Preparation and *In Vitro* Permeation of Chlopheniramine Maleate (CPM) from Gel through Rat Skin

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Abstract

Chlopheniramine maleate (CPM), is one of the H₁-receptor antagonist, widely used in allergic diseases, like skin rash and pruritis. CPM 3% w/w was successfully loaded in 2% w/w sodium alginate (SA) as a gel base, and to be considered as a selected formula. It was found that the diffusion of CPM through the skin of albino rat was increased as the concentration of CPM increased from 2% w/w sodium alginate. More over, the addition of Triethanolamine 5% w/w, to sodium alginate 2% w/w, loaded by CPM 3% w/w, increased the amount of CPM diffuse through the skin of albino rat. While the addition of PEG 1000 2% w/w, and urea 5% w/w, separately to sodium alginate 2% w/w, loaded by CPM 3% w/w, hindered significantly P<0.05 the amount of the drug diffused through the skin of the rat. The selected formula of sodium alginate 2% w/w as a base loaded by CPM 3% w/w was physically acceptable, with shelf life approximately 3.3 years.

Key words: chlopheniramine maleate, gel, skin permeation

Introduction

Gels are semi solids consisting of dispersions made up of either small inorganic particles or large organic molecules enclosing and interpenetrated by a liquid (1). The delivery of the drug into and through the skin is recognized an effective means of therapy for local dermatological and systemic disease. In recent years, the development of transdermal permeation has been attracting an attention due to several advantages, such as better control of blood levels, reducing systemic toxicity and avoid first pass metabolism (2). Sodium alginate, a naturally occurring poly saccharide has been widely used as a disintegrant and gelling agent in pharmaceutical preparations (3). It has several unique properties that have been enabled it to be used as a gel matrix for delivery of many drugs (4). Chlorpheniramine maleate as a potent H₁-receptor antagonist can be indicate for many types of allergy such as rhinitis and pruritis, it can prevent but does not reverse histamine mediated response (5). This study aimed to both suggest new alternative dosage form for enhancing topical penetration of CPM, and to evaluate the potential and transdermal absorption.

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Received: 20/8/2008
Accepted: 16/12/2008
Experimental

Materials and Equipments:
Chlorpheniramin maleate CPM, supplied by Samraa drug industry SDI, Sodium alginate, triethanolamine TEA, from (Hopkins and William LTD, England), Sodium carboxymethylcellulose NaCMC, diethyl ether, glycerin, from (BDH chemical limited, Pool, England), Formaldehyde 37% (v/v), urea, from (Fluka AG, Switzerland), Polyethylene glycol PEG1000, methyl and propyl hydroxyl benzoate, from (Merk-Shuchardt, Germany), UV- spectrophotometer, Carywin UV, Varian, Australia USP dissolution apparatus, magnetic stirrer, ultrasonic cleaner, VWR cpley, England, Water bath shaker, hot air oven, memmert, Germany

Preparation of sodium carboxymethylcellulose (NaCMC) 5%w/w gel base:
Simply, the method employed for base was fusion method. It was carried out by incorporation
CPM equivalent to 1%w/w in the following base content:
NaCMC powder 5gm.
Glycerol 15gm.
Methylhydroxybenzoate 0.1gm.
Purified water to 100gm.
The base was prepared by mixing NaCMC with glycerol in a glass mortar, while methylhyd-roxybenzoate (methyl paraben) was dissolved in 40ml. of distilled water using heat to about 70°C with vigorous stirring by stirrer for 15 minutes, and cooled, then the later mixture was mixed with polymer-glycerol mixture and stirring until clear gel-base was gained. Then the CPM was incorporated to the base with 5 minutes continuous traturation and stirring to obtain homogenous clear drug-gel solution.

Preparation of sodium alginate (SA) 2%w/w gel base:
Sodium Alginate 2gm.
Glycerol 15gm.
CaCl2 0.2gm.
Propylhydroxybenzoate 0.2gm.
Distilled water to 100gm.
The same principle of procedure was done as in preparation of Na-CMC gel base. The polymer of sodium alginate was mixed with glycerin in a glass mortar and the mixture was poured in small amounts to the vehicle with stirring, while calcium chloride was dissolve in small amount of water and added to the vehicle with stirring then complete the volume with distilled water with 5 minutes continuous stirring, until translucent—white clear gel was formed.

