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Original Research Article

FORMULATION AND EVALUATION OF ETHOSOMAL GEL OF MICONAZOLE NITRATE FOR FUNGAL INFECTION BY COLD METHOD

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ABSTRACT

The present study investigates the entrapment of miconazole in ethosomal vesicles prepared by cold method. The composition includes phospholipid, ethanol and polyethylene glycol. Drug entrapment efficiency (DEE), *invitro* drug diffusion studies, FT-IR and stability studies of the prepared ethosomes were investigated. Vesicle size and zeta potential of the ethosomes were measured by photon correlation spectroscopy using a Malvern Zetasizer. *Invitro* drug release studies were performed using modified franz diffusion cell in phosphate buffer, pH 7.4 for 8 h while drug content was determined by UV. DEE was ranked from 72.23±0.19 to 86.69±0.12 %. Highest DEE was seen with F4 ethosomal formulation with a vesicle size of 178.5±5.6nm. FT-IR studies confirmed that there was no chemical interaction between drug and excipients used in the formulation. The selected formulation (F4) was incorporated in to gel using Carbopol 934 and evaluated for pH, washability, extrudability study, spreadability, viscosity, % assay and % drug release. Selected ethosomal gel (EG2) showed a drug content of 95.56±0.12 % and drug release of 95.45±2.45 % in 8 hrs. This study disclosed that EG2resides at targeted site for a relatively longer period of time thereby signifying the improved patient compliance.

Key words: Ethosomes, Miconazole, Ethanol, Carbopol 934, In-vitro drug diffusion.

INTRODUCTION:

Targeting drugs by a carrier system has been a central theme of research in therapeutics. Several approaches have been investigated to deliver the drugs via topical route. (Pratik et al., 2014). Ethosomes are soft malleable vesicles constituting phospholipids, ethanol (relatively high concentration) and water (Touitou et al., 1999). They act as non-invasive delivery carriers for targeting drugs to deep skin layers. Hence, when integrated into a vesicles membrane, ethosomes promote the vesicle to penetrate the stratum corneum (Bendas et al., 2007). The utility of ethosomes as a carrier of antiviral drug acyclovir was previously tested by Essa and coworkers for the topical treatment of herpetic infection. They demonstrated significant improvement of ethosomal 5% acyclovir system as compared to a 5% acyclovir cream by performing two-

armed, double-blinded, randomized clinical trial (Essa et al., 2003). In another study, enhanced drug delivery via ethosomal carrier was observed by an increase in depth and fluorescent activity (Godin et al., 2003). A synergistic mechanism was suggested between ethanol, vesicles and skin lipids by Touitou and coworkers for elucidating the role of ethosomes in promoting enhanced drug delivery (Touitou et al., 2001). It was proposed that ethanol effect resulted in an interaction of ethanol with the lipid molecules in the polar head group region and exhibits reduction in the transition temperature of lipids in the stratum corneum, which ultimately increases their fluidity and decreases the density of lipid multilayer. This effect is followed by the ethosomal effect which involves the penetration and permeation of lipids due to the malleability and fusion of ethosomes with skin lipids. This step resulted in the release of drug into the deep layers of skin. It should be noted that since ethanol imparted flexible characteristics to vesicles, it allowed the ethosomal vesicles easier and deeper penetration into the deeper layers of the skin. The release of the drug in the deep layers of the skin and its transdermal absorption could then be the result of a fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway (Elsayed et al., 2006). The pharmaceutical technology in recent years has witnessed the formulation of modified liposomes for skin mediated drug delivery and in this regard, considerable attention has been paid to vesicular approaches involving transfersomes and ethosomes. These approaches utilize non-toxic and biodegradable chemicals which prolong half-life of a drug in order to provide a sustained drug delivery release effect (Verma et al., 2003; Cosco et al., 2008). Ethosomal systems are very efficient in delivering substances in terms of quantity and depth by increasing cell permeability/lipid fluidity (Touitou et al., 2000; Sinha et al., 2004; Pardeike et al., 2009). Miconazole is an imidazole antifungal agent and commonly applied topically to the skin or mucus membranes to cure fungal infections. It works by inhibiting the synthesis of ergosterol, a critical component of fungal cell membranes. It can also be used against certain species of Leishmania protozoa (which are a type of unicellular parasite), as these also contain ergosterol in their cell membranes. In addition to its antifungal and antiparasitic actions, it also has some limited antibacterial properties. It is marketed in various formulations under various brand names. Miconazole is also used in Ektachrome film developing (along with surfactants such as Photo-Flo) in the final rinse of the Kodak E-6 process and similar Fuji CR-56 process, replacing formaldehyde. Miconazole is also identified as a final rinse additive in their formulation of the process C-41RA rapid access color negative developing process. Miconazole is mainly used externally for the treatment of athlete's foot, ringworm and jock itch. Internal application is used for oral or vaginal thrush (yeast infection). In addition, the oral gel may also be used for the lip disorder angular cheilitis (Sarinnate et al., 2007; Agarwal et al., 2002). Hence, the objective of the present study involves the formulation of ethosomes containing Carbopol 934 and phospholipids as vesicle forming agent along with miconazole to observe its effect at targeted site for a relatively longer period of time with pappas model of drug release kinetics.

