form the base line. Blood samples were collected at predetermined time intervals of 1, 2, 5, 8 and 12 h from the retroorbital venous plexus of the rats post-administration. Samples were collected in sterilized plastic tubes, centrifuged at 5000 rpm for 10 min and their sera carefully collected into sterile eppendorf tubes, and securely sealed. Sabouraud dextrose agar (SDA) was prepared and treated according to the manufacturer's specification by mixing 65 g with 1L of sterile distilled water and sterilized at 121 °C for 15 min. The media was allowed to cool to 50 °C and later dispensed into 90 mm sterile agar plates and left to set. The sterile SDA plates were inoculated with the test culture *C. albicans* previously standardized to MacFarland suspension. A sterile cork borer was used to make six wells (8 mm in diameter) on each of the SDA plates and 3 drops of sera from the various groups were introduced into the wells. The plates were allowed to stand at room temperature (25 ± 2 °C) for 5 min followed by incubation at 25 °C for 48 h. The IZDs were measured in triplicate for each sample.

2.2.7. Statistical analysis

Results were computed using Microsoft excel 365 edition. Mean and standard deviation values were computed and presented in the representative tables.

3. Results

3.1. Percentage Yield

The percentage yield of the nanosponges is presented in Table 2. The results showed a fair yield with the least percentage of 35.80 % and highest percentage of 60.76 %.

Table 2 Percentage yield of formulated nanosponges

	Percentage yield			**Encapsulation efficiency**			
Formulation	**Recovered yield (g)**	**Weight of drug and solid excipients(g)**	**Percentage yield (%)**	**Encapsulation efficiency (%) After preparation**	**Encapsulation efficiency (%) After 6 months**	**Z-average (nm)**	**Polydispersity index**
NS1	1.91	3.50	54.57	84.00	82.00	266.5	0.540
NS2	2.75	5.00	55.00	77.00	77.00	781.1	0.799
NS3	3.95	6.50	60.76	90.00	88.00	229.0	0.380
NS4	4.85	9.00	53.89	98.00	93.33	348.3	0.344
NS5	1.79	5.00	35.80			366.1	0.357

3.2. Characterization of Nanosponges

3.2.1. Encapsulation efficiency

The results of the encapsulation efficiency determination are presented in Table 2. From the results obtained, all formulations exhibited a high encapsulation efficiency with NS4 possessing the highest percentage. Also, after a six-month period, there were only slight reductions in the values obtained showing a high degree of stability of the nanosponges.

3.2.2. Particle size and polydispersity index

The result of the particle size and polydispersity index are presented in Table 2. From the results obtained, all the formulations were within the nanometer size range with highest value of 781.1 nm obtained for NS2 and lowest value of 229.0 nm obtained for NS3. Also, the particles had PDI values between 0.3-0.7 indicating intermediate dispersity index.

3.2.3. Imaging of the nanosponges

The SEM images of the nanosponges are presented on figures 1. The images depict sponge-like structures with varying degrees of cavitation.

