Formation of polymeric films containing supersaturated levonorgestrel contraceptive drug by transdermal metered dose aerosol

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Due to the benefits of transdermal sprays, formulation of levonorgestrel as a transdermal metered dose aerosol was studied in this work. Simultaneously, super-saturation was evaluated as an effective method to increase the transfer of active ingredients to the skin. By using anti-nucleant polymers and solvent evaporation after spraying, we could form films containing supersaturated drug after spraying the formulation and inhibiting crystallization. Film formation and visual properties of films were investigated. The kinetics of weight loss and super saturation after film formation was calculated and finally, the rate of drug release from cellulose acetate membrane was assessed. In the formulations containing higher amounts of polyvinyl pyrrolidone (PVP) and 4 mg of levonorgestrel, 84.31% of drug was released during 4 h. While, in formulation with 2 mg drug, the maximum 95.79% of the drug was released during that time. According to these results, this kind of transdermal metered dose aerosol could be suggested as a suitable form for delivery of levonorgestrel through the skin.

Key words: Transdermal delivery, levonorgestrel, topical aerosol, supersaturation.

INTRODUCTION

Transdermal delivery is generally considered as a noninvasive and acceptable route of drug administration that provides continuous penetration of drugs through the intact skin (Fan et al., 2008; Chen et al., 2010). In this drug delivery approach, number of physical, chemical and biochemical methods have been suggested to improve the transportation of drug through the skin. All these attempts are aimed to reduce the barrier properties of stratum corneum or increase the diffusion properties of the drug (Raghavan et al., 2000; Wokovich et al., 2006).

Enhancing permeation into the skin via supersaturation of the drug has previously been shown to improve the efficiency of topical drug release (Raghavan et al., 2001a; Valenta and Auner, 2004; Moser et al., 2001).

Supersaturation is achieved when a compound is solubilised at a concentration which is greater than the saturated equilibrium solubility. The increased concentration of drug in a vehicle above saturation leads to a greater thermodynamic activity which proportionally increases the rate at which the drug can pass through the skin (Reid et al., 2008; Jones et al., 2009). It has been shown that metered dose aerosol (MDA) formulations can be applied for formation of topical films by solvent evaporation to induce supersaturation of therapeutic agents (Lulla et al., 2004). Supersaturation in these transient systems is driven by evaporation of the propellant during dose actuation and disappearance of the co-solvent once the dose has reached the skin. Although these

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these types of systems are considered to have high levels of organic solvents, due to their highly volatile nature and short time spent on the surface of the skin, they are not thought to pose an irritation risk (Jones et al., 2009).

Transdermal delivery of estrogens and progestogens for contraception and hormone replacement therapy has been evaluated in numerous researches (Agrawal and Pruthi, 2011; Burkmann, 2007; Raynaud, 2005). The use of long acting implants and patches are currently two conventional methods that are available in the pharmaceutical markets for this indication (Chrisman et al., 2006; Mansour et al., 2011; Toole et al., 2002; Caruso, 2003). In this way, application of topical metered dose aerosols to provide greater transdermal dosing flexibility and overcome some of the dosing limitations of current transdermal delivery dosage forms has been proposed in the recent years (Fraser et al., 2007).

The aim of this study was to investigate topical formulations that enhance release of levonorgestrel as a contraceptive drug from polymeric films via transient supersaturation and to determine the degree of supersaturation for this system after application. Levonorgestrel is a synthetic derivative of the progestosterone hormone. It is thought to prevent pregnancy in three different ways, depending on the stage of the menstrual cycle at which unprotected intercourse occurs: it suppresses ovulation, inhibits the fertilization of any egg already released, and may also cause changes to the endometrium to prevent a fertilized egg implanting (Schindler et al., 2003). Metered dose aerosols that contained levonorgestrel, hydroxyfluoroalkane as propellant, ethanol as volatile cosolvent and a film forming polymer was developed to deliver drug. Efficacy of poly vinyl pyrrolidone (PVP) and hydroxy propyl methyl cellulose (HPMC) to form films containing supersaturated drug were compared and attempts were made to determine the differential scanning (DS) by testing drug release from the formulations through regenerated cellulose membranes.

**MATERIALS AND METHODS**

Levonorgestrel was kindly gifted by Iran hormone Co. (Iran). Absolute ethanol (99.7 to 100%) was purchased from Bisdestan (Iran). HPMC (methocel E5) was from Colorcon (England) and PVP K30 was purchased from Fluka (Switzerland). 1,1,1,2-tetrafluoroethane (HFA 134a) propellant was kindly donated by INEOS Fluor (UK). Tween 80 was supplied by Merck (Germany). Regenerated cellulose membrane (RCM) (2000 Da molecular weight cut-off) was purchased from Spectrum (USA).

**Formulation and preparation of metered dose aerosol**

A solution was prepared by mixing film former polymer (PVP or HPMC), the drug substance and pure ethanol and was left to stir overnight to allow the polymer to solvate. An aliquot of the solution was dispensed into an aerosol container (Bespak Europe Ltd, UK). A 63 µl valve was immediately crimped onto each container and the canister was filled with HFA 134a through the valve using a 3,4-methylenedioxyamphetamine (MDA) filler (Pamasol Willi Mader AG, Switzerland) until the desired weight was obtained. The compositions of a series of formulations are shown in Table 1. The concentration of levonorgestrel was adjusted to 33 µg per puff. The concentration of pure ethanol was fixed to a maximum of 10.0% (w/w), to minimize the effect on the vapor pressure and consequently on the release velocity of the liquefied propellant throughout the orifice. To evaluate film forming of polymers, sprays were applied onto a plastic plate and the films were characterized after evaporation of solvents.

**Optical microscopy**

The morphology of produced films was studied with an optical microscope (Euromex, Netherlands) equipped with a camera system (Sony, Japan). The samples were prepared by spraying the formulations into glass plates and were observed by 40× lens and then took photographs by the camera.

**Measurement of evaporation rate and degree of saturation**

Thirty actuations from every metered dose aerosol were applied to an aluminum stage on an analytical balance (Sartorius, Germany) and monitored for weight loss after application. Weight of the formulation was plotted against time and the rate of solvent evaporation was calculated using a line of best fit over at least three time points. The final weight of the film was compared to the weight at a set time point to calculate the weight of the remaining ethanol at that time, and this was used to determine the concentration of drug, as described previously by Jones et al. (2009). By comparing this value with the saturated solubility of the drug in ethanol, degree of saturation was obtained using equation 1:

\[
DS = \frac{WD_{App} / (WF_t - WF_{Final})}{CSS}
\]

Where WD_{App} (mg) was the weight of the drug applied, WF_t (g) was the weight of the formulation at the time point t, and WF_{Final} (g) was the final weight of the formulation after 4 h. This gave a concentration (mg of drug/g of solvent) at time t, which was then divided by the saturated solubility concentration of the drug in the solvent, CSS (mg/g). If the concentration at time t was greater than the saturated solubility, then the formulation was classified as supersaturated. The degree of saturation was plotted against time to assess degree of saturation kinetics over the time of the experiment.

**Drug release studies**

The in vitro drug release was evaluated using United States Pharmacopeia (USP) 23 dissolution test apparatus 5 (paddle over disk). It was performed using a dissolution tester (Erweka, Switzerland) and the dissolution medium comprised 500 ml...
Table 1. Composition of formulations for preparation of transdermal metered dose aerosols.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polymer type</th>
<th>Amount of polymer (%)</th>
<th>Amount of drug (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>PVP K30</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>F2</td>
<td>PVP K30</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>F3</td>
<td>PVP K30</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>F4</td>
<td>PVP K30</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>F5</td>
<td>PVP K30</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>F6</td>
<td>PVP K30</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>F7</td>
<td>PVP K30</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>F8</td>
<td>PVP K30</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>F9</td>
<td>HPMC</td>
<td>0.10</td>
<td>1</td>
</tr>
<tr>
<td>F10</td>
<td>HPMC</td>
<td>0.10</td>
<td>1.5</td>
</tr>
<tr>
<td>F11</td>
<td>HPMC</td>
<td>0.10</td>
<td>2</td>
</tr>
<tr>
<td>F12</td>
<td>HPMC</td>
<td>0.10</td>
<td>2.5</td>
</tr>
<tr>
<td>F13</td>
<td>HPMC</td>
<td>0.20</td>
<td>1</td>
</tr>
<tr>
<td>F14</td>
<td>HPMC</td>
<td>0.20</td>
<td>1.5</td>
</tr>
<tr>
<td>F15</td>
<td>HPMC</td>
<td>0.20</td>
<td>2</td>
</tr>
<tr>
<td>F16</td>
<td>HPMC</td>
<td>0.20</td>
<td>2.5</td>
</tr>
</tbody>
</table>

of 5 microgram poly sorbate 80/g water maintained at a temperature of 35.0°C and a paddle rotation speed of 50 rev/min. For sample preparation, firstly, the synthetic cellulose membrane was soaked in deionized water. Then 5 puffs of aerosol were applied to the disk surface and the film surface was covered with membrane and was sealed. The disk containing the sample was submerged into the dissolution medium. Five milliliters of sample were collected at predetermined time intervals over 4 h. The drug concentration was measured by an ultra violet (UV) spectrophotometer (Spekol, Germany) according to USP monograph for levonorgestrel.

Differential scanning calorimetry (DSC)

Thermal behavior of raw materials and the selected films containing active substance were studied quantitatively and qualitatively by differential scanning calorimetry (DSC 204 F1, Netzsch, Germany). The samples (7 to 12 mg) were accurately weighed into standard aluminum pans and sealed. Thermograms were recorded during heating and cooling runs at a scan rate of 10°C min⁻¹ between 25 and 300°C.

RESULTS AND DISCUSSION

Supersaturation of a drug in a topical formulation is one approach by which skin penetration enhancement can be achieved without the use of exogenous chemical enhancers and expensive complicated technologies. In a supersaturated state, the saturation solubility of the compound in its formulation exceeds the equilibrium solubility and the driving force for diffusion is elevated and therefore a higher flux across the skin membrane can be achieved. However, such a system must remain physically stable, and minimal crystallization of the drug should occur during the permeation process. The addition of anti-nucleant polymers to supersaturated formulations prevents crystal nucleation or growth and thus maintains the elevated thermodynamic activity. Also, anti-nucleant polymers can extend the time of supersaturated solution stability and also facilitate the generation of higher levels of supersaturation. Addition of PVP and HPMC to supersaturated solutions has been reported to be effective in crystal growth inhibition of drugs (Megrab et al., 1995).

In the present study, antinucleant effects of these two polymers were investigated for formation of topical films containing supersaturated levonorgestrel. For this purpose, primarily, formulations containing levonorgestrel, film-forming polymer and ethanol were prepared and behavior of each component within the solution was assessed by visual solubility experiments. It was found that all formulations resulted in the formation of clear solutions after stirring for 24 h. However, formulations which contained relatively higher concentrations of drug or polymer than the amounts mentioned in the table did not form transparent solutions.

After addition of propellants to the solutions and preparation of metered dose aerosols, it was found that the formulations F11 to F16 containing higher levels of HPMC was not stable and the drug and polymer were precipitated in the cans. Appearance of other films was evaluated following actuation of spray onto a transparent plastic plate. Also, optical microscopy was applied for detailed evaluation of film integrity. The results showed
that all of the formulations could produce tough films with smooth surfaces. However, some fine air bubbles could be detected in all films under microscope as presented in Figure 1. In the case of F11, which contains HPMC films, some agglomerations was detected that could be attributed to the formation of drug nuclei after supersaturation.

The weight loss profiles of selected metered dose aerosols after application were plotted as a function of time to explore supersaturation kinetics. As shown in Figure 2, there were three obvious gradients in the weight loss profiles of PVP formulations. One immediately post dose application, a second after approximately 2 min and the third after 10 min. These three regions in the evaporation profile have previously been defined as Hydrofluoroalkane loss (first region) followed by co-solvent loss (second region) and finally hardening of the film (no weight loss in the third region) (Jones et al., 2009; Stein and Myrkdal, 2006). The trend of weight loss is relatively similar in the formulations containing PVP and the difference between graphs may be related to the different weights of solid materials in the formulations. However, there is a moderate decrease in the rate of weight loss with increase of polymer in the formulations. This relatively slower rate of weight loss from F1 to F8 would be attributed to the proportion of ethanol to polymer. Ethanol molecules must diffuse through the polymer to reach the surface of the film and escape. Increasing the viscosity of a solution via the addition of PVP will reduce the molecular diffusion and possibly lead to ethanol depletion at the air-liquid interface and thus a reduction in evaporation rate (Aronson et al., 2004). Weight losses in the HPMC films were much faster than PVP films and the slope of graphs is very sharp (Figure 3). In these formulations, second phase of weight loss profile could not be detected and it seems that ethanol evaporation occurs in a short time. It could be related to the low concentrations of HPMC in the formulations and lower affinity of ethanol to this polymer (Kim et al., 2006).

Monitoring the evaporation of solvents enabled theoretical calculation of the degree of drug supersaturation in the films over time (Figures 4 and 5). The saturation kinetics was different as a consequence of the altered evaporation profiles and film compositions after loss of the HFA. Formulation containing PVP were supersaturated after dose actuation and during 60 min of study; while the HPMC formulations were highly supersaturated during 10 min after actuation.

Anti-nucleant polymers such as PVP and HPMC are thought to prevent crystallization through an increase in solution viscosity which slows molecular diffusion and prevents seed nucleation (Raghavan et al., 2001b). However, a chemical interaction between PVP and the drug caused by the adsorption and the orientation of the polymer at the solid/liquid interface of the crystal as it forms makes the antinucleant capability of a polymer more efficient (Meghab et al., 1995; Sekikawa et al., 1978). Although some drug nuclei were observed in the films generated by the HPMC during the time course of the experiments, the drug in the films did not necessarily return to saturated drug concentrations immediately. The quantity of drug remaining in solution is dependent upon the rate at which the drug recrystallizes and thus the potentialenhanceddrugreleasecouldstillexist. Although
Figure 3. A comparison of weight loss of films after actuation from selected metered dose aerosols containing different weight ratios of HPMC.

Figure 4. Theoretical degrees of saturation drug (DS) after dose delivery from metered dose aerosols containing different weight ratios of PVP.

it was critical for HPMC formulations, melting endotherm of drug was not observed in DSC thermo-grams (Figure 6). It was attributed to the difference between crystalline structures of unprocessed sample of levonorgestrel and drug nuclei in the film. Observing such an endotherm was not expected for PVP films and it was not detected. The profiles of drug release from the films confirmed these findings and it was deduced that release of drug
Figure 5. Theoretical degrees of saturation drug (DS) after dose delivery from selected metered dose aerosols containing different weight ratios of HPMC.

Figure 6. DSC thermograms of raw materials and selected films containing PVP and HPMC and levonorgestrel.
Figure 7. Profiles of drug release from unprocessed levonorgestrel and selected polymeric films containing PVP or HPMC.

Figure 7. Profiles of drug release from unprocessed levonorgestrel and selected polymeric films containing PVP or HPMC.

from PVP films was drastically higher than both release from HPMC films and dissolution of unprocessed drug (Figure 7). In the formulations containing higher amounts of PVP and 2.5 mg of levonorgestrel (F8), 84.31% of drug was released during 4 h while in formulation with 2 mg drug (F7), the maximum 95.79% of the drug was released during that time. In other word, PVP could play its role as an antinucleant polymer for supersaturation of levonorgestrel, and it could probably control the release of drug by a combination of physical properties and supersaturation kinetics.

Conclusion

Transdermal metered dose aerosol could be considered as an efficient dosage form for delivery of supersaturated levonorgestrel as a potent contraceptive. This drug delivery system was specifically formulated such that the solution sprayed onto the skin was readily taken into the skin, and rapidly evaporates from the surface, thereby leaving the surface of the skin dry within less than 1 min of application. Furthermore, utilization of PVP as antinucleant resulted in the formation of satisfactory films capable to provide supersaturation of drug and improvement of drug release.

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