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 H- 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 , enhanced the amount of CPM diffuse through the skin of albino rat . Mean 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 wards: chlopheniramine maleate , gel , skin permeation

الخلاصة

الكلورفنيرامين ماليبيت هو احد المثبطات لمستقبلات هه 1 والمستعملة بشكل واسع في الطفح الجلدي والحكة الخارجية .لقد نجحت المحاولات بتركيب الكلورفنيرامين ماليبيت 3%(وزن \وزن(في 2% (وزن\وزن(من الجينات الصوديوم كقاعدة هلام , واعتبرت كتركيبة مختارة . لقد وجد ان تخلل الكلورفنيرامين ماليبيت من خلال جلد الجرذ الابرش يزداد بزيادة تركيز العقار في 2%(وزن\وزن(من الجينات الصوديