DESIGN AND EVALUATION OF A TRANSDERMAL PATCH WITH ATORVASTATIN

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Abstract
A transdermal patch loaded with atorvastatin was developed in order to improve dyslipidaemia therapy and had good drug release for several days. This transdermal system can be used as a new alternative for the treatment of hypercholesterolemia. Transdermal patches (TP) were generated by the plate casting technique; the evaluated patches were generated using a factorial design to obtain the optimum formulation. The patches were characterized by differential scanning calorimetry (DSC), scanning electron microscopy (SEM), bioadhesion, post-wetting-bioadhesion, tensile strength, drug release studies and in vitro percutaneous absorption studies. The results obtained for optimal TP formulation showed good properties such as no interactions between drug and excipients in DSC studies, 1063.05 gf of bioadhesion and 995.9 gf for post-wetting-bioadhesion, the tensile strength of 1307.5 gf, thickness of 0.43 mm, the SEM studies showed a porous matrix with no precipitates of the drug, good drug release adjusted to Higuchi model and a flux of atorvastatin of 8.6 µg/cm²/h for percutaneous in vitro absorption. The optimal TP proved the release of atorvastatin during several days.

Rezumat
A fost dezvoltat un plasturie transdermic cu atorvastatin pentru a îmbunătăți terapia dislipidemiei și pentru a obține o cedare îmbunătățită a substanței active pe parcursul mai multor zile. Acest sistem transdermic poate fi folosit ca o nouă alternativă pentru tratamentul hipercolesterolemiei. Plasturile au fost evaluată utilizând un design factorial pentru a obține formularea optimă. Patch-urile au fost caracterizate prin calorimetrie de scanare diferențială (DSC), microscopie electronică de scanare (SEM), bioaderență, studii de cedare a substanței active și studii de absorbție percutanată in vitro.

Keywords: transdermal patch, polyvinylpyrrolidone, human skin, atorvastatin, Eudragit E100®

Introduction
The hypercholesterolemia is characterized by high levels of cholesterol in the blood and is associated with high risk of atherosclerosis, heart attacks or stroke. This is why nowadays hypercholesterolemia is one of the leading causes of death in the world. People with hypercholesterolemia will have to take the treatment for life; however, the treatment is oral and presents some drawbacks such as irritation of the gastrointestinal tract, drug interactions with food, variability associated with the oral route, “first-pass” metabolism and multiple dosages are required. All these may cause some omission and generate therapeutic ineffectiveness [5, 16, 23, 33]. Transdermal drug delivery systems (TDDS) – such as a transdermal patch – improve the treatment of hypercholesterolemia because they allow the drug to be released into the systemic circulation directly [23], these systems have a controlled and constant delivery of drugs through the skin. This action reduces the frequency of dose, they are easy to apply and remove, they have a reduction of variability in absorption, these systems are non-invasive, painless and comfortable, and they avoid the first pass hepatic metabolism [5, 16, 33]. Atorvastatin calcium is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), being used to treat hypercholesterolemia. This enzyme catalyses the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol [13, 30]. Atorvastatin calcium was used because it has appropriate characteristics in order to be formulated in a trans-dermal patch: low molecular weight (558.64 g/mol), high lipid solubility, low initial dosage in some cases, high first-pass metabolism, low...
bioavailability ≈ 14%, food decreases of Cmax with 25%. These last characteristics make atorvastatin calcium a much more interesting drug candidate to be formulated in a transdermal patch, trying to improve its bioavailability and release for several days [9, 14]. For this reason, the aim of this study was to develop a patch that allows the drug delivery during several days.

Materials and Methods

In this research we used analytical grade reagents that comply with the Analytical Chemistry Society (ACS) specifications and were as follows: atorvastatin calcium (Biocon Limited India), Eudragit® E100 (Helm de México), polyvinylpyrrolidone K30 (PVP K30) (Drogueria Cosmopolita, Mexico), methyl (Meyers), triacetin and propylene glycol (Sigma--Aldrich, Germany), distilled water Mili-Q (Milipore Inc., Germany), dibasic sodium phosphate (Ferment) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (Sigma Aldrich, Germany). 

