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Preparation and *In-Vitro* Evaluation of Minoxidil Niosomal Gel by Thin Film Hydration Method Using Different Surfactants.

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ABSTRACT

Present study has been an effort towards improving the skin penetration of Minoxidil by encapsulating it in niosomes. Niosomal formulations of Minoxidil were prepared by thin film hydration method using two different surfactants like sorbitan monoesters (span 40, 60) and polysorbate (tween 60) with cholesterol. The prepared niosomes were evaluated for size, shape, entrapment efficiency and *in-vitro* drug release. Niosomes were spherical in shape and size range was found to be 4.10±1.05 to 6.74±2.99 µm. The entrapment efficiency was found in the range of 36.74±0.84 to 61.16±0.36 % at the end of 24hrs. Among all the Minoxidil niosomes the promising formulation were found to be of span 60 and cholesterol in the ratio of 1:0.2 and were incorporated into carbopol gel. The prepared gel was characterised for appearance, pH, drug content, viscosity and *in-vitro* drug release. The *in-vitro* drug release study was carried out using phosphate buffer saline (PBS) pH 7.4 and was found to be 42.16±1.94% at the end of 12 hrs.

Keywords: Niosomes, Minoxidil, Skin permeation, Carbopol gel, *In-vitro* drug release.

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INTRODUCTION

Androgenic alopecia is a common form of hair loss in both men and women. Minoxidil a pyrimidine derivative (2,6-diamino-4-(piperidin-1-ium-1-olate) is the only topical medical treatment with proven efficacy for the treatment of androgenetic alopecia. About 0.3 to 4.5% of a topical dose of Minoxidil is absorbed from intact scalp. Common side effects associated with Minoxidil therapy include scalp dryness, irritation, burning, redness and allergic contact dermatitis [1, 2].

Since decades researchers are developing to use the drugs in an efficient manner to treat various diseases. Their aim was to reduce the undue exposure of drug to the entire body by striving this through reduced dose, reduced dosage frequency, reduced side effects, greater patient compliance and maximum concentration of the drug at the site of action. Localized drug delivery system helps to achieve these advantages by topical administration through ophthalmic, rectal, vaginal and epidermal (skin) routes. Recently, transdermal drug delivery system has gained importance by systemic medication through topical application to the intact skin surface [3, 4].

Skin disorders critically need new treatment options. The skin is a natural barrier against particle penetration for topical delivery, and also it offers a potential approach for the delivery of therapeutics, especially in diseased skin and via the opening of hair follicles. Recent there is an expansion into dermatological treatment with localized drug delivery directly into the targeted skin region, via novel carriers.

Nanocarriers are the drug delivery systems that are used to deliver drugs to various parts of the body, including the skin. Application of the drug to skin surface is used to elicit a local response. Encapsulating dermetalogically active drugs in nanocarriers may offer a number of benefits compared to directly loading drugs into traditional multiphase semi-solid dosage vehicles [5].

Niosomes are microscopic lamellar structures obtained on admixture of non-ionic surfactant of alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media [6].

Niosomes are promising vehicle for drug delivery and have advantages in many aspects, such as chemical stability, high purity, content uniformity, low cost, convenient storage of non-ionic surfactants. Due to availability of large number of surfactants incorporation of various drugs in niosomes become easy that can minimize the drug degradation after administration [7, 8].

Niosomes are also effective in delivering anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine [9].

Hence in a present study an attempt will be made by developing and characterizing niosomal gel formulation of Minoxidil to show effective treatment in androgenic alopecia.

MATERIALS AND METHOD

Materials:

Minoxidil was gifted by Dr. Reddy's Laboratories, Hyderabad. Span 40, span 60, tween 60, disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate, chloroform, methanol, glycerol and triethanolamine were purchased from SD Fine Chemicals Ltd, Mumbai. Carbopol 934 was purchased from Himedia, Mumbai. Propylene from Loba Chemie. Pvt. Ltd, Mumbai. Propylene glycol was purchased from Universal Laboratories Pvt. Ltd, Mumbai. All other materials were of analytical grade and were used as received.

