Bulletin of Pharmaceutical Research 2014;4(1):1-8
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF TOPICAL GEL CONTAINING HAIR GROWTH PROMOTERS FOR THE TREATMENT OF ANDROGENIC ALOPECIA

Eby George* and Manju Maria Mathews

Dept. of Pharmaceutics, Nirmala College of Pharmacy, Muvattupuza-686 673, Ernakulam, Kerala, India

**E-mails*: ebykunnappillil@gmail.com, manjumaria4@rediffmail.com
Tel.: +91 9249985184.

Received: December 18, 2013 / Revised: January 06, 2014 / Accepted: January 07, 2014

The objective of present work was to develop and evaluate a Minoxidil emulgel and compare its properties with Minoxidil gels. When gel and emulsion are used in combination the dosage form is referred as Emulgel. For preparing the emulgel, first, Minoxidil was dissolved in solvent system comprising water and propylene glycol in ratio 35:15 with liquid paraffin as oil phase. The prepared *w/o* emulsion was then mixed with carbopol gel solution in 1:1 ratio and finally neutralized with triethanolamine to form emulgel. Total eight formulations were prepared of which four were gels and other four were Emulgels. The gels were evaluated for physicochemical parameters, *in vitro* drug release and *ex vivo* permeation study. Among developed formulations, F1 showed 56.30% cumulative release after 8 h, whereas F6 showed 72.31% release after 8 h.

Key words: Minoxidil, Emulgel, Spreadability, Carbopol 934, Alopecia.

INTRODUCTION

Androgenetic alopecia occurs in both men and women and is characterized by the progressive loss of hair from the scalp in a defined pattern. Alopecia means hair loss which is the most common problem of modern societies, which create much economical and psychological effect; affecting about 70% males and 30% females. Recently, a great effort has been made to treat hair loss or alopecia. One of the most common types of alopecia is androgenic alopecia and alopecia areata (Kaur *et al* 2010).

Chemically, Minoxidil is 2, 4-diamino-6-piperidinopyrimidine-3-oxide**,** soluble in water to the extent of approximately 2 mg/ml, is more readily soluble in propylene glycol or ethanol, and is nearly insoluble in acetone, chloroform, or ethyl acetate (Lowenthal and Affrime, 1980). Minoxidil was introduced in the early 1970s as a treatment for hypertension. Hypertrichosis was a common side-effect in those taking Minoxidil tablets and included the regrowth of hair in male balding. Topically applied Minoxidil was shown to improve blood flow in human balding scalp. A topical formulation of Minoxidil then was developed to exploit this side effect (Gupta *et al* 2012). This led to the development of a topical formulation of emulgels which are, emulsions, either of the oil-in-water or water in oil type; gelled by mixing with a gelling agent. There is no marketed formulation of minoxidil emulgel till date. Therefore, present research has been undertaken with the aim to develop an emulgel formulation of minoxidil.

MATERIAL AND METHODS

Materials

Minoxidil (Yarrowchem Products, Mumbai), Carbopol 934 (Himedia laboratories Private Ltd, Mumbai), Propylene glycol, Triethanolamine, Ethanol, Propyl paraben, Span 80, Light liquid paraffin and mentha oil were purchased from Nice Chemicals, Kochin. All other chemicals and reagents used were of the analytical grade.

De-ionized distilled water was used throughout the study.

Methods

Determination of solubility of Minoxidil

The solubility studies were performed in distilled water, by adding excess amount of drug in each case and keeping the flasks containing excess amount of drug containing phosphate buffer pH 7.4 on a rotary shaker for 24 h. After 24 h, solutions were analyzed spectro-photometrically at 275.6 nm, which was the absorption maxima determined earlier and drug concentrations were calculated.

Determination of partition coefficient

n-Octanol and water were pre-saturated with each other for 24 h before experiment. To the pre-equilibrated buffer (10 ml), known quantity of drug was dissolved. Ten ml of octanol was added to equal volume of drug solution in a separating funnel. The system was kept for 24 h with intermittent shaking. Finally, water layer was separated, clarified by centrifugation and assayed.

Drug-Excipient interaction study

The infrared (IR) spectra were recorded using an FTIR spectrophotometer by the KBr pellet method in the wavelength region between 7800 and 350 cm^{-1}. The spectra obtained for Minoxidil and physical mixtures of Minoxidil with polymer were compared to check compatibility of drug with carbopol 934.

Preparation of Minoxidil gels

Required amount (1 g) of Minoxidil was dissolved in solvent mixture (Ethanol:water:: 1:1). The required amount of carbopol 934 was weighed and transferred to the solvent mixture. Allow the polymer to swell completely without constant stirring. After complete swelling, the dispersion was constantly stirred at 500 rpm for about 2 h. Later, the speed was reduced to avoid air entrapment. After 2 h, the hydrogel solution containing the drug was neutralized by the addition of the alkali triethanolamine to obtain gel with maximum viscosity.

Preparation of Minoxidil emulgel

The emulgel was formulated in three different steps. Step 1 was formulation of emulsion either *o/w* or *w/o*. Step 2 was formulation of gel base. Now, step 3 involves incorporation of emulsion into gel base with continuous stirring. Different formulations were prepared using varying amount of gelling agent and penetration enhancer.

The method only differed in the process of making gel in different formulations. The preparation of emulsion was same in all the formulations. The gel bases were prepared by dispersing Carbopol 934 in distilled water separately with constant stirring at a moderate speed using mechanical shaker. Formulations F5, F6, F7, and F8 were prepared by carbopol 934 as gelling agent. The pH values of all the formulations were adjusted to 6-6.5 using triethanolamine (TEA). The oil phase of the emulsion was prepared by dissolving Span 80 in light liquid paraffin while the aqueous phase was prepared by dissolving Minoxidil in purified water. Methyl paraben was dissolved in propylene glycol and mixed with aqueous phase of water.

Mentha oil was also mixed in oil phase. Both the oily and aqueous phases were separately heated to 70°C to 80°C, then the aqueous phase was added to the oil phase with continuous stirring until it got cooled to room temperature (Mohamed, 2004). The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtain the Emulgel **(Table 1)**.

Evaluation of gels/emulgels

Following parameters were used for the evaluation of gels/emulgels:

Physicochemical characteristics

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Grittiness

All the formulations were evaluated microscopically for the presence of particles. If no appreciable particulate matter is seen under light microscope, the gel preparation fulfils the requirement of freedom from particular matter and from grittiness as desired for any topical preparation.

Measurement of pH

The pH of Minoxidil gel formulations were determined by using digital pH meter. One gram of gel was dissolved in 100 ml of distilled water for pH measurement in triplicate and average values were calculated.

Table 1. Formulation plan of Minoxidil gel and emulgels

Ingredients (*% w/w*)	Minoxidil gels				Minoxidil emulgels			
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Minoxidil	1	1	1	1	1	1	1	1
Carbopol-934	0.5	1	1.5	2	0.5	1	1.5	2
Propylene glycol	15	15	15	15	15	15	15	15
Triethanolamine	0.4	0.5	0.6	0.7	0.4	0.5	0.6	0.7
Ethanol	30	30	30	30	5	5	5	5
Propyl paraben	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Span 80	-	-	-	-	12	12	12	12
Mentha oil	-	-	-	-	3	4	5	6
Light liquid paraffin	-	-	-	-	25	25	25	25
Distilled water	51.6	51.0	50.4	49.8	35	35	35	35

Drug content studies

Minoxidil gel (500 mg) was taken and dissolved in 50 ml of phosphate buffer pH 7.4. The volumetric flasks were kept for 2 h and shaken well in a shaker to mix it properly.

The solution was passed through the Whatman filter paper and filtrates were analyzed for drug content spectrophotometrically at 285 nm against corresponding gel concentration as blanks.

Viscosity studies

The measurement of viscosity of formulations was done with a Brookfield Viscometer. The gels were rotated at 10 and Emulgels at 20 rpm using spindle no. 64. At each speed, the corresponding dial reading was noted (Martinez *et al* 2007).

Spreadability

The spreadability was determined by parallel plate method which is widely used for determining and quantifying the spreadability of semisolid preparations. Various formulations (1 g) were pressed between two 20 × 20 cm horizontal plates, the upper of which weighed 125 g. The spread diameter Φ was measured after 1 min. Under these experimental conditions, the term semi stiff was applied to samples with <50 mm and semi fluid to those with >50 mm but <70 mm. The results were expressed in terms of the spreading area as a function of the applied mass (Garg *et al* 2002).

