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FORMULATION AND EVALUATION OF KETOCONAZOLE PRONIOSOMAL GEL

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ABSTRACT

Proniosomes are water-soluble carrier particles that are coated with surfactant and can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. Provesicular systems, such as proniosomes which is one of the advancement in nanotechnology minimize problems of vesicular systems. This research mainly emphasizes on formulating Ketoconazole Proniosomal gels with span surfactants, cholesterol, soya lecithin and alcohol as aqueous phase. These are prepared by coacervation phase separation method and the prepared formulations are characterized for FTIR studies, Encapsulation efficiency, size distribution and In vitro release studies were carried. FTIR studies showed that there was no interaction between API and used excipient. The encapsulation efficiency of Proniosomal formulations are in the range of 55% to 69.94%. Morphological size and shape of the vesicles are characterized by using optical microscopy and scanning electron microscopy, particles are found to be spherical, size of the particles are in the range of 3.29µm to 30µm and permeation studies showed good control release for prolonged period of time. Span20 Non lecithin formulation showed highest amount of drug release of 74% in 24 hours.The results suggest that ketoconazole proniosome formulations can be used for Topical drug delivery system for the treatment of skin infections.

KEYWORDS: Proniosomal Gel, Ketoconazole, Nanotechnology, Formulation.

INTRODUCTION

Niosomes play an increasingly important role in drug delivery as they can reduce toxicity and modify pharmacokinetic and bio-availability. Topically applied niosomes can increase the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of the drug. Ketoconazole is a broad spectrum Imidazole derivative useful in the treatment of superficial and systemic fungal infections.[1] It is having broad spectrum activity against systemic and superficial mycoses. It is readily but incompletely absorbed after oral dosing and it varies among individuals.Common side effects associated with Ketoconazole therapy include mild burning at the application site, severe allergic reactions, blisters, irritation, pain or redness.[2]

The major barrier of the drug absorption through skin is the stratum corneum, the top layer of the epidermis. Low molecular weight (≤500 Da), lipophilicity, and effectiveness at a low dosage are the ideal characteristics of the drugs for transdermal delivery.[3] Thus, the therapeutic effectiveness of the existing drugs is improved by formulating them in an advantageous way.

In the past few decades, considerable attention has been focused on the development of a new drug delivery system. To increase permeability, chemical and physical approaches have been examined to lower stratum corneum barrier properties. These approaches include tape stripping, iontophoresis, electroporation and vascular systems, such as liposomes and niosomes. Among these approaches, liposomes and niosomes are widely used to enhance drug permeation across the skin in cosmetic and dermatologic fields.[4] Vesicles can play a major role in modeling biological membranes, and in the transportation and targeting of active agents. Different types of pharmaceutical carriers, e.g., Particulate, polymeric, macromolecular or cellular carrier is present. Particulate type carrier, also known as the colloidal carrier system, includes lipid particles, microspheres, nanoparticles, polymeric micelles and vesicular systems.[5] Niosomes (non-ionic surfactant vesicles) are microscopic lamellar structures obtained on an admixture of non-Ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol (CHO) with subsequent hydration in aqueous media.[6,7] The encapsulation of drugs in niosomes can minimize drug degradation and inactivation after administration; prevent undesirable side effects, increase drug bioavilability and targeting to the pathological area.[8] Surfactants also act as penetration enhancers as they can remove the mucus layer and break functional complexes. Niosomes as delivery devices

have also been studied with anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine.[9]

Hence, in the present investigation, an attempt is made to develop and characterize proniosomal gel formulation of Ketoconazole to treat fungal infections of the skin more efficiently.

MATERIALS AND METHODS

Materials

Ketoconazole was a gift sample from M/s. Yarrow Chemicals Limited, Mumbai, Hyderabad. Soya lecithinwas obtained from Ms. Sigma Chemicals Limited, Hyderabad and dialysis membrane-50 (Molecular Weight. cut off 12000 to 14000) was obtained from M/s. HiMedia Laboratories, Mumbai. Cholesterol was obtained from M/s. Finar Chemicals Limited, Ahmedabad. Span 20 and Span 40 were obtained from M/s. LobaChemiePvt.Ltd., Mumbai. All other materials used in this study were of analytical grade.

Preparation of ketoconazole Proniosomal Gel

Niosomes were prepared by coacervation-phase separation methodology.[10] Precisely weighed amounts of drug, cholesterol and soya lecithin are taken in a wide mouth ampoule and to this add 0.5 ml of alcohol to it. Once after adding all the ingredients warming them and then mixed with a glass rod and the temperature was maintained (60-70^{o}C)for 5 minutes till it get completely dissolved. After that the aqueous solution(glycerol) was added to it and warm on water bath until it gets transparent solution and then convert into proniosomal gel on cooling.this formulation has been stored in a dark place for characterization.

