FORMULATION AND EVALUATION OF COATED MICRONEEDLES FOR THE TREATMENT OF HAIRLOSS

ABSTRACT

Coated micro needles have been shown to deliver proteins and DNA into the skin in minimum invasive manner. Still detailed studies of preparing coated micro needles and their breadth of applicability are lacking. Androgenic alopecia is the most commonly known form of nonscaring alopecia in humans. Till now, in India minoxidil is marketed as topical solution in aqueous vehicle in treatment of alopecia. High percentage of alcohol present in marketed formulations as a permeation enhancer was known to damage hair, hair follicle and scalp epidermal cells due to dehydration. The goal of the study was to enhance permeation of drug with the aid of microneedles, thus reducing the concentration of alcohol and damage of scalp cells. Stainless steel microneedle roller (1 mm, 142 microneedles per roller) was purchased. Microsyringe was used to coat each individual needle present on the roller. Coated microneedles were studied for coating uniformity, in-vitro drug release and ex-vivo drug release. Drug release profile of coated microneedles was found to be comparable with marketed solution of minoxidil of the same strength. Accelerated stability study of one month at accelerated temperature and humidity condition showed insignificant rate of degradation.

KEYWORDS Micro needle, coating, minoxidil, drug release, micro syringe, transdermal drug delivery.
1. INTRODUCTION

Microneedles are hybrid between hypodermic needles and transdermal patches. They are generally one micron in diameter and range from 1-100 microns in length. It is smaller than hypodermic needle; it hurts less when it pierces skin and offer several advantages when compared to conventional needle technologies. Microneedles can be fabricated to be long enough to penetrate the stratum corneum, but short enough not to puncture nerve endings. Thus reduces the chances of pain, infection, or injury. It was reported that the flux of small compounds like diclofenac, methyl nicotinate and calcein was increased by microneedle arrays. In addition, microneedles also have been examined to enhance the flux of permeation for large compounds like fluorescein isothiocyanate-labeled Dextran, insulin, bovine serum albumin, plasmid DNA and nanospheres. Microneedles may create microconduits sufficiently large to deliver drug-loaded liposomes into the skin. A variety of materials used for the preparation of microneedles are silicon, metal and polymers. Type of molecules that can be delivered via microneedles are hydrophilic drugs, larger size drug molecules and even small particulate carrier system.

Alopecia is a very common problem seen both in men and women. A person suffering from hair loss hesitates to present himself in public. Till now, in India minoxidil is marketed as topical solution in aqueous vehicle in treatment of alopecia which offers application in place for 4 hrs in order to be effective. Minoxidil has a serum half-life of 4.2 hrs. The slow penetration of minoxidil means that serum concentration of minoxidil will never reach high therapeutic levels, particularly when applied to frontal area, where stratum corneum and entire epidermis is thicker and have high melanin content. High percentage of alcohol (90%) is used as a penetration enhancer in the manufacturing of minoxidil formulations which causes dehydration of hair, hair follicle and scalp epidermal cells. At this high concentration of ethanol, it causes death of epidermal cells and actually prevents hair growth.

Hence to enhance the penetration of minoxidil and reduce the side effects of ethanol, minoxidil was coated on microneedles. Thus coated microneedles for scalp can be used to increase serum level of minoxidil without an increase in drug concentration.

2. MATERIAL AND METHOD

2.1. Material

Stainless steel microneedle roller (1 mm; 142 microneedles per roller) was purchased from Coherent medical system, New Delhi; Minoxidil was obtained as a gift sample from Manish Pharmaceuticals, Bhosari; Propylene glycol (LOBA chemicals), ethanol (LOBA chemicals) and other ingredients used were of analytical grade.

2.2. Method

All the experiments were performed in triplicate and average values were reported.

2.2.1. Preformulation study of minoxidil

2.2.1.1. Description

The minoxidil was observed for its color, odor and appearance.
2.2.1.2. Melting point determination

The melting point of minoxidil was determined by capillary method using melting point apparatus (Veego VMP I). The capillary was sealed at one end and a very fine powder of drug (10 mg) was filled in capillary. Due care was taken to maintain the uniform heating of silicon bath, in which the capillary containing the drug was placed. The temperature at which the column of the substance collapsed was recorded as a melting point of the substance under test and was compared with reported standard.

2.2.1.3. Fourier transform infra-red (FTIR) spectroscopy

The IR spectrum of minoxidil was recorded using Fourier transform infra-red spectrophotometer (FTIR 4100 Jasco Japan). Sample preparation was done by mixing the drug with potassium bromide (1:300), triturating it in glass mortar. A transparent pellet of the mixture was formed and placed in the sample holder and scanned over a frequency range 4000 – 400 cm⁻¹. The spectrum obtained was compared with reported standard.

2.2.1.4. Differential scanning calorimetry (DSC)

The DSC thermogram of minoxidil was recorded using differential scanning calorimeter (Mettler star SW 9.01). Approximately 2-5 mg of drug sample was heated in an aluminum pan (Al-Crucibles, 40 Al) at a heating rate of 10⁰C/min under a stream of nitrogen at flow rate of 50 ml/min. The thermogram obtained was compared with reported standards.

2.2.1.5. UV Visible Spectroscopy

2.2.1.5.1. Determination of λmax of minoxidil

Accurately weighed minoxidil (10 mg) was transferred to a separate 100 ml volumetric flask and dissolved with phosphate buffer pH 7.4 and diluted upto the mark with the same to obtain a standard stock solution having concentration of minoxidil (100µg/ml). An aliquot (1 ml) of stock solution was diluted with phosphate buffer pH 7.4 upto 10 ml to give 10 µg/ml and scanned for UV range from 200-400 nm on UV/Visible spectrophotometer (Jasco V-630, Japan) against phosphate buffer pH 7.4 as blank.

2.2.1.5.2. Development of standard curve of the drug using UV visible spectrophotometer in phosphate buffer pH 7.4

Stock solution was prepared as described above and aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml and 1.2 ml samples were withdrawn and diluted with phosphate buffer pH 7.4 to make solutions of the concentration 2, 4, 6, 8, 10 and 12 µg/ml respectively. The absorbance of each dilution was measured at 285 nm against phosphate buffer pH 7.4 as a blank.

2.2.1.6. Partition Coefficient

The partition co-efficient of the drug was determined in n-octanol as an oil phase and phosphate buffer pH 7.4 as an aqueous phase. The oil phase and aqueous phase were presaturated with each other for atleast 24 h before the experiment. An accurately weighed quantity of drug (10 mg) was dissolved in 10 ml of oil
phase and shaken for sometime against 10 ml of aqueous phase in a separating funnel. The mixture was allowed to stand for 24 h. The separated n-octanol phase was assayed by UV spectroscopy to determine minoxidil concentration and hence the amount partitioned into the aqueous phase.

\[ K_{o/w} = \frac{\text{Concentration in octanol}}{\text{Concentration in phosphate buffer pH 7.4}} \]

2.2.1.7. Solubility study

The solubility of minoxidil was determined in methanol, ethanol, propranol, butanol, propylene glycol and water. An excess quantity of minoxidil was added to each vial containing 1 ml of solvent. The mixture was stirred and sonicated to facilitate proper mixing of the drug. The mixture was shaken for 72 hrs at 40±0.5°C in a rotary orbital shaker (REMI, Mumbai.). The mixture was then allowed to stand for 24 h to attain equilibrium. Further the mixture was centrifuged at 3,000 rpm for 15 min, followed by filtration through whatman filter paper. The filtrates were diluted with phosphate buffer pH 7.4 and quantified by UV Spectrophotometry at 285 nm.

2.2.1.8. Selection of polymer for coat formation

Hydroxyl propyl methyl cellulose, ethyl cellulose, sodium carboxy methyl cellulose, carbopol and eudragit E 100 were used to prepare coating solutions. Various concentrations of 1%, 2%, 3%, and 4 % of each polymer were prepared in mixture of ethanol and propylene glycol (3:2); the resulting solutions were examined for their coating ability.

