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Formulation and Evaluation of Oxiconazole Nitrate Niosomal Gel for Transungual Delivery

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Original Research Article

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Abstract: Poor response of fungal nail infection to topical treatment with antimycosis is probably related to poor drug permeation into the infected nail. Niosomes are the nano carriers which play an increasingly important role in drug delivery as they can reduce toxicity and modify pharmacokinetics and bio availability. Oxiconazole nitrate is a potent antifungal drug used in the treatment of fungal infections. The aim was to develop Oxiconazole nitrate Noisome using thin film hydration method and optimized for molar quantities of span 60 and cholesterol to impart desirable characteristics. And the formulation was evaluated for entrapment efficiency and invitro drug release. The entrapment efficiency was found in the range of 76.05-94.64%and invitro drug release in the range of 49.03-67.26%. Oxiconazole Nitrate Noisome formulated with span 60 and cholesterol in the ratio of 1.5:0.2 were found to be promising and were incoparated into 1% carbapol gel. The formulated gel was evaluated for various physicochemical parameters and antifungal activity. The invitro drug release study was carried out using phosphate buffer saline pH 7.4 and was found to be 67.95% in 6 hours. Gel formulation containing Noisome loaded with oxiconazole showed prolonged action than the non niosomal form and it can be developed successfully to improve antifungal activity.

Keywords: Oxiconazole, Noisome, transungual delivery, in vitro drug release study, anti fungal activity.

INTRODUCTION

The most common route of administration is oral drug delivery system, but it have significant disadvantage in case of the drug which shows a high hepatotoxicity. Especially in case of the anti-fungal drug, In order to overcome this limitation and for better action most anti-fungal drugs can be formulated in form of topical formulations. Every medical condition demands an accurate and appropriate treatment. As a matter of fact, the thought of resolving the patient's disease with the least harm done to patient's health is said to be basic goal of any therapy [1].

Topical versions of skin and nail diseases is desirable in terms of patient acceptability and reduction if side effects associated with systemic drug delivery. This is particularly the case for nail diseases as they are frequently difficult to cure and require long periods of treatment. The nail plate is a highly keratinized tissue, which is characterised by low permeability to diffusing substance.

The nail diseases are widely spread in the population, particularly among elderly and immune-compromised patients. Although the architecture and composition of nail plate severely limits penetration of drugs and in addition to that only a fraction of topical drug penetrates across the nail, oral therapies are accompanied by systemic side effects and the drug interations. For the successful treatment of nail disease the applied active drug must permeate through the dense keratinized nail plate and reach deeper layers, the nail bed and the nail matrix [2].

Horny structure of nail plate is responsible for penetration of drug across it. As it is hard enough the penetration becomes difficult, only a fraction of topical drug penetrates across it. Hence the effective therapeutic concentration is not achieved. The nail plate may appear abnormal as a result variety of diseases occurs. These diseases can be cured by achieving desired therapeutic concentration of drug by nail drug delivery system.

The success of local topical therapy for onychomycosis depends on the achievement of effective chemical concentrations into/through the human nail plate; therefore, a suitable anti-fungal drug

must be coupled with an appropriate delivery method. The method should maximise the effect of the active principle by aiding its diffusion into the nail bed to levels exceeding the Minimum Inhibitory Concentration (MIC) against local infection by dermatophytes. Thus, a suitable carrier may be needed to enhance drug penetration through the nail barrier. Dermatologists and Podiatrists have long used mechanical methods of enhancing nail penetration, including nail abrasion and nail avulsion, but these methods have varying results in addition to being invasive and potentially painful. Thus, current research focuses on less invasive chemical and physical modes of nail penetration enhancement [3].

Oxiconazole nitrate is a potent anti-fungal drug, used in the treatment of fungal infection. Oxiconazole nitrate has a broad-fungicidal or fungistatic activity against a number of pathogenic fungi including candida albicans. Its antifungal activity is due to the inhibition of the ergosterol biosynthesis, which is critical for cellular membrane integrity. It acts to destabilize the fungal cytochrome P450 51 enzyme (also known as Lanosterol 14-alpha demethylase) [4]. This is vital in the cell membrane structure of the fungus. Its inhibition leads to cell lysis. Oxiconazole has also been shown inhibition of DNA synthesis and suppresses intracellular concentrations of ATP. Like other imidazole antifungals [5].

