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FORMULATION, OPTIMIZATION AND EVALUATION OF BIFONAZOLE LOADED PRONIOSOMAL GEL FOR TREATMENT OF FUNGAL INFECTION

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ABSTRACT
The present proniosomal gel investigation was aimed to minimize the adverse effect associated with present topical bifonazole formulation and made to enhance its bioavailability and sustained release by novel proniosomal drug delivery system. Bifonazole is an imidazole and triazole class of antifungal drug which mainly recommended to treat fungal infection. Proniosomes were prepared by coacervation phase separation method using different concentration of non-ionic surfactant (tween, span) and evaluated various parameter like entrapment efficiency, drug content, viscosity, in-vitro drug release, anti-fungal studies and stability studies. The result shows that formulation(F7) containing span 60 showed highest drug content (95.92%), entrapment efficiency(99.14%), in-vitro drug release(84.48%) compared to other formulation. The PH and viscosity of prepared proniosomal gel formulation were found to be 6.8 and 9546cps.

KEYWORDS: Bifonazole, Fungal infection, Proniosomes, coacervation phase separation method, Non-ionic surfactant, In-vitro drug release, Anti-fungal activity.

INTRODUCTION[1]
The aim of Novel Drug Delivery System is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug delivery system should deliver drug at a rate control by the necessarily of the body over a specified term of treatment. A number of novel drug delivery system has emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity if selective uptake can be achieved. Consequently a number of vesicular drug delivery system such as liposomes, niosomes, transfersomes and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of this system that allow drug targeting and sustained or controlled release of conventional drug medicines.

Advantages of vesicular drug delivery[2]
1. Prolong the existence of the drug in systemic circulation, and perhaps reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
2. Improves the bioavailability especially in the case of poorly soluble drugs
3. Both hydrophilic and lipophilic drugs can be incorporated
4. Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems Lipophilic biocarriers: Liposomes, Emulsomes, Virosomes, Enzymosome, Vesosomes, Ethosomes, Transferosomes.

Non-lipoidal biocarriers: Niosomes, Bilosomes

Approaches for improvement of vesicular sytem[3]
Pro vesicular drug delivery: Pro vesicular drug delivery developed to overcome the stability problems associated with vesicular drug delivery systems composed of water soluble porous powder as a carrier and drug is dissolved in an organic solvent to produce free flowing granular product. It can avoid many of the problems associated with aqueous vesicular dispersions.

Types of pro vesicular drug delivery system
1. Proliposomes

2. Proniosomes

Proniosomes[4]

Proniosomes are dry product which could be hydrated easily immediately before use would avoid many of the problems. These dry formulations of surfactant coated carrier can be measured as needed and rehydrated by brief agitation in hot water to form niosomal dispersion. These are considered superior drug delivery system because of their lower cost, greater stability, non-toxicity, biocompatible, biodegradable and non-immunogenic, as it is non-ionic in nature.

Mechanism of action[5]

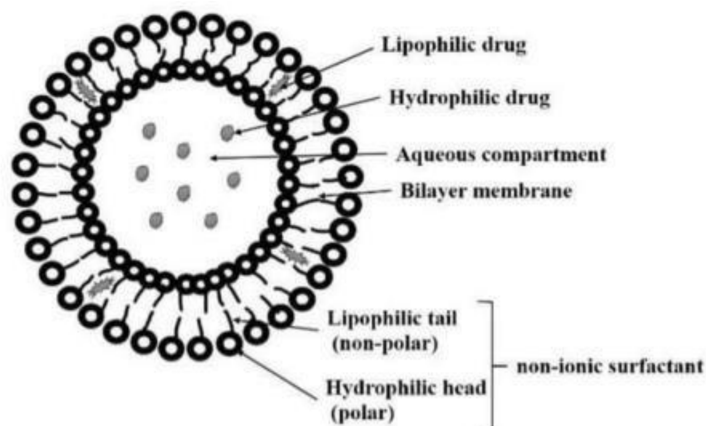
The exact mechanism of penetration of drug in the vesicles through the skin are not yet explored, but the penetration will depends on the nature and type of the drug used, vesicle formed and hydration temperature for the conversion of proniosomes to niosomes. The lipid used in the preparation, act as carrier that will form depot at the site of action and hence sustains the action. The rate limiting step in the penetration of drug through the transdermal drug delivery is the lipid(Ceramides) part of stratum corneum which packed tightly as bilayer by hydrogen bonding. The hydrogen bonding will strengthen and stabilize the lipid bilayer and as a result will impart the barrier property of stratum corneum. Proniosomes will hydrate to niosomes when applied to skin surface, the niosomes formed adsorbs fuses and loosens the ceramides by competitively breaking the hydrogen bond network leading to thermodynamic activity at the surface. That will increase by the concentration gradient and hence increases the diffusion pressure for the driving of drug through the stratum corneum.

Components of proniosomes[13]

Span and tween (Maintain HLB level, increase the fluidity of membrane), Cholesterol and Lecithin (penetration enhancer), Maltodextrin (holds the drug), Methanol and Chloroform (influence on vesicle size and permeability of drug).

Type of proniosomes[3]

1. Dry granular proniosomes
2. Liquid crystalline proniosomes



Advantages

1. Both the non-ionic surfactants and phospholipids in proniosomes can act as penetration enhancers and helps in diffusion of the drug.
2. Avoid problems associated with liposomes like degradation by hydrolysis or oxidation as well as sedimentation.

Disadvantages

1. Leaking of entrapped drug
2. Aggregation
3. Hydrolysis of encapsulated drug

Drug profile[6,7]

Bifonazole

1. Molecular formula - $C_{22}H_{18}N$
2. Molecular weight - 310.391
3. Chemical Name - 1 – ([1,1'- Biphenyl]-4-yl(phenyl) methyl)-1H-imidazole
4. Melting point – 142^0C
5. Category - Anti-fungal

Pharmacokinetics

1. Absorption - Well absorbed into infected skin layers; increased in inflamed skin
2. Metabolism - Hepatic
3. Elimination half - life 8 - 50 hours
4. Excretion - 45%isexcreted in urine and40% is eliminated in feces

Pharmacodynamic

Bifonazole is a type of antifungal medicine known as an imidazole. It kills fungi and yeasts by interfering with their cell membranes.

MATERIALS AND METHODS[12]

Materials

Drug – Bifonazole, Cholesterol, Soya lecithin, Ethanol and Methanol, Glycerol, Carbopol 934, Span 60, Span 40, Tween 60, Tween 40, Sodium hydroxide, Potassium dihydrogen ortho phosphate.

Methods

Carriers, non-ionic surfactants and membrane stabilizers are used for proniosomal preparation. Three different methods of preparation of proniosomes are

1. Slurry method
2. Coacervation phase separation method
3. Slow spray coating method

Procedure[12]

Coacervation phase separation method

Coacervation is usually defined as the spontaneous formation of a dense liquid phase from a macromolecular solution of poor solvent affinity. In coacervation the loss of salvation arises from the interaction of complementary macromolecular species. Coacervation is a phenomenon in which a macromolecular aqueous solution separates into two immiscible liquid phases. The denser phase, which is relatively concentrated in macromolecules is called coacervate and is in equilibrium with the relatively dilute macromolecular liquid phase. Liquid-solid separation is known as precipitation also it means coacervation.

Step1: Addition of lecithin, cholesterol, surfactant and drug Step 2: Addition of solvent

Step 3: Warmed on water bath at 60-70^{0}C Step 4: Add aqueous phase

Step 5: Finally, proniosomes are prepared.

Formulation table

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8
Drug (mg)	10	10	10	10	10	10	10	10
Cholesterol (mg)	75	75	75	75	75	75	75	75
Lecithin (mg)	250	250	250	250	250	250	250	250
Tween 40 (mg)	600	450	-	-	-	-	-	-
Tween 60 (mg)	-	-	600	450	-	-	-	-
Span 40 (mg)	-	-	-	-	600	450	-	-
Span 60 (mg)	-	-	-	-	-	-	600	450
Ethanol (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

RESULTS AND DISCUSSION

Preformulation studies[8]

Preformulation studies on the obtained sample of drug were performed for identification and compatibility studies.

Physical appearance

The drug (bifonazole) powder was examined for its organoleptic properties like color and odour.

