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Evaluation of Transdermal Targeted Niosomal Drug Delivery of Terbinafine Hydrochloride

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Abstract: The objective of present study is to develop a targeted delivery to the fungal affected cells. Niosomes of terbinafine hydrochloride were formulated by thin film hydration method using different ratios of non ionic surfactant (tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. The prepared formulations were evaluated for its vesicle size (by AFM), entrapment efficiency (by dialysis method) *in vitro* release studies and antifungal activities. Increase in surfactant concentration, increased the entrapment efficiency (up to 84.92%) and the formulation with surfactant cholesterol ratio 2:1 in each group of surfactant showed good entrapment. Niosomal preparation were tested for *in vitro* antifungal activity using the strain *Aspergillus niger* and compared with pure drug solution (as standard). All the niosomal formulations showed gradual increase in zone of inhibition due to the controlled release of medicament. The best formulation with maximum zone of inhibition and sustained release of drug (tween 40 nisomes) incorporated into gel bases and evaluated. The studies revealed that gel containing total niosomes possess maximum zone of inhibition values (12mm) initially followed by sustained release (12mm-16mm) comparing to gel containing drug entrapped niosomes, gel containing pure drug and marketed preparation.

Key Words: Niosomes, Targeted drug delivery, Vesicle size, Entrapment efficiency.

Introduction

The present work aims to deliver the drug terbinafine hydrochloride to the fungal affected cells. This targeting can be achieved by formulating drug as niosomal transdermal gel, which prolongs the circulation of drug. Niosomes are microscopic lamellar structures formed on admixture of cholesterol and single alkyl chain non ionic surfactant with subsequent hydration in aqueous media [1]

Recently niosomes are gaining popularity in the field of topical and transdermal drug delivery because of its special characteristic features like increasing penetration of drugs, acting as local depot to provide sustained release and serving as a solublizing matrix for both hydrophilic and lipophilic drugs)[2,3]. By delivering the drug terbinafine hydrochloride in topical gel form systemic toxicity can be reduced. Terbinafine hydrochloride is effective against dermatophyte infections, microsporum canis of the skins and nails particularly in the treatment of pityriasis (tinea) vesicolor due to malassezia furfur. In conventional form only less than 5% of the dose is absorbed but by administering the drug in niosomal gel form better effect can be obtained by increasing absorption [4]

Materials and Methods

Terbinafine Hydrochloride was obtained as gift sample from Dr.Reddys laboratories, Hyderabad, India. Cholesterol (Loba chemie, India), Tween 20 and Tween 80 (SISCO laboratories, India) Tween 40 and Tween 60 (Hi Media, India) were obtained from the local market. All reagents used were of analytical grade.

Preparation of Niosomes

Niosomes were prepared by thin film hydration technique using different grades of surfactants (tween 20, 40, 60 & 80) and cholesterol in different ratios as per the composition shown in Table: 1.The surfactants and cholesterol were dissolved in diethyl ether and solvent was then evaporated under reduced pressure using rotary flash evaporator (Super

fit rotary vacuum) at 150 rpm for 5- 10 min. This intermittent vortexing at 50°C results in deposition of thin layer of solid mixture on the sides of the flask. Then it was hydrated with aqueous phase containing the drug (25mg) in 20ml of distilled water. It was then vortexed again and heated at 60-70°C for 1 hour. The resulting multilamellar vesicles were cooled in an ice bath and then sonicated using probe type Ultrasonicator (Vibronics P, India) for 3min at 150V. This resulted in unilamellar vesicles of niosomes which were stored at 4°C in a refrigerator. For each formulation plain niosomes were prepared without the drug using the same procedure.[5]

Entrapment Efficiency

Entrapment efficiency was determined by Dialysis method[6]. Niosomal dispersion (5ml) was placed inside the dialysis tube and it was suspended suitably in a beaker containing 100ml diffusion medium (distilled water) which was constantly stirred at 37°±1°C on a magnetic stirrer for 4 hours. Samples were withdrawn at various time intervals and assayed spectrophotometrically at 283nm using UV Spectrophotometer. (Shimadzu –UV Pharmaspec 1700, Japan). The time required to release the unentrapped drug was noted.[7] Entrapment efficiency was calculated by subtracting the amount of the drug dialyzed from the total drug used in the formulation.[8]

Drug Content Analysis [8]

The amount of drug in the formulation was determined after lysing the nisomes using n-propanol (50%).