Among several nanovesicular carriers, niosome is selected here as a carrier of choice owing to its dominance over conventional liposomes with respect to stability and cost-effectiveness. Niosome contains several concentric bilayer membrane mainly composed of nonionic surfactants and cholesterol enclosing aqueous phase in the core. Niosomes are known to improve the solubility, bioavailability, and stability of some poorly soluble drugs along with an ability to provide sustained release for prolonged drug action [6].

MATERIALS

Oxiconazole nitrate was purchased from Yarrow chem. Pvt Ltd Mumbai. Span 60 and Cholesterol were purchased from Chemdyes, Corporation, Rajcot. Carbapol 934 was purchased from Yarrow chem Pvt Ltd., Mumbai. Disodium hydrogen orthophosphate and Potassium dihydrogen orthophosphate were purchased from SD Fine Chemicals Ltd., Mumbai. All other materials were of analytical grade.

METHOD

Niosomes were prepared by a thin film hydration method using a lipid mixture consisting of surfactant span 60 and Cholesterol, at different specified ratios as given in Table-1. Surfactant, Cholesterol and drug were dissolved in 5 ml Methanol and 5ml of chloroform. The lipid mixture was then transferred to a 100 ml round bottom flask, and the solvent was evaporated under reduced pressure at a temperature of 55-65°C, using a rotary flash evaporator until the formation of a thin lipid film. The formed film was hydrated with 10 ml of Phosphate buffer saline pH 7.4. The hydration was continued for 1 hr, while the flask was kept rotating at 55-65°C in the rotary evaporator [7].

Table-1: Formulation table of Noisome

Formulation code	Drug (mg)	Span 60 (mg)	Cholesterol (mg)	Methanol (ml)	Chloroform (ml)
F1	100	100	20	5	5
F2	100	150	20	5	5
F3	100	200	20	5	5
F4	100	100	25	5	5
F5	100	150	25	5	5
F6	100	200	25	5	5
F7	100	100	30	5	5
F8	100	150	30	5	5
F9	100	200	30	5	5

Determination of drug-excipient compatibility by Fourier Transform Infra Red (FTIR) spectroscopy

The compatibility studies were carried out by FTIR to determine the interaction of Oxiconazole nitrate with other excipients used in the formulation. Physical mixture of the drug with excipients in the ratio (1:1) was prepared and the samples were analysed in FTIR spectrum. The FTIR spectrums of drug with all excipients were compared with standard FTIR spectra of the drug.

Evaluation of oxiconazole nitrate niosomes

Niosome formulation with different Surfactant and Cholesterol (by using 3^2 factorial design) were evaluated and characterized by the following methods and the best among it was selected for Noisome formulation and further evaluation.

Determination of Encapsulation Efficiency

Percent encapsulation efficiency (EE) was determined by centrifugal method. The Oxiconazole nitrate containing niosomes were separated from unentrapped drug by centrifuging at 14,000 rpm at 4◦ C for 30 min. The supernatant was taken and diluted with

phosphate buffer pH 7.4. The drug concentration in the resulting solution was assayed at 205 nm using UV spectrophotometer. The percentage of drug encapsulation was calculated by the following [8]:

$$\%EE=\frac{\text{Total drug-Unentrapped drug}}{\text{Total drug}}\times 100$$

Invitro-drug diffusion study

The invitro drug diffusion studies were carried out in an open diffusion tube .which was opened at both the ends. Cellophane membrane (previously soaked in Phosphate Buffer pH 7.4 for overnight) and was fixed to the one end of tube. The sample (1ml) was added uniformly on the tube such that the preparation occupies inner circumference of tube. The whole assembly was fixed in such a way that the lower end of tube containing gel was just touched (1-2 mm deep) the surface of diffusion medium i.e., 50ml pH 7.4 phosphate buffer and 50 ml methanol contained in beaker which was placed in water bath and maintained at 37±2ºC. The cellophane membrane acts as a barrier between the Noisome and pH 7.4 phosphate buffers (sink condition). A quantity of 1 ml samples were withdrawn from receptor fluid at the time interval of 15min, 30min, 45min,1, 2, 3, 4, 5, 6hrs. 1 ml solvent was replaced each time and the released drug was estimated spectrophotometrically at 205 nm.