2.2.1.9. Drug-excipient compatibility study

2.2.1.9.1. FTIR Spectroscopy

The physical mixture of minoxidil and eudragit E 100 (1:1) was prepared and kept at accelerated temperature and humidity conditions in triple stability chamber at 30°C/65 RH and 40°C /75 RH for one month.

The FTIR spectra of physical mixture containing drug and polymer were recorded after 1 month using Fourier transform infra-red spectrophotometer (FTIR 4100 Jasco, Japan) with diffuse reflectance principle. The spectrum was scanned over a frequency range 4000–400 cm\(^{-1}\). The resultant spectra were compared for any spectral changes.

2.2.1.9.2. Differential Scanning Calorimetry (DSC)

The physical mixture of minoxidil and eudragit E 100 (1:1) was prepared and the DSC thermogram of minoxidil was recorded using differential scanning calorimeter (Mettler star SW 9.01). Approximately 2-5 mg of drug sample was heated in an aluminum pan (Al-Crucibles, 40 Al) at a heating rate of 10°C/min under a stream of nitrogen at flow rate of 50ml/min. The thermogram obtained was compared with reported standard for any shift in endothermic peak.
2.2.2. Formulation and development

2.2.2.1. Preparation of coating solution

Coating solution was prepared by dissolving eudragit E 100 in ethanol. Propylene glycol was added to the eudragit solution. To this 0.1 gm (2%) of drug was added and the mixture was stirred at 500 rpm on magnetic stirrer for 30 min. The resulting solution was used to coat the microneedles. (table 1)

2.2.2.2. Coating of microneedles

The coating solutions of various concentration of eudragit E 100 were prepared as described above. Using a microsyringe the coating solution was deposited on each individual needle. To minimize coat formation on the surface of the roller, approximately half of the microneedles were coated and roller was inverted and allowed to air dry for 10 minutes. Due to gravitational effect the coating solution was retained preferably on the microneedles. Then after, remaining needles were individually coated and allowed to air dry for 10 minutes in the inverted position. After complete coating of all the microneedles, the roller was then allowed to dry in hot air oven at 37°C for 30 minutes. The total coating solution consumed was calculated by subtracting final weight of coating solution from initial weight after coating the microneedles.

2.2.3 Evaluation of coated microneedles

2.2.3.1. Measurement of coating dimension

Length of coated microneedle was calculated and compared with uncoated microneedle with the help of motic microscope.

2.2.3.2. Percent Drug content

To determine the drug content entire roller of coated microneedles was soaked in 3 ml of phosphate buffer pH 7.4 until all the coat was dissolved. The drug content was determined by UV Visible Spectrophotometry at λmax 285 nm after appropriate dilutions.

2.2.3.3. In-vitro drug release study \[14-16\]

The in vitro drug release study of coated microneedles was carried out in Keshary–Chien diffusion cell using a cellophane membrane. Receptor compartment was filled with 100 ml of phosphate buffer pH 7.4. Coated microneedle roller was rolled once on the cellophane membrane. The donor compartment was then placed on the membrane. The temperature was maintained at 37±0.5°C. The solution on the receptor side was stirred by externally driven teflon coated magnetic bars. An aliquot of 1 ml was withdrawn at predetermined time interval from the receptor compartment and immediately replaced with 1 ml of phosphate buffer pH 7.4. The drug concentration on the receptor fluid was determined by UV spectrophotometer at 285 nm. Marketed minoxidil solution (1 ml) was applied on the cellophane membrane. The treated membrane was mounted on Keshary-chien diffusion cell in similar manner as described above. An aliquot of 1 ml was withdrawn at predetermined intervals of time. The concentration was determined by UV Spectrophotometry at 285 nm after appropriate dilutions. Drug release profiles of coated microneedles and marketed preparation were compared.

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2.2.3.4. *Ex-vivo* drug permeation study\textsuperscript{[17]}

The abdominal hairs of albino mice, weighing 22-25 g were shaved using a razor after sacrificing the mice with excess chloroform inhalation. The abdominal skin was surgically removed and adhering subcutaneous fat was carefully cleaned. The skin was allowed to hydrate for 1 h in phosphate buffer pH 7.4. The skin was then mounted on Keshary-chien diffusion cell such that stratum corneum was facing the donor compartment. The *ex-vivo* permeation study was carried out for coated microneedles as well as for marketed solution of minoxidil in essentially the same manner as described under *in-vitro* drug release study by replacing cellophane membrane with abdominal skin of albino mice.

2.2.3.5. Accelerated stability studies

The coated microneedles stored in plastic casings were placed in a triple stability chamber maintained at \(40^\circ\text{C} \pm 2^\circ\text{C}\), 75 ± 5% RH for 30 days. The coated microneedles were withdrawn at 0, 10, 20 and 30 days. Drug content and microneedle morphology of coated microneedles were evaluated at each time interval.

3. RESULT AND DISCUSSION

3.1. Preformulation Study of minoxidil

3.1.1. Description: The received sample of minoxidil was white in color, odorless and crystalline.

3.1.2. Melting Point: Melting point of minoxidil was found to be \(248^\circ\text{C}\) which was in accordance with reported value.

3.1.3. Infrared spectroscopy

The IR spectrum of the minoxidil was obtained on Jasco 4100 spectrophotometer by KBr disk method (fig.1). The result showed the presence of characteristic peaks as shown in table 2, which were compared with standard spectrum. It was confirmed that the drug molecule was 2, 4 diamino-6-piperidimopyrimidine-3-oxide (minoxidil).

3.1.4. Differential Scanning Calorimetry (DSC)

The thermogram of the minoxidil (fig.2) showed sharp endothermic peak at 247.85°C. The endothermic peak at this region was due to the melting of minoxidil. From DSC it was confirmed that the melting point of the drug was 248°C

3.1.5. Ultraviolet Spectroscopy

3.1.5.1. Determination of λ\textsubscript{max}

Minoxidil had a characteristic ultra-violet absorption spectrum in phosphate buffer pH 7.4. Standard solution of minoxidil (10 µg /ml) in phosphate pH 7.4 was scanned in the range of 200-400 nm. The \(\lambda\text{max}\) was obtained at 285 nm. The spectrum obtained (fig.3) complied with reported standards.
3.1.5.2. Development of standard curve of the drug using UV Spectrophotometer

The standard curve of minoxidil constructed in phosphate buffer pH 7.4 using UV spectrophotometer was found to be linear with $R^2$ value 0.99 (fig.4).

3.1.6. Determination of Partition Coefficient

The partition coefficient of drug was found to be 1.22 which was comparable to reported value of 1.24 (table 3).

3.1.7. Solubility studies

It was clear from the solubility studies that the solubility of minoxidil in water was least amongst the selected solvents; whereas it was found maximum in propylene glycol. Also it was observed that the solubility of minoxidil was inversely proportional to carbon chain length of the monohydric alcoholic solvents considered for study (table 4). Highest solubility of minoxidil in propylene glycol was observed which may be due to presence of two hydroxyl group in the solvent. Also propylene glycol was used as a moisturizing agent in many topical formulations. Hence it was chosen to eliviate dehydration of dermal cells caused due to ethanol.

3.1.8. Selection of polymer

From the various coating solutions prepared, all the polymers failed to coat microneedles except eudragit E 100. There was no coat formation observed on the microneedles when coated with hydroxyl propyl methyl cellulose, ethyl cellulose, carbopol and sodium carboxy methyl cellulose. Therefore eudragit E 100 was selected as coat forming polymer.

From the literature it was found that eudragit E 100 showed maximum solubility in alcohols.[18] Hence ethanol was selected as solvent for eudragit E 100.

