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EFFECT OF SURFACTANTS ON THE FORMULATION OF NOVEL NANO VESICULAR FATTY ACID STRUCTURES OF MICONAZOLE; ENHANCED TOPICAL AND ANTIFUNGAL ACTIVITY

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Abstract

Background: Transcutaneous drug delivery is the most desirable method to improve efficacy and increase patient tolerance. Infections have increased over the past two decades. Superficial skin infections are treatable with conventional herbs while deep root infections cannot be treated due to disruption of the stratum corneum. the skin.

Objective: This study aimed to encapsulate the antifungal miconazole nitrate (MN) in advanced novasomes to improve skin penetration and clinically transform therapeutic improvement. Method : Novasomes with free fatty acid (FFA) as an internalization promoter were prepared by ethanol injection method and novasomes were characterized by percentage entrapment efficiency (EE%), particle size (PS), polydispersity index (PDI) and zeta potential (ZP), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Differential Microscopy Calorimetry (DSC), MIC (Minimum Control Concentration), Agar Diffusion Method have been extensively studied.

Result: The optimized MN7 formulation with 30 mg of lipid components and Span 60 oleic acid at a ratio of 2:1 (w/v) shows EE% = 97.45%, PS = 154 nm, PDI = 0.019, and ZP = ± 14 mV. In addition, MN7 showed greater inhibition of Candida albicans growth compared to MN suspension using the resazurin reduction test, The drug MN7 novasomes had significant results against C.albicans with a maximum zone of inhibition of 23.667±0.667mm. The MIC of MN7 was lower than that of unloaded novasomes (12.5 and 25 mg/mL respectively). The cell capacity remained above 85% which shows that it is non-toxic

Introduction

Topical medication delivery techniques have gained traction, as a result of the increased frequency of chronic dermatological diseases needing compliance and targeted administration [1]. Globally, the prevalence of superficial fungal infections of the skin, nails, and hair has grown. In poor and underdeveloped nations, it is believed that over 40 million individuals have been infected with fungi. Antifungal medicines, both topical and systemic, are used as treatment options. Because of the risks of traditional systemic therapy, such as herbal toxicity, drug-drug combinations, and greater medical costs, fungal agents applied topically are typically recommended [2]. Because the therapeutic efficacy of a medication administered locally is largely determined by its ability to enter and permeate the skin, novel drug delivery techniques that can cross the skin barrier are urgently needed (brick and mortar-like structure). Vesicular systems are one of the most common systems utilised for this purpose [3].

Miconazole nitrate (MN) is a broad-spectrum imidazole antifungal drug that disrupts the cell membrane by blocking the activity of the enzyme lanosterol 14-demethylase, which is involved in the production of ergosterol, the major sterol component of the fungal cell membrane. [4], It has a poor water solubility. Despite being a nitrate salt, it is insoluble in water and has an octanol-water partition coefficient of around 6.25, indicating that it is lipophilic [5]. MN is frequently used topically to treat fungal infections as well as Gram-positive bacilli and cocci infections [6]. The vertical sublayer of the epidermis stratum corneum (SC), which can function as a physical barrier to substances touching the skin, is a key challenge in skincare. To circumvent the absorption restriction given by SC, appropriate carrier systems such as topical administration of different medicines are necessary [7-10]. Previous challenges have been stated for MN skin delivery include, among others, conventional liposomes [11, 12], ethosomes [12], noisomes and microemulsions [13]. These systems, however, have problems with stability and cytotoxicity [14-18]. To get around this problem, MN was encased in novasomes.
Novasome technology is a novel encapsulation technique developed by IGI labs NOVAVAX to address effectiveness and efficiency issues with current drug delivery systems. Novasomes are thought to have a better liposomal or niosomal structure, which is made up of a mixture of cholesterol, free fatty acid (FFA), and monoester of polyoxyethylene fatty acid [19]. Novasomes have a variety of characteristics; as a multi-layered vesicle with a high capacity central core in a small size range, they may deliver a large number of active substances. Several vaccines manufactured as novasomes have been authorized [20, 21].
MN was designed to be incorporated into novasomes as a unique potential nanosystem in this research. Furthermore, the superiority of novasomes over niosomes in topical administration has not yet to be explored. As a result, the objective of this study was to see how effective novasomes are in increasing skin penetration when compared to niosomes using MN as a model medication. To explore the impact of different formulation variables on the characteristics of the prepared novasomes regarding entrapment efficiency percent (EE%), particle size (PS), polydispersity index (PDI), and zeta potential (ZP) were investigated and to choose the optimum formulation. Further, the deformability index (DI) of the optimum novasomes was determined and compared with traditional niosomal formulation to measure the elasticity of the vesicles. Moreover, microbiological efficacy using resozurine dye reduction assay for the optimum novasomes compared to MN suspension was accomplished to determine the inhibition efficacy of MN against candida albicans. Furthermore, in vivo skin deposition tests of the optimum novasomes were performed compared to traditional niosomal formulation and MN suspension.

Material and methods

Miconazole nitrate was kindly provided by CCL pharmaceutical Pvt Ltd. Cholesterol was purchased from Alfa Aaeser Germany. Oleic acid, sodium hydroxide, potassium phosphate monobasic and steric acid were purchased from Dae jung chemical Korea. Span 60, span 80, tween 80 and dimethyl

sulfoxide (DMSO) were purchased from Sigma Aldrich Germany. Ethanol was purchased from Merck Darmstadt United States. All are other chemicals and solvents were of analytical grade and were received without further modification.

Preparation of MN loaded novasomes

The technique of ethanol injection used by Kakkar and Kaur was used to create MN loaded novasomes. [22]. Changes in surface active agent (SAA) type, FFA ratio, SAA to FFA ratio (w/v), and total lipid content were used to create novasomes. In a 62°C water bath, ethyl alcohol was used to dissolve MN (20 mg), SAA, FFA, and cholesterol (30 mg). This was then gently injected into a five-fold bigger vehicle of phosphate-buffered saline (PBS, pH 7.4) that had been magnetically agitated at the same temperature and stirring for the same amount of time. When a sudden turbidity was noticed, novasomes dispersions were produced, and particles size decrease was achieved by swirling for two hours. At 4°C, each of the ready formulations was stored until it was used. [23].