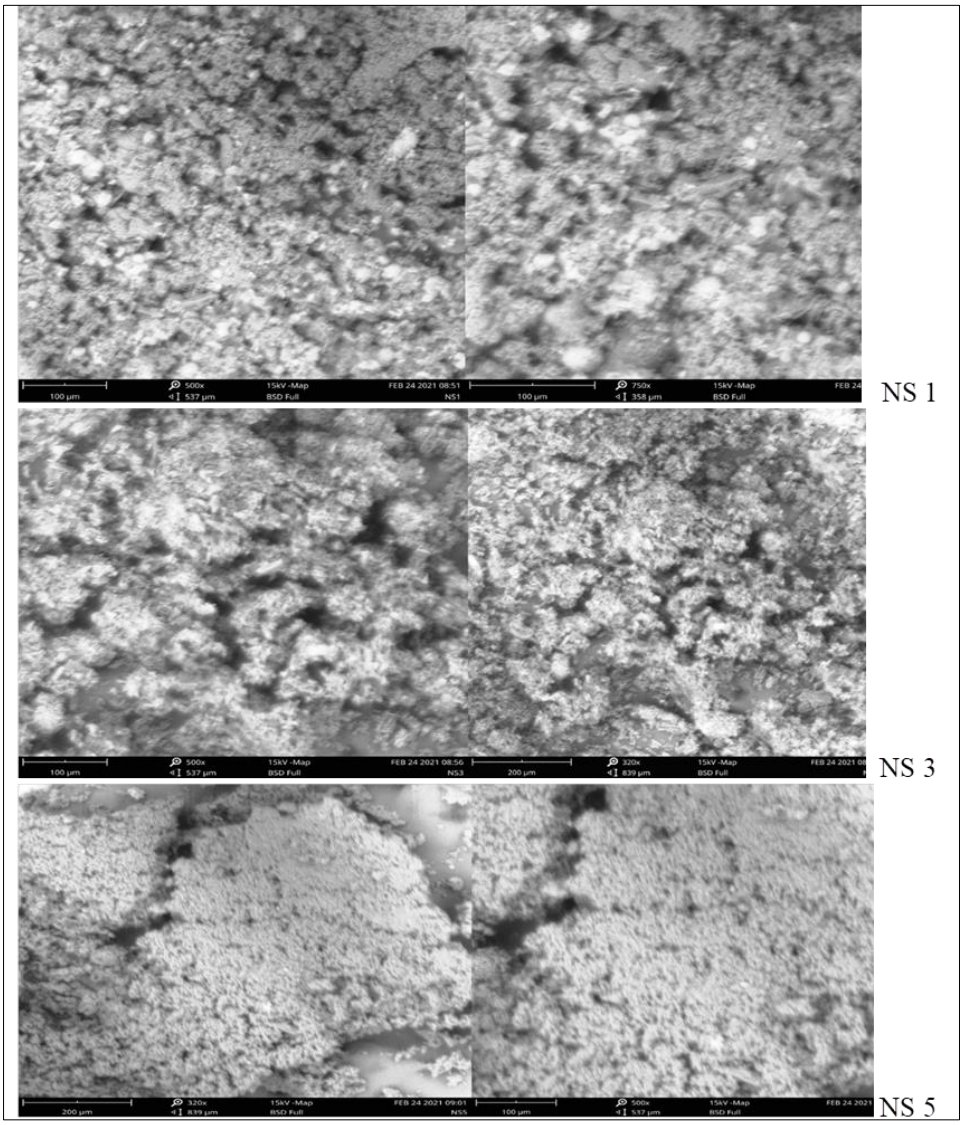

Figure 1 SEM Images of selected formulations

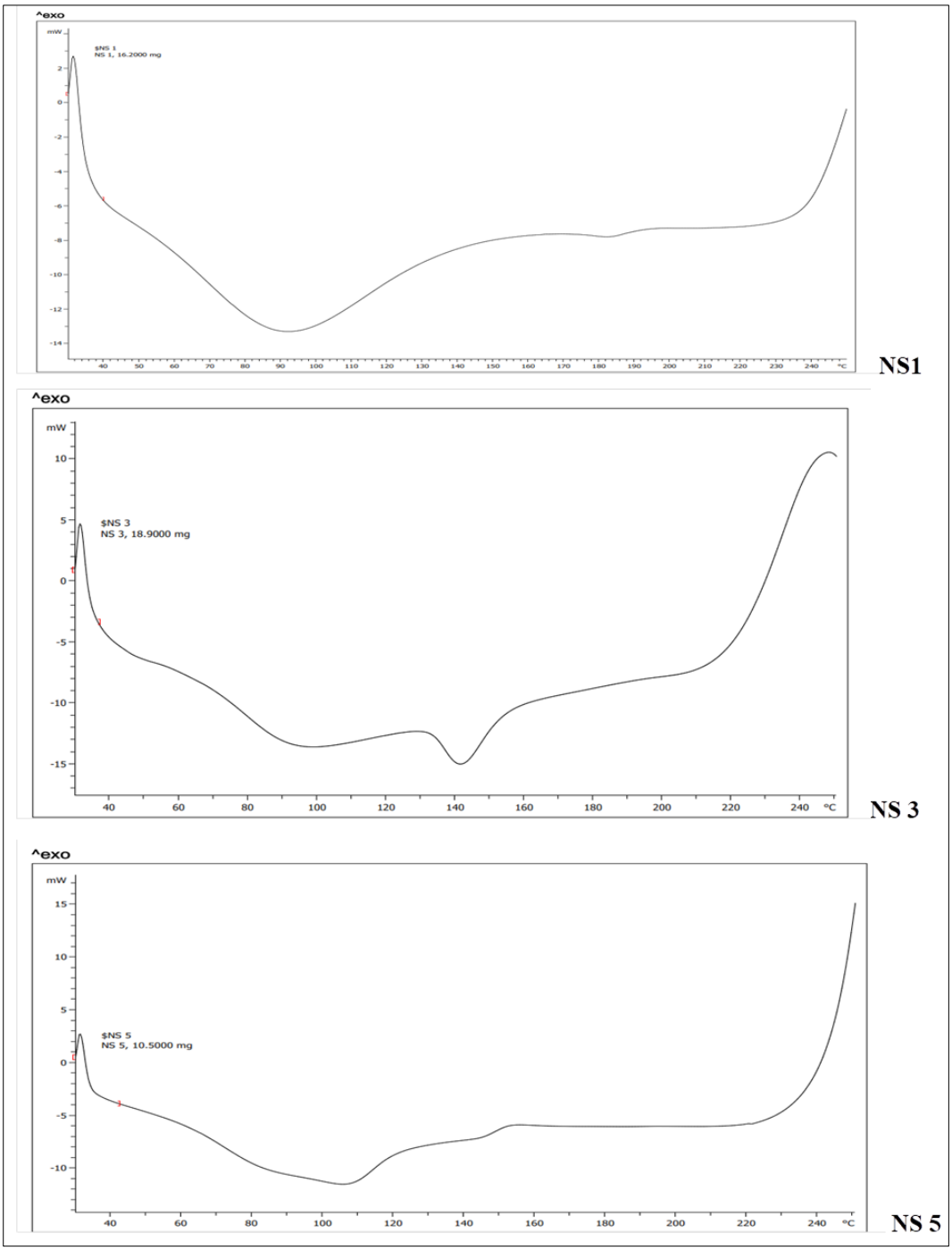

Figure 2 DSC thermograms of formulations

Table 3 Enthalpies and peak exotherms of formulations

Formulation	Peak (°C)	Enthalpy (mJ)	Tg
NS1	91.86	-5578.18	32.52
NS3	142.02	-8843.29	243.97
NS5	221.69	-9233.11	

3.2.4. pH evaluation

The pH evaluation of the formulations is presented in table 4. The results obtained showed a stable pH over a six-month period.

Table 4 Time-based pH evaluation

Batches	pH (± S.E.M) (after formulation)	pH (± S.E.M) (1 month)	pH (± S.E.M) (3 months)	pH (± S.E.M) (6 months)
NS1	5.45 ± 0.00	5.18 ± 0.00	5.52 ± 0.09	5.15 ± 0.04