PREPARATION OF OXICONAZOLE NITRATE NIOSOMAL GEL

Gel was prepared using Carbopol 934.The appropriate quantity of Carbopol 934 powder was dispersed into distilled water under constant stirring with a glass rod, taking care to avoid the formation of indispersable lumps and allow to hydrating for about 24hrs at room temperature for swelling. Niosomel gel formulations were prepared by incorporation of Noisome (optimized formulation) containing drug (separated from unentrapped drug) were mixed into the carbopol gel with a mechanical stirrer. The dispersion was neutralized using triethanolamine.

CHARACTERISATION OF OXICONAZOLE NITRATE NIOSOMAL GEL

Dynamic light scattering (DLS)

Particle size of niosome was determined using Malvern particle size analyzer version 7.01.For particle size analysis, niosomes were suspended in double distilled water and one drop was placed on clean slide and the particle size was observed. The average particle size, zeta potential was calculated and significant value ($P<0.05$) was calculated by using Graph pad prism 5.1 software.

Drug content

The Oxiconazole nitrate gel 100 mg dissolved in 100 ml solution (50 ml methanol+50 ml phosphate buffer). The Volumetric flask containing gel solution was shaken for 2 hr on mechanical shaker in order to get complete solubility of drug. From this 1ml was pipetted out and made up to 10ml with pH 7.4 phosphate buffer. This solution was filtered and estimated spectrophotometerically at 205 nm.

pH, Homogeneity, Spreadability of gels are evaluated [9].

Determination of zone of inhibition

Candida albicans were employed for testing antifungal activity using the well plate method. The culture was maintained on sabouraud´s agar medium was inoculated with 72 hrs old 0.2 ml suspension of *Candida albicans* in the petri dish and allowed to set by keeping undisturbed for 15 mints. The wells were punched in the petri dish and filled with 0.05 ml of sample and in another plate pure drug solution was placed carefully. The plate were kept for diffusion at +_40ºc for 1 hr, and incubated at 30ºc for 48 hrs. After completion of incubation the zone of inhibition in millimeter were measured [4].

RESULT AND DISCUSSION

FTIR Spectroscopy

The major peaks observed in drug spectrum were also observed in spectrums of drug with excipients; therefore it could indicate that there was no incompatibility between drug and different excipients (Table-2, Fig-1 & 2).