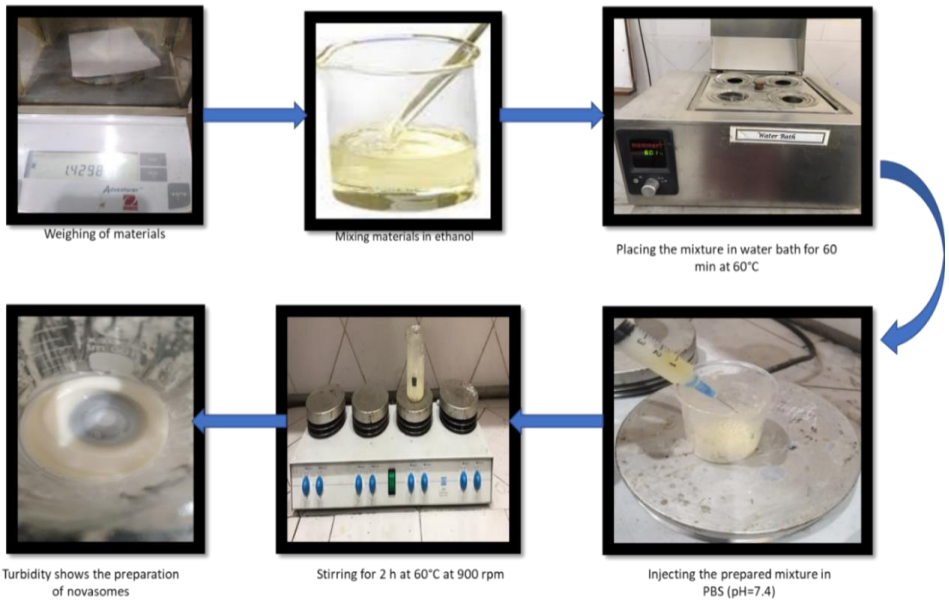


Fig.. Steps of novasomes formation

Table 3.1: Composition of Novasomes formulation

Formulations	Type of SAA	Type of FFA	Ratio of SSA:FFA	Cholesterol (mg)
MN_1	Span 80	Oleic acid	2:1	30
MN_2	Span 80	Oleic acid	1:1	15
MN_3	Span 60	Steric acid	2:1	12.5
MN_4	Span 60	Steric acid	1:1	12.5
MN_5	Tween 80	Oleic acid	2:1	30
MN_6	Tween 80	Oleic acid	1:1	30
MN_7	Span 60	Oleic acid	2:1	30
MN_8	Span 60	Oleic acid	1:1	30
MN_9	Span 80	Steric acid	2:1	30
MN_{10}	Span 80	Steric acid	1:1	30

***In vitro* characterization of MN loaded novasomes**

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to detect the surface morphology of all novasomes, as well as the microstructure of the sample [24].

Particle size analysis
Zeta-sizer 2000 (Malvern Instrument Ltd., UK). was used to measure the particle size of the various formulated MN laden novasomes. Before the measurements, the Novasomes formulations were diluted. Each sample was quantified three times and the average value was calculated.

Zeta potential
The same instrument was used to assess the zeta potential (zeta-sizer). The Formulations were diluted properly before zeta-sizer testing, and the ZP of MN loaded novasomes was determined by monitoring their electrophoretic mobility in an electrical field. The results were chosen based on the average of the three observations.

Polydispersity index (PDI)
Photon correlation spectroscopy was used to estimate the PDI in 10 mm diameter cells at 25°C using a Malvern Zeta-sizer Nano (Malvern Instruments, Malvern, Worcestershire, UK) at an angle of 90o. To achieve a sufficient scattering intensity for PDI measurement, one millilitre of each formulation was diluted with 15 mL distilled water. The results were calculated as averages.

Entrapment Efficiency
The percentage of MN EE in novasomes was calculated using an indirect assessment of MN free amount (un-entrapped) [25]. 1 millilitre of each novasomes formulation was centrifuged at 4°C for one hour at 20000 rpm in a cooling centrifuge. After diluting the clear supernatant, the MN content was determined using a UV/VIS spectrophotometer set to max = 422 nm. This equation was used to determine the MN EE percent:

$$EE(\%) = \frac{Total\ amount\ of\ MN - Unentrapped\ MN}{Total\ amount\ of\ MN} \times 100$$

Fourier transform infrared spectroscopy (FTIR)
Using an attenuated total reflectance-FTIR spectrophotometer, the FTIR spectra of MN, oleic acid, span 60, cholesterol, drug unloaded novasomes, and drug loaded novasomes (MN7) were determined. It's used to look at how excipients and drugs interact in the chain, and 20 scans were taken for each spectrum between 4000 and 800 cm-1 wave number, with a resolution of 10 cm-1.

Differential scanning calorimetry (DSC)
Novasomes were thermally characterised using a differential scanning calorimeter to determine the thermal stability of MN loaded and unloaded novasomes.

Thermogravimetric analysis (TGA)
By measuring the weight change that occurs when the novasomes are heated at a constant rate, thermogravimetric analysis was used to determine the thermal stability of novasomes and their proportion of volatile components.

Drug release
The drug release from various MN loaded novasomes and MN aqueous solution was studied using the USP Dissolution test equipment II (paddle apparatus). The cellulose membrane dialysis technique was used, with a consistent volume of MN loaded novasomes (equal to 2 mg MN) put in a glass cylinder (2.5 cm in diameter and 7.5 cm in length). From one end, each tube was securely wrapped with a cellulose membrane that had been presoaked in PBS (pH = 7.4) and connected to the shaft instead of baskets. The shafts were then dropped into beakers containing 50 mL of PBS (pH = 7.4) as a dissolving media until the cylinder's surface came into contact with the dissolution medium's surface. These beakers were water jacketed inside the dissolving apparatus's vessels to maintain a

consistent temperature during the experiment. At 37 degrees Celsius, the cylinders were set to revolve at a constant pace (100 rpm). During the experiment, the containers were covered to prevent the dissolving media from evaporating. 5 mL samples were taken and replenished with new medium at specified time intervals (0.5, 1, 2, 3, 4, 5, 6, 7,8, 24 h) to maintain sink conditions, and spectrophotometrically measured at max 422 nm using PBS (pH = 7.4) as a blank. The results were calculated as averages (n = 3± SD) [26].