NS2	5.67 ± 0.00	5.76 ±0.01	5.22 ± 0.01	5.40 ± 0.01
NS3	5.72 ± 0.01	5.68 ± 0.01	5.18 ± 0.02	5.38 ± 0.01
NS4	5.67 ± 0.02	5.68 ± 0.00	5.20 ± 0.04	5.37 ± 0.00
NS5	5.71 ± 0.01	5.70 ± 0.00	5.26 ± 0.01	5.39 ± 0.00

3.2.5. X-ray Diffraction studies

The results of the X-ray diffraction studies for the formulations are presented in Fig. 3.

From the results obtained, it was observed that the formulations had similar diffraction patterns but varying intensities. The differences in intensities could be attributed to the drug:polymer ratio of the formulations.

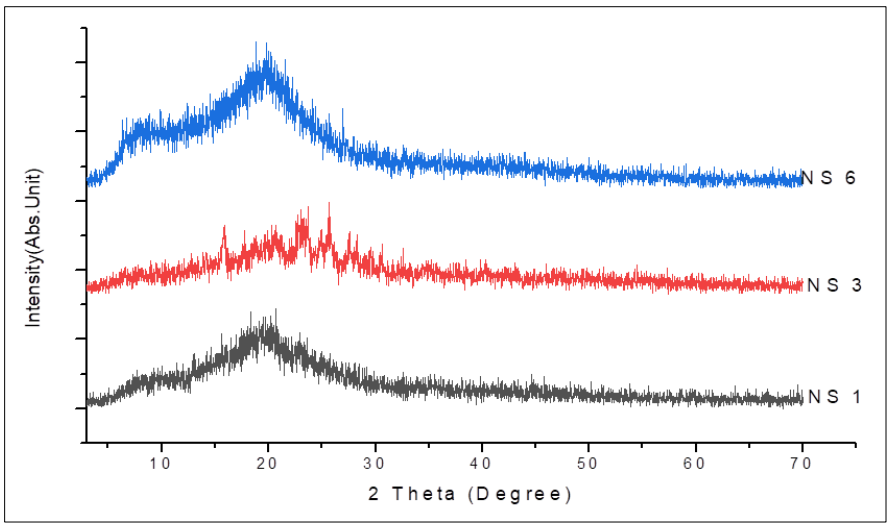

Figure 3 XRD studies

3.2.6. Beer's plot of miconazole in 0.1N HCl

The result of the Beer's plot is presented in figure 4.