Transdermal patches preparation

The transdermal patches (TP) were prepared by dissolving the appropriate amount of Eudragit E100® in methanol and then the rest of the ingredients were added to each formulation (Table I). The films obtained were dried at room temperature for 72 hrs. We used a factorial design to find the optimal formulation; the software used was Statgraphics. All experiments were performed in triplicate for each formulation. 

Uniformity of drug content

Transdermal patches samples (with an area of 6.25 cm²) were dissolved in methanol in extraction tubes, leaving them under constant agitation for 24 hours to ensure the complete extraction of the drug. After this process samples were filtered (filter CRM globe Nylon 0.45 μm) and analysed by UV-Vis spectrophotometry (Velab model VE-5100UV, USA). The drug content was determined at 246 nm (see Table I) [6, 7, 27]. The method was previously validated, complied with the specifications for linearity parameters (r² > 0.99, slope coefficient CV < 2%), accuracy and repeatability (coefficient of variation of replicates CV < 2%).

Differential scanning calorimetry (DSC)

The DSC studies allowed the determination of excipient-excipient and drug-excipients interactions in the formulations evaluated. DSC studies were performed using a Mettler Toledo model dsc822e, (USA), with a heating rate of 10°C/min, from -25°C to 250°C with an inert atmosphere of nitrogen for the excipients, formulation and drug [25, 27, 31]. Scanning electron microscopy (SEM)

Samples were examined by SEM using a JEOL microscope (model JSM-6010LA, USA). The samples were previously coated by the sputtering technique under the following conditions: sputter 7 Amps 300 s. This study was performed in order to analyse the surface of the optimal patch and the excipients.

Tensile strength

The texturometer (Brookfield CT3 Texture analyser, USA with TexturePro CT Software) with a loading charge of 4.5 kg, was used for this test and determinations were made for each patch of the experimental design. Patches with an area of 8.4 cm² were held with tweezers in the texturometer in the following conditions: pre-test velocity of 2 mm/s, an activation charge of 6.8 g and a test speed of 0.5 mm/s, the probe being withdrawn at a speed of 4.5 mm/s, determining the necessary force for the patch to be broken [25, 27, 31].

Bioadhesion test and post wetting-bioadhesion

The studies were carried out using the same texture analyser. The skin samples from abdominoplasty (donated by the Hospital Angeles Inn Chapultepec), were placed and glued with cyano-acrylate at the bottom of the texturometer, and at that moment the cylindrical probe (perplex cylinder 1.27 cm²) started to descend to the transdermal patch (1.27 cm²) at a pre-test velocity of 2 mm/s until the patch came into contact, a loading force of 6.8 gf a speed of 0.5 mm/s. Finally, the cylinder was removed at a speed of 4.5 mm/s until a separation distance of 100 mm was obtained [27]. All experiments were performed in triplicate for each formulation.

Drug release study

The studies were made in the Mayasa disolutor, model APPM-0250, Mexico, the apparatus number 5 USP (paddle over disc method). The conditions were 500 mL phosphate buffer pH = 5.5 referring to physiological skin pH [31], at 37.5°C under stirring (50 rpm). 3 mL samples were taken at 5, 10, 15, 20, 30 minutes and 1, 2, 3, 4, 5, 6, 7 hours. Subsequently, the amount of drug released as a function of time was quantified by a spectro-photometric method at 246 nm [25, 26, 27]. The R-squared value from the kinetic release profiles was calculated with the following equations [4]:

Zero order model:

Q_t = Q_0 + K_0 t,

were Q_0 = the amount of drug dissolved in time t, Q_0 = the initial amount of drug in the solution (most times, Q_0 = 0) and K_0 is the zero order release expressed in units of concentration/time.
In the thermograms, the endothermic peaks of atorvastatin were observed at 50°C and 130°C, the first corresponding to the loss of water or dehydration (probably indicating the presence of the hydrated form), which is reported between 50°C and 130°C and the second is due to its reported drug melting point between 160.64°C - 182°C [1, 3, 8]. As for PVP K30, it presents two endothermic peaks (54.83°C and 149.85°C), the first is the loss of water and the second is the glass transition temperature (Tg) [1, 12, 17]. In the case of Eudragit® E100, it has an endothermic peak at 58.45°C corresponding to its Tg [32].