Figure 3.3 Drug release study by paddle apparatus

Optimization of MN loaded novasomes

The main objective of desirability is to predict the optimum levels for the factors under investigation and to assist in selecting the formula of choice. The criteria for the optimum formula selection was accomplished based on the smallest (PS and PDI) and the highest (EE% and ZP (as absolute value)). The solution with desirability outcome close to one was chosen. To confirm the performance of this model, the chosen formula was formulated, evaluated, and compared in relation to the predicted outcomes.

The impact of FFA addition on the physico-chemical characteristics of the vesicular system was done by comparing the optimum novasomes to the suspension of drug in 5% CMC solution prepared by the method previously mentioned with the same amounts of the optimum novasomes but without FFA, then both of them were evaluated with respect to the mentioned responses (Table 3.1).

Effect of storage on the optimized novasomes

For 90 days, the optimum novasomes formulation was stored at 4°C and 25°C. On the day (0, 45, and 90), samples were drawn from each formulation. The effect of storage was assessed by comparing the initial outcomes with the post storage findings regarding EE%, PS, PDI, and ZP [27]. Statistical analysis was computed by Student's *t*-test utilizing SPSS® program 22.0. The difference at $P \leq 0.05$ was considered significant.

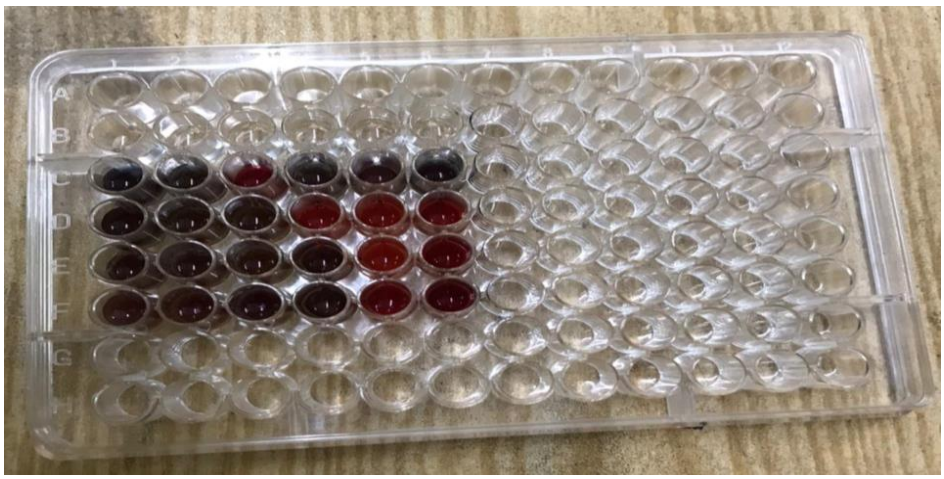
Microbial efficacy of MN loaded novasomes for the treatment of candida albicans

Minimuminhibitory concentration (MIC) assay via resazurin reduction technique

The MIC value, which was calculated using a micro-well dilution technique, reflects the lowest doses that fully inhibit the growth of Candida albicans (ATCC). The inoculums were primed and maintained at a concentration of 106 CFU/mL. In a ninety-six-well plate, DMSO was used to dilute the optimal novasomes loaded with miconazole nitrate MN7, novasomes made without the drug, and miconazole nitrate drug [28]. Every well of the microplate was filled with 40 µL of brain heart infusion (the growth medium), 10 µL inoculum, and 50 µL diluted formulae. DMSO was used as a negative control, then the plates were incubated at 37°C for 24 h, followed by 10 µL of resazurin dye addition, then

incubated in the dark for an hour at 37˚C, any change in color as a result of dye was calculated at 492 nm *via* the microtiter plate reader (Tecan Sunrise absorbance reader, UK) [29]. The percentage of inhibition was obtained as follows:

$$The\ percentage\ of\ inhibition\ = 1 - \left(\frac{mean\ of\ test\ wells}{mean\ of\ control\ wells}\right) x\ 100$$



FigureMicro-well dilution technique

Agar well diffusion method

Agar well diffusion method was used to determine the antifungal activity of novasomes loaded miconazole nitrate (MN_7). Macconkey agar plate surface was inoculated by Candida albican (ATCC#) by streak plate method. A hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and a volume (20 µL) of optimum novasomes (MN_7), novasomes with unloaded drug and miconazole nitrate drug poured into each well. Then, agar plates were incubated for 48hrs [30].