Methods:

Preparation of niosomes:

Niosomes were developed using surfactants (span 40, span 60 and tween 60) and cholesterol, at different specified ratio by thin film hydration method. Lipid mixture of surfactant and cholesterol along with the drug were dissolved in 10ml of chloroform, then transferred to 100ml of round bottom flask, than the solvent was evaporated under reduced pressure at a temperature of 55-65ºC using rotary flash evaporator till the formation of thin lipid film. The formed film was hydrated for 1hr with 20ml of PBS pH 7.4, while the flask was kept rotating at 55-65ºC in the rotary evaporator (photograph 1). The hydrated niosomes were sonicated (photograph 2) using a bath sonicator for 20min to obtain niosomal dispersion containing both free and unentrapped drug of varying size [10, 11].

Table 1: Formulations of Minoxidil Niosomes

Ingredients	**Formulation code**								
	TMS_1	**TMS_2**	**TMS_3**	**TMS_4**	**TMS_5**	**TMS_6**	**TMT_7**	**TMT_8**	**TMT_9**
Ratios (Drug: Surfactant: Cholesterol)	1:1:0.2	1:1.5:0.3	1:2:0.4	1:1:0.2	1:1.5:0.3	1:2:0.4	1:1:0.2	1:1.5:0.3	1:2:0.4
Minoxidil*	100	100	100	100	100	100	100	100	100
Span 40*	100	150	200	--	--	--	--	--	--
Span 60*	--	--	--	100	150	200	--	--	--
Tween 60*	--	--	--	--	--	--	100	150	200
Cholesterol*	20	30	40	20	30	40	20	30	40
Chloroform#	10	10	10	10	10	10	10	10	10
PBS (pH 7.4)#	20	20	20	20	20	20	20	20	20

***** mg, # ml

Evaluation of Minoxidil niosomes:

Morphological characterization:

The formed vesicles were seen by optical microscopy in 45X resolution. The niosomal suspension placed over a glass slide and fixed over by drying at room temperature and observed for the formation of vesicles [12]. By scanning electron microscope the detailed surface characteristic of the selected Minoxidil niosomal formulation was observed.

Fourier transform infrared spectroscopy study:

Infrared spectrum of Minoxidil, excipients and formulation (TMS_4) were determined by using potassium bromide dispersion method.

Entrapment efficiency:

Entrapment efficiency of niosomes was obtained by exhaustive dialysis method using niosomal suspension of measured quantity into a dialysis tube to which dialysis membrane was securely attached on one side. The dialysis tube was suspended in 100ml PBS pH 7.4 containing 10% v/v methanol, which was stirred on a magnetic stirrer. At every hour entire medium (100ml) was replaced with fresh medium (for about 6-7hrs) till the absorbance reached a constant reading indicating no drug is available in unentrapped form. Absorbance of withdrawn samples were checked at 287nm. Amount of entrapped drug was determined by subtracting amount of unentrapped drug from the total drug encapsulated [13].

$$\text{Percentage entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

In-vitro drug release study

The *in-vitro* release of Minoxidil from niosomal formulations were carried out by membrane diffusion technique. The niosomes left after removal of un-entrapped drug were dialyzed into a beaker containing 100ml of PBS pH 7.4 containing 10%v/v methanol to maintain the sink condition which act as receptor compartment. The receptor compartment was agitated using magnetic stirrer and temperature maintained at 37±0.5ºC. Periodically aliquots of 5ml sample were withdrawn and replaced with same volume of medium. The withdrawn sample was analyzed at 287nm using UV spectrophotometer [14, 15]. The tests were carried out in triplicate.

Formulation of Minoxidil niosomal gel:

Selected Minoxidil niosomal suspension (TMS_4) which is equivalent to 2%w/w Minoxidil, was incorporated into gel base made of carbopol 934 (0.5%), triethanolamine (qs) and solvent mixture (ethanol: propylene glycol: water in the ratio of 50:30:20) [16, 17].

Evaluation of niosomal gel:

Physical appearance:

The formulated gel was examined for clarity, color, homogeneity and presence of foreign particles.

pH:

Accurately weighed quantity of gel (2.5gm) was dispersed in 25ml of distilled water and analyzed for pH using digital pH meter [18].

Viscosity measurement:

Using Brookfield programmable DV III ultra viscometer (spindle no. CP 52), viscosity of the preparation was determined with an optimum speed of 0.01 rpm.