Table 1: Composition of Proniosome gel formulation.

FORMULATION	F1	F2	F3	F4
Drug	200mg	200mg	200mg	200mg
Span 20	1000mg	1000mg	-	-
Span 40	-	-	1000mg	1000mg
Lecithin	200mg	-	200mg	-
Cholesterol	100mg	200mg	100mg	200mg

EVALUATION OF KETOCONAZOLE PRONIOSOMES

Morphological characterization

The prepared formulation gel was viewed under phase contrast optical microscope.After the vesicle formation was confirmed by optical microscopy in 40× resolution. The proniosomal

gel suspension was placed on a glass slide by covering with the cover slip and observed the formed vesicles from the microscope by using a digital camera[11] [Figure 1]

Fourier transform infrared spectroscopy study(FTIR)

Infrared spectrum of Ketoconazole, excipients and formulation was determined using the KBr disc/pellet method. The sample was grounded gently with anhydrous KBr and compressed to form pellet. The scanning range was 400 and 4000 cm-1.

Scanning electron microscopy (SEM)

Particle size of proniosomes is a important factor. The surface examination and size distribution of proniosomes were observed by SEM.On the scanning electron microscope the aluminum stub was kept in a vacuum chamber (XL 30 ESEM with EDAX, Philips, Netherlands). The morphological assuming of the samples was examined using a secondary electron detector (working pressure of 0.8 torr, acceleration voltage-30.00 KV) XL 30, (Philips, Netherlands).[12,13]

Encapsulation efficiency (Centrifugation method)

For this evaluation of the loading capability of Proniosomal system, a entire quantity of proniosomal gel was distributed in phosphate buffer pH 7.4 and then it changed to creamy solution to make niosomes. Then the dispersion was centrifugedat 18000 rpm for 40 min at 50C (Remi cooling centrifuge).After that clear fraction was used for the determination of free drug by victimisation of the subsequent equation

%Encapsulation efficiency = (1-unencapsulated/total drug)×100.

Particle size determination

The mean particle size was determined by particle size analyzer (Malvern). The instrument used to measure the particle size based on the laser diffraction theory. The apparatus consists of a He-Ne laser beam of 11.5nm focused with a minimum power of 5 mW using a Fourier lens to a point at the center of multielement detector and a sample holding unit (Su cell). The sample was placed and then stirred using a stirrer before determining the vesicle size. The vesicle solution was diluted about 100 times in the deionized water. Diluted gel suspension was added to sample dispersion unit containing stirrer and stirred at high speed in order to reduce aggregation and laser beam was focused.

In vitro release studies

TheIn vitro release studies of proniosomal gel were performed by using Franz diffusion cell. The capacity of the compartment was 15 ml. The area of the donor compartment exposed to receptor compartment was 1.41cm^2.Cellophane membrane was soaked in phosphate buffer pH 7.4 for 3 hr before carrying the experiment. The dialysis cellophane membrane (MMCO14KDC)[14] was seated between the donor and receptor compartment. A weighed quantity of proniosomal gel was kept on one side of the dialysis membrane and was placed in phosphate buffer pH 7.4 which acts as receptor medium.[15,16] The receptor compartment was enclosed by a vessel to maintain the temperature at 37^{o}C. Stable heat was provided by using a hot plate with a magnetic stirrer bearing a magnetic bead which helps in stirring of the receptor fluid. At every interval of sampling, 3ml sample were withdrawn and then replaced by equal volumes of fresh buffer on every occasion and analyzed spectrophotometrically at 270nm.[17, 18]

Microbiological assay

The microbiological assay of ketoconazole was carried out by cup plate method. The potato detrose agar medium was prepared, sterilized and inoculated with candida albicans micro-organism immediately poured the inoculated medium into petri plates to give a depth of (4 to 5) mm uniformly and kept aside for solidification. Small holes of 10 mm diameter were made on solidified potato agar petri plates by using sterilized cylinder shaped borer. 500 µl of the prepared standard solutions and sample solutions (i.e equivalent to 1 µg/ml and 5 µg/ml drug concentration) were added into each cavity. These petri plates are left for 30 minutes at room temperature as a pre-incubation diffusion to minimize the effects of variation in time between different solutions. Prepared petri plates were incubated for 24 hours at 32-35 ^{0}C and measured the diameter of circular inhibited zones.