3.1.9. Drug Polymer Compatibility study

3.1.9.1. FTIR Spectroscopy

The IR spectrum of a physical mixture of minoxidil and eudragit E 100 was recorded on Jasco FTIR-1100 spectrophotometer by KBr disk method (fig.5,6). The result showed the presence of all characteristic peaks compared with standard peaks. No significant change in spectrum of drug was observed. This indicated no strong interaction between the drug and the polymer.

3.1.9.2. Differential Scanning Calorimetry (DSC)

The thermogram of the physical mixture of minoxidil and eudragit E 100 showed sharp endothermic peak at temperature of 248.14°C. The endothermic peak at this region was due to the melting of minoxidil. From DSC it was confirmed that the selected polymer (eudragit E 100) was compatible with minoxidil. (fig.7)
3.2. Formulation of coated microneedle

If dip coating method would have adopted for coating of microneedles, which was easier as compared to coat each individual needle, this would have resulted in coating of roller surface in addition to microneedles. The amount coated on roller surface would have not been effectively administered while actual application. Hence this would have resulted in unadministerable portion of formulation in turn increased cost of manufacturing. Therefore coating of each individual microneedle, inversion of roller for drying and further coating was adopted for efficient formulation development.

The physical appearance of the coat after observation under motic microscope showed complete coat formation over the microneedle surface and less at the base. The coated microneedles were evaluated for appearance, coating dimensions, drug content and percent cumulative drug release. (table 5)

In formulations F1 and F2, the eudragit E 100 was used in low concentration as compared to formulations F3 and F4. Both the formulations F1 and F2 showed uniform but very thin coat formation when observed under microscope. Also during inversion and drying, coat was observed to get concentrated at the tip of the microneedles. Hence in formulations F3 and F4 the polymer concentration was increased. It was found that formulation F4 showed uniform coat formation with better thickness as compared to F1 and F2 but showed the tendency of getting concentrated at the tip during inversion and drying. Whereas formulation F3 showed uniform and thicker coat formation and reduced tendency of gathering at the tip. This may be due to increased viscosity of coating solution due to higher polymer concentration as well as increased concentration of propylene glycol. Hence formulation F3 was finalized based upon coating efficiency.

The drug content and drug release results were found to be comparable with each other.

3.3. Evaluation of coated microneedles

3.3.1. Motic image: As it was clear from fig. 8 that the plain microneedles showed sharp tips whereas coated microneedles showed blunt tips. This represented presence of coat on microneedles.

Under motic microscope, length of uncoated microneedle and coated microneedle was found to be 518.6 µ and 626.9 µ respectively which further confirmed coat over the microneedle (fig.9)

3.3.2. Percent drug content:

The drug content of all the formulations were comparable with each other. (table 5)

3.3.3. In-vitro drug release study

To understand the characteristics of drug release from coated microneedles, an invitro release study of formulation F3 was carried out. The drug release of minoxidil from coated microneedle formulations was also compared with the marketed formulation (fig.12). It was found that percentage drug release from coated microneedles and marketed formulation was 94.82% and 89.71% respectively. The F3 microneedle formulation was found to follow Matrix drug release kinetics (R value 0.9969; fig.10) where as marketed formulation was found to follow Hixson Crowell drug release kinetics (R value 0.9953; fig.11). The flux of
drug permeation through cellophane membrane of both the formulations, coated microneedles (F3) and marketed formulation was found to be 67.59 µg/min/cm² and 63.88 µg/min/cm² respectively.

From the above observations it was found that percentage drug release and flux of drug permeation of microneedle formulation and marketed formulation showed insignificant differences.

**3.3.4. Ex-vivo drug permeation study**

To study the effect of skin anatomy on drug release from the coated microneedle formulation (F3) the *ex vivo* study was conducted (fig.13). Also the results were compared with marketed formulation.