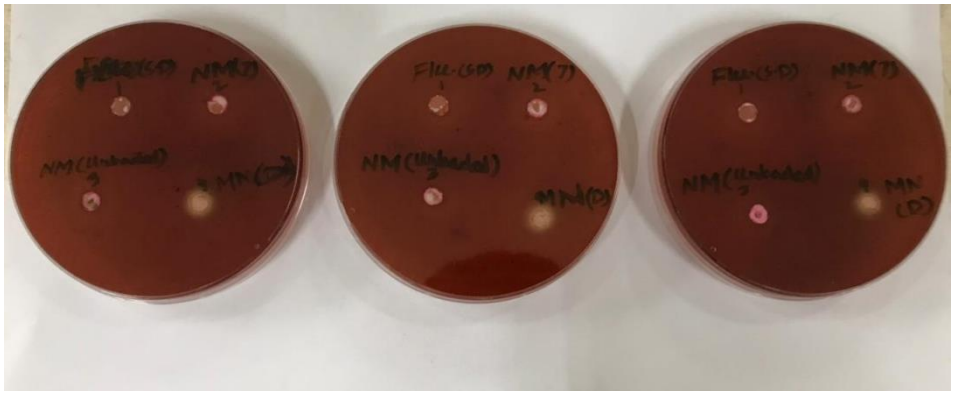


Figure Streak plate method

Cell viability studies

The cell viability tests of formulation employing Caco-2 cells were evaluated using the Resazurin assay. Caco-2 cells (25103/well) were seeded on 24-well plates using minimal necessary medium (MEM) with 5% heat inactivated foetal bovine serum (FBS). The cells were incubated at 37 degrees Celsius in a humidified environment containing 5% CO2. After 14 days, the monolayer of Caco-2 cells was verified. The cells were then placed in MEM without FBS for 24 hours before being washed with 25 mM HEPES buffered saline (HBS) at pH 7.4. For 6 and 24 hours, the fresh MEM medium was replaced with or without MEM dispersion of the formulation. 250 µl of 2.2 mM resazurin solution was added to each well after washing with new HBS buffer, and incubated for 3 h at 37 0C. The fluorescence was observed in the dark using a microplate reader (TECAN Spark®, Tecan Trading

AG, Zurich, Switzerland) after the 24-well plate was gently shaken for 5 minutes. MEM and 1% Triton X-100 were used as positive and negative controls, with each condition being evaluated in three different ways. The findings are based on three separate trials. The percentage of cell viability was calculated using the calculation below.

$$Cell\ viability\ (\%) = \frac{Average\ absorbance\ value\ of\ each\ sample}{Average\ absorbance\ value\ of\ low\ control} \cdot 100$$

Result and discussion

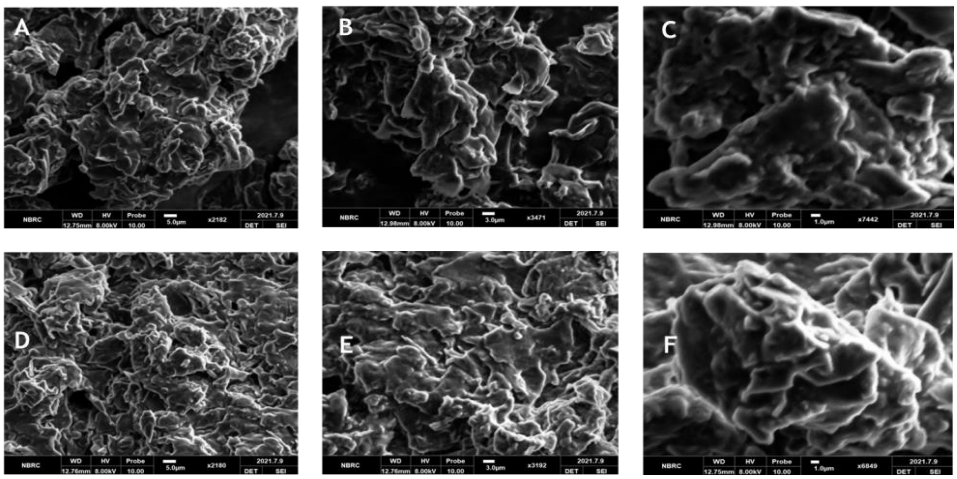
Entrapment Efficiency (EE%)

The entrapment efficiency of all the formulations ranged from 49.02% to 97.45%. Formulation MN_7 showed the highest entrapment efficiency while MN_1 showed the least entrapment efficiency value 49.02% as shown in Table 4.1. It can be seen that the formulations with tween 80 as SAAs and formulations in which steric acid was used with combinations of any surfactant showed less EE% but the formulation with oleic acid and span 60 has given relatively higher EE% and MN_7 showing the EE% of 97.45% indicated that MN_7 formulation has highest capability to entrap larger amount of drug. The same study performed with same results by Mosallam et al; in the preparation of novasomes for improving the topical delivery of terconazole.

Formulation code	EE%
MN_1	49.02
MN_2	78.45
MN_3	80.98
MN_4	67.49
MN_5	61.45
MN_6	90.19
MN_7	97.45
MN_8	92.31
MN_9	82.34
MN_{10}	91.56

Scanning electron microscopy (SEM)

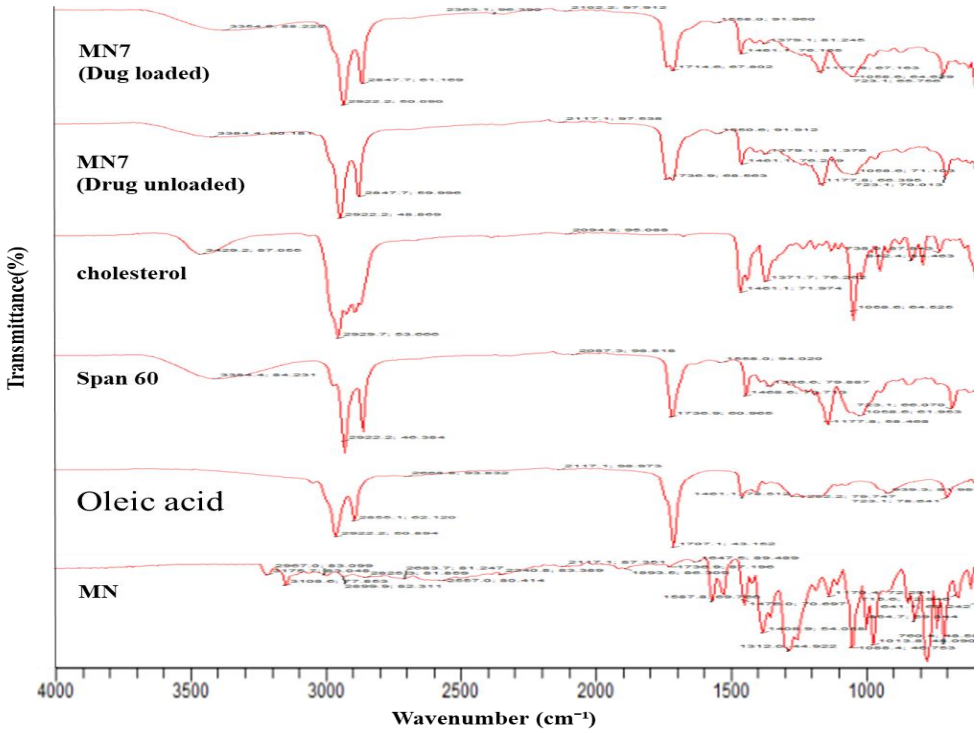
The SEM of unloaded formulations of novasomes showed creamy appearance due to utilization of cholesterol and oleic acid in the preparation as shown in fig (A,B,C)The drug loaded novasomes also showed creamy appearance but showed different surface appearance indicated the loading of drugs as shown in fig. (D,E,F)



Fig…… SEM images of drug loaded novasomes MN_7 (A, B,C) and drug unloaded novasomes (D, E, F) at different resolution.