Solubility estimation

Solubility of Bifonazole pure drug was tested in distilled water, methanol and phosphate buffer pH 6.8.

S. NO	Medium	Solubility profile
1.	Methanol	Freely soluble
2.	DMSO (Dimethyl sulfoxide)	Freely soluble
3.	Phosphate buffer pH 6.8	Sparingly soluble
4.	Distilled Water	Poorly soluble

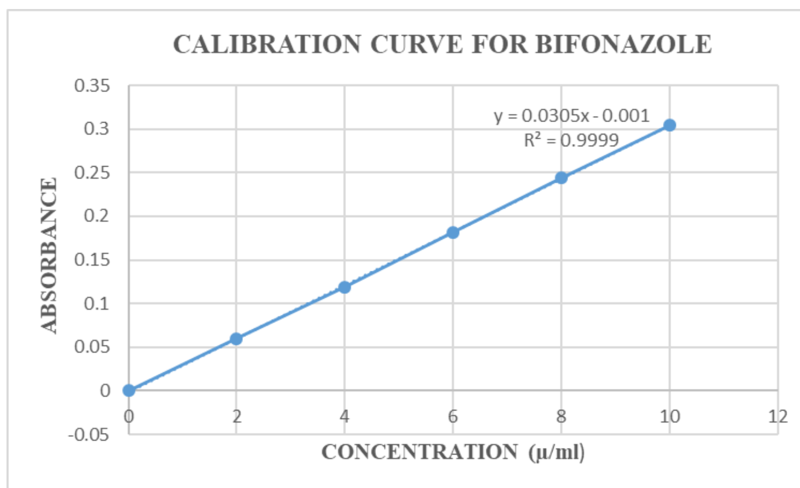
Determination of melting point

Small amount of drug was loaded in a capillary tube where one of capillary tube was closed and kept in the melting point apparatus and temperature was noted when drug melts.

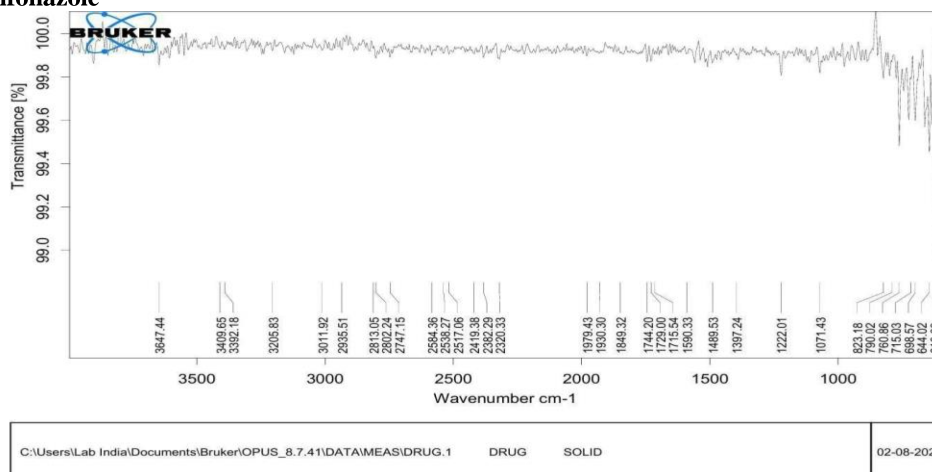
S. No.	Melting Point (°C)	Average
1.	143	142°C
2.	141	
3.	142	

Determination of wavelength of maximumabsorbance

10mg of Bifonazole was weighed and transferred to 100 ml volumetric flask. The drug was dissolved in 5 ml of methanol and the volume was made upto 100 ml with phosphate buffer pH 6.8.10 ml from this above solution was again diluted with phosphate buffer pH 6.8 upto 100 ml.10 ml from the above solution was pipetted into 100 ml volumetric flask. The volume was made upto 100 ml using phosphate buffer pH 6.8 to get a concentration of 10 µg/ml. This solution was then scanned at 254nm in UV-Visible spectrophotometer between 200-400 nm and obtained the absorption maximum (λmax).



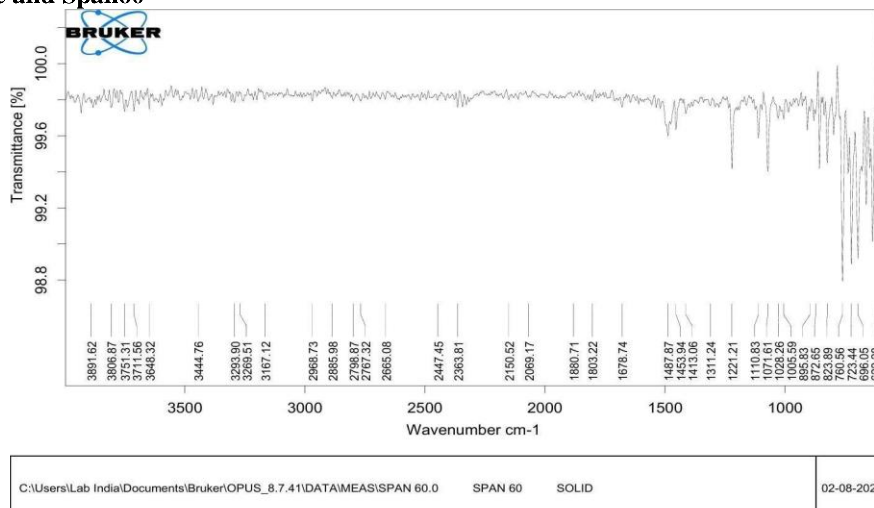
Ftir study of bifonazole



Ir spectrum of bifonazole

S. No.	Types of vibrations	Wave number
1.	C-H	3205.83 cm^{-1}
2.	C=N	1590.33 cm^{-1}
3.	C-N	1222.01 cm^{-1}
4.	C-C	1071.43 cm^{-1}

Ftir of Bifonazole and Span60

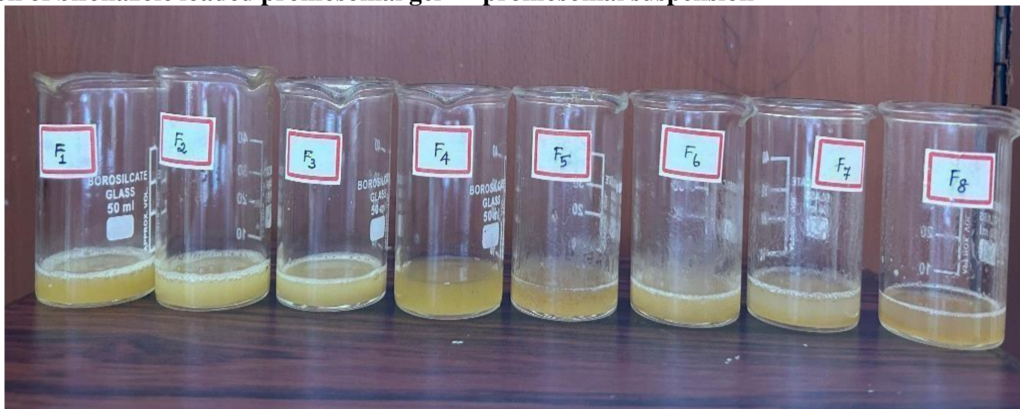


Ir of Bifonazole and Span 60

S. NO	Types of vibrations	Wave number
1.	C-H	3293.90 cm^{-1}
2.	C=O	1678.74 cm^{-1}
3.	O-H	3444.76 cm^{-1}
4.	C-O	1221.21 cm^{-1}

The peak observed in the FT-IR spectrum of Bifonazole and span 60 showed no shift and no disappearance of the characteristic peaks of drug. This suggest that there is no interaction between the drug and the span 60.

Formulation of bifonazole loaded proniosomal gel[11] proniosomal suspension



Optimized proniosomal gel

Characterization of proniosomal formulation[9,11]

The physical appearance of the prepared gels were tested by visual observations after the gel had been set in the container.