3.3. *In vitro* drug release studies

The results of the *in vitro* drug release studies for the formulations are presented in Fig. 5. from the results obtained, it was noticed that there was a sustained release of miconazole nitrate for all the formulations. The pure drug itself, had a gradual increase in release until it peaked at 2 hours, and quickly declined afterwards. However, the various formulations NS1-NS4 increased gradually, but did not release maximally (100 %) before reaching a plateau which they sustained for several hours.

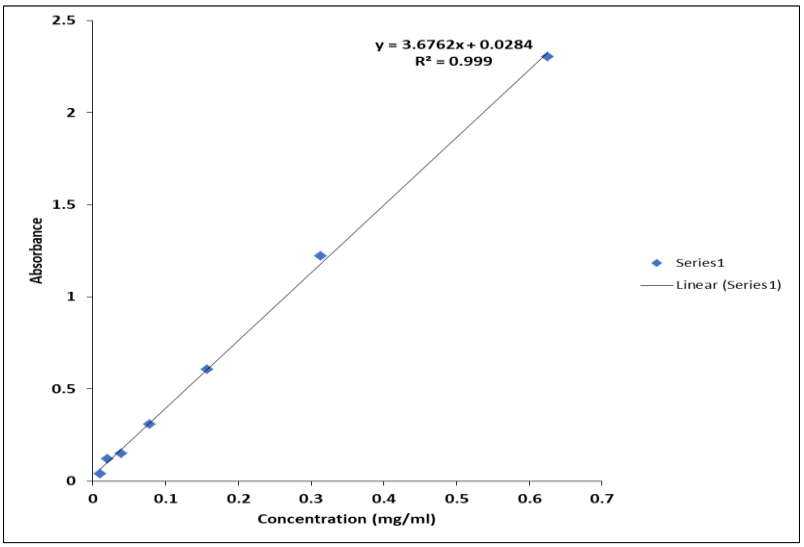

Figure 4 Beer's plot of miconazole in 0.1N HCl

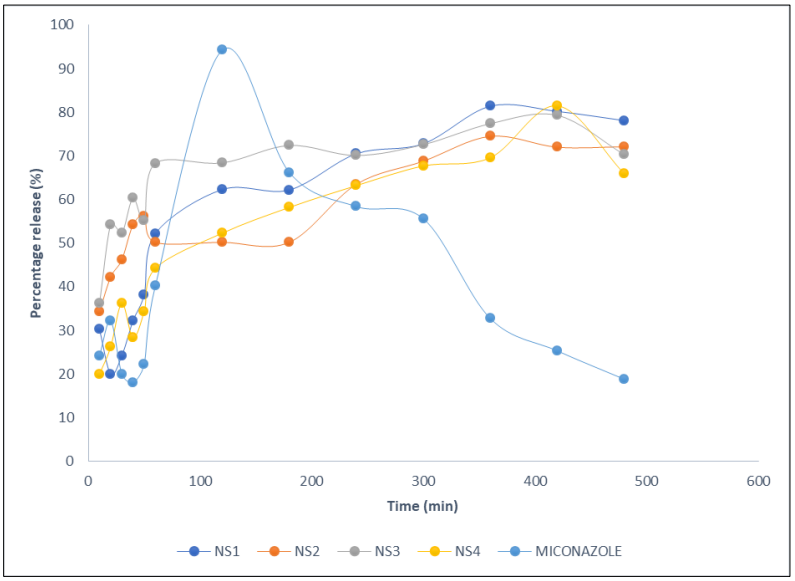

Figure 5 *In vitro* release studies

3.4. Calibration plot of miconazole nitrate against *Candida albicans*

The results of the calibration plot determination for miconazole nitrate against *Candida albicans* to be used in the determination of blood serum levels using the bioassay method is presented in the figure 6.

3.5. Antimycotic evaluation of the formulations

The evaluation of the formulations showed that they all had a concentration dependent effect on the IZDs against *C. albicans*. However, it was observed that the blank formulation also had some activity against *C. albicans* (Table 5).