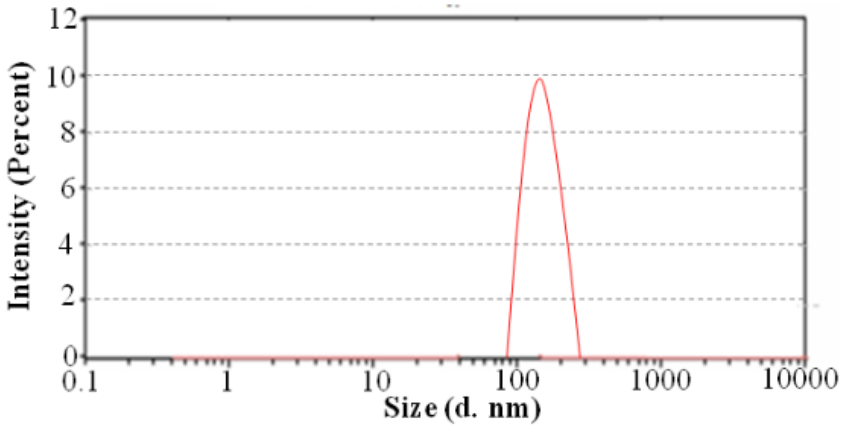
Fourier transforms infrared spectroscopy (FTIR)

The Miconazole nitrate showed stretching of N-H group of benzene ring at 2899 cm^{-1} and C-C stretching of dichlorobenzene group at 1587 cm^{-1} and 1476 cm^{-1} as shown in figure. The stretching of -OH group of cholesterol was observed at 3429 cm^{-1}. Asymmetric stretching of C-H bond of cholesterol was observed at 2929 cm^{-1}. The oleic acid showed asymmetric and symmetric stretching of CH_2 group at 2922 cm^{-1} and 2855 cm^{-1} as shown in figure. The SPAN 60 showed O-H stretches at 3384 cm^{-1}, aliphatic C-H stretching at 2922 cm^{-1} and stretching of CH3 group at 1468 cm^{-1}. The peak of cyclic 5- membered ring in Span 60 was observed at 1736 cm^{-1}.



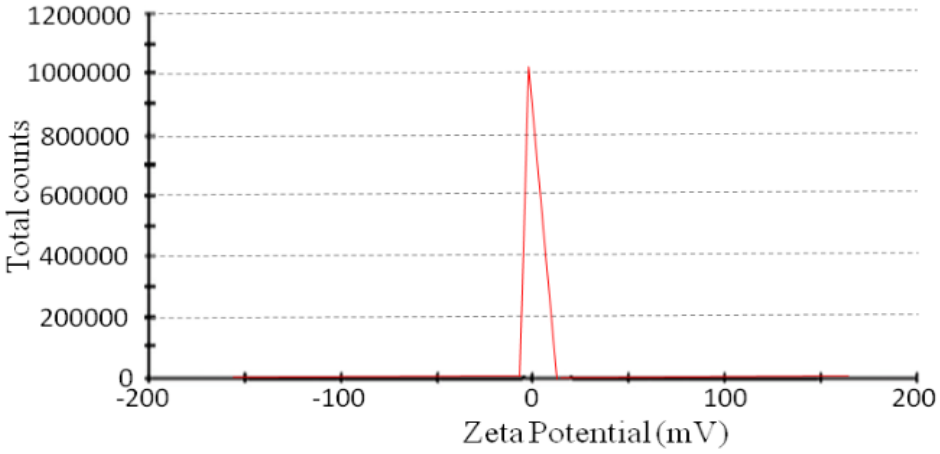
Particle size

The figure showed the particle size distribution of novasomes of MN_7 formulation. The average particle diameter of MN_7 formulation was 154 nm.



Zeta potential

The stability of formulation depends on zeta potential as it opposes aggregation. Zeta potential of prepared formulation MN_7 was ranged from -9 to +21mV. The value of zeta potential ±30 mV was enough for the system's stability.



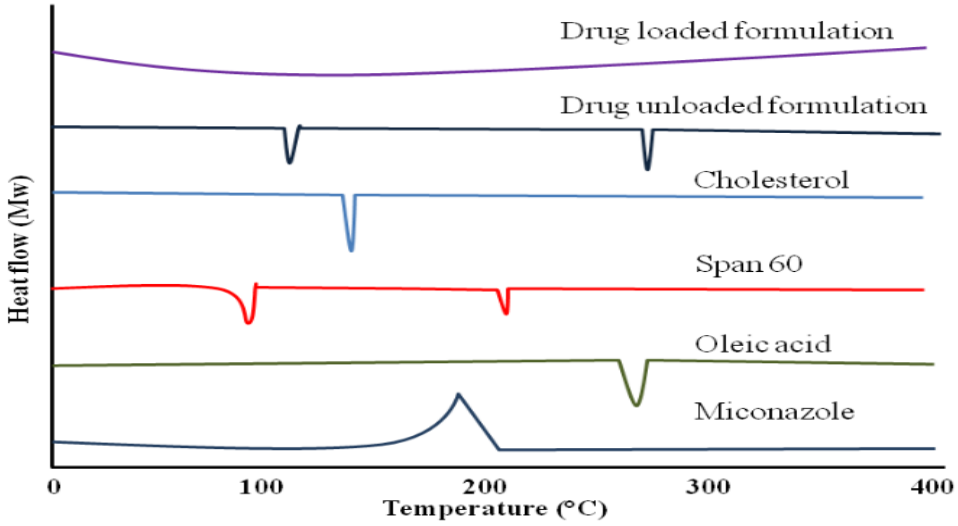
Polydispersity index (PDI)

The polydispersity index of the prepared formulation was less than 0.019.