Physical appearance of proniosomal gel

Formulation code	Colour
F1	Pale yellow
F2	Whitish yellow
F3	Whitish yellow
F4	Pale yellow
F5	Dark yellow
F6	Pale yellow
F7	Light pale yellow
F8	Dark yellow

Entrapment efficiency[11]

The proniosomal suspension (0.3g) was taken in a test tube and reconstituted with 10 ml Isotonic phosphate buffer of pH 6.8. This aqueous solution was sonicated in a sonicator bath. The drug containing niosomes were separated from the dispersion by centrifugation at 3000 rpm for 30 min at 20 °C. The supernatant (1 ml) was taken and diluted with phosphate buffer (in 10 ml volumetric flask). And again, from stock solution, 1 ml was withdrawn and transferred to a 10 ml volumetric flask and made upto the mark with buffer. The drug concentration in the resulting solution was assayed by UV-visible spectroscopy method. The percentage of drug encapsulation was calculated by the following formula

EE% = [(Ct- Cr)/Ct] X 100

Where, EE = Entrapment Efficiency,

Ct = Concentration of total drug,

Cr = Concentration of unentrapped drug

Entrapment Efficiency was proceeded using centrifuge

and analysed using UV- Spectrophotometer.

Entrapment Efficiency of proniosomal suspension

Formulation code	Entrapment efficiency (%)
F1	97
F2	98.41
F3	98.4
F4	97.14
F5	99.02
F6	98.57
F7	**99.14**
F8	98.25

The % Entrapment Efficiency was found to be in the range of **97 to 99.14.** Increase in concentration of surfactant increases the entrapment efficiency of proniosomes due to high fluidity of the vesicles.

Drug content[9]

Drug content is one of the important evaluation parameter for any type of dosage form which is determined by UV spectrophotometer. A specific quantity (10 mg) of proniosomal dispersion was taken and dissolved in 100 ml of phosphate buffer of pH 6.8. The volumetric flask containing dispersion was shaken for 2hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at 254 nm using phosphate buffer (pH 6.8) as blank.

Table 9.13: Drug content of proniosomal gel.

Formulation code	Drug content (%)
F1	54.05
F2	49.30
F3	74.74
F4	53.51
F5	88.53
F6	81.48
F7	**95.92**
F8	89.07

The % drug content of all formulations was in the range of **49.30-95.92%** indicating uniform distribution of drugs in all formulations.

***In-vitro* drug release[11]**

No air bubbles were seen between the egg membrane and the liquid surface of PBS. The temperature was maintained at 37 °C±0.5 at 50rpmusing a magnetic stirrer. 0.5 ml of the sample was withdrawn from the receptor chamber side tubeat the time interval of 15 min, 30 min, 45 min, 1h, 2h, 4h, 6h, 8h, 12h, and 24h and equilibrated with a new or fresh dissolution medium to maintain a sink state. An *in-vitro* drug release study was performed using Franz diffusion cell assembly. An in vitro drug release study was performed by using Franz diffusion cell assembly. It consists of two compartments, one of the receptor chambers containing a Phosphate Buffer Saline(PBS) of pH 6.8 and another donor compartment containing proniosomal gel of 5 mg of the drug. The egg membrane was placed in contact with PBS filled in the receptor compartment to avoid disruption in the ongoing process; it was ensured that Suitable dilution was carried out and was spectroscopically analyzed at a λ max of 254 nm using UV-visible spectroscopy.

Formulation code	% Cumulative drug release
F1	60.03%
F2	54.05%
F3	52.15%
F4	53.17%
F5	70.91%
F6	76.78%
F7	**84.48%**
F8	80.38%

The optimized proniosomal formulation showed sustained drug release. The cumulative % drug release at 8hrs for optimized formulation was found to be **84.48%**

Characterization of optimized proniosomal gel[9,10,11]

pH

The pH measurements are performed using a digital pH meter. Before measurements, the pH meter was calibrated and readings were taken by dipping the glass electrode into the gel formulations.

The pH of the optimized Bifonazole proniosomal gel was found to be **6.8**

Viscosity

The measurement of the viscosity of the prepared proniosomal gel was done with a Brookfield Viscometer (DV-E). 10g of gel formulation was measured by rotating the spindle 64 at 12 rpm at 37 °C because gel comes under the High Viscosity (HA) category.