In vitro drug release studies

Before experiment, the cellophane membrane was washed in the running water and then soaked in distilled water for 24 h to remove glycerin present on it. The *in vitro* diffusion studies of prepared gels were carried out in hollow tube diffusion cell using prehydrated cellophane membrane and phosphate buffer pH 7.4 (100 ml) as receptor compartment. 500 mg of each of formulation was spread uniformly on the membrane (Yamaguchi *et al* 1996).

The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5°C. The solution on the receptor side were stirred by externally driven teflon coated magnetic bars. At predetermined time intervals, 5 ml of solution from the receptor compartment was pipetted out and immediately replaced with fresh 5 ml phosphate buffer.

The drug concentration on the receptor fluid was determined spectrophotometrically at 285 nm against appropriate blank. Calculation of percentage drug release was done using the formula:

$$\% \text{ drug release} = \frac{(\text{Conc. of drug (in mg)} \times \text{Volume of receptor compartment}) \times 100}{\text{Label claim (amount of drug in donor compartment)}}$$

Ex vivo evaluation

Ex vivo release study was conducted using preserved or fresh chicken skin from slaughter house. The skin was then soaked in sodium bromide solution for 5-6 h and washed with water so as to remove adhering fat tissue. The

epidermis was thoroughly washed with water, dried at 25% relative humidity, wrapped in aluminium foil and stored in freeze until further use. For *ex vivo* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Franz diffusion cell with the stratum corneum (SC) facing the donor compartment. The sample was applied on the skin and then fixed in between donor and receptor compartment of Franz diffusion cell. The receptor compartment contained phosphate buffer pH 7.4 an the temperature of the medium was thermostatically controlled at 37±1.0°C by surrounding water jacket and the medium was stirred with bar magnet using magnetic stirrer. Aliquots, withdrawn at predetermined intervals of time, were spectrophotometrically estimated at 285 nm against their respective blank formulation treated in the same manner.

Kinetic data analysis: Drug release models (Singhvi and Singh, 2011; Dash *et al* 2010; Sharma *et al* 2011)

Zero order release kinetics

Zero order release kinetics refers to the process of constant drug release from a drug delivery device such as oral osmotic tablets, transdermal systems, matrix tablets with low-soluble drugs and other delivery systems. In its simplest form, zero order release can be represented as:

$$Q = Q_0 + K_0 t \qquad \text{Eq. 1}$$

where Q is the amount of drug released or dissolved (assuming that release occurs rapidly after the drug dissolves), Q_0 is the initial amount of drug in solution (it is usually zero), and K_0 is the zero order release constant. The plot made was cumulative % drug release *vs* time (zero order kinetic model).

First order release kinetics

The rate laws predicted by the different mechanisms of dissolution both alone and in combination, have been discussed by Higuchi.

$$\text{Log } C = \text{Log } C_0 - kt / 2.303 \qquad \text{Eq. 2}$$

where, C_0 is the initial concentration of drug and K is first order constant. The equation in resemblance to the other rate law equations, predicts a first order dependence on the concentration gradient (*i.e.* Cs - Ct) between the static liquid layer next to the solid surface and the bulk liquid.

Higuchi Model

Ideally, controlled drug-delivery systems should deliver the drug at a controlled rate over a desired duration. It has been shown that in the case of hydrophilic matrices, swelling and erosion of the polymer occurs simultaneously, and both of them contribute to the overall drug-release rate.

Higuchi tried to relate the drug release rate to the physical constants based on simple laws of diffusion. Release rate from both a planar surface and a sphere was considered. Higuchi was the first to derive an equation to describe the release of a drug from an insoluble matrix as the square root of a time-dependent process based on Fickian diffusion.

$$Q_t = k_H(t)\ 0.5 \qquad \text{Eq. 3}$$

where, Q_t is the amount of drug released in time t, and k_H is the release rate constant for the Higuchi model.

Determination of diffusion exponent

To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model:

$$M_t/M_\infty = K_t\, n \qquad \text{Eq. 4}$$

where M_t/M_∞ is fraction of drug released at time t, k is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms as given in table for cylindrical shaped matrices.