Invitro Release Study

Invitro release studies were carried out by dialysis method using an open ended cylinder and cellophane paper as dialyzing membrane (previously soaked in glycerin, water mixture (1:3) for 15minutes). All the dialysis studies were performed in distilled water (100ml) maintained at 37°±1° C and stirred continuously at 100rpm by using Magnetic stirrer. Total niosomal formulation was taken for the dialysis study and the receptor compartment has been changed immediately at the time when unentrapped drug was completely dialyzed. Then the release study was carried out for the entrapped drug from the vesicle. The collected samples were analysed spectrophotometrically at 283 nm using distilled water as blank in a UV-visible spectrophotometer (Shimadzu –UV Pharmaspec 1700, Japan).The in vitro release studies were also carried out for the pure drug by same method**.** [9]

Microbiological studies

The prepared niosomal formulations having good entrapment (in each group of surfactant) were selected for the microbiological studies. The invitro antifungal activities were carried out by cup-plate method using sabouraud dextrose agar[10]. The strain *Aspergillus niger* NCIM 545 was used. Along with each formulation, the plain niosomes were also tested for antimicrobial activity and compared with the standard solution of terbinafine Hydrochloride. The plates were incubated at 25°C and the results were observed for 72 hours at 24 hours intervals. The zone of inhibition for each sample was recorded[11].

Preparation of Gel

On the basis of *in vitro* release studies and the antimicrobial studies, the niosomal dispersion showing sustained release and maximum zone of inhibition was selected for incorporation into a gel base. Two gel bases, Carbopol 934(1%w/w) and Sodium carboxy methyl cellulose (3%w/w) were used. Three formulations were made with gel containing pure drug (0.25 %).Niosomal gel I (gel containing total niosomes 0.25 %) Niosomal gel II (gel containing only entrapped drug 0.25 %w/w).

Evaluation of the prepared gels

The prepared gels were evaluated for their pH, drug content, *in vitro* release and antimicrobial studies. Finally the gel selected for further studies based on the efficiency of antimicrobial activities.

Results and Discussion:

The prepared niosomes revealed that they are discrete in size (20-60 nm) and shape. The Scanning Electron Microscopic photographs of the prepared niosomes are shown in figure 1.The entrapment efficiency increased with increase in surfactant concentration and decreased with increase in cholesterol concentration. Among all the formulations, F14 formulation (with tween 60 & cholesterol ratio 2:1) showed highest entrapment efficiency of 84.92 %. The results are shown in Table 2.

The impact of surfactant and cholesterol in the entrapment efficiency and release rate is significant. As entrapment efficiency increases (with increase in surfactant concentration) more time is taken for maximum drug release or the drug release is prolonged. Similarly as the entrapment efficiency decreases (for the formulations with increased cholesterol ratio) less time was required for maximum drug release. The results are shown in figure 2. As concentration of cholesterol increased in the formulations F_5, F_{10}, F_{15} and F_{20} the entrapment

efficiency was decreased and there was maximum drug release in 24 hours than the other formulations which had less cholesterol ratio (F_5 - 96%, F_{10} - 92%, F_{15} – 73%, F_{20} – 90%). It confirms as per the report gives by J.N. Khandhar et al [12].

As the concentration of surfactant increased, niosomes showed high entrapment efficiency in all the tween grades. At the same time the release was also sustained, in all the tween grades 20, 40, 60 and 80, when the surfactant concentration increased from 0.5 to 2 in F_4, F_9, F_{14}, and F_{19} the release was 88%, 75%, 58%, and 85% respectively at the end of 24 hours compared to other formulations with less surfactant and it was shown in figure - 2

The niosomal formulations with good entrapment (i.e surfactant cholesterol ratio 2:1) in each group of surfactant were selected for *in vitro* antifungal activity and compared with standard (pure drug solution).These tests showed that the pure drug solutions showed maximum zone of inhibition 45 mm and it was constant in 24 hrs itself, where as all other niosomal formulations showed less zone of inhibition than pure drug. The formulations F_9, F_{14} and F_{19} showed gradual increase in zone of inhibition during the study period due to the controlled release of medicament. The results were shown in table 3.

The niosomal formulations stored at 4°± 2°C (68 -75% at the end of 12weeks) was found to be more stable when compared to the niosomes stored at room temperature (30 – 40% at the end of 12 weeks).