Content uniformity:

The drug content of the formulated gel was determined by dissolving accurately weighed quantity of gel equivalent to 10mg of drug in 100ml volumetric flask and volume was made up to the mark with methanol. Filtered the content through Whatmann filter paper no 41. 5ml of the solution was taken into 25ml volumetric flask and volume was adjusted up to mark with methanol. The Minoxidil content in gel was determined by Shimadzu UV-Visible spectrophotometer at 287nm. The drug content was determined from calibration curve of Minoxidil. The tests were carried out in triplicate.

In-vitro drug diffusion studies:

The drug diffusion study was carried out using an apparatus consists of a glass cylinder with both the ends open. One end of the cylinder was fixed with pre-soaked (24hrs in distilled water) dialysis membrane, using an adhesive. Gel equivalent to 10mg of Minoxidil was placed in the cell (donor compartment) and the cell was immersed in a beaker having 100ml of PBS pH 7.4 containing 10% v/v methanol (to maintain sink condition), act as receptor compartment [19]. The assembly was fixed in such a way that the lower end of the cell containing gel was agitated using a magnetic stirrer at the temperature of 37±0.5ºC. At each interval aliquots of 5ml sample were withdrawn from receptor compartment and replaced with same volume of fresh buffer. The withdrawn samples were analyzed by UV/Visible spectrophotometer at 287nm [16, 20]. The tests were carried out in triplicate.

RESULTS AND DISCUSSION:

In the present study Minoxidil niosomes were formulated by thin film hydration method using non-ionic surfactants (span40, span60 and tween60) and cholesterol in different proportions (1:0.2, 1.5:0.3 and 2:0.4). The prepared Minoxidil niosomes were evaluated for particle size, shape, entrapment efficiency and *in-vitro* drug release. Finally, the best formulation was incorporated into the gel for topical uses.

Development of Vesicles:

The prepared vesicles were analyzed under 45X magnification. The niosomes were observed as spherical with smooth surface. Size variation was seen with the addition of cholesterol into the vesicular suspension. The correlation between niosomes size and cholesterol content has been characterized to the decrease in surface energy with increase in hydrophobicity that results in smaller vesicles [21]. The diameter of the prepared niosomes was found to be in the range of 4.10 to 6.74µm (Table 2). Finally the surface morphology was observed using scanning electron microscope (Figure 1).

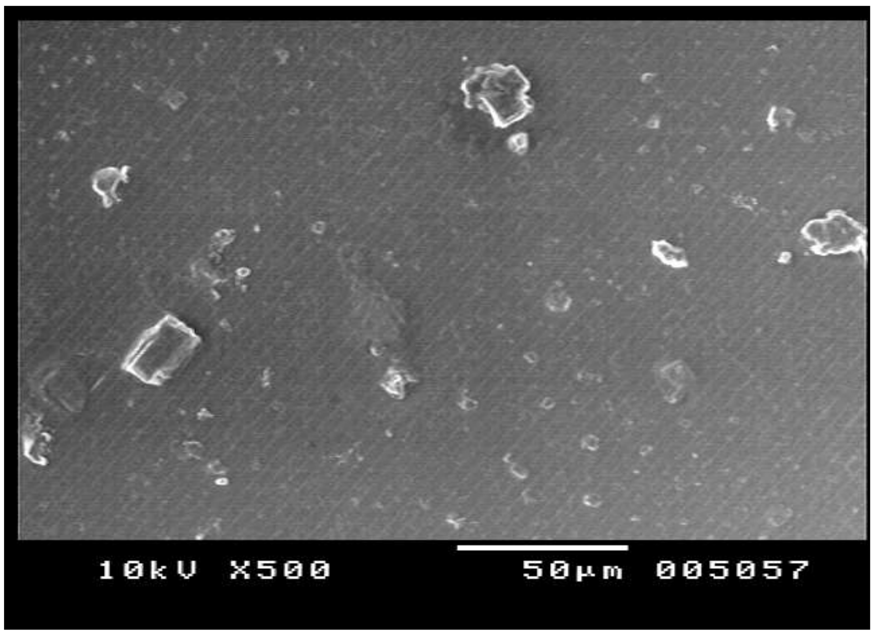

Figure 1: SEM image of niosomes

Correlation of drug solubility, entrapment efficiency and particle size:

Compared to hydrophilic drugs, lipophilic drugs are preferentially up taken by niosomes as a result they are highly loaded and partitioned at the lipid phase of the vesicles. Particle size of non-ionic surfactant vesicles increases with encapsulation of drug may be by interaction of drug with surfactant head groups or increasing the charge or mutual repulsion of the surfactant bilayers [22]. It is expected, due to good solubilising environment provided by hydrocarbon chains, lipophilic drugs were located between the fatty acyl side chains of bilayer membrane [23].