RESULTS AND DISCUSSIONS

Optical microscopy

Proniosomal gel formulation exists as a liquid crystalline state upon hydration converts to niosomes, found to be spherical in photo micrographia on 100 X magnification (as shown in Figure1)

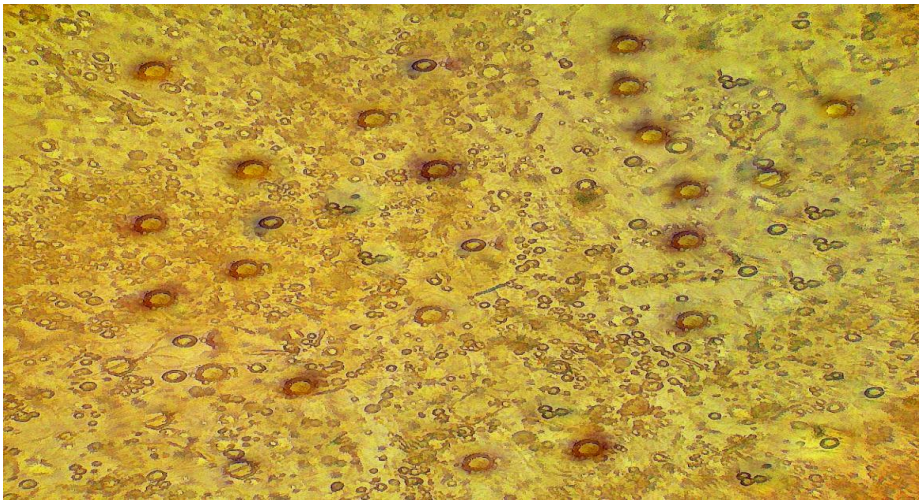

Figure 1: Photomicrographia of Niosomes on 40x magnification under optical microscope.

Scanning electron microscopy

Optimized span 20 formulation were studied using scanning electron microscopy.Particle size of proniosomes may be issue of high importance. The surface morphology and size distribution of proniosomes were studied by SEM (Figure 3) Scanning electron microscopy images for pure drug ketoconazole are examined (Figure 2). They are found to be in crystal form. In the figure 3Proniosomal ketoconazole gel formulations are examined the image purely indicating spherical shaped particles with a size range of 3.29 to 30.9µm.

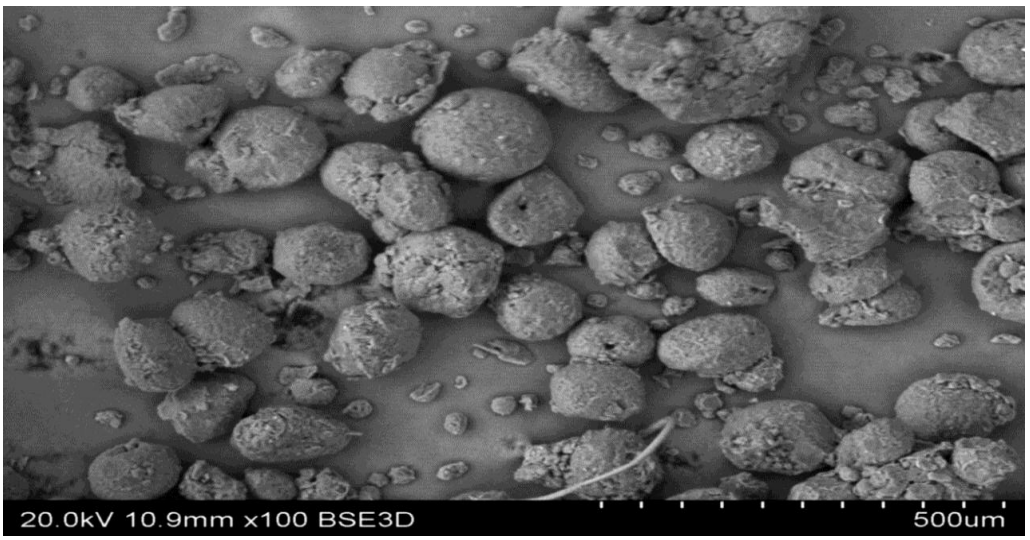

Figure 2: SEM image of ketoconazole pure drug.