Formulation code	PDI
MN_1	0.341
MN_2	0.192
MN_3	0.367
MN_4	0.278
MN_5	0.034
MN_6	0.178
MN_7	0.019
MN_8	0.369
MN_9	0.176
MN_{10}	0.267

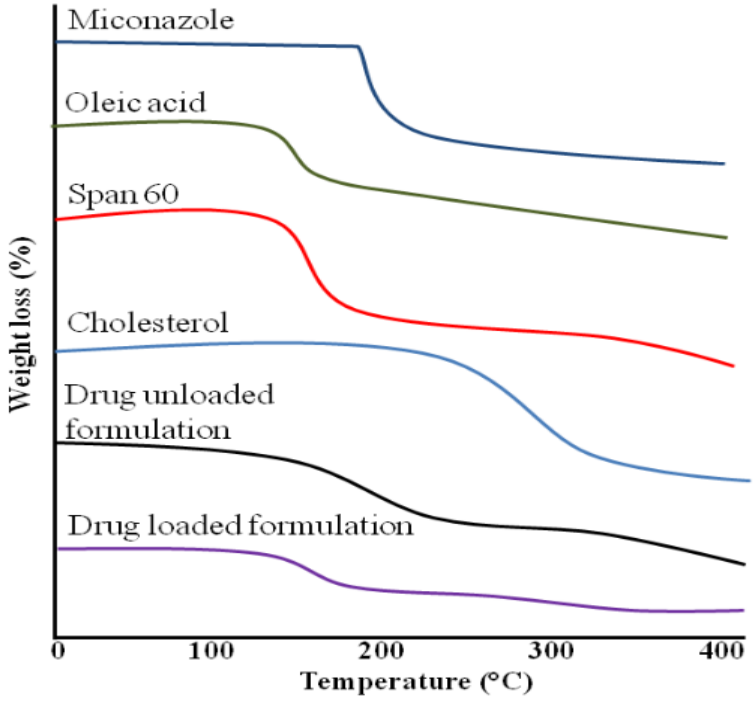
Differential scanning analysis (DSC)

The MN showed an exothermic peak at 190 ℃. Oleic acid showed endothermic peak 280℃. Span 60 showed two endothermic peaks at 100℃ and 210℃. Cholesterol showed endothermic peak at 140℃. Drug unloaded formulation showed two endothermic peaks at 110℃ and 290℃. Drug loaded formulation MN_7 showed no peak which indicates that drug and excipients uniformly distributed and our prepared formulation was stable.



Thermogravimetric analysis (TGA)

Thermogravimetric analysis of MN showed weight loss at 200℃ and a rapid weight loss was observed between 200℃ to 300℃ as shown in figure. The weight loss of oleic acid was observed at 140℃ as shown in figure. A rapid weight loss of oleic acid was observed between 162℃ to 190℃. The weight loss of span 60 was started at 162℃ and continues till 400℃. The weight loss of cholesterol was started at 240℃ as shown in figure. The TGA of drug unloaded and loaded formulation showed weight loss started at 154℃ which showed thermal stability of formulation.

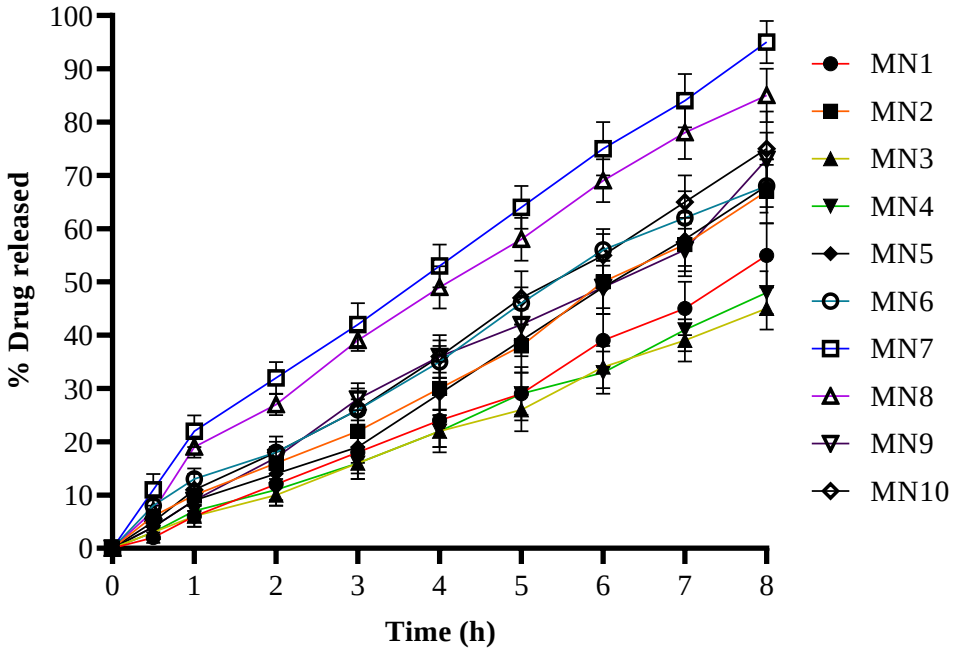


In vitro Drug release

The release pattern of different MN loaded novasomes as well as drug solution was illustrated in figure. The % MN released from solution was very high, about 97% MN released in first 4 hr. On the other hand the prepared MN loaded novasomes succeeded to retard the drug release compared with the drug solution. The release of MN from all novasomes was biphasic, relatively initial showing a fast release phase followed by a slower phase. This may be attributed to rapid portioning of surface adsorbed MN with hydrophilic nature into the releases medium accounting for burst effect observed. This indicates that MN loaded novasomes are expected to show a rapid onset of action due to its initial fast release phase followed by sustained drug delivery due to its slower phase.