It was observed that percentage drug release of formulation F3 was 96.04% whereas of marketed formulation was 85.39% (fig.16). The F3 microneedle formulation was found to follow Peppas drug release kinetics (R value 0.9935; fig.14) where as marketed formulation was found to follow Hixson Crowell drug release kinetics (R value 0.9684; fig.15). The flux of drug permeation through cellophane membrane of both the formulations, coated microneedles (F3) and marketed formulation was found to be 62.78 µg/min/cm² and 60.55 µg/min/cm² respectively.

From the above observation it was found that there was no significant difference in drug release and flux between coated microneedle and marketed formulation through abdominal skin of albino mice.

**3.3.5. Accelerated stability study**

It was observed that the coated microneedle formulation showed no significant change in appearance as well as drug content when the samples were kept at accelerated condition of 40°C ± 2°C, 75 ± 5% RH for 30 days. The rate of degradation was calculated to be 0.036 mg/day at above mentioned accelerated condition. To predict the stability of the formulation, study at an additional accelerated temperature and humidity was needed. (table 6, fig.17)

**5.1. TABLES**

**Table 1:** Composition of minoxidil coated microneedle

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Name of ingredient</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Minoxidil (2%)</td>
<td>0.1 mg</td>
<td>0.1 mg</td>
<td>0.1 mg</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>2</td>
<td>Eudragit E 100</td>
<td>0.1 mg</td>
<td>0.1 mg</td>
<td>0.15 mg</td>
<td>0.15 mg</td>
</tr>
<tr>
<td>3</td>
<td>Propylene glycol</td>
<td>1 ml</td>
<td>2 ml</td>
<td>2 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>4</th>
<th>Ethanol</th>
<th>4 ml</th>
<th>3 ml</th>
<th>3 ml</th>
<th>4 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Total volume</td>
<td>5 ml</td>
<td>5 ml</td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

**Table 2:** Observations of FTIR Spectrum of minoxidil

<table>
<thead>
<tr>
<th>Wave number Standard (cm(^{-1}))</th>
<th>Wave number observed (cm(^{-1}))</th>
<th>Group identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>3470,3445,3430, 3385, 3280</td>
<td>3423.99</td>
<td>N-H stretch</td>
</tr>
<tr>
<td>3280, 3040</td>
<td>3268.75</td>
<td>H- bonded N-H</td>
</tr>
<tr>
<td>2975,2955,2880</td>
<td>2944.77</td>
<td>Aromatic and aliphatic C-H stretch</td>
</tr>
<tr>
<td>1650,1618</td>
<td>1646.91</td>
<td>Aromatic C-H stretch</td>
</tr>
<tr>
<td>1568, 1485, 1475,</td>
<td>1554.34</td>
<td>Aromatic O-C stretch</td>
</tr>
<tr>
<td>1460, 1450</td>
<td>1363.43</td>
<td>N-H bending</td>
</tr>
<tr>
<td>1260, 1248, 1225</td>
<td>1233.25</td>
<td>Aromatic C-N stretch</td>
</tr>
</tbody>
</table>

**Table 3:** Observations of partition coefficient study

<table>
<thead>
<tr>
<th>Phase</th>
<th>Absorbance</th>
<th>Concentration Abs./slope</th>
<th>Dilution factor</th>
<th>Concentration (µg/ml)</th>
<th>Concentration mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>For organic phase</td>
<td>0.4426</td>
<td>26.03</td>
<td>100</td>
<td>26030.0</td>
<td>26.03</td>
</tr>
<tr>
<td>For aqueous phase</td>
<td>0.1186</td>
<td>2.1178</td>
<td>100</td>
<td>2117.8</td>
<td>0.211</td>
</tr>
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</table>