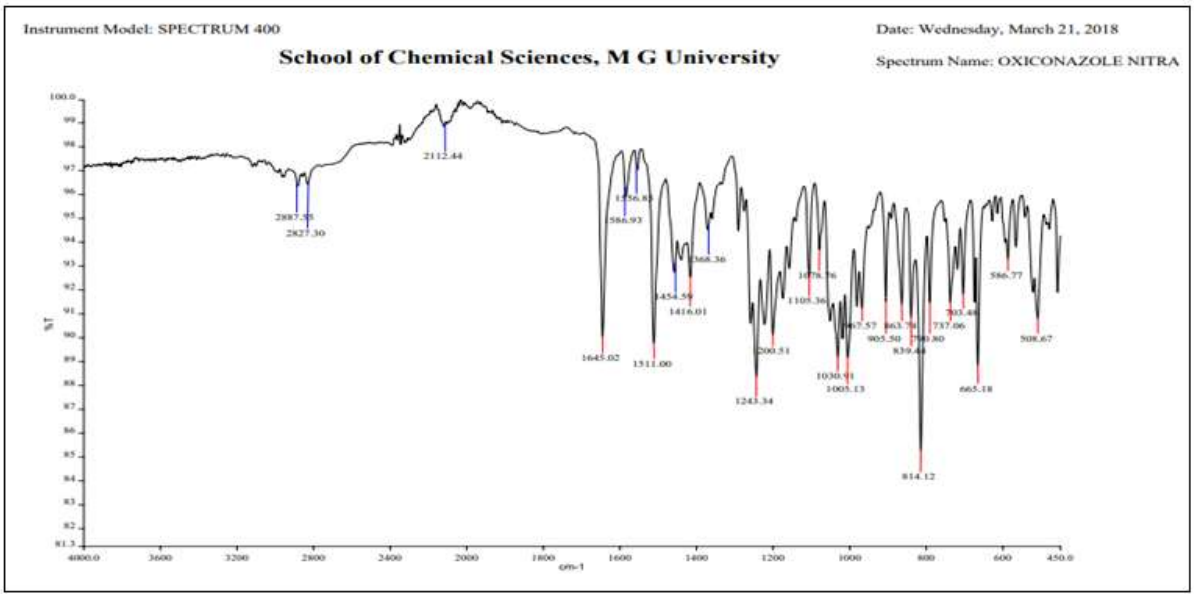


Fig-1: IR Spectrum of Oxiconazole nitrate

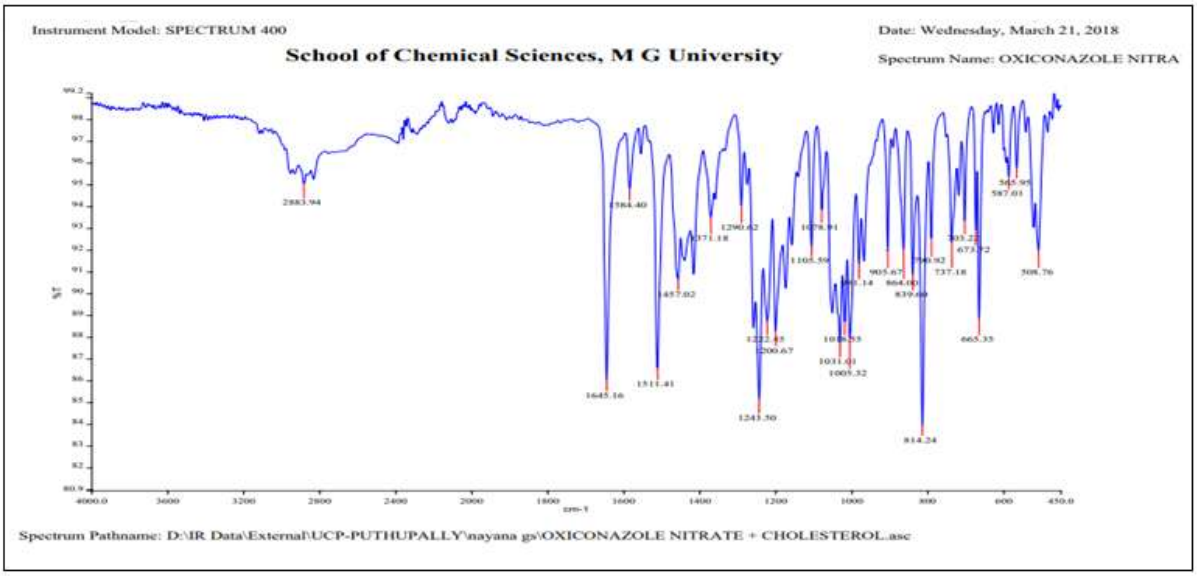


Fig-2: FTIR Spectrum of Oxiconazole nitrate with excipient

Table-2: interpretation of IR spectra of Oxiconazole nitrate

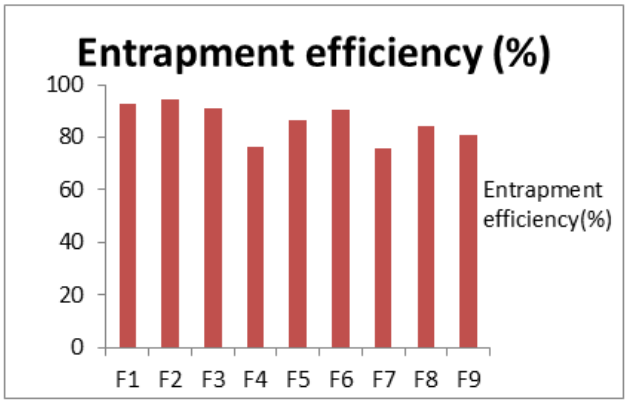
Group	Characteristic peak (cm^{-1})	Observed peak(cm^{-1}
C-H streching	3000-2850	2827
C=C Streching	1700-1400	1457
N-O streching	1500-1550	1511
C-Cl streching	850-550	814
C=N streching	1690-1640	1645.12

Invitro drug release and entrapment efficiency

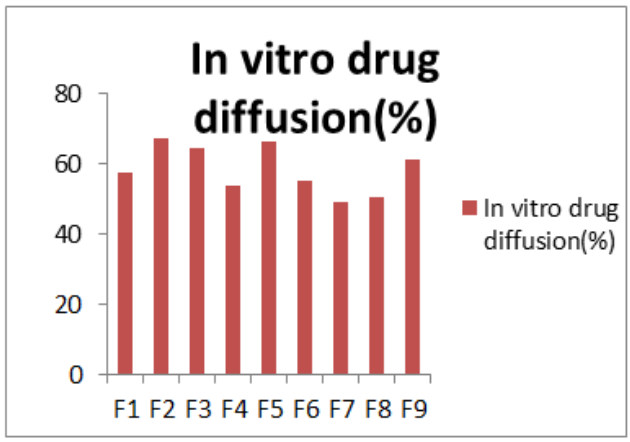
By using 3^2 factorial designs 9 formulations are prepared (by using different ratios of cholesterol and span 60) and entrapment efficiency and in vitro drug release are the responses.

Table-3: Responses of invitro drug release and entrapment efficiency

Formulation code	Responses	
	Y1: invitro drug diffusion (%)	Y2: % Entrapment efficiency (%)
F1	57.48	78.04
F2	67.26	94.64
F3	64.39	91.12
F4	53.9	76.42
F5	66.32	89.35
F6	55.14	90.31
F7	49.03	76.05
F8	50.65	86.68
F9	61,43	81.05



Graph -1: Entrapment efficiency of 9 formulations.



Graph-2: In vitro drug release of 9 formulations

Increase in mole fraction of span60 resulted increased entrapment efficiency and in vitro drug release of niosome, a further increase results decrease the entrapment efficiency. On higher Cholesterol level a reduction in entrapment efficiency resulted probably due to the competition between cholesterol and drug for packing space within the bilayer. Based on the in vitro drug release and entrapment efficiency, the formulation F2 was found to be the best formulation and which is selected for the preparation of gel.

EVALUATION OF OXICONAZOLE NITRATE NIOSOMAL GEL

Zeta potential and polidispersity index

Zeta potential analysis revealed about the stability of prepared Niosome is -16mv. Niosomes with Zeta Potential values greater than +20 mV or less than -20 mV typically have high degrees of stability. Polydispersity index (PDI) value was used to characterize the monodispersed and polydispersed nature of Niosomes. Polydispersity index value obtained (0.269) indicated the low level of non uniformity in the prepared Niosome.