The gel formulations were evaluated for its drug content (carbopol gel – 98%, sodium carboxy methyl cellulose – 96%) which showed uniform distribution of drug in the gel. Release studies showed that the gel containing pure drug (carbopol based gel – 98.63% in 210min, sodium carboxy methyl cellulose – 98.14% in 150min) and marketed cream released (99.49% in 150min) the drug within short period where as niosomes containing gel showed sustained release of drug in 24 Hrs (carbopol based gel 76.59% and sodium carboxy methyl cellulose based gel – 77.37%). The results were shown clearly in figure 4.

Among the gel containing niosomes, the gel with total niosomes showed initial burst of drug (due to availability of unentrapped drug) and after 1.5 hours showed sustained release (due to gradual release of medicament). Further, antifungal activity of the niosomal gel prepared with sodium carboxy methyl cellulose showed better zone of inhibition than the gel prepared with carbopol. The antifungal activity of niosomal gel prepared with sodium carboxy methyl cellulose showed sustained release of medicament by showing gradual increase of zone inhibition measured at regular intervals when compared to marketed products and pure drug gel. The results are shown in table 4 and in figure 5.

Table 1: COMPOSITION OF NIOSOMES

FORMULATION	SURFACTANT	RATIO OF	
		SURFACTANT	CHOLESTEROL
F_1	Tween 20	0.50	1
F_2	Tween 20	1.00	1
F_3	Tween 20	1.50	1
F_4	Tween 20	2.00	1
F_5	Tween 20	1.00	2
F_6	Tween 40	0.50	1
F_7	Tween 40	1.00	1
F_8	Tween 40	1.50	1
F_9	Tween 40	2.00	1
F_{10}	Tween 40	1.00	2
F_{11}	Tween 60	0.50	1
F_{12}	Tween 60	1.00	1
F_{13}	Tween 60	1.50	1
F_{14}	Tween 60	2.00	1
F_{15}	Tween 60	1.00	2
F_{16}	Tween 80	0.50	1
F_{17}	Tween 80	1.00	1
F_{18}	Tween 80	1.50	1
F_{19}	Tween 80	2.00	1
F_{20}	Tween 80	1.00	2

TABLE-2 ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

FORMULATION SURFACTANT	**ENTRAPMENT EFFICIENCIES (IN PERCENTAGE)**				
	S/C 0.5:1	**S/C 1:1**	**S/C 1.5:1**	**S/C 2:1**	**S/C 1:2**
Tween 20	F1 18.49	F2 21.88	F3 18.31	F4 23.21	F5 13.96
Tween 40	F6 66.21	F7 74.73	F8 73.45	F9 76.74	F10 71.55
Tween 60	F11 79.04	F12 79.47	F13 82.35	F14 84.92	F15 80.86
Tween 80	F16 53.46	F17 57.79	F18 61.07	F19 64.93	F20 61.52

TABLE N: 3 ANTIFUNGAL ACTIVITY – *Aspergillus niger*

Samples	**Zone of inhibition (in mm)* ± S.D**		
	24hrs	**48hrs**	**72hrs**
Standard	**45 ± 0.25**	**45 ± 0.14**	**45 ± 0.14**
F4 (P)	**25 ± 0.11**	**25 ± 0.13**	**25 ± 0.12**
F4	**34 ± 0.31**	**34 ± 0.27**	**34 ± 0.27**
F9 (P)	**26 ± 0.42**	**26 ± 0.15**	**26 ± 0.15**
F9	**36 ± 0.47**	**38 ± 0.18**	**40 ± 0.16**
F14 (P)	**12 ± 0.05**	**12 ± 0.29**	**12 ± 0.29**
F14	**16 ± 0.12**	**20 ± 0.33**	**24 ± 0.01**
F19 (P)	**20 ± 0.19**	**20 ± 0.41**	**20 ± 0.02**
F19	**35 ± 0.60**	**37 ± 0.68**	**37 ± 0.56**