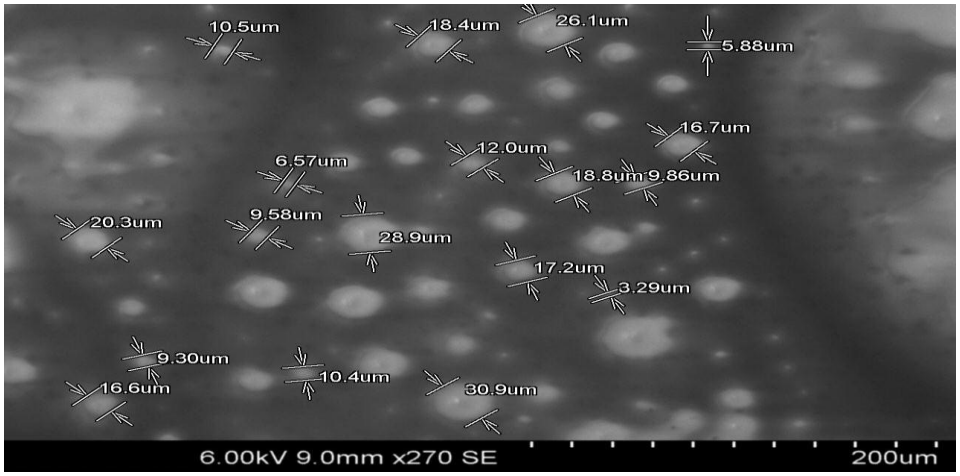

Figure 3: SEM image of proniosome formulation(F1).

FTIR Studies

FTIR studies were done for API and span 20 optimized ketoconazole formulations as shown in figure below (4 and 5). From these studies it was concluded that there is no noticeable change in the peak of the spectrums when the ingredients were analyzed individually as well as in the form of drug and excipients mixture. Individual peaks of ketoconazole were clearly establish without any interaction of excipients used in these formulation at 3387 ±4cm-1 (N-H stretch of amines), 2924 ± 3cm-1(O-H stretch of carboxylic acid), 1466±3cm-1(C-H rock of alkanes), 1366±3cm-1 (N–O symmetric stretch nitro compounds)and 828cm-1 (C-Cl of alkyl halides). FTIR studies of drug and excipients results showed that there was no interaction between the ingredients.

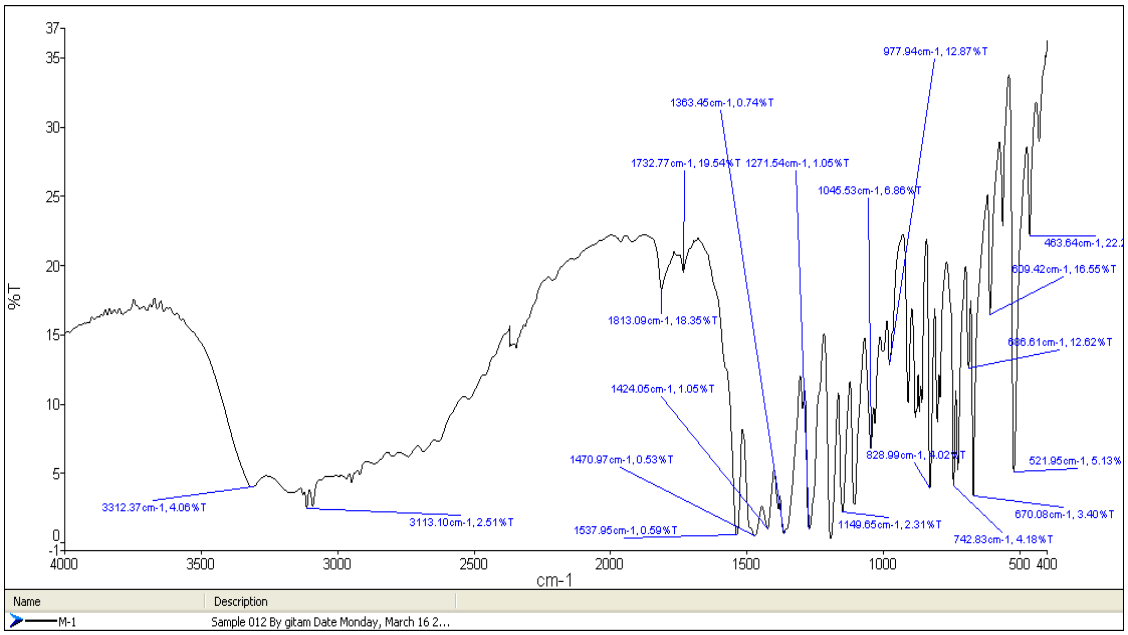

Figure 4: FTIR spectra of pure drug.