In vitro release of CPM from gel base:
A small glass container with 3cm. in diameter of its opening mouth was modified in order to be filled with one gram of each formula, which was containing equivalent weight of 1%w/w of CPM. The mouth of container was covered with the filter paper which secured in place with rubber band. The dialysis cell was inverted in 500ml. of phosphate buffer pH 7.4 contained in a beaker of the dissolution apparatus. The system maintained at 37°C, the samples were withdrawn after 1, 2, 3, 4 and 5 hours, and replaced with an equal volume of fresh buffer solution. The sample were analyzed for their CPM content using uv-spectrophotometer at λmax 261 nm.

Preparation of the diffusion membrane:
The albino rat (4-6 week old male) was sacrificed by ether inhalation, then the skin was shaved lightly with an electrical clipper, taking care to prevent any damage to the skin, a rectangular section of abdominal skin several centimeters in each dimension was excised using a sharp blades. The defatting procedure, of the skin was carried out by weeping the skin with a cotton tip soaked in diethyl ether to remove the subcutaneous fat and scraping the dermal side to remove the muscles and blood vessels, the adhering fat was again removed by another cotton tip soaked in diethyl ether, and kept in phosphate buffer pH 7.4 for 2 hours in a water bath maintained at 37°C, to allow water soluble uv absorbing materials to leach out. The buffer was changed three times during this period with fresh amounts. Then the prepared skin for diffusion was stored in phosphate buffer for 24 hours in the refrigerator at 2°C before use.

In vitro diffusion of CPM through rat skin membrane:
One gram of each formula containing CPM was introduce in a small container and the epidermal surface of the rat skin was stretched over the mouth of the container with diameter 3cm. and legated with rubber band, the diffusion cell then inverted and immersed in 500ml of phosphate buffer at pH 7.4 contained in a beaker of dissolution
apparatus. The system was maintained at 37°C and the buffer solution was stirred at 100 r.p.m. during 5 hours of the study. Samples of 5 ml. were pipetted from the collection medium after 1, 2, 3, 4, and 5 hours replaced with an equal volume of freshly prepared phosphate buffer pH 7.4 at 37°C. The samples were analyzed using uv-spectrophotometer at λ max 261 nm.

**Effect of the temperature on the pH of the gel:**

The pH of the gel was measured every week for one month, by taking one gram of the gel from each stored sample at 40, 50, and 60°C, and shaken up with 10mls. of distilled water. The pH of the final solution was measured and recorded.

**Statistical analysis**

The significance between mean values was analyzed by student t-test, P-value of less than 0.05 was considered significant for all analyzed data shown in the results of this study.

**Results and Discussions**

**Effect of gel bases on the release of CPM:** Table 1. and figure 1, show the amount of drug release from gel bases, the results indicated that the drug released is significantly increased P<0.05 as a function of polymer type used in an order of 4%w/w SA > 4% NaCMC, this result may be referred to the hygroscopic effect of cellulose derivatives that affect water entrapment in the cross linking gel of 4%w/w NaCMC more than that of 4% w/w SA, since this amount of water may hinder another water molecules diffuse inside gel structure and then more drug releasing occurred. This result is in consistent with results obtained by Cetin T. et. al (13).

**Table 1. Effect of different bases on the release rate constant (K) of CPM**

<table>
<thead>
<tr>
<th>Type of Bases</th>
<th>Amount of CPM released (mg)</th>
<th>CPM released %</th>
<th>K (mg.min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4%w/w SA</td>
<td>9.06 ±0.09</td>
<td>90.6</td>
<td>0.427</td>
</tr>
<tr>
<td>4%w/w NaCMC</td>
<td>6.77 ±0.11</td>
<td>67.7</td>
<td>0.401</td>
</tr>
</tbody>
</table>

Each value represents the mean SD (n=3 readings in each group *)

**Effect of different enhancers and their concentrations on the diffusion:**

In order to evaluate best release profile of CPM from selected formula, different enhancers, urea 5%w/w, polyethylene glycol (PEG1000) 2% w/w, and triethanolamine (TEA) 1%, 2.5%, and 5% w/w were used on diffusion of CPM through rat skin.