**Table 4:** Observations of solubility study in selected solvent

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.232</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.100</td>
</tr>
<tr>
<td>Propranol</td>
<td>0.090</td>
</tr>
<tr>
<td>Butanol</td>
<td>0.070</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>0.382</td>
</tr>
<tr>
<td>Water</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Table 5: Evaluation of coated microneedle formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Appearance</th>
<th>Drug content (%)± S.D.</th>
<th>Percent cumulative release ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Uniform</td>
<td>98.20±1.80</td>
<td>90.04±0.023</td>
</tr>
<tr>
<td>F2</td>
<td>Uniform</td>
<td>97.50±2.20</td>
<td>90.73±0.12</td>
</tr>
<tr>
<td>F3</td>
<td>Uniform</td>
<td>98.80±1.60</td>
<td>94.82±0.98</td>
</tr>
<tr>
<td>F4</td>
<td>Uniform</td>
<td>97.94±1.23</td>
<td>90.71±0.076</td>
</tr>
</tbody>
</table>

Table 6: Observations of accelerated stability study (mean±SD, n=3)

<table>
<thead>
<tr>
<th>Time</th>
<th>Appearance</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (0 day)</td>
<td>Uniform</td>
<td>98.80±1.60</td>
</tr>
<tr>
<td>10 day</td>
<td>Uniform</td>
<td>98.20±1.20</td>
</tr>
<tr>
<td>20 day</td>
<td>Uniform</td>
<td>97.90±1.80</td>
</tr>
<tr>
<td>30 day</td>
<td>Uniform</td>
<td>97.70±2.60</td>
</tr>
</tbody>
</table>

5.2. FIGURES

Fig. 1: Infrared spectrum of minoxidil
Fig. 2: DSC thermogram of minoxidil

Fig. 3: Ultra-violet absorption spectrum of minoxidil in phosphate buffer pH 7.4
**Fig. 4:** Standard plot of minoxidil in phosphate buffer pH 7.4

**y = 0.056x + 0.036**

**R² = 0.99**

**Fig. 5:** The spectra of physical mixture of minoxidil with eudragit E 100 at 30°C/65 RH after one month.

**Fig. 6:** The spectra of physical mixture of minoxidil with eudragit E 100 at 40°C/75 RH after one month.
Fig. 7: DSC thermogram of physical mixture of minoxidil and eudragit E 100 (1:1)

Fig. 8: Motic image of: a) plain microneedle and b) coated microneedle
Fig 9: Length difference: a) plain microneedle and b) coated microneedle

Fig 10: In-vitro drug release profile of minoxidil coated microneedles

Fig. 11: In-vitro drug release profile of marketed minoxidil formulation

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Fig. 12: Graph of cumulative percent drug release vs time of coated microneedles and marketed formulation

Fig. 13: Abdominal skin tissue of mice after microneedle application for drug permeation study

Fig. 14: Ex-vivo drug release profile of minoxidil coated microneedle
Fig. 15: *Ex-vivo* drug release profile of marketed minoxidil formulation

Fig. 16: Graph of cumulative percent drug permeated vs time of coated microneedles and marketed formulation

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6. CONCLUSION

Coated microneedle formulations showed great potential in transdermal drug delivery systems. Alopecia presented common problem in both the genders. It was also learnt that various areas on scalp presented differences in drug permeation due to differences in concentration of fat cells under the skin. As minoxidil was the most commonly used drug for topical application to promote hair growth, the same was chosen for the study.

Determination of melting point, IR spectrum, λmax by UV Visible spectrophotometry, partition coefficient and DSC study of the received drug sample confirmed that the drug was minoxidil. The solubility of the drug was determined in various solvents showed maximum solubility in propylene glycol. Hence propylene glycol was selected as solvent for the drug. Eudragit E 100 was able to form complete coat over microneedle as compared to other other polymers under consideration. Also eudragit E 100 was found compatible with minoxidil. Hence eudragit E 100 was selected as coat forming polymer. During formulation coated microneedles were dried at 37°C for 30 min. This resulted in evaporation of solvent system composed of ethanol and propylene glycol. Hence undesirable effects of ethanol were reduced. The drug release profile of coated microneedle was found comparable to marketed solution of minoxidil. Hence it was concluded that microneedle formulation showed comparable drug permeation while the concentration of ethanol, which was used as permeation enhancer in marketed formulation was reduced. It was found that detailed stability study was required to predict the stability of the formulation.

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8. REFERENCES