RESULTS AND DISCUSSION

Solubility and partition coefficient study

From the solubility studies, the drug concentration was found to be 2.5 mg/ml in water. The logarithmic value of partition coefficient (log P) was found to be 1.25. The results obtained also indicated that the drug possessed sufficient hydrophilicity and lipophilicity, which fulfils the requirements of formulating it into a gel and emulgel formulation (data not produced).

Physicochemical properties

All formulations were found to be free of grittiness, homogeneous, without phase separation with white viscous creamy preparation with a smooth homogeneous texture and glossy appearance **(Table 2, Figure 1).**

Table 2. Physicochemical characteristics of formulations

Formulation	Homogeneity	Grittiness	Colour	Phase separation
F1	+++	-	Transparent	-
F2	++	-	Transparent	-
F3	+++	-	Transparent	-
F4	+++	-	Turbid	-
F5	+++	-	White to cream in colour	Slight separation of oil phase
F6	+++	-	White to cream in colour	-
F7	+++	+	White to cream in colour	-
F8	+++	-	White to cream in colour	-

Excellent +++, Good ++, absent -, present +

Fig. 1. Prepared gels and emulgels of Minoxidil

pH, viscosity and drug content

The pH of the formulations was in the range of 6.34 to 7.52, which lies in the normal pH range of the skin and would not produce any skin irritation. This may be due to the addition of base triethanolamine to the resultant gel and emulgel solution during mixing so as to neutralize the acidic groups present in the polyacrylate chains of carbopol polymer. There was no significant change in pH values as a function of time for all formulations**.** The viscosity of gels and Emulgels were found to increase with increase in the concentration of the polymer used. The viscosity of emulgels was higher as compared to corresponding gels since the emulgels was formulated by finally mixing emulsion with the carbopol gel in 1:1 ratio. The emulgels showed comparatively high % drug content than that of the corresponding gel formulations. This indicated homogenous distribution of drug throughout the emulgels which could be due to high entrapment of drug in the internal phase of emulsion **(Table 3)**.

Table 3. Results of pH, viscosity and drug content studies

Formulation code	pH*	Viscosity* (centipoise)	Drug content* (%)
F1	7.12	47450	96.34
F2	7.47	49863	93.60
F3	7.52	51729	89.37
F4	6.83	53194	95.77
F5	7.19	9990	97.50
F6	6.34	11192	95.42
F7	6.56	12212	94.93
F8	6.82	12502	89.43

*Each reading is an average of three determinations

Spreadability

As per results of spreadability studies, the spreading area was found to decrease with increase in viscosity, since spreadability and viscosity are inversely proportional. The emulgels were found to show excellent spreadability since they are less viscous and the presence of oil phase in emulgels reduces the shearing stress **(Table 4)**.

***In vitro* drug release**

The release of Minoxidil from the gels and Emulgels was varied according to concentration of polymer. The release of the drugs from gel

Table 4. Results of spreadability studies

Formulation code	Spread diameter* (φ) mm	Spreading area (S)
F1	65	3295.5
F2	61	2902.38
F3	54	2274.48
F4	50	1950
F5	90	6318
F6	70	3822
F7	51	2028.78
F8	56	2446.08

*Each reading is an average of three determinations

formulations ranked in the order F1 > F2 >F3 > F4, Where the amounts of the drug released after 8 h were 76.42%, 68.22%, 62.31%, 59.69% respectively. Drug release from the emulsified gel formulation can be ranked in the following descending order: F6 > F5 >F7> F8 where the amount of the drug released after 8 h were 64.35%, 53.96%, 52.32%, 51.32% respectively. The progressive increase in the amount of drug released from the formulations attributed to gradual decrease with increase in concentration of polymer. It has been concluded that, if we increase the concentration of polymer, the diffusion of drug through the membrane also decreases **(Figure 2, 3)**.