Sonication:

Sonication was carried out for producing a lipid vesicle of known size, where cavitation (bubble formation) was the principal effect has been shown to be responsible for many physical effects of ultrasound on lipid membranes. The prepared niosomes were sonicated for 20min [24].

Entrapment efficiency:

Improper selection of process variables like hydration medium, hydration time, vacuum and speed of rotation of flask may results inappropriate hydration which intern results into formation of fragile niosomes or drug leakage from niosomes. The rotational speed of the flask influences on the uniformity and thickness of the lipid film. Hydrating temperatures used effect the phase transition of the system, which must be above the gel liquid phase transition temperature of the system in turn the effect reflects interm of increased entrapment efficiency. Cholesterol (CHO) was used to form stable niosomes, as known it abolish the gel to liquid phase transition of liposomal and niosomal systems, resulting in niosomes that are less leaky. In case of niosomes formulated without cholesterol, about 55-85% of the entrapped drug was leaked. CHO is amphipathic in nature and present in almost all vesicles and biomembranes, influences membrane properties such as ion permeability, aggregation, fusion process, elasticity, shape and size. Increase in cholesterol concentration, leads to an increase in the entrapment of Minoxidil in niosomes, which is attributed due to the property of cholesterol to cement the leaking space in the bilayer membranes. Surfactant concentration directly affects the entrapment efficiency and the time taken for drug release. The entrapment efficiency was in the range of 29.99 to 47.94% (Table 2), determined by separating the unentrapped drug using dialysis [25, 26].

Table 2: Evaluation of Minoxidil Niosomes

Sl. No.	Formulation code	Particle size ± SD (µm)	Percentage entrapment efficiency*	Cumulative percent drug released* (After 24 hrs)
1	TMS_1	5.01 ± 1.80	29.99 ± 1.28	61.160 ±0.361
2	TMS_2	4.86 ± 1.74	32.74 ± 1.38	56.721±1.029
3	TMS_3	4.71 ± 1.65	35.29 ± 0.88	51.972 ±0.583
4	TMS_4	5.01 ± 1.80	41.96 ± 0.44	54.579 ±1.036
5	TMS_5	4.40 ± 1.42	42.45 ± 1.45	49.861 ±0.346
6	TMS_6	4.10 ± 1.05	46.27 ± 1.45	45.550 ±0.556
7	TMT_7	6.74 ± 2.99	38.92 ± 1.32	43.929 ±0.387
8	TMT_8	5.77 ± 2.48	45.98 ± 0.74	40.938 ±0.795
9	TMT_9	5.32 ± 1.90	47.94 ± 1.17	36.748 ±0.843

*Values represented as mean ± SD (n=3)

***In- vitro* drug release:**

The choice of medium was important to provide sufficient solubility of the drug. Drug is insoluble in water. Initially, a PBS pH 7.4 was used due to better stability of Minoxidil. Later a medium with

10% methanol was considered to be appropriate for maintaining required sink condition during diffusion studies. The volume of receptor medium used was 100ml. The *in-vitro* drug release of the prepared niosomes was found to be in the range of 36.74±0.84 to 61.16±0.36% at the end of 24hrs (Table 2). For the determination of the release kinetics, the *in-vitro* drug release data was fitted to First order plots, Higuchi diffusion plots and Peppas log-log plots [27, 28]. The regression coefficient values for this entire graph showed good linearity. Slope values of the Peppas log-log plot were also calculated, were in the range of 0.83 to 0.93 in almost all the cases, suggesting that the drug was released by non-fickian (anomalous) release mechanism. Among all the prepared Niosomal formulations TMS_1, TMS_2 and TMS_4 showed higher drug release. TMS_4 showed highest entrapment efficiency as compared to TMS_1 and TMS_2. Also the particle size of TMS_4 was less compared to TMS_1. Considering all characteristics, TMS_4 was found to be the best formulation having vesicle size of 4.56±1.55µm, entrapment efficiency of 41.96±0.44% and drug release of 54.57±1.03% at the end of 24hrs. Finally the promising formulation TMS_4 was centrifuged and pellets were obtained and incorporated into a carbopol gel.