The maximum release of MN1 and MN2 in 8hrs was 55% and 67% respectively. The MN3 showed maximum release at 8 hrs was 45% as shown in grapgh.MN4 and MN5 showed the maximum release at 8 hrs was 48% and 58% respectively as shown in graph. Maximum release of MN6 and MN7 at 8 hrs was 68% and 95 % respectively. MN8, MN9 and MN10 showed maximum release of 85%, 73% and 75% respectively at 8 hrs.

Formulations containing span 60 i.e; MN3, MN4, MN7 and MN8 showed better release than the formulations containing span 80 and tween 80 i.e MN1, MN2, MN5, MN6, MN9 and MN10. Similarly formulations with oleic acid i.e; MN1, MN5, MN6, MN7, and MN8 showed better release than the formulations containing stearic acid.



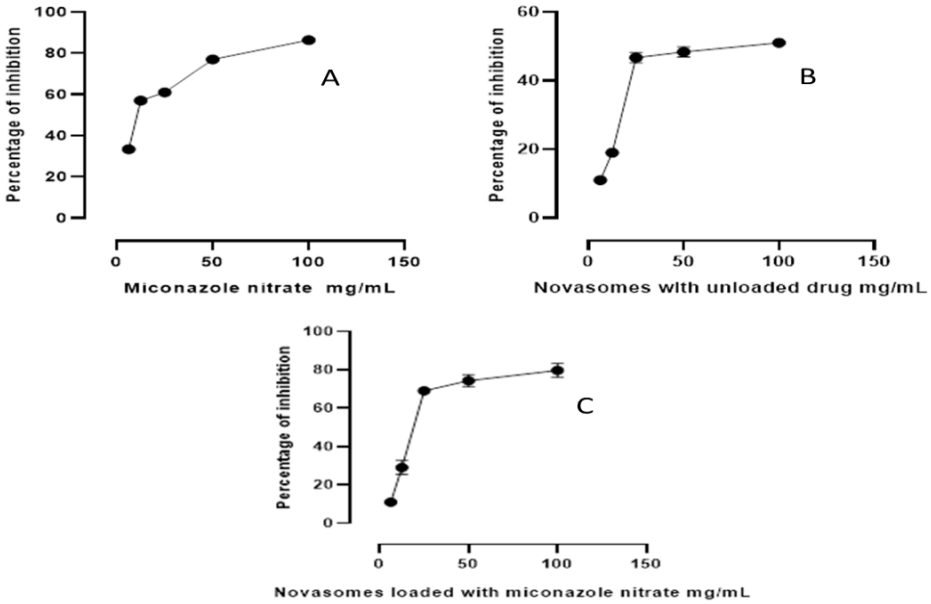
Release kinetics

The value of R^2 for zero order ranged from 0.9903 to 0.9985. The value of R^2 for first order ranged from 0.9443 to 0.9840. Indicated the developed novasomes showed the zero order release kinetics. The value of R^2 for Higuchi Model ranged from 0.8216 to 0.9403 while the value of R^2 for Hixon-Crowell Model ranged from 0.9611 to 0.9931 as shown in table….. As indicated in table the value of n ranged from 0.778 to 1.176 indicated that release mechanism for the formulation was non-fickian. Reffrences????

Formulation	Zero order		First order		Higuchi		Korsmeyer peppas			Hixon-crowell	
	R^2	k_0	R^2	k_1	R^2	k_H	R^2	N	k_{KP}	R^2	k_{HC}
MN_1	0.9907	6.438	0.9635	0.081	0.8286	14.755	0.9966	1.149	4.920	0.9740	0.025
MN_2	0.9933	8.108	0.9601	0.110	0.8536	18.705	0.9944	1.063	7.233	0.9739	0.033
MN_3	0.9974	5.535	0.9840	0.067	0.8574	12.763	0.9981	1.049	5.067	0.9899	0.021
MN_4	0.9954	5.780	0.9787	0.071	0.8537	13.324	0.9966	1.063	5.156	0.9857	0.022
MN_5	0.9882	8.083	0.9443	0.108	0.8216	18.508	0.9962	1.176	5.878	0.9611	0.033
MN_6	0.9904	8.886	0.9777	0.126	0.8956	20.700	0.9934	0.911	10.428	0.9874	0.038
MN_7	0.9748	12.472	0.9697	0.221	0.9403	29.357	0.9978	0.778	18.532	0.9850	0.062
MN_8	0.9805	11.356	0.9818	0.188	0.9308	26.662	0.9978	0.806	16.082	0.9931	0.054
MN_9	0.9903	8.583	0.9656	0.119	0.8668	19.861	0.9903	0.995	8.662	0.9773	0.036
MN_{10}	0.9985	9.261	0.9639	0.133	0.8661	21.396	0.9986	1.022	8.905	0.9797	0.039

Minimum inhibitory concentration (MIC) assay via resazurin reduction technique

Candida albicans was selected for the *in vitro* antifungal test, as it is the prime reason for the superficial and disseminated fungal infections in humans. Resazurin reduction assay offers an advantage over the agar diffusion technique due to its ability to quantify the activity of *C .albican.* It measures the cell activity through the quantified colorimetric estimation of the intracellular formazan compound which is released upon the reduction of resazurin. In vitro antifungal activity of MN suspension, novasomes unloaded drug, and MN_7 is shown in figures.

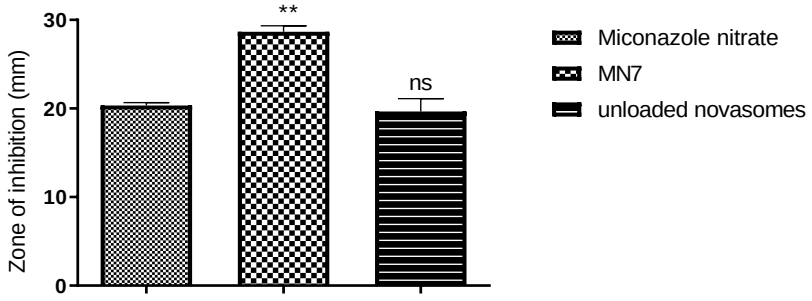


Figure…. Microbial efficacy of (A) MN suspension, (B) novasomes unloaded drug and (C) MN_7 for the management of Candida albican infection. Data are presented as mean ± SD (n = 3).