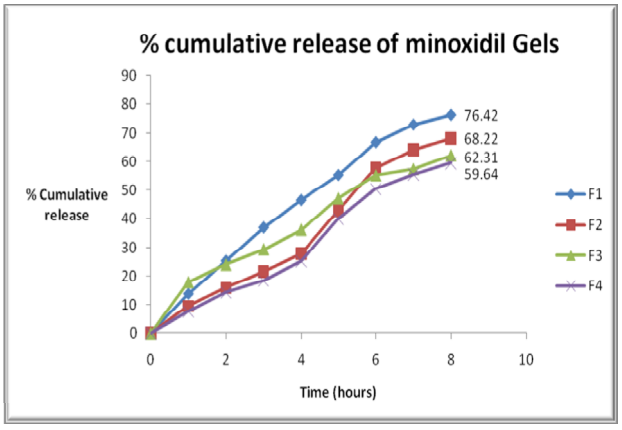

Fig. 2. Comparative drug release profiles of formulations F1-F4

Kinetics of drug release

The release kinetics data indicated that the release of drug from Emulgel F6 best fits to zero order release model because the correlation coefficient values were higher in case of zero order equation and the release from gel F1 fits to Higuchi model. The release rate is independent of the concentration of the drug. The release exponent value of Korsmeyer-Peppas Equation

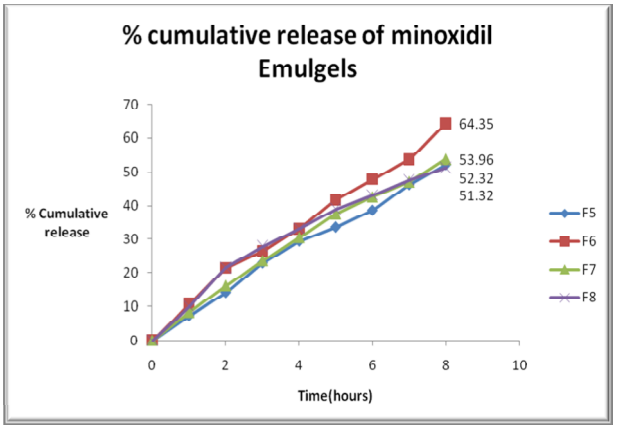

Fig. 3. Comparative drug release profiles of formulations F5-F8

for F1 and F6 was 0.845 and 0.840 suggesting that the Emulgel followed anomalous transport or non-fickian diffusion (Zero order release).

Determination of mechanism of release from Diffusion exponent (n)

The value of diffusion exponent, n = 0.845 and 0.840 for F1 and F6 indicated anomalous non-Fickian diffusion of drug from both gels and emulgels. Fick's laws of diffusion describe the spatial and temporal variation of the molecules in the aqueous solution. In Fickian diffusion, the drug flux or the rate of permeation through a unit material is proportional to the concentration gradient. A fundamental criterion for Fickian diffusion is that the surface concentration attains equilibrium value immediately and remains constant throughout the sorption process i.e. polymer chain at surface must instantaneously reach saturation. Although Fickian diffusion theories have been thoroughly developed, most of the polymer-solvent systems do not obey such a simplified description. Diffusion process in which the mean square displacement (MSD) of drug grows non linearly

with time are referred to as Anomalous or non-Fickian *i.e.* the release pattern is irregular and is independent of drug concentration. In reality, the mean square displacement does not increase linearly with time in anomalous diffusion and does no longer exist.

These complexities associated with the transport mechanisms are also associated with the physical properties of the polymers. The kinetics of release is affected by the viscosity of swollen polymers. This process is evident from the *in vitro* drug release data of both gels and emulgels *i.e.* the release of minoxidil profoundly decreased with increase in polymer concentration. The diffusion exponent calculated by Korsmeyer-Peppas plot signifies that the mechanism of drug release from both gel and emulgel follows Anomalous diffusion (**Table 5**).

Table 5. Regression co-efficients (R^2) values of kinetic models for formulation F1 and F6

Formulation code	R^2 values			Diffusion exponent (n)	Drug release mechanism
	Zero order	First order	Higuchi model		
F1 (Gel)	0.983	0.992	0.993	0.845	Anomalous transport (non-Fickian)
F2 (Emulgel)	0.994	0.975	0.977	0.840	Anomalous transport (non-Fickian)

Ex vivo permeation study

Best formulations (F1 and F6) selected were subjected to *ex vivo* release study through chicken skin using Franz diffusion cell. The *ex vivo* release displayed better estimate of drug permeation characteristics through animal skin. Minoxidil emulsified in oil phase showed higher release through skin as compared to minoxidil solubilized in hydrophilic gel matrix of carbopol. The presence of oil phase enhanced the drug permeation through stratum corneum, which is considered to be the main barrier to the permeation of drugs through skin. The amount of drug permeated through skin after 8 hours from F1 and F6 was 56.3% and 72.31% (Das and Ahmed, 2007) **(Figure 4)**.