The viscosity of the optimized proniosomal gel was found to be **9546 cps.**

Spreadability

0.5 g of gel was placed within a circle of 1 cm diameter pre- marked on a glass plate over which a second glass plate was placed. A weight of 50 g was allowed to rest on the upper glass plate. Due to spreading of the gels, the diameter was increased and noted.

The time in seconds taken in order to separate the two slides was measured as spreadability.

S= m XL/t

Where,

S – Spreadability m – weight of load

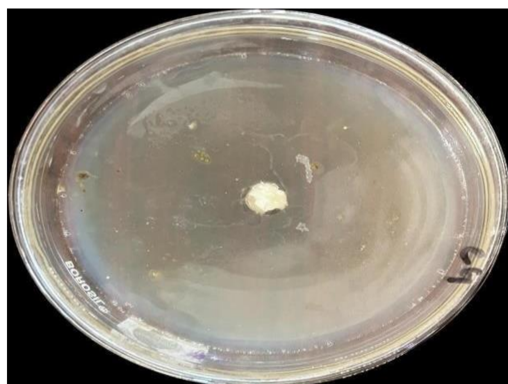
L – length travelled by upper slide

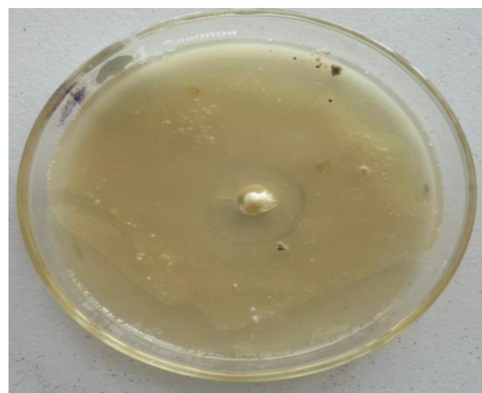
t – time in seconds.

The Spreadability for the optimized proniosomal gel was determined and was found to be **4 cm**.

Antifungal studies

The micro-organism used for antifungal studies is *MOULD* which is taken from the retard bread. Zone of inhibition for optimized proniosomal gel after 24hours is **1.5 cm.**





Stability studies of optimized formulation

The optimized proniosomal gel were kept at room temperature and it was subjected to stability studies as per ICH guidelines.

Table 9.16. Data for stability studies.

Parameter	Condition: at room temperature	
	Initial	**After 1 month**
Visual Appearance	Light Pale yellow	Light Pale yellow
Homogenecity	Homogenous	Homogenous
PH	6.8	6.8
Viscosity	8048	8048
Spreadability	5.19 cm	5.12 cm
Entrapment Efficiency (%)	97.21%	96.26%
Drug Content (% w/v)	93.97%	92.14%
In-vitro Drug Release (%)	82.07%	81.45%

SUMMARY AND CONCLUSION

The purpose of this research was to prepare Bifonazole loaded proniosomes for sustained release of drug and incorporate it into topical gel delivery system to reduce the side effects by site specific targeting. The Chemical compatibility study of Bifonazole with excipients was carried out using FTIR Spectrometer. It revealed no interaction between the drug and excipients. Calibration curve was plotted for Bifonazole and it was found that the solutions show linearity (0.999). Solubility of the pure drug was determined. Bifonazole pure drug in distilled water was found to be insoluble. The entrapment efficiency of the optimized Bifonazole loaded proniosomal formulation was observed to be 99.14%.

The drug content was found to be 95.92%. In vitro release of optimized Bifonazole loaded proniosomal formulation was found to be 84.48%. The shape and surface morphology of optimized Bifonazole loaded proniosomal formulation was observed in Scanning Electron Microscopy. It showed that the proniosomes were spherical and discrete in morphology. The optimized formulation was subjected to room temperature, accelerated stability study (temperature 40°C±2°C and RH 75±5%) and refrigerator temperature (4±20C). The results show no significant change in appearance, drug entrapment efficiency and drug content of optimized formulation after one month. It is concluded that coacervation phase separation technique is a best method for the successful incorporation of poorly water soluble drug Bifonazole into proniosomes with high entrapment efficiency. The administration of the drug as gel type formulation enhances its penetration across stratum corneum and reduce the side effects by site specific targeting.

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