*** n= 3**

TABLE NO: 4 ANTIFUNGAL ACTIVITIES OF GELS

Samples	**Zone of inhibition (in mm)***			
	24hrs	**48hrs**	**72hrs**	**96hrs**
A (Niosomal gel containing entrapped drug 0.25%)	10 ± 0.16	12 ± 0.20	14 ± 0.16	15 ± 0.31
B (Marketed cream 1%)	20 ± 0.25	20 ± 0.05	20 ± 0.15	20 ± 0.16
C (Gel containing the Pure drug 0.25%)	15 ± 0.10	15 ± 0.01	15 ± 0.11	15 ± 0.77
D (Niosomal gel containing both entrapped and unentrapped drug 0.25%)	12 ± 0.11	14 ± 0.28	15 ± 0.10	16 ± 0.32

* n=3

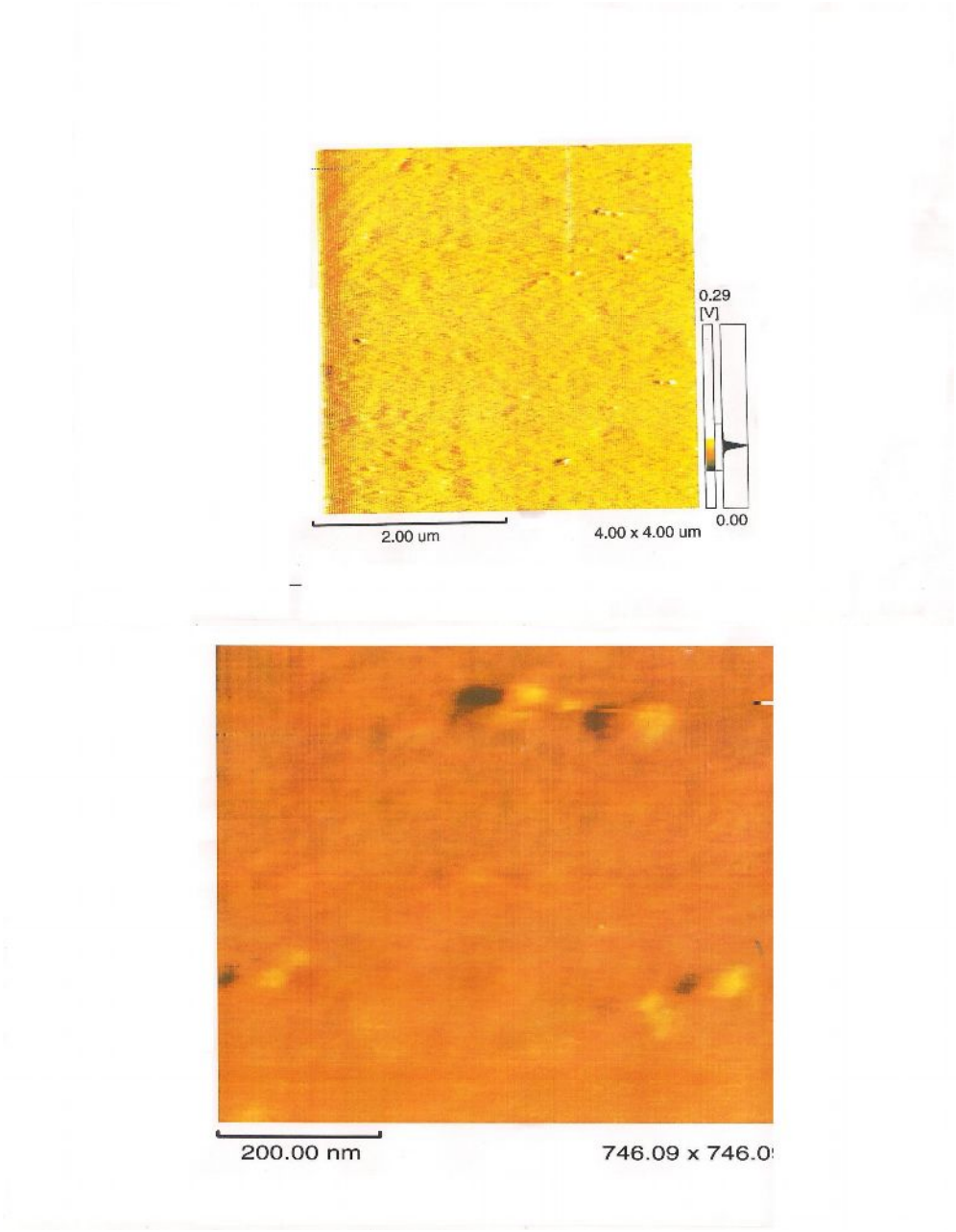


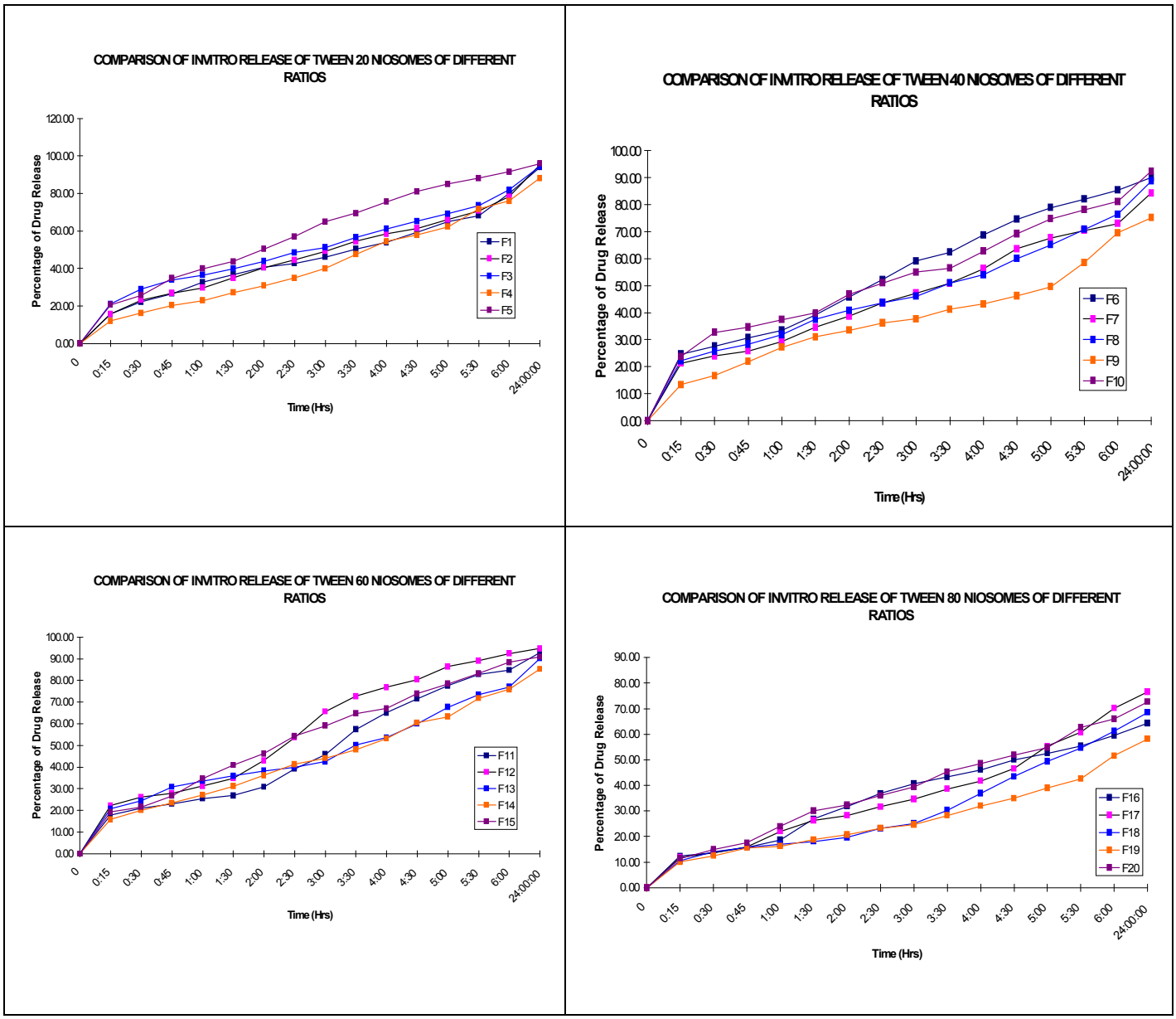
Figure 1- Scanning Electron Microscopic Photographs of prepared noisome

Conclusion:

In this work niosomes were prepared by altering the surfactant and the cholesterol concentration. From this work it is concluded that by increasing surfactant concentration entrapment efficiency increases and maximum time was required for the release of drug from those entrapped vesicles. At the same time with increasing cholesterol concentration entrapment efficiency decreases and only less time was required to release the drug. This was confirmed from antifungal studies also, in which zone of inhibition of the formulations with increased entrapment was sustained showing gradual increase in zone due to the controlled release of the medicament

Gels prepared with Sodium carboxy methylcellulose containing total niosomes showed an initial burst release at 1.5 hrs followed by sustained release comparing with the gel containing only entrapped drug where sustained release alone occurred. Anti fungal activities also confirmed these results. So by formulating the drug as niosomal transdermal gel (containing total niosomes) better effect can be obtained owing to its increased penetration.