The optimal TP shows two endothermic peaks in which the first one is around 50°C - 130°C and the second one to 212°C - 227°C. Both peaks are related to atorvastatin. The value obtained in the last peak is increased due to the combination of polymers possibly giving more stability to the formulation of the TP [26].
Figure 1.
Thermograms of TP and excipients

Scanning electron microscopy
Microscopy studies are helpful to observe the surface morphology of the films generated, as well as to evaluate the physical stability of the formulations. In general, based on the micrographs performed for the solid and active excipients (see Figure 2) that were used as reference, the free drug was not observed in the optimal formulation, indicating that the drug was homogeneously dispersed. Therefore, the physical appearance of the patch was maintained in good condition. It was also observed that the matrix had pores as can be observed in Figure 2D [27].

![Micrographs of the films](image)

Figure 2.
Average results of bioadhesion, post wetting bioadhesion, and tensile strength

Bioadhesion
Bioadhesion studies are extremely important because using the appropriate bioadhesives it is possible to target the drug to specific sites and therefore the formulation remains longer at the site of application. Coupled with a controlled release of the drug, the frequency of drug administration can be reduced [32] and consequently, the bioavailability of the drug is improved.

The results of bioadhesion for each transdermal atorvastatin patch of the design can be seen in Table II and Figure 3. Regarding the bioadhesion studies, in Figure 3, it can be observed that the increase in the amount of PVP K30 decreases the bioadhesion. The PVP K30 is not a good bioadhesive as evidenced by the studies of Islam et al. and Saroj et al., for this case [11, 24]. The triacetin plasticizer had a positive effect on bioadhesion as its organic esters interact with
Eudragit® E100 conferring bio-adhesive properties [29]. For good bioadhesion properties it has to be used a lower amount of PVP K30 and triacetin plasticizer.

**Table II**

Average results of bioadhesion, post wetting bioadhesion, and tensile strength

<table>
<thead>
<tr>
<th>TP Formulations</th>
<th>PVP k30 (mg)</th>
<th>P</th>
<th>B (gf)</th>
<th>PB (gf)</th>
<th>TS (gf)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>PEG® E400</td>
<td>673.33 ± 153.60</td>
<td>687.39 ± 157.42</td>
<td>131.83 ± 132.15</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>PEG® E400</td>
<td>542.78 ± 98.19</td>
<td>881.44 ± 234.55</td>
<td>167.83 ± 152.20</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>800</td>
<td>PEG® E400</td>
<td>593.35 ± 243.08</td>
<td>606.28 ± 207.48</td>
<td>98.83 ± 81.56</td>
<td>0.76 ± 0.16</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>Triacetin</td>
<td>750.39 ± 271.42</td>
<td>679.67 ± 141.80</td>
<td>40.83 ± 16.78</td>
<td>0.71 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>600</td>
<td>Triacetin</td>
<td>753.17 ± 236.77</td>
<td>877.11 ± 195.08</td>
<td>140.17 ± 164.41</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>6</td>
<td>800</td>
<td>Triacetin</td>
<td>672.17 ± 166.91</td>
<td>667.17 ± 146.05</td>
<td>84.33 ± 62.64</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>Optimal TP</td>
<td>638.83</td>
<td>Triacetin</td>
<td>1063.05 ± 147.03</td>
<td>995.9 ± 199.38</td>
<td>1307.5 ± 317.26</td>
<td>0.43 ± 0.01</td>
</tr>
</tbody>
</table>