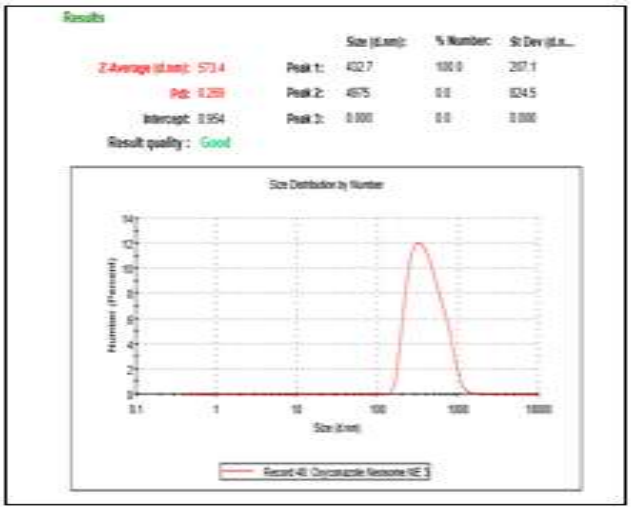


Fig-3: Particle size distribution

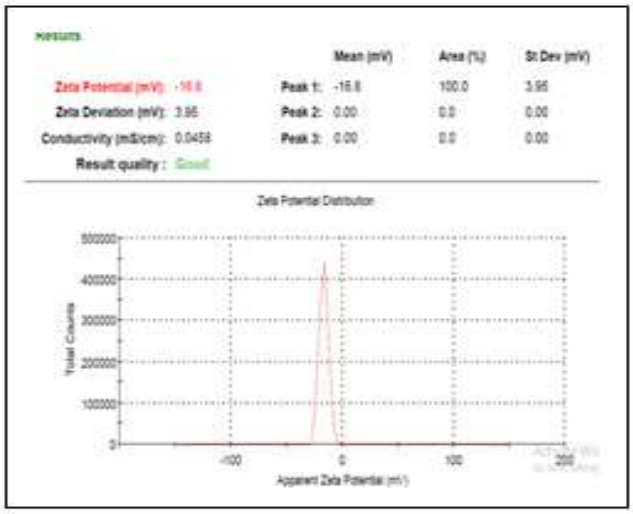


Fig-4: Zeta potential analysis

pH, Homogenisity, spredability, Drug content

pH of niosomal gel was found to be 6.6, that suit with skin pH and have good homogenicity and free of gritty particles. The values of spredability (31.32gm cm/sec) indicate that the gel is spreadable by small amount of shear**.** Drug content of niosomal gel formulation was 85.18%.

In vitro drug diffusion of niosomal gel

Table-4: in vitro diffusion of Oxiconazole nitrate niosomal gel

Time in hrs	In vitro drug diffusion (%)
0	0
0.25	4.015
0.5	9.540
0.75	12.473
1	18.88
2	27.970
3	39.94
4	51.85
5	59.81
6	67.95

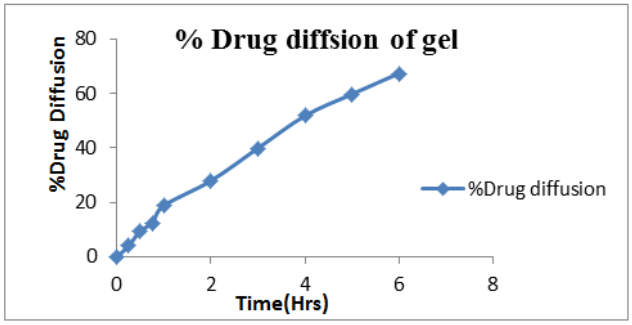


Fig-7: Invitro drug diffusion of Oxiconazole nitrate niosomal gel Vs time

In vitro drug diffusion of Oxiconazole nirate niosomal gel after 6 hrs is 67.95%. From the kinetics of drug release the diffusion of drug from the formulation follow zero order kinetics.

Fig-8: Zone of inhibition of oxiconazole nitrate niosomal gel

Antifungal activity (zone of inhibition)

The zone of inhibition of Oxiconazole nitrate niosomal gel is compared with pure drug solution of Oxiconazole nitrate. The zone of inhibition obtained for niosomal gel formulation (4.2) is more than the pure drug solution (2.2), so the niosomal gel have sufficient antifungal activity.

CONCLUSION

Oxiconazole nitrate are prepared by thin film hydration method using different ratio of Cholesterol and Span 60 by using 3^2 factorial design show a maximum of 67.26 % drug diffusion at 6^{th} hour and having entrapment efficiency 94.64%. Oxiconazole nitrate was successfully entrapeed with in the non ionic surfactant vesicle. The present study showed that niosomal gel is a suitable carrier for the delivery of antifungal drug (oxiconazole ntrate).

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