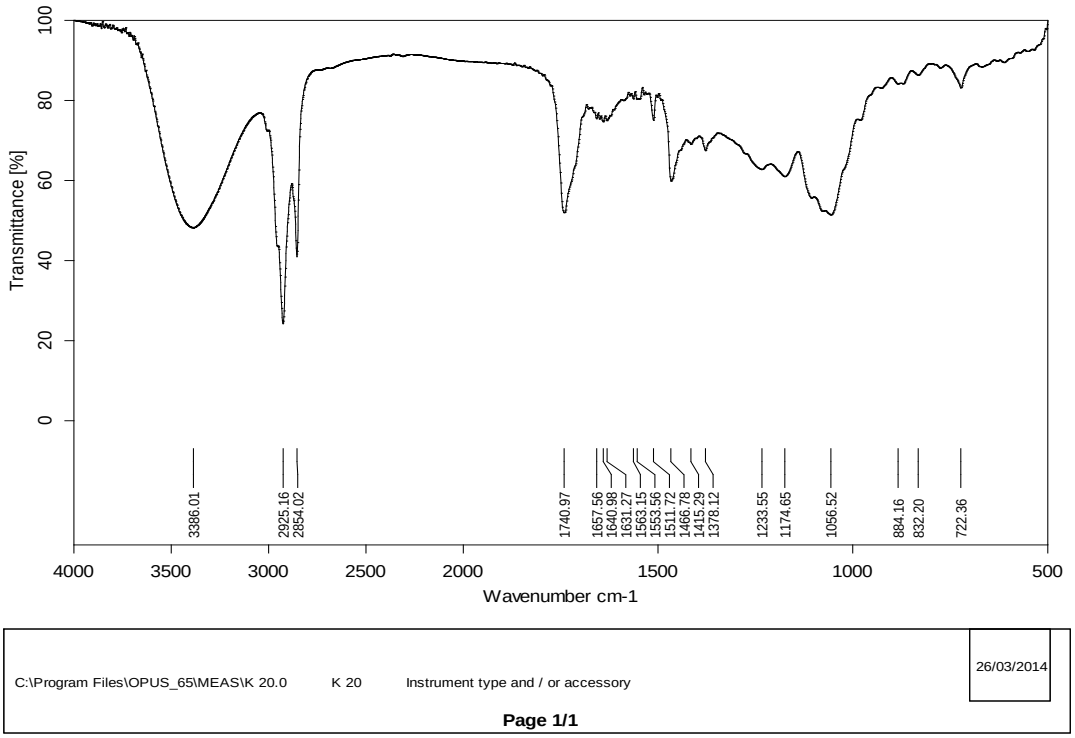

Figure 5: FTIR spectra of span 20(F1) formulation.

Encapsulation efficiency

Span 20(F1) among of all formulations shown highest encapsulation efficiency and F4 showed least encapsulation efficiency. The %Encapsulation efficiency of the formulations are listed in the below Table 2.

Table 2: Percentage Encapsulation efficiency values of various formulations.

Formulation	%Encapsulation Efficiency
F1	69%
F2	64%
F3	58%
F4	55%

Particle size determination

The particle size distribution analysis was performed by using particle size analyzer (Malvern) and the results (Figure 6) showed that the average particle size of the proniosome vesicle for formulation F1 was found to be 11.5nm The results indicated that vesicle size is in nano particulate range and the size distribution is uniform.

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Measurement Results

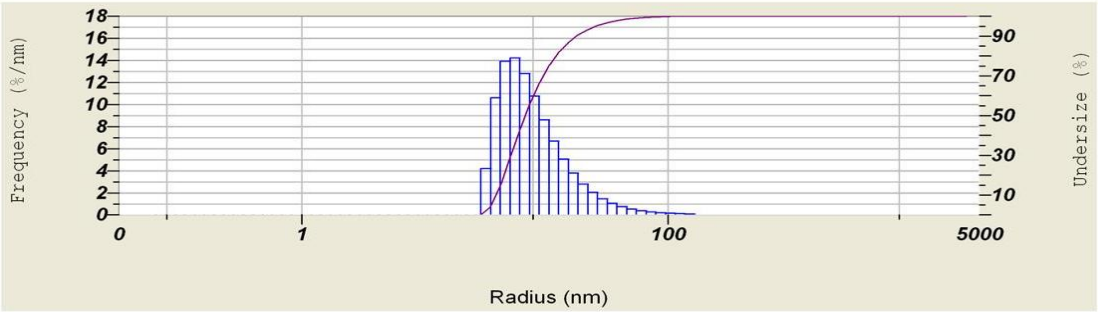
Date	:	Tuesday, February 10, 2015 10:48:31 AM
Measurement Type	:	Particle Size
Sample Name	:	git pharmy 4
Scattering Angle	:	90
Temperature of the holder	:	25.0 °C
T% before meas.	:	43061
Viscosity of the dispersion medium	:	0.895 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Volume
Count rate	:	0 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	20.0 nm	11.5 nm	14.5 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	20.0 nm	11.5 nm	14.5 nm