Table 3: Evaluation Parameters of Minoxidil Niosomal Gel (TMS_4)

Sl. No.	Parameter	Result
1	Appearance	Off-white
2	Homogeneity	Good
3	pH*	8.64 ± 0.165
4	Percent drug content*	97.40 ± 0.815
5	Viscosity* (cps)	8835

*Values represented as mean ± SD (n=3)

Niosomal gel:

In this study carbopol was selected as gelling agent for the preparation of Niosomal gel due to its hydrophilic nature and bioadhesive properties, this increase the resistance time of a drug by interacting with the mucosa at the site of absorption [29]. Niosomes stability can be enhanced after incorporating into the gel base by prevention of fusion between niosomes. Drug skin retention in case of Niosomal gel may be higher due to, creation of reservoir effect for dug in skin and thereby increasing the drug retention capacity into the skin.

The prepared Niosomal gel formulation (TMS_4) was characterised for appearance, pH, viscosity, drug content and *in-vitro* drug release. Color of the gel was off white, pH was 8.64±0.16 and viscosity was 8835cps. The drug content of the niosomal gel was found to be 97.40±0.81%. The formulated niosomal gel was also subjected to *in-vitro* drug release study and the release data was compared with Minoxidil plan gel and marketed gel (Manexil).

The Minoxidil niosomal gel, plain gel and marketed gel released 42.16±1.94, 76.47±0.69 and 81.22±1.01% of drug respectively at the end of 12hrs. To determine release kinetic the in-vitro release data was fitted to First order plots, Higuchi diffusion plots and Peppas log-log plot. The regression coefficient values for this entire graph showed good linearity. Slope values of the Peppas log-log plot were also calculated. Drug was released by anomalous diffusion (coupling of diffusion and erosion mechanism) where it showed release exponent for Minoxidil niosomal gel, plain gel and marketed gel as 0.889, 0.733 and 0.724 respectively.

IR study:

The IR spectrum of the pure drug shows characteristic peaks at 1210, 1231, 1550 and 1640 cm^{-1} due to N-O, C=C, NH_2 and C=N stretching groups respectively. Formulation TMS_4 also showed similar peaks at 1211.30, 1547.16, 1627.92 and 1234.44 cm^{-1} respectively for the above groups. This confirms undisturbed structure of the drug in the formulation. Hence, there was no drug-excipients interaction (Figure. 2, 3).