*P: plasticizer; B: Bioadhesion; PB: post wetting; TS: tensile strength

**Figure 3.**

Graphs surface response for each of the tests performed

*Post wetting-bioadhesion*

Post-wetting bioadhesion has the same importance as bioadhesion, except that this test considers wetting of the TP by transpiration or by external conditions such as environmental variables and washing. The results obtained from the design are shown in Table II and Figure 3. The design was also analysed with the Statgraphics Centurion XVII program, obtaining the surface response graph. The plasticizer triacetin enhances the bioadhesion of the films in comparison with PEG® E400, this property being attributed to several mechanisms of bioadhesion, adsorption, diffusion and electrostatic force when the patches are wetted [21]. However, in the case of PVP an intermediate amount of PVP K30 also increases the bioadhesion due to the formation of a strong gel that penetrates deeply into the mucin molecules [24] because PVP K30 interacts with water allowing a better adhesiveness, but it is interesting that at lower or higher PVP K30 proportions, this bioadhesive property is lost, (p < 0.05) [32].

**Tensile strength**

If the TP does not have a good resistance to rupture, there may be problems of safety, therapeutic efficacy and they will not supply the adequate dose to the patient [27, 29]. The results obtained are found in Table II.

In terms of tensile strength (Figure 3), it was observed that PEG® E400 gave a higher hardness to the patches and an intermediate amount of PVP K30 was proper for a better resistance to rupture. The resistance to rupture increased when using PEG® E400 (Table II and Figure 3). In general, the films formed by PEG® E400 are more rigid and resistant with Eudragit E100 because the films can be completely plasticized using PEG® E400 [21], in the case of triacetin, Eudragit® E100 interacts with the organic esters bonds of triacetin and makes a more bioadhesive and flexible TP [2, 20, 29]. As for the PVP K30 the tensile strength of the PTs may change due to the nature of the polymer or plasticizer used. A soft and weak polymer is characterized by a low breaking strength and low elongation, a hard and brittle polymer is defined by a moderate shear strength and low elongation, a soft and hard polymer is characterized by high shear strength and moderate elongation, whereas a hard polymer is characterized by its high breaking strength and high elongation [2]. Thus, at high or low concentrations this property is diminished.
In vitro drug release study

The importance of the test is to predict the rate and duration of drug release and ensure the constant release of drug from the polymer matrix of TP [10, 27]. The release plots for each of the formulations are shown in Figure 4.

In the profiles, we can observe that the PVP K30 plays an important role in the release (p < 0.05), its hydrophilic characteristics that help to capture water from the medium and thus to hydrate the matrix more rapidly, allowing a faster drug release [32], Figure 4 shows the profiles of each formulation with the respective errors bars. The effect of the PVP K30 can be corroborated with Figure 5 in the Pareto diagram where the PVP K30 has a positive effect on the released drug (p < 0.5), which means that when increasing the PVP K30 in the formulation, the release of atorvastatin calcium will be increased.

In terms of its kinetic profile, the drug release from patches (formulations 2 - 6) was adjusted to Peppas model that means anomalous diffusion of the drug. The drug release is controlled by diffusion and erosion. Optimal transdermal patch and formulation 1 were adjusted to Higuchi model [181]. This model is used to describe the drug release from porous matrices (Table III), which is the case of Eudragit® E100 as a matrix that has porous characteristics [4, 32]. The pores of the matrix can be observed in microscopy studies (Figure 2B).

Another important aspect observed is that the plasticizer triacetin allows a better release than PEG® E400. This is why the matrix formed with triacetin is not as rigid as the one of PEG® E400 and this allows carrying a faster process of solvation, letting the polymer matrix to release a higher percentage of drug.

Table III

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Order 0 ($r^2$)</th>
<th>Order 1 ($r^2$)</th>
<th>Higuchi ($r^2$)</th>
<th>Peppas ($r^2$)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.570</td>
<td>0.363</td>
<td>0.861</td>
<td>0.823</td>
<td>0.577</td>
</tr>
<tr>
<td>2</td>
<td>0.594</td>
<td>0.396</td>
<td>0.810</td>
<td>0.872</td>
<td>0.682</td>
</tr>
<tr>
<td>3</td>
<td>0.402</td>
<td>0.297</td>
<td>0.652</td>
<td>0.772</td>
<td>0.568</td>
</tr>
<tr>
<td>4</td>
<td>0.381</td>
<td>0.309</td>
<td>0.636</td>
<td>0.795</td>
<td>0.603</td>
</tr>
<tr>
<td>5</td>
<td>0.412</td>
<td>0.313</td>
<td>0.666</td>
<td>0.752</td>
<td>0.521</td>
</tr>
<tr>
<td>6</td>
<td>0.501</td>
<td>0.346</td>
<td>0.745</td>
<td>0.829</td>
<td>0.632</td>
</tr>
<tr>
<td>Optimal TP</td>
<td>0.930</td>
<td>0.782</td>
<td>0.997</td>
<td>0.986</td>
<td>0.710</td>
</tr>
</tbody>
</table>