The MIC and MBC determination as presented in table 6 showed different levels of activity for all the formulations. NS1 had an MIC of 0.31 mg/ml and an MBC of 2.5 mg/ml; NS2 had an MIC of 0.31 mg/ml and an MBC of 2.5 mg/ml; NS3 had no MIC within the range assayed while its MBC was 0.31 mg/ml; NS4 also had no MIC within the range assayed while its MBC was 0.31 mg/ml; NS5 had an MIC of 1.25 mg/ml and an MBC of 2.5 mg/ml; Miconazole nitrate had no MIC or MBC within the range of concentrations examined.

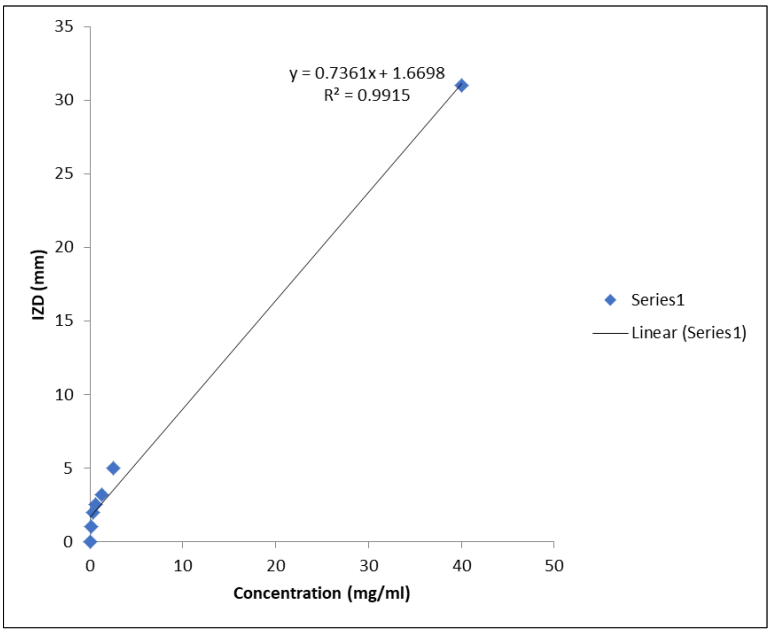

Figure 6 Calibration plot of miconazole nitrate against *Candida albicans*

3.6. *In vivo* release studies of formulations

The *in vivo* release evaluation of the formulations is presented in Figures 7 - 9. All the results obtained for different concentrations of the formulation showed a concentration dependent increase in the drug release into the plasma.

Table 5 Results of antimicrobial activity of formulations against *C. albicans*

Conc.(mg/mL)	Formulation / inhibition zone diameter (mm)					
	NS1	NS2	NS3	NS4	NS5	MZ
5	27.5±2.1	29.5±3.5	31±1.4	28.5±2.1	13.5±0.7	28.5±0.7
2.5	24±0	24±0	30.5±2.1	27±1.4	11.5±0.7	27±1.4
1.25	22.5±0.7	23.5±0.7	25.5±0.7	25±1.4	10.5±2.1	25±1.4
0.63	21±1.4	23±0	23.5±0.7	23±1.4	8±1.4	24±0.7
0.31	21±1.4	22±0	23±1.4	23±1.4	7.5±0.7	22±0

Key: results expressed as Mean ± SEM; NS1-NS5: formulations

Table 6 Minimum inhibitory concentration (MIC) determination and minimum biocidal concentration (MBC)

Conc. (mg/mL)	NS1		NS2		NS3		NS4		NS5		MZ	
2.5	-	-*	-	-*	-	-	-	-	-	-*	-	-
1.25	-	+	-	+	-	-	-	-	-*	+	-	-
0.63	-	+	-	+	-	-	-	-	+	+	-	-
0.31	-*	+	-*	+	-	-*	-	-*	+	+	-	-
0.13	+	+	+	+	-	+	-	+	+	+	-	-

Key: N1-N5: formulations, (-): no growth/inhibition, (+): growth/no inhibition, (*): indicates MIC and MBC respectively