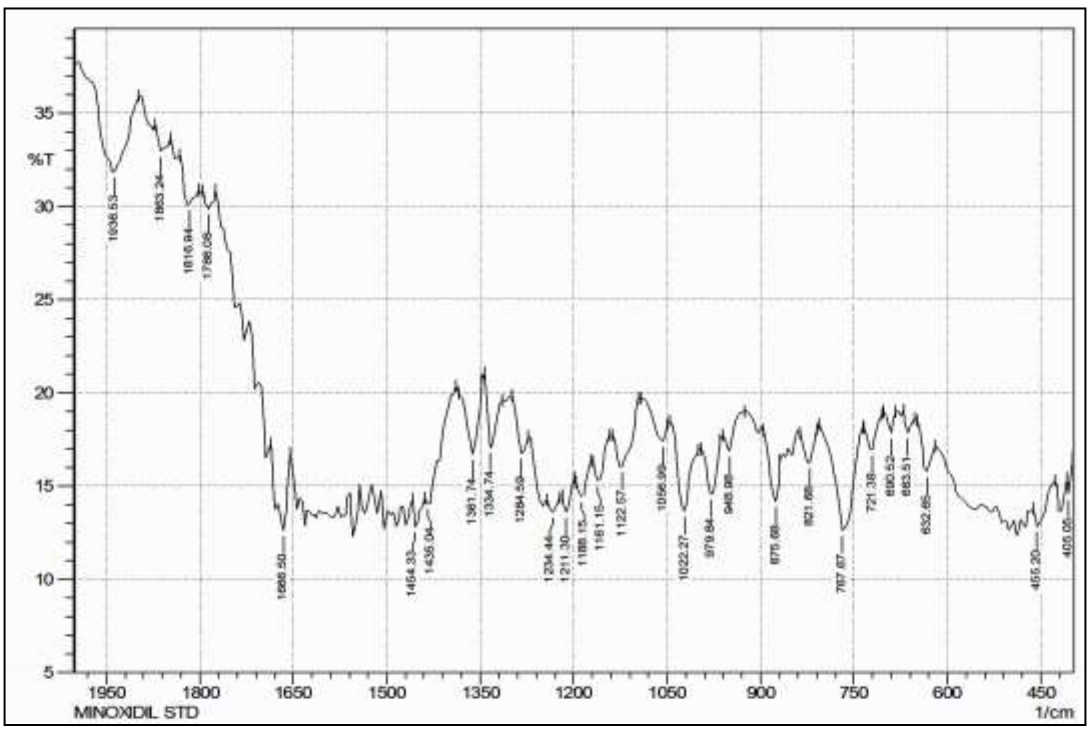

Figure 2: IR spectrum of Minoxidil

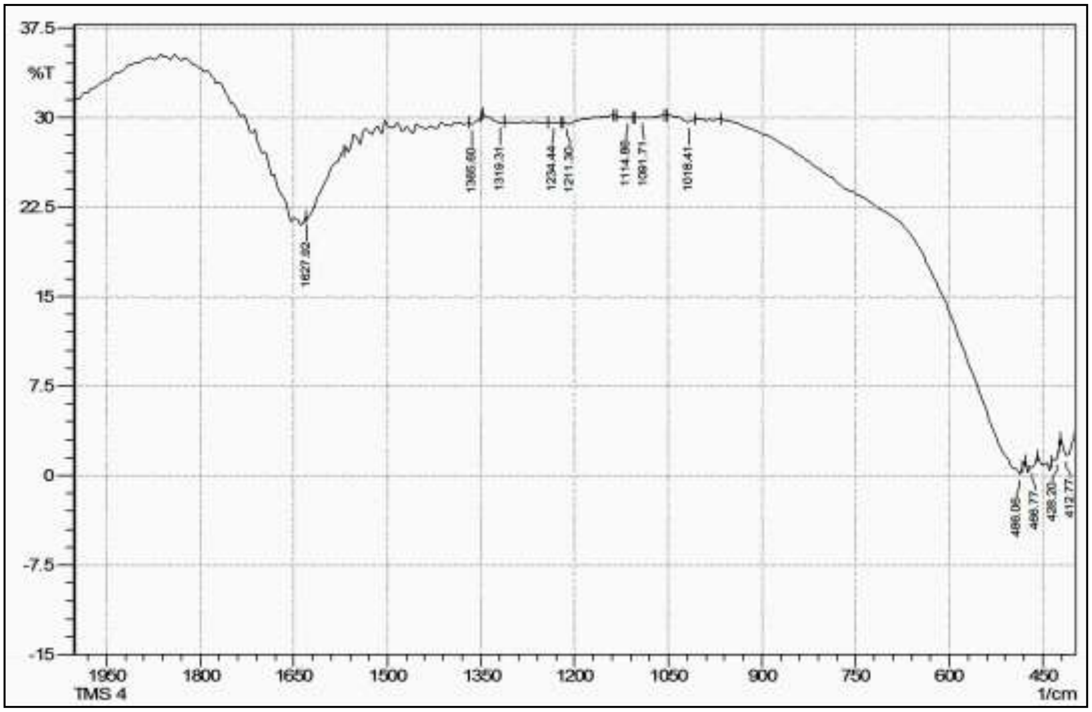

Figure 3: IR spectrum of formulation TMS_4

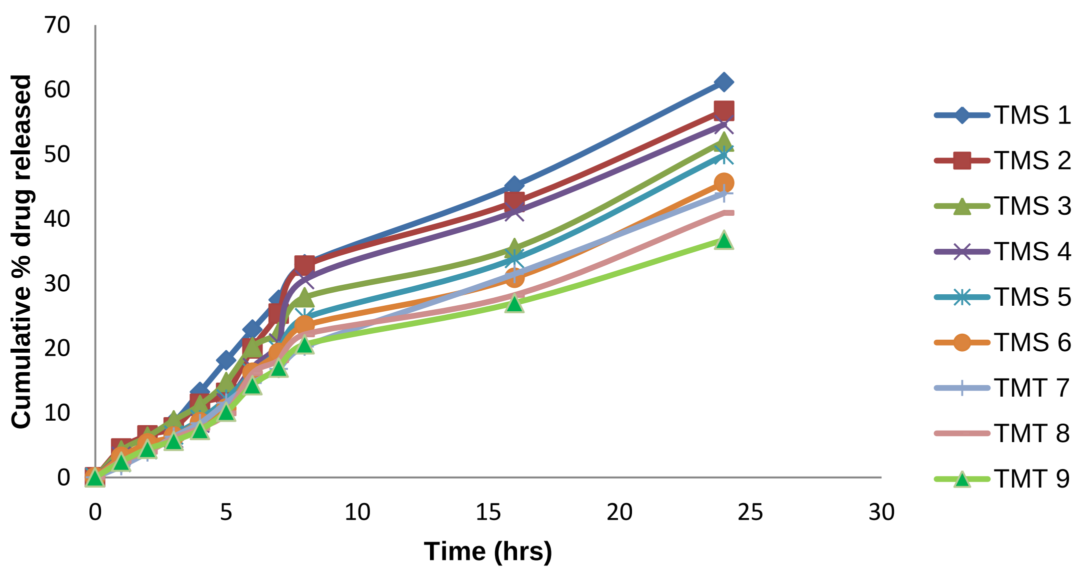

Figure 4: *In vitro* drug release profiles of