EXPERIMENTAL

Materials

Miconazole nitrate was obtained as a gift sample from Dr. Reddy's, Hyderabad. Phospholipid, polyethylene glycol, Carbopol 934 was purchased from Sigma-Aldrich Chem, Germany. High purity 99.9% Ethanol were obtained from SD Fine chemicals, Mumbai, India. All other chemical and materials were of analytical grade. Triple distilled water was generated in house.

Formulation of miconazole loaded ethosomes by cold method

According to this method, the ethanolic vascular system was composed of phospholipid, ethanol, polyethylene glycol, drug and distilled water to 100% (V/V). Phospholipid, polyethylene glycol was dissolved along with the drug in ethanol. This mixture was heated to 40^0C ± 1^0C and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 40^0C ± 1^0C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 4^0 C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator. Six ethosomal formulae were presented in Table 1.

Table 1 Different composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid (mg)	Ethanol (ml)	PEG (mg)	Water (ml)
F1	50	250	10	10	100
F2	50	250	20	10	100
F3	50	250	30	10	100
F4	50	250	40	10	100
F5	50	250	50	10	100
F6	50	250	60	10	100

Evaluation of miconazole loaded ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation.

Vesicle size and zeta potential

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a Malvern Zetasizer.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the eppendorf tubes and centrifuged at 17000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain SLN without budesonide was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 278 nm. The entrapment efficiency was determined by the following equation,

EE% = (Total drug) - (free drug)/ Total drug X 100.

Formulation of ethosomal gel

The incorporation of the drug loaded ethosomes (equivalent to 2%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w.

Evaluation of gel

Physical characteristic

The **Physical** Characteristic was checked for gel formulations (homogeneity and texture).

Determination of pH

The pH of the gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Extrudability study: The gel formulations were filled into collapsible metal tubes or

aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Spreadability

An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. It is determine by formula given below.

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)
m = weight tied to the upper slide (20 grams)
l= length of glass slide (6cms).
t = time taken is seconds.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25^0C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 $\pm/1^0$C) before the measurements.

***In-vitro* drug release studies using the prehydrated cellophane membrane**

The cellophane membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 200 ml dissolution medium which was stirred at 50 rpm maintained at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 278 nm for Miconazole and using UV visible spectrophotometer. The release of Miconazole was calculated with the help of Standard curve of Miconazole.

Release kinetics

In-vitro diffusion has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where ft is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug products model dependent (curve fitting), statistic analysis and model independent methods can be used.

In order to elucidate mode and mechanism of drug release, the *invitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low soluble drugs, coated forms, osmotic systems

etc., where the drug release is independent of concentration.

$$Qt = Qo + Kot \quad (1)$$

Where, Qt is the amount of drug released in time t, Qo is the initial amount of the drug in the solution and Ko is the zero order release constant

The first order Eq. (2) describes the release from the system where release is concentration dependent e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

$$\log Qt = \log Qo + K1\ t /2.303 \quad (2)$$

Where Qt is the amount of drug released in time t, Q is the initial amount of drug in the solution and K1 is the first order release constant.

Higuchi described the release of drug from insoluble matrix as a square root of time as given in Eq. (3)

$$Qt = KH \sqrt{t} \quad (3)$$

Where, Qt is the amount of drug released in time t, KH is Higuchi's dissolution constant.

The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model).

Korsemeyer-Peppas

The curves plotted may have different slopes, and hence it becomes difficult to exactly pin-point which curve follows perfect zero order release kinetics. Therefore, to confirm the kinetics of drug release, data were also analyzed using Korsemeyer's equation.

$$Q_t/Q_\infty = k_{KP}.t^n$$

Where Q_t/ Q_∞ is the fraction of drug released at time t, k_{KP}a constant compromising the structural and geometric characteristics of the device and n is the release exponent.