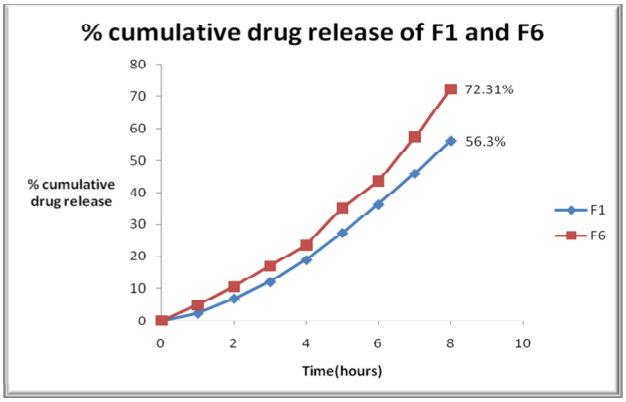

Fig. 4. Comparison of *ex vivo* permeability study of F1 and F6

CONCLUSION

The results of present studies confirmed the feasibility of using Minoxidil emulgels over Minoxidil gels for developing an effective and safe topical delivery system for the treatment of androgenetic alopecia. Hence, an effective Emulgel of Minoxidil is recommended as being more promising than Minoxidil gels.

ACKNOWLEDGEMENT

We would like to thank Prof. Dr. S. Kuppuswamy (Head of the Department, Pharmaceutics, Nirmala College of Pharmacy, Muvattupuzha, Kerala) and Prof. John Joseph (Principal, Nirmala College of Pharmacy, Muvattupuzha) for kind support and help in conducting research work.

REFERENCES

Das MK, Ahmed AB. Formulation and *ex-vivo* evaluation of rofecoxib gel for topical application. *Acta Pol. Pharm.* 2007;64(5):461-7.

Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol. Pharm*. 2010;67(3):217-23.

Garg A, Aggarwal D, Garg S, Singla AK. Spreading of semisolid formulations: An update. *Pharm. Tech.* 2002; 84-105.

Gupta S, Shahi S, Tadwee I, Zadbuke N, Tribhuvan S, Sonawane U. Enhanced topical formulation of Minoxidil gel for alopecia condition. *Int. J. Pharm. Res. Allied Sci.* 2012;1(1):41-7.

Kaur LP, Garg R, Gupta GD. Development and evaluation of topical gel of Minoxidil from different polymer bases in application of alopecia. *Int. J. Pharm. Pharm. Sci*. 2010; 2(3):43-7.

Lowenthal DT, Affrime MB. Pharmacology and pharmacokinetics of Minoxidil. *J. Cardiovasc. Pharmacol.* 1980;2(2):S93-106.

Martinez MAR, Gallardo JLV, de Benavides MM, Lopez-Duran JDG, Lara VG. Rheological behavior of gels and

meloxicam release. *Int. J. Pharm.* 2007;333(1-2):17-23. [DOI: http://dx.doi.org/10.1016/j.ijpharm.2006.09.045]

Mohamed MI. Optimization of chlorphenesin emulgel formulation. *AAPS J.* 2004;6(3):81-7. [DOI: 10.1208/aapsj060326]

Sharma R, Walker RB, Pathak K. Evaluation of the kinetics and mechanism of drug release from Econazole nitrate nanosponge loaded carbapol hydrogel. *Ind. J. Pharm. Edu. Res*. 2011;45(1):25-31.

Singhvi G, Singh M. Review: *In vitro* drug release characterization models. *Int. J. Pharm. Stud. Res.* 2011; II(I):77-84.

Yamaguchi Y, Sato H, Sugibayashi K, Morimoto Y. Drug release test to assess quality of topical formulations in japenese market. *Drug Dev. Ind. Pharm.* 1996; 22(7):569-77. [DOI: 10.3109/03639049609063210]

*****