Since surfactant also showed some antifungal activities by increasing concentration of surfactant, better effect can be obtained than in conventional topical dosage form.



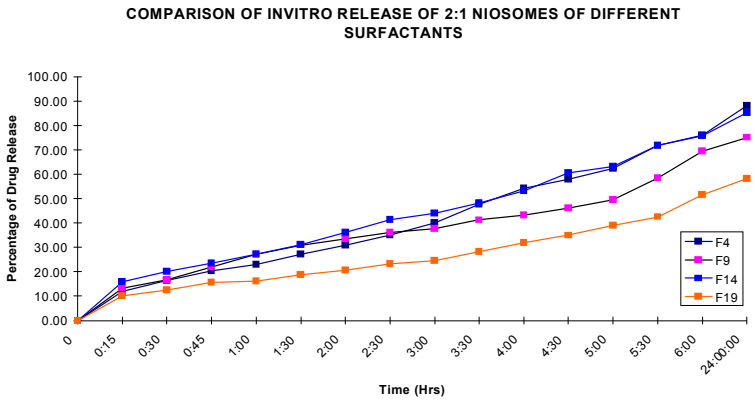
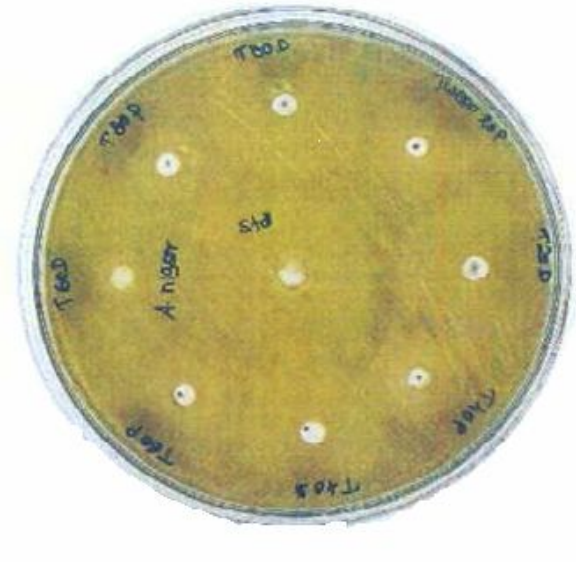
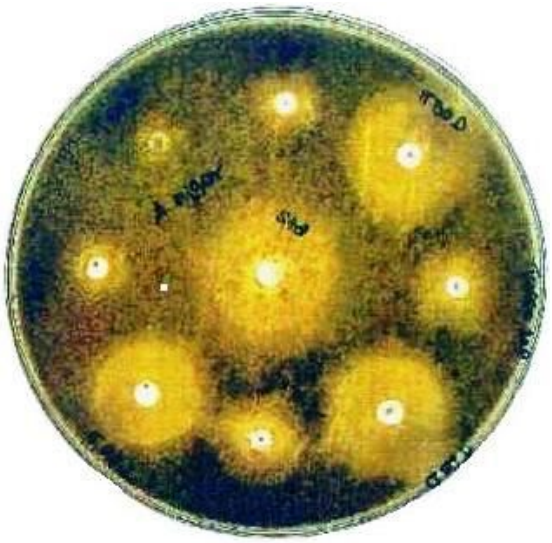


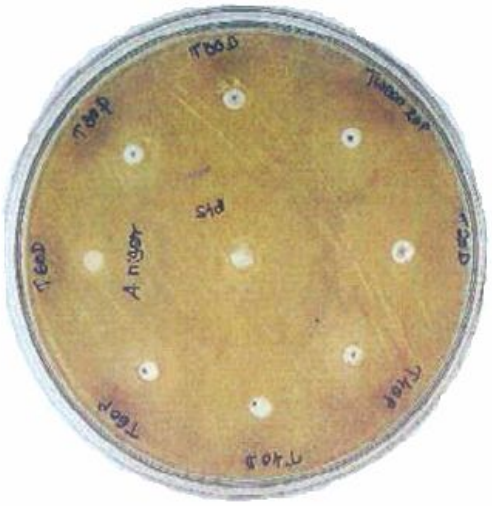
Figure 2- Comparison of release profiles of Tween 20, Tween 40, Tween 60, Tween 80 of different ratios