The slope of the linear curve gives the 'n' value. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When n = 1, the release rate is independent of time (typical zero order release / case II transport); n = 0.5 for Fickian release (diffusion/ case I transport); and when 0.5 < n < 1, anomalous (non-Fickian or coupled diffusion/ relaxation) are implicated. Lastly, when n > 1.0 super case II transport is apparent. 'n' is the slope value of log M_t/M_∞ versus log time curve.

Stability studies

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer.

RESULT AND DISCUSSION

From the preformulation studies, it was found that miconazole was freely soluble in methanol, ethanol and 7.2 phosphate buffers, slightly soluble in chloroform, 0.1N NaOH and 0.1N HCL. IR spectra of pure miconazole were given in fig 1. Melting point was determined by melting point apparatus and found to be 159-162°C. The absorption maxima of miconazole were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (LABINDIA UV 3000 +) using concentration range of 5-25µg/ml miconazole in 7.2 phosphate buffers fig 2 & 3.

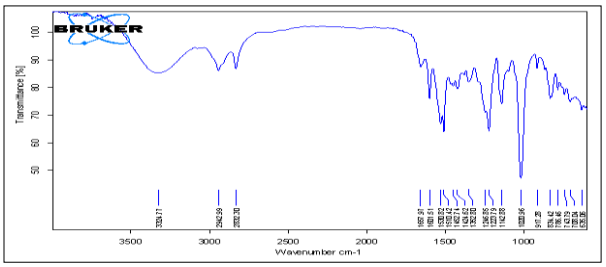

Fig. 1 IR spectra of miconazole

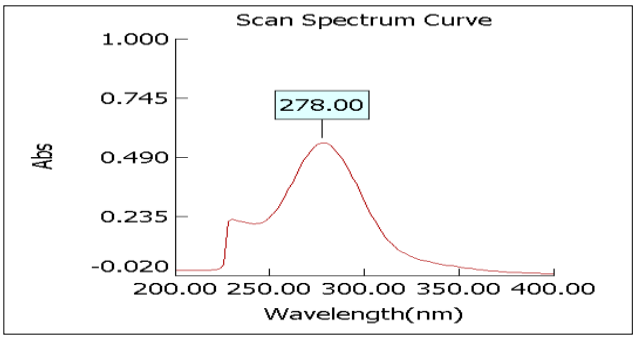

Fig. 2 Determination of λ max of miconazole

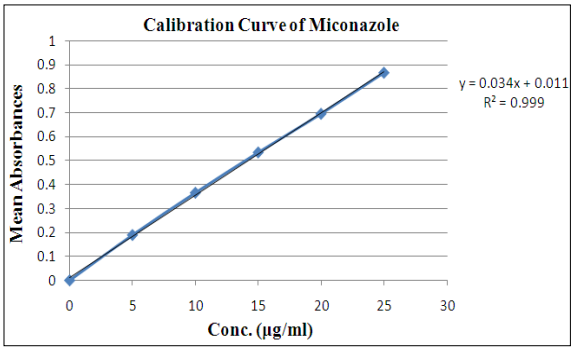

Fig. 3 Calibration curve of miconazole

To observe the shape of the prepared ethosomes formulation by using optical microscope fig 4. Vesicle size and zeta potential of the ethosomes were measured by photon correlation spectroscopy using a Malvern Zetasizer and Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium by UV spectrophotometer the results shown in table 2 and fig 5 & 6.