Cumulant Operations

Z-Average	:	0.9 nm
PI	:	6.448



No.	Radius	Frequency	Cumulation	No.	Radius	Frequency	Cumulation	No.	Radius	Frequency	Cumulation	No.	Radius	Frequency	Cumulation
1	0.17	0.000	0.000	27	4.05	0.000	0.000	53	96.74	0.179	99.728	79	2311.41	0.000	100.000
2	0.19	0.000	0.000	28	4.57	0.000	0.000	54	109.30	0.125	99.853	80	2611.48	0.000	100.000
3	0.22	0.000	0.000	29	5.17	0.000	0.000	55	123.49	0.087	99.940	81	2950.51	0.000	100.000
4	0.24	0.000	0.000	30	5.84	0.000	0.000	56	139.52	0.060	100.000	82	3333.55	0.000	100.000
5	0.28	0.000	0.000	31	6.60	0.000	0.000	57	157.63	0.000	100.000	83	3766.32	0.000	100.000
6	0.31	0.000	0.000	32	7.45	0.000	0.000	58	178.10	0.000	100.000	84	4255.28	0.000	100.000
7	0.35	0.000	0.000	33	8.42	0.000	0.000	59	201.22	0.000	100.000				
8	0.40	0.000	0.000	34	9.52	0.000	0.000	60	227.34	0.000	100.000				
9	0.45	0.000	0.000	35	10.75	4.188	4.188	61	256.86	0.000	100.000				
10	0.51	0.000	0.000	36	12.15	10.606	14.794	62	290.20	0.000	100.000				
11	0.57	0.000	0.000	37	13.72	13.889	28.683	63	327.88	0.000	100.000				
12	0.65	0.000	0.000	38	15.50	14.188	42.871	64	370.44	0.000	100.000				
13	0.73	0.000	0.000	39	17.52	12.793	55.664	65	418.54	0.000	100.000				
14	0.83	0.000	0.000	40	19.79	10.727	66.391	66	472.87	0.000	100.000				
15	0.94	0.000	0.000	41	22.36	8.586	74.977	67	534.26	0.000	100.000				
16	1.06	0.000	0.000	42	25.26	6.656	81.633	68	603.62	0.000	100.000				
17	1.19	0.000	0.000	43	28.54	5.043	86.676	69	681.98	0.000	100.000				
18	1.35	0.000	0.000	44	32.25	3.755	90.432	70	770.52	0.000	100.000				
19	1.52	0.000	0.000	45	36.44	2.759	93.190	71	870.55	0.000	100.000				
20	1.72	0.000	0.000	46	41.17	2.005	95.195	72	983.57	0.000	100.000				
21	1.95	0.000	0.000	47	46.51	1.445	96.640	73	1111.26	0.000	100.000				
22	2.20	0.000	0.000	48	52.55	1.033	97.673	74	1255.52	0.000	100.000				
23	2.48	0.000	0.000	49	59.37	0.734	98.407	75	1418.52	0.000	100.000				
24	2.81	0.000	0.000	50	67.08	0.519	98.927	76	1602.68	0.000	100.000				
25	3.17	0.000	0.000	51	75.79	0.366	99.293	77	1810.74	0.000	100.000				
26	3.58	0.000	0.000	52	85.62	0.256	99.549	78	2045.81	0.000	100.000				

1 / 1

Figure 6: Particle size distribution of formulation F1.

In vitro release studies using dialysis membrane

The release studies(as shown in figure 7) for formulation prepared from span 20 (F1) showed highest amount drug release of 77% and F2 formulation showed 70% of drug release for 24 hours. In similar way F3 and F4 also showed around 64% and 58% of drug release, while marketed ketoconazole marketed formulation showed only 75% of drug release for 24 hours of study.