Finally, these studies (Figure 4) show that in 7 h the optimal formulation disperse 100% of the drug from TP. However, these studies consider a hyperhydration which would not be real at the application site of the TP which is the skin; therefore, the studies should be complemented with percutaneous absorption studies.

In vitro percutaneous absorption studies

It is important to determine the kinetics of percutaneous drug absorption, because it can provide a good prediction of skin absorption in vivo. For this reason, the use of human skin in vitro is a good alternative. In addition, these studies have a low-cost, a short test time and high reproducibility [15, 19]. We calculated the cumulative amount (mg) of atorvastatin and the amount accumulated per patch area.
The latter was obtained by dividing the cumulative amount with 2.19 cm² (area of skin exposed to the TP) and these values were plotted in order to obtain the transdermal penetration profiles as a function of time (Figure 6). The parameters obtained like flow (F), which is the amount of active ingredient which crosses the membrane per unit area in a given (µg/cm²/h)[28] for optimal TP was F = 8.6 µg/cm²/h. The permeability constant (kp) was 5 x 10⁻⁴ cm/h, this parameter allowing us to determine the amount of active substance contained in the transdermal patch that passes through every cm² of the membrane at a given time (cm²/h) [18, 27, 28]. Finally, the lag time (tL) that can be defined as the amount of drug released in a given period of time and where the release becomes constant through the skin [18, 27, 28], in the case of TP was 17.33 h. Based on these results, the optimum TP formulation with a patch area of 16.53 cm² (≈ 4 cm x 4 cm) allowed to release a 10 mg dose of atorvastatin for 13.6 days thus giving a more effective therapeutic effect compared to tablets.

**Conclusions**

The optimal formulation had good tensile strength characteristics with a value of 1307.5 gf, meaning that the formulation cannot break easily in comparison with the other formulations and ensures the therapeutic efficacy. The bioadhesion and post-wetting bioadhesion values were 1063.05 gf and 995.9 gf, respectively. These results are better in comparison with the other formulations which guarantees that the patch adheres properly to the skin in order to carry out its therapeutic effect. The drug content in the optimal formulation was of 98.7%, in general, all formulations complied with this parameter. The thickness of the optimal patch formulation was of 0.43 mm which turns out to be quite comfortable for the patient. The SEM showed a porous matrix without precipitated drug and DSC studies showed no interactions between the drug and the excipients. A good drug release was obtained from the optimal formulation patch which fits the Higuchi model that describes the drug release from porous matrices. The optimal patch had a flux value throughout the skin of 8.6 µg/cm²/h and a lag time of 17.33 h. The results indicate that it is possible to formulate a TP to increase the release of atorvastatin calcium throughout the skin for 25 days. Our research group is working on the possibility of combining this characterized transdermal patch with a biodegradable microneedle array in order to generate a new alternative that allows improving the drug release for dyslipidaemia treatment.

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7. Escobar Chávez J.J., Estudio de la penetración a través de la piel de naproxeno sódico utilizando agentes promotores de penetración (azona y transcutol), y de dicolunocato de clorhexidina mediante iontoforesis. FESC UNAM, México; 2006.


14. Khan F.N., Dehghan M.H.G., Enhanced Bioavailability of Atorvastatin Calcium from Stabilized Gastric


27. Serrano Cañada P., Desarrollo y caracterización de un parche transdérmico de pravastatina acoplado a microagujas como promotor físico de la penetración transdérmica. [Asesor: José Juan Escobar Chávez, Ph.D], UAM Xochimilco. México; 2014.