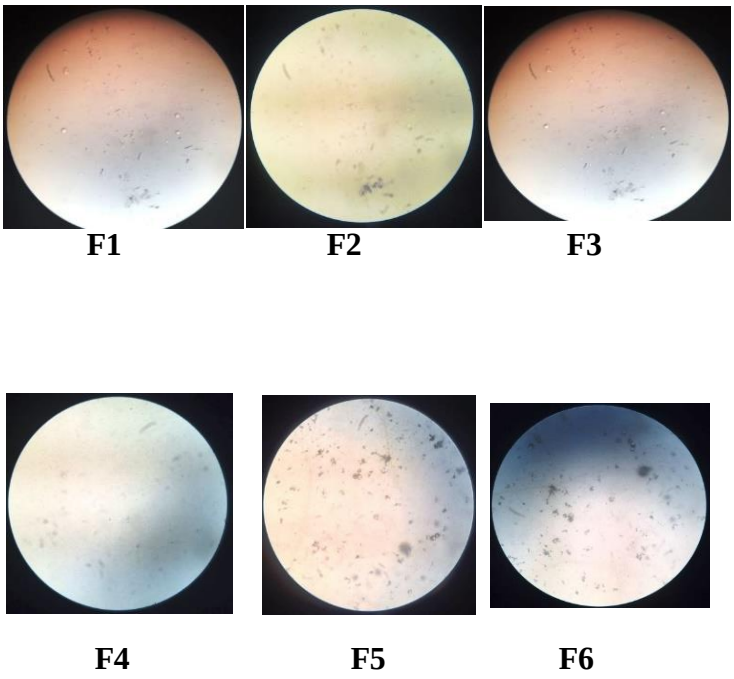

Fig. 4 Microscopic observations of prepared ethosomes formulations

Table 2 Result for vesicle size and entrapment efficiency of drug loaded ethosomes

F. Code	Vesicle size	EE (%)
F1	278.4±8.5	80.40±0.68
F2	265.6±7.6	78.59±0.11
F3	254.3±9.4	82.85±0.25
F4	178.5±5.6	86.69±0.12
F5	245.5±11.4	76.55±0.22
F6	230.5±14.2	72.23±0.19

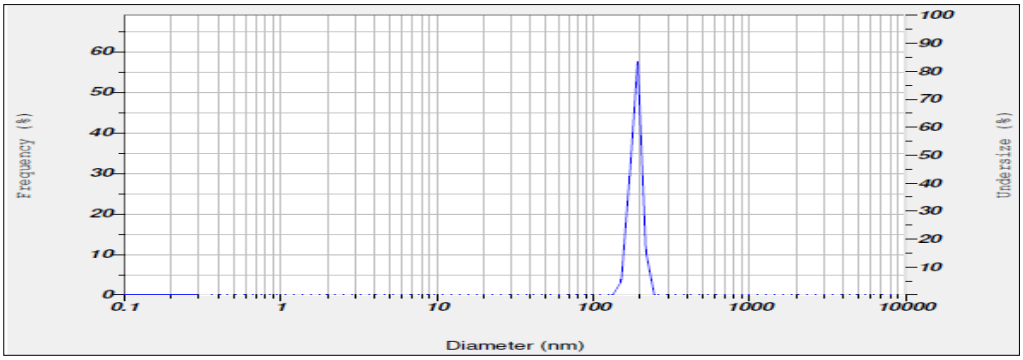

Fig. 5: Vesicle size of optimized formulation F4

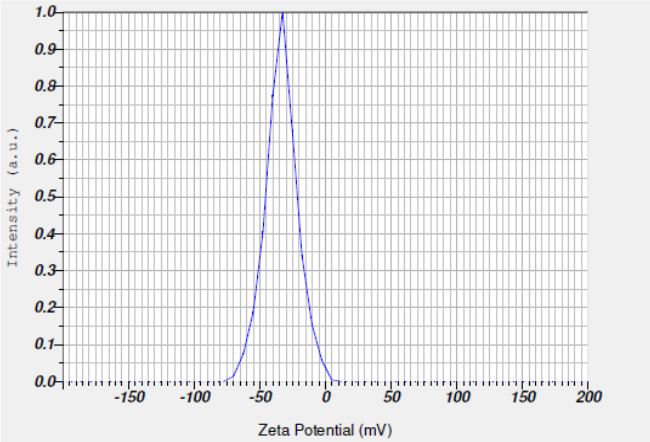

Fig. 6 Zeta potential of Optimized formulation F4 (-33.6)

Results of evaluation of ethosomal gel formulation (EG1- EG3) of optimized formulation (F4) were incorporated into three different carbapol gel concentration 0.5, 1 and 2% w/w respectively. Formulation EG2 was found to be good Table 3. Results of *In-vitro* drug release from optimized formulation (EG2) are given in table 4 was found 95.45±2.45 after 8 hrs. The *in vitro* drug release data of the formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Korsmeyer's -pappas models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of formulation was maximum i.e 0.924 hence indicating drug release from formulations was found to follow Korsmeyer's -pappas model of drug release kinetics table 5 & 6 and Fig 7-9.