Aspergillusniger24 hours activity



Aspergillus niger – 72 hours activity



Aspergillus niger – 48 hours activity

Aspergillus niger – 96 hours activity

Figure 3- Antifungal activity of terbinafine hydrochloride niosomes

Note: Std- indicates standard; T20 P, T40 P, T60 P, T80 P- indicates plain niosomes without drug in each surfactant type (surfactant: cholesterol 2:1); T20 D, T40 D, T60 D, T80 D- indicates niosomes containing terbinafine hydrochloride (surfactant: cholesterol 2:1)

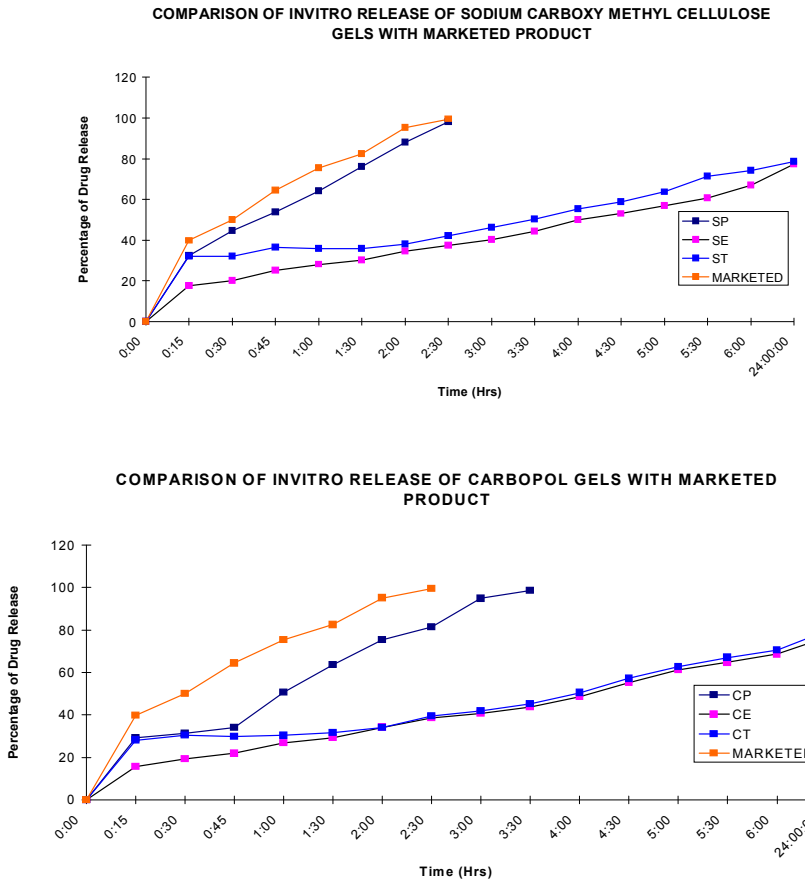
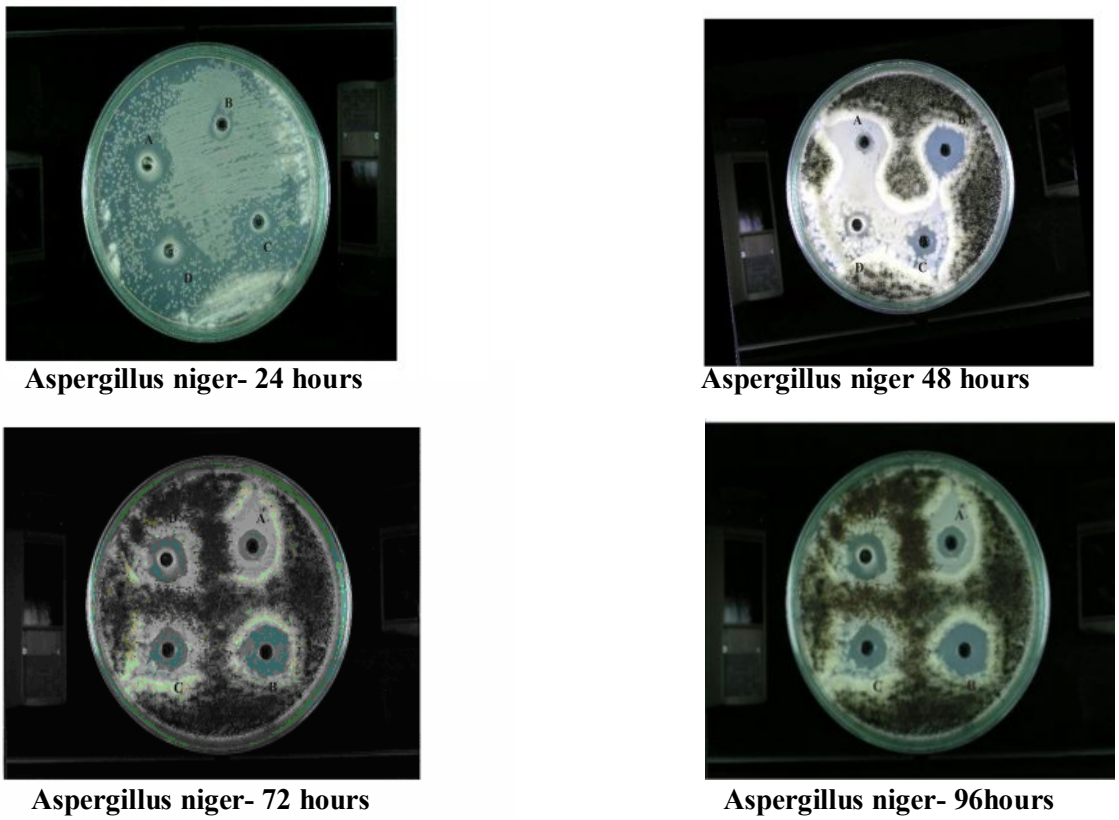