**Skin irritation test:**

Skin male albino rats weighing approximately 500gm. were used to study the irritation test of the selected formula, on the rat skin. The dorsal side of the rat was carefully shaved and two circular areas of 2.5 cm. in diameter in each animal were done. Then 0.8% w/v aqueous solution of formalin as standard irritant to one circular area, and 5% w/w TEA gel formula contain 3% w/w CPM to the other circular area for three rats, and 2% w/w sodium alginate gel containing 3% w/w CPM to other circular areas of other three rats. The fresh gel samples and formalin solution were applied for 7 days. Finally, the application sites were graded to the visual scoring scale always by the same investigator.

**Stability study:**

The estimation of the shelf life of a selected formula 3% w/w CPM kept in a collapsible tubes at room temperature and oven maintained separately at 40, 50, and 60°C, samples were taken every seven days for 4 weeks. Each sample of the gel equivalent to 250mg. CPM in 50 ml. of phosphate buffer at pH 7.4. These samples were quantitatively transferred to volumetric flasks and appropriate dilutions were made with the same buffer, then the resulting solution were filtered with 0.45µ filter paper. The absorbance of each collected sample was calculated for CPM content at λ max 261 nm.
Figure (1). The effect of different bases on the release of CPM 1% w/w at pH 7.4 and 37°C

Each value represents the mean SD (n=3 readings in each group *)

Effect of polymer concentrations on the release of 1% w/w CPM gel:

Table 2, and figures 2 and 3, demonstrate the effect of SA concentrations on the release profiles of the CPM through rat skin. It was seen that the drug released from SA at different concentration and diffused through the filter paper was not affected by the concentration of the polymer, since no significant increase in the drug release, this behavior gives an impression that the drug release from SA followed zero-order kinetics in these concentrations, since water uptake by polymer is not affected by the concentrations of the polymer itself. Meanwhile the plot of the amount of the drug released versus square root of time demonstrates that there is a linear relationship of the drug release followed Higuchi principle in the diffusion process from semisolids in percutaneous absorption. These results were in agreement with the results obtained from the permeation of carvedilol transdermal patches.

Table 2. Effect of Sodium Alginate (SA) concentrations on the rate constant (K) of CPM 1% w/w phosphate buffer pH 7.4 at 37°C.

<table>
<thead>
<tr>
<th>Sodium alginate Concentration</th>
<th>Amount of CPM (mg./Shr.)*</th>
<th>CPM released %</th>
<th>Rate constant (K) (mg.min^-1/2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% w/w</td>
<td>8.934±0.17</td>
<td>89.3</td>
<td>0.4983</td>
</tr>
<tr>
<td>2% w/w</td>
<td>9.435±0.14</td>
<td>94.3</td>
<td>0.5713</td>
</tr>
<tr>
<td>4% w/w</td>
<td>9.060±0.04</td>
<td>90.6</td>
<td>0.4273</td>
</tr>
</tbody>
</table>

Figure (2). The effect of polymer concentration on the release of CPM 1% w/w through rat skin at pH 7.4 and 37°C

Figure (3). The kinetic analysis of CPM 1% w/w release from different polymer concentration at pH 7.14 and 37°C

Effect of CPM concentrations on the diffusion process:

Table 3, and figure 4, illustrate the effect of different CPM concentration 1% w/w and 3% w/w on the amount of CPM diffused through the rat skin, using 2% w/w SA as a gel base, the results showed that the amount of CPM diffused during the period of application (5 hours), increased as a function of increasing drug concentration. This behavior confirmed that the drug diffusion followed first-order mechanism, and the penetration rate is proportional to the concentration, since this diffusion depend on many factors, among them, the partition coefficient (K) and the...
concentration of the drug \(^{14}\). In this experiment the rate limiting step of the drug diffusion through the rat skin can't be estimated, because there are two types of partitioning, one of the partition of the drug for the skin (Ds), and the other for vehicle (Dv), or gel base. So these two magnitudes of the two diffusion coefficient Ds and Dv, determines whether the release from vehicle or skin is the rate limiting step, and by this approach, the concentration of incorporated drug in the gel base may solve this problem, regardless the diffusion or partition coefficient \(^{11,14}\).

Table 3. Effect of CPM concentration on the diffusion rate constant (K) using 2%w/w Sodium Alginate (SA) gel.