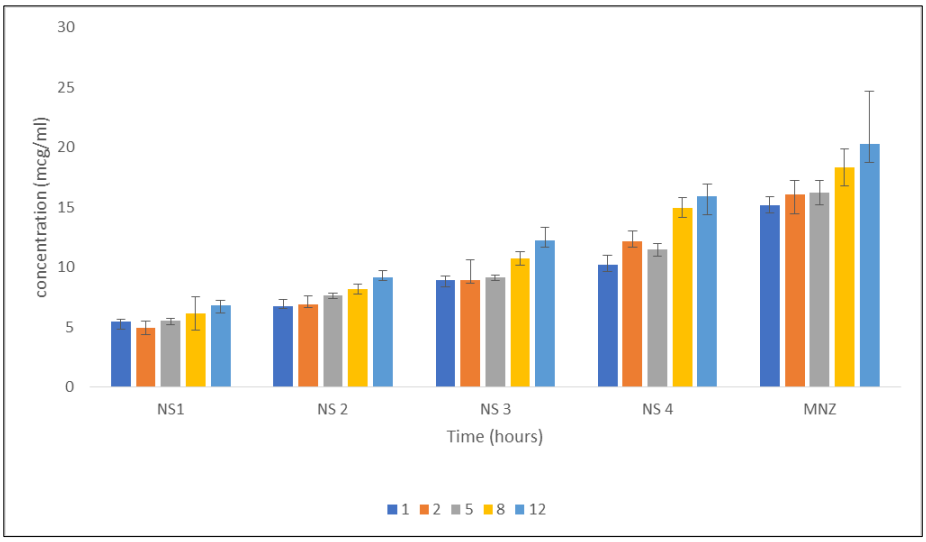

Figure 7 *In vivo* release studies of miconazole nanosponges (3 mg/kg)

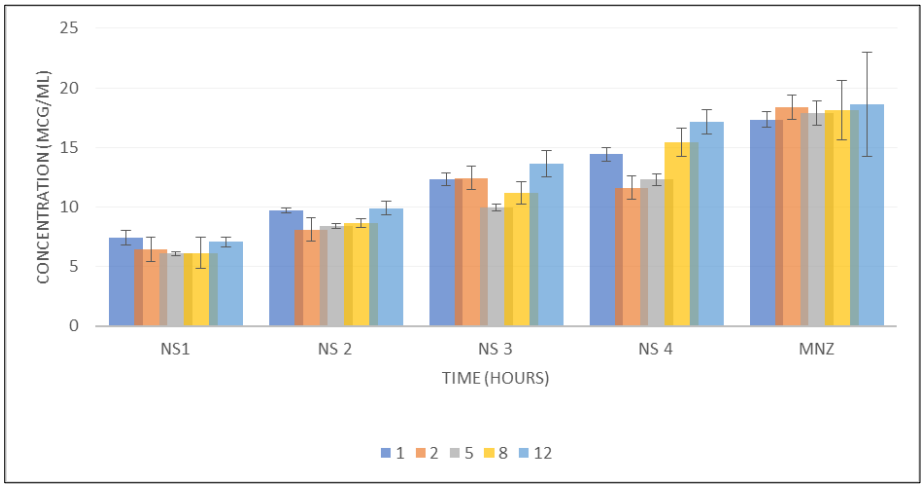

Figure 8 *In vivo* release studies of miconazole nanosponges (4 mg/kg)

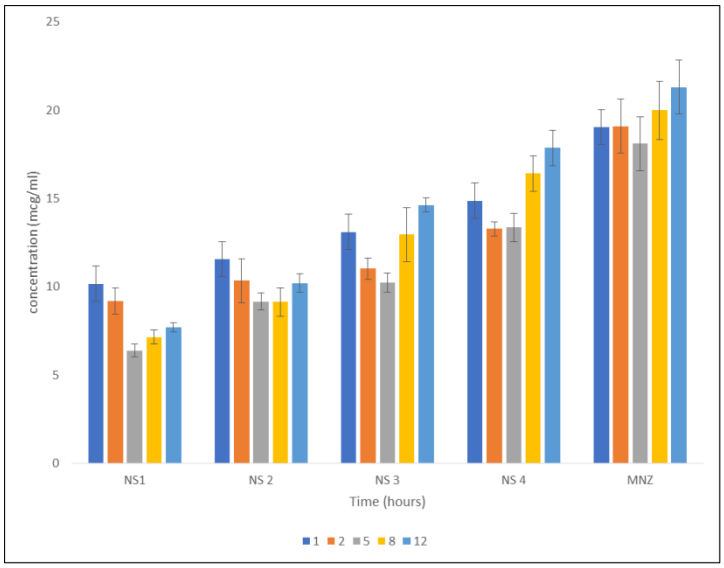

Figure 9 *In vivo* release studies of miconazole nanosponges (5 mg/kg)

4. Discussion

There was no direct relationship between the percentage of drug loading and recovered yield. However, the presence of drug in nanosponges may have increased the percentage yield as evidenced in the difference in percentage yields of loaded and unloaded nanosponges.