formulations using Span 40, Span 60 and Cholesterol

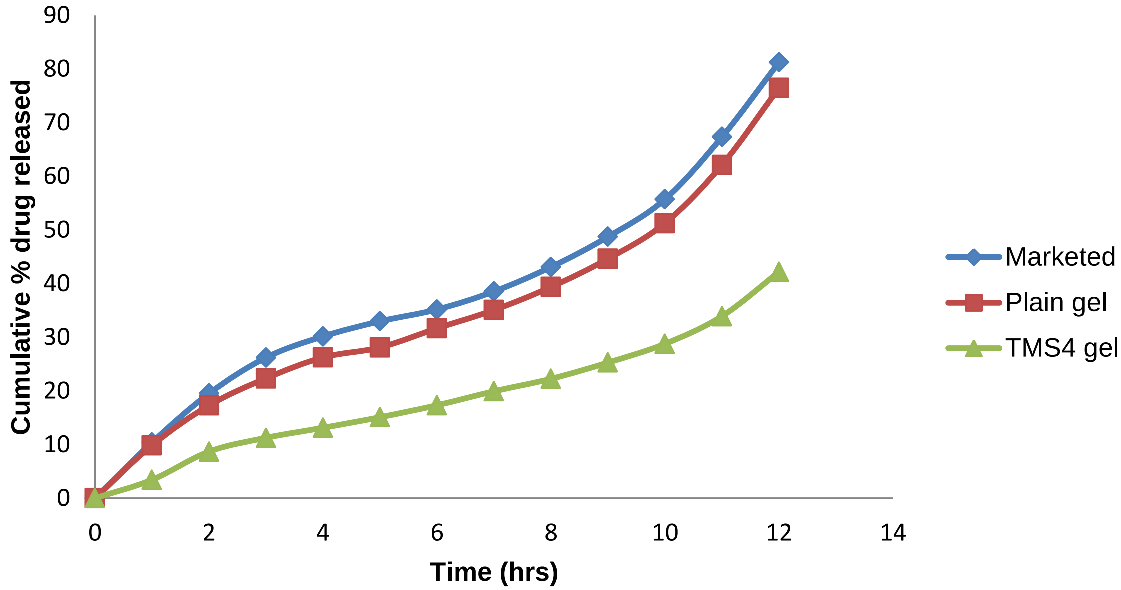

Figure 5: *In vitro* release plot of Minoxidil plain gel, Marketed gel and Minoxidil niosomal gel (TMS_4)

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