<table>
<thead>
<tr>
<th>CPM concentration %</th>
<th>CPM amount diffused mg./5hr. *</th>
<th>Rate constant (mg.min (^{-1/2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%w/w</td>
<td>7.54±0.09</td>
<td>0.582</td>
</tr>
<tr>
<td>3%w/w</td>
<td>11.76±0.21</td>
<td>0.8217</td>
</tr>
</tbody>
</table>

Each value represents the mean SD (n=3 readings in each group) *

Table (4). Effect of different enhancers on the diffusion rate constant (K) of CPM 3%w/w through the rat skin.

<table>
<thead>
<tr>
<th>Enhancer type</th>
<th>CPM amount diffused mg./5hr.</th>
<th>Rate constant (mg.min (^{-1/2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No addition</td>
<td>11.765±0.11</td>
<td>0.8217</td>
</tr>
<tr>
<td>Urea 5%w/w</td>
<td>5.648±0.24</td>
<td>0.3659</td>
</tr>
<tr>
<td>PEG1000 2%w/w</td>
<td>6.820±0.12</td>
<td>0.4807</td>
</tr>
<tr>
<td>TEA 2.5%w/w</td>
<td>13.138±0.16</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Estimation of irritation property of selected formula:
The selected formula which was introduce to specify the irritation test consist of 3%w/w CPM loaded in 2% w/w SA and fortified by 5% w/w TEA as an enhancer. After 7 days of gel application on the dorsal shaved skin of albino rat, it was seen that a
Permeation of chlorpheniramine maleate from gels

recognized redness area on the skin developed during this period, while the application of the same formula free from 5% w/w TEA showed no appearance of this irritation. This observation may be related to the irritation effect of TEA itself at this concentration, since most of the quaternary ammonium surfactant are strongly cationic irritant enhancers \(^{(18)}\).

Figure 5. The effect of different enhancers on the diffusion process of CPM 3% w/w through rat skin at \(37^\circ\)C and

\[
\frac{1}{(\text{Absolute temp.})} \times 1000
\]

Figure 6. Accelerated degradation of CPM in a selected formula at different exaggerated temperatures

Stability study

Determination of the shelf life

The results of this study showed CPM followed first order kinetic degradation, when the selected formula kept in collapsible tubes maintained separately at 40, 50, and 60°C, the contents of these tubes for CPM amount were determined every seven days for 4 weeks, and the rate of degradation at 25°C (K25°C) was found to be \(0.872 \times 10.\text{day}^{-1}\), when Arrhenius plot was constructed as a logarithm of degradation rates constants for above exaggerated temperatures against reciprocal of absolute temperatures of CPM storage as shown in figures 6 and 7. To estimate the shelf life, the following expression was used to estimate 90% of the drug content remain at that time.

\[
t_{90\%} = 0.105/0.872 \text{ day} = 1204 \text{ days}
\]

So the calculated shelf life for a selected formula was found to be about 3.3 years.

Figure 7. Arrhenius plot for estimation of shelf life of CPM of a selected formula

Effect of temperatures and storage time on the pH, color, and odor of 3% w/w CPM gel

The result after 30 days storage at different storage exaggerated temperatures of CPM gel, revealed that slight increase in the pH of CPM gel from 4.25 to 4.6, which may be attributed to the ionization of CPM that releases chlorpheniramine base, which belongs to the basic properties of this types of antihistamines \(^{(19)}\). More over no change in the original translucent white color or appearance of unpleasant odor was observed. These results indicated no probability of physical instability, or growth of micro-organisms in the selected formula.
Conclusions
Concerning the results obtained, one can conclude the followings:
1. Maximum CPM release was achieved, when 4% w/w of SA was introduced as a gel base.
2. The diffusion of CPM through the skin of the rat was increased as a function of increasing CPM concentrations, loaded in 2% w/w SA gel base.
3. Addition of 5% w/w TEA to 2% w/w SA loaded by 3% w/w CPM, enhances the amount of the drug diffused through the skin of the rat.
4. Addition of 2% w/w PEG1000, and 5% w/w urea, separately to 2% w/w SA loaded by 3% w/v CPM, decreases significantly P< 0.05 the amount of the drug diffused through the skin of the rat.
5. There was a marked irritation spots recognized, when TEA 5% w/w used as an enhancer in the selected formula, compared with no effect when this enhancer is avoided.
6. The selected formula of SA 2% w/w as a base loaded by 3% w/v of CPM was acceptable with calculated shelf life about 3.3 years.
This formula may need a further clinical study on volunteers to ensure its therapeutic, and economic value.

References