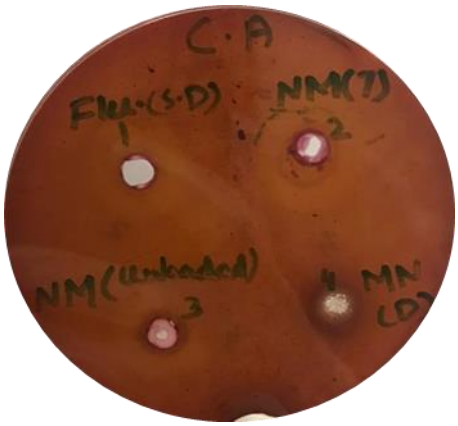
The MIC for MN_7 was lower than that of the unloaded novasomes (12.5 and 25 mg/mL respectively). The lower the MIC value, the higher the efficacy of the formulation. It is revealed that unloaded novasomes also exhibited remarkable antifungal activity with 48% percent fungal growth inhibition at 25 mg/ml dose. This could be due to oleic acid which contains fixed bend C=C bonds. Hence, it occupies a wider cross section when enters into the fungal membrane. The presence of oleic acid in formulation enhanced the permeation and mobility of formulation into fungal membrane resulting in higher fungicidal action. Therefore, it is noted that increased oxidative stress arising from the inclusion of polyunsaturated lipids in the membrane will lead to the antifungal action of FFA. M N_7 accomplished lower MIC in comparison to MN suspension which could be attributed to the high discharge and eventual diffusion of MN from MN_7 with synergistic antifungal action of FFA compared to MN suspension.

Agar well diffusion method

It is manifested that MN7 produced maximum zone of inhibition compared to unloaded novasomes and Miconazole nitrate (23.667±0.667, 19.667±1.453 and 22.5±0.33 mm). Whereas drug unloaded novasomes also showed remarkable antifungal activity non-significant to Miconazole nitrate respectively. In antifungal analysis just medicated novasomes MN_7 had valuable results against C.albicans with maximum zone of inhibition.



Fig…..Values are expressed as mean ± SEM, (*n= 5*), ns (non-significant) compared to Miconazole nitrate suspension.

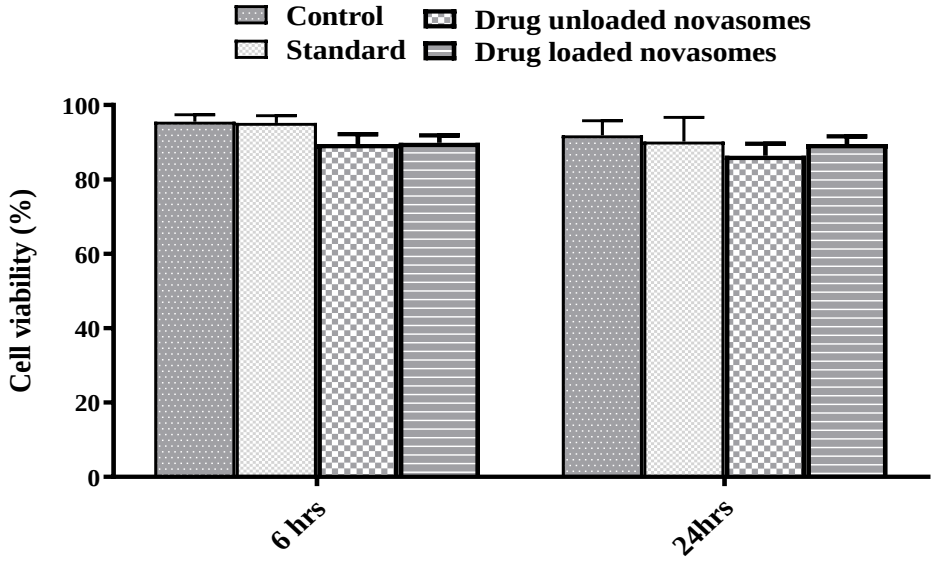


Fig…. Antifungal activity of MN_7, unloaded novasomes and Miconazole nitrate by agar well diffusion method.

Cell viability studies

Cell viability studies did not reveal a significant difference among the different formulations. The viability of cells remained above 85% showing that the formulation is non-toxic and safe. The viability of caco-2 cells (colon cancer cells) treated with novasomes remained close to a 100% when compared to the viability if the cells treated with MEM medium only.

Cells that have been treated with the formulation showed the viability ranging around 90% after 6hrs and around 85 after 24 hrs. the difference between 6 and 24 hrs was not significant as shown in the fog. The results indicate that novasomes dosage forms have a slightly different toxicity profile compared to control nevertheless it could be assume that the developed novasomes are save to use since the toxicity profile does not differ significantly from the control.



CONCLUSION

Miconazole nitrate novasomes were effectively produced utilising span 60 as SAA and oleic acid as FFA in a 2:1 (w/v) ration with 30 mg of cholesterol. The novasomes were made utilising the injection method, and characterizations were performed in order to find the optimum novasome formulation. The greatest EE percent of MN_7 was discovered. The MN_7 formulation was likewise the best in terms of particle size and zeta potential. Miconazole nitrate was found to be compatible with the SAA and FFA, as well as cholesterol, according to the FTIR. SAA and FFA have thermal compatibility,

according to DSC and TGA. MIC assays and zone of inhibition investigations using the agar-well method revealed that the formulation has a good antifungal impact, while cell viability tests revealed that the novasomes formulation is non-toxic and safe to use. As a result, novasomes may be described as a promising and effective method for encapsulating drugs and enhancing penetration.

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