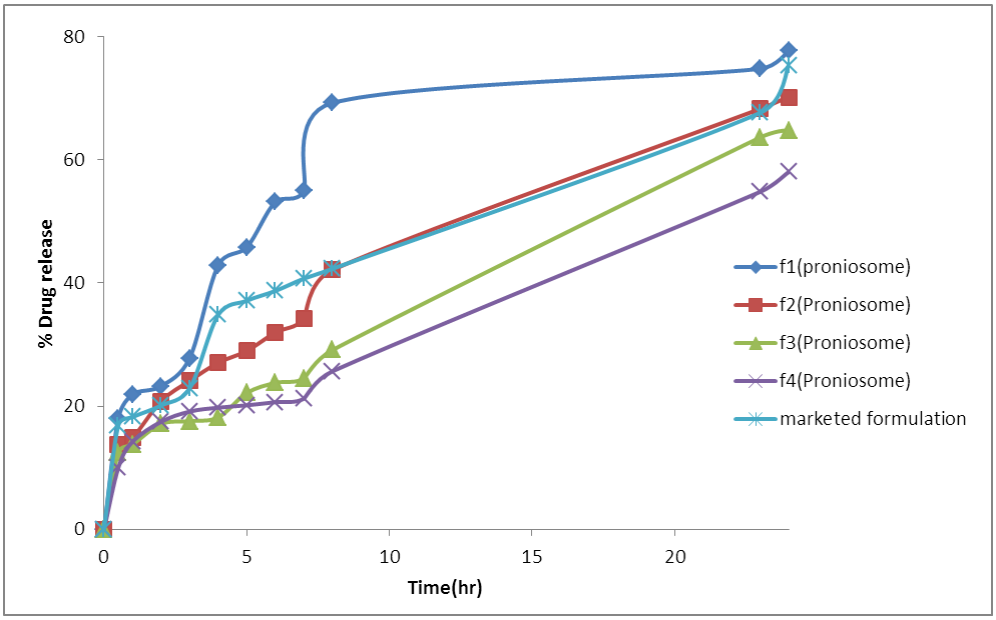


Figure 7: Comparative In vitro release profile of various proniosome gel formulations.

Microbiological assay (In vitro antifungal activity)

The results in Table 3 showed that the percentage inhibition. The inhibition was found to be linear in the concentration range of 1 to 5 µg/ml. Among them the formulation F1 showed highest percentage with 106% zone of inhibition after 24 hours at 5µg/ml concentration Graphs from all formulations (as shown in figure 8) showed linearity with straight lines on plot between log concentration and % inhibition and showed that on increasing drug concentration the percentage of inhibition also increased. The results also showed that there was a steady release of the drug and is capable of inhibiting microorganism *candida albicans*for 24 hrs. This indicated that the prepared proniosome formulation are efficient and also can inhibit the growth of microorganism *candida albicans*to high drug diffusion.

Table 3: Percentage Zone of Inhibition for prepared formulation

Formulation	Concentration (µg/mL)	log concentration	Inhibition diameter		% Inhibition
f1	1	0.00	26	30	86.67
	5	0.70	32	30	106.67
f2	1	0.00	22	30	73.33
	5	0.70	29	30	96.67
f3	1	0.00	20	30	66.67
	5	0.70	27	30	90
f4	1	0.00	18	30	60
	5	0.70	27	30	90
marketed	1	0.00	24	30	80
	5	0.70	30	30	100

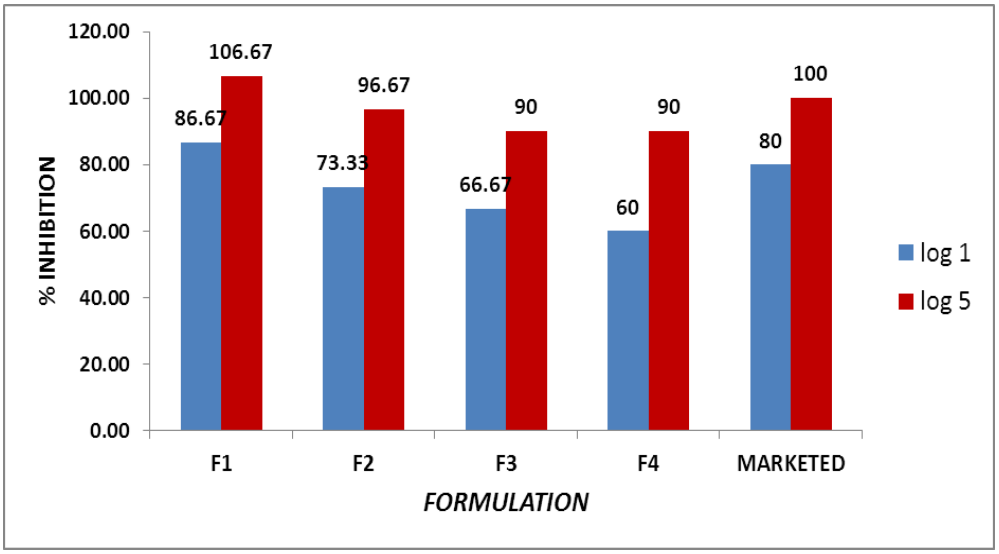


Figure8: Microbial assay of prosiomal formulation.