Table 3 Results of evaluation of gel formulation

Code	Homogeneity and Texture	pH	Spreadability (gm.cm/sec)	Viscosity (cps)	% Assay
EG1	**Good**	7.3±0.21	13.15±0.12	2056	90.25±0.21
EG2	**Good**	7.1±0.15	14.22±0.25	2345	95.56±0.12
EG3	**Good**	7.2±0.04	16.32±0.36	2565	91.25±0.25

Table 5 In Vitro drug release data for EG2

Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
0.5	0.707	-0.30103	25.65	1.409	74.35	1.871
1	1.000	0	45.58	1.659	54.42	1.736
2	1.414	0.30103	68.98	1.839	31.02	1.492
4	2.000	0.60206	76.65	1.885	23.35	1.368
6	2.449	0.778151	85.58	1.932	14.42	1.159
8	2.828	0.90309	95.45	1.980	4.55	0.658

* Average of three determinations

Table 6 Regression analysis data of ethosomal gel formulation

Formulation	Zero order	First order	Pappas plot
EG2	R^2 = 0.830	R^2 = 0.891	R^2 = 0.924

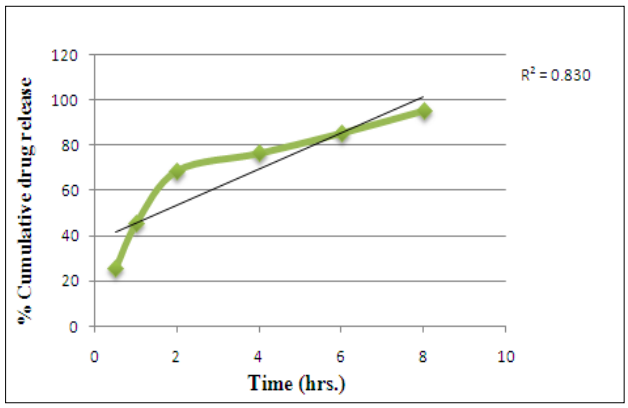

Fig. 7 Cumulative % drug released vs time (Zero Order Plots)

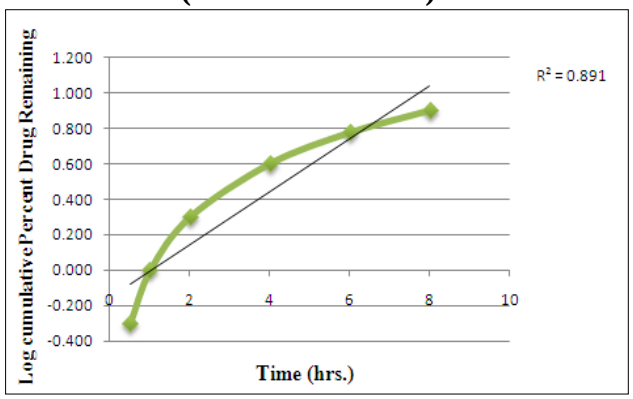

Fig. 8 Log cumulative % drug remaining vs time (First Order Plots)

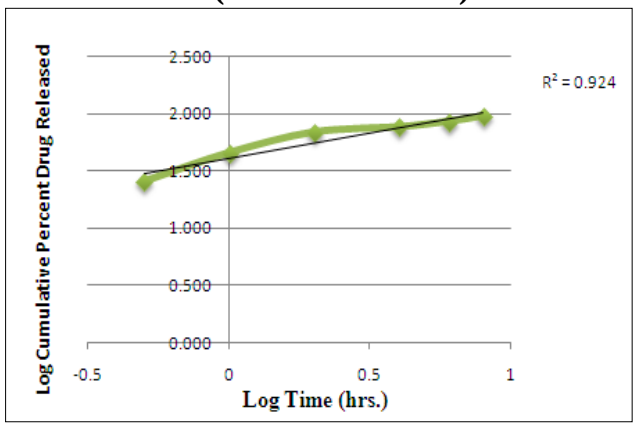

Fig. 9 Log cumulative % drug released vs log time (Peppas Plots)

The particle size of the ethosomes was found to increase at RT, which may be attributed to the aggregation of ethosomes at higher temperature. At 45±2°C the aggregate i.e. these ethosomes were unstable at higher temperature like 45±2°C .Percent efficiency of ethosomes also decrease at higher temperature like 45±2°C.

Conclusion

Miconazole loaded ethosomal formulation was successfully prepared by loading phospholipids and ethanol and ethosomal gel based formulations were prepared with hydrophilic polymer Carbopol 934. It can serve as a useful vehicle for the delivery of miconazole through the affected part of the skin for extended period of time. This study also revealed that ethosomal gel (EG2) resides at targeted site for a relatively longer period of time with a Korsmeyer's - pappas release profile. It signifies the improved patient compliance.

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