Figure 4– In vitro release profile of terbinafine hydrochloride carbo gels sodium carboxymethyl cellulose gels with marketed product



Aspergillus niger- 24 hours **Aspergillus niger 48 hours**

Aspergillus niger- 72 hours **Aspergillus niger- 96hours**

Figure 5- Antifungal activity of terbinafine hydrochloride gels

References

1. Vyas and Khar S.P.Vyas and R.K.Khar, 2004, Niosomes, Targetted & Controlled Drug delivery Systems, Novel Carrier Systems, 250,286. Vallabh Prakashan,Delhi.
2. Namedo Alok and Jain, N.K., Niosomes as drug carriers,Ind J.Pharm.Sci.,1996,58(2),41-46.
3. Vanlerberghe, G. and Morancias, J.L., Niosomes in perspective, S.T.P. Pharma Sciences, 1996,6(1),5-11.
4. AHFS, Drug information, American Society of Health System – Pharmacists 2004, 3331-3333.
5. Chengjlu Hu, David .G Rhodes, Pro niosomes, A novel drug carrier preparation, International Journal of Pharmaceutics ,20006, 206. 110-122
6. D.Gopinath, D.Ravi, B.R.Rao Ascorbyl Palmitate vesicles (Aspasomes) formation characterization and applications, International Journal of Pharmaceutics edition,2004, 271, 96-113.
7. Nidhi N, Dubey, Joly R.Parikh, Preparation and optimization of niosomes containing Piroxicam, Indian Pharmacist, Oct-2006,97-103.
8. Majusha Malhotra , N.K.Jain, Review Article – Niosomes As Drug Carriers Indian Drugs,1996, 31(3), 81-86
9. Abbas Pandakhty, Jaleh Varshosaz, Invitro study of poly oxyethylene alkyl ether niosomes for delivery of insulin International Journal of Pharmaceutics, 2006 (Article in press).
10. Indian Pharmacopoeia, Vol II 1996 "Microbiological tests and assays", A-90.
11. Simone Gondaves Cardoso, Elfrides .E.S Schaporal, Microbiological assay for Terbinafine Hydrochloride in tablets & creams, International Journal of Pharmaceutics, 2000 203, 109-113.
12. J.N. Khandare, Jiwandas Bobade, "Preparation and evaluation of reverse phase evaporation and multilamellar niosomes as ophthalmic carriers for acetazolamide" international journal of pharmaceutics 2005, 306, 71 – 82.

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