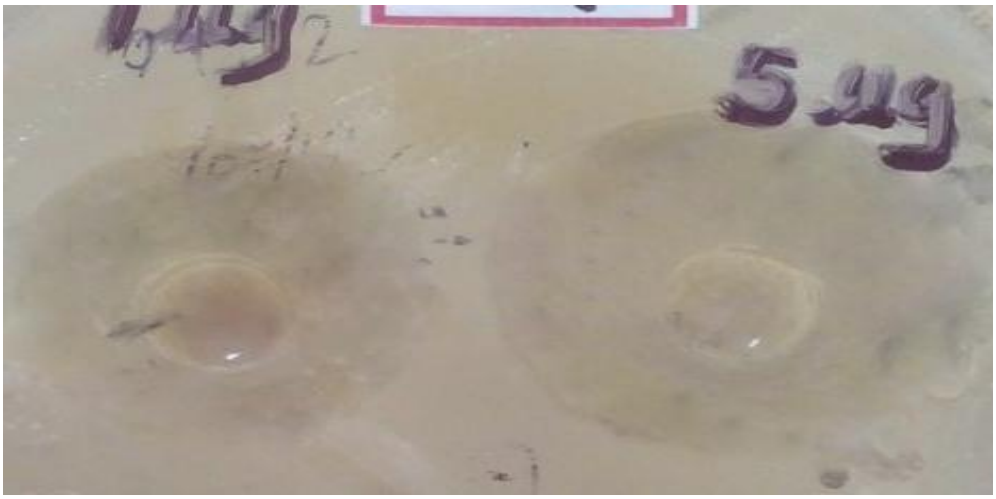


Figure 9: Microbiological assay studies and Zone of Inhibition for Formulation F1.

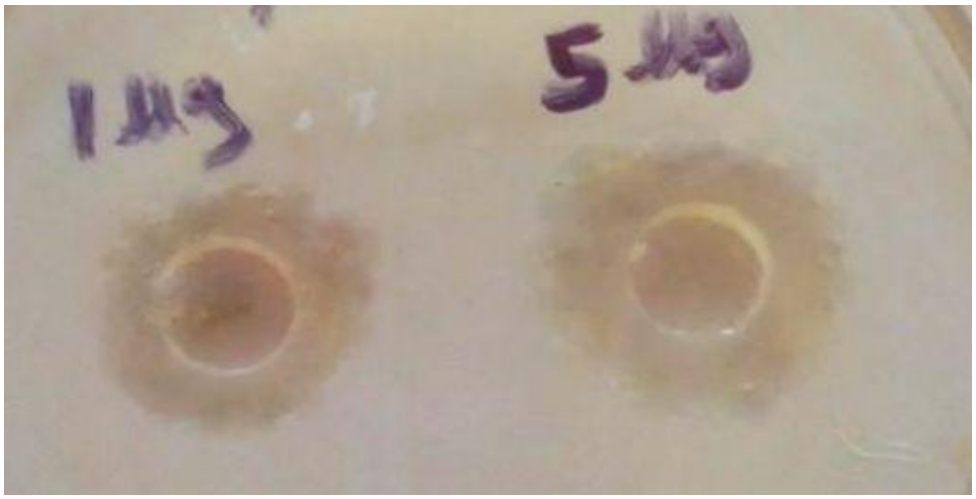


Figure 10: Microbiological assay studies and Zone of Inhibition for marketed formulation

CONCLUSION

In conclusion we are able to conclude that proniosome ketoconazole formulation shows continuous drug release out of all formulation made span 20 (F1) shows the highest quantity of encapsulation of drug compared of all alternative formulations. FTIR studies are also showed result that there is no interactions between the API and all the used excipients. SEM result conclude the size of the particle of F1 Formulation of proniosomal gel. Microbiological study also showed that there is a steady release of the drug and is capable of inhibiting microorganism *candida albicans* for 24 hrs. This indicated that the prepared proniosome formulation are efficient and also can inhibit the growth of microorganism *candida albicans* due to high drug diffusion.

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