Encapsulation efficiency results showed stability of the nanosponges as the values were relatively the same after preparation and six months later. This result shows that the formulations were stable throughout the evaluation period and there was little or no drug degradation or expulsion from the polymer matrix.

Particle size results revealed that higher ratios of miconazole to ethylcellulose led to smaller sized nanosponges with PDI values of combinations lower with higher ratios too. This result is similar to the results obtained by Srivivas and Reddy [20] who observed a decrease in particle size with increase in drug-polymer ratio. They explained that thickness of polymer wall was directly proportional to polymer concentration and inversely proportional to polymer size, with higher polymer concentrations yielding larger-sized particles. Also, Penjuri *et al.* [19] showed optimum yield at a particular concentration above which increasing polymer concentrations led to a decrease in percentage yield and entrapment. Possible reasons could be due to a reduced concentration of polymer in the aqueous phase. In addition, Abbas *et al*., [21] who formulated fluconazole nanosponges with ethylcellulose and Polyvinyl Alcohol also showed decreasing yield concentrations of nanosponges with increasing polymer concentrations.

Morphology determination of the nanosponges revealed densely packed structures with a spongy-appearance and presence of cavities. The level or degree of sponginess varied with drug-polymer concentration. Higher drug-polymer ratios e.g NS3 (0.66:1) had more cavities than NS1 (0.5:1). For the NS5, containing no drug, there were little or no visible cavities in the densely packed sponges. The presence of drugs may have led to an alteration in the polymer structure to accommodate them hence the difference in images obtained dependent on drug concentration [20].

XRD results obtained for NS1, NS3 and NS5 were similar with higher polymer concentrations showing higher intensities thus indicating more crystallinity. Also, the similarities of the patterns depict molecular dispersion of the drug in the polymer matrix without affecting the polymer structure.

The MIC and MBC determinations of the formulations showed the ability of the drug to exert a fungicidal effect. This result is in line with literature evidence of miconazole nitrate possessing fungicidal effect [16]. The results as mentioned earlier, showed lower MBC values for formulations containing higher concentrations of the drug (NS3 and NS4). Nanosponges have pores that permit diffusion of drug out of their membrane. This thus would not impede drug release from the formulations. The activity observed with the blank formulation (NS5) may be as a result of antimycotic activity of some of the excipients used e.g. ethanol.

In vitro drug release showed a sustained release pattern for the miconazole containing nanosponges, while the miconazole pure drug showed an initial burst release before the release declined sharply. This result is consistent with previous reports on the release patterns of drugs from nanosponges. The polymers, owing to their nature, tend to retard drug release causing them to release gradually thereby sustaining the action of the drug [19].

For the *in vivo* evaluation, the Bioassay method was used. It is a cheap, reliable, and convenient method of analyzing plasma drug content especially for antibiotics [22-24]. The results obtained showed that there was a concentration dependent increase in amount of drug found in the plasma with different dose concentrations (3, 4 and 5 mg/kg) exhibiting the same pattern of release. the higher plasma concentrations observed with miconazole nitrate was because of the drug being solubilized in tween 80 prior to administration to the animals. Miconazole absorption is dissolution rate limited. The results obtained also showed a time-dependent increase in concentration of drug in the plasma. this can be attributed to the long half-life of the drug (24 hours) thus allowing it to accumulate in the blood before elimination.

5. Conclusion

Miconazole nitrate nanosponges were successfully formulated and evaluated *in vitro* and *in vivo*. The results obtained showed an ability of the nanosponges to sustain the release of the drug *in vitro*, up till 12 hours. *In vivo* administration of the drug also showed a comparable bioavailability of the formulations with the solubilized drug. Results obtained were dose-dependent with higher doses showing higher blood concentrations. Among the formulations made, NS4 with the highest amount of loaded drug, showed the best release profile and the highest plasma concentration.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

Animal experiments were carried out in accordance with the guidelines of the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka and the National Institute of Health (NIH) guide for care and use of laboratory animals (Pub No: 85-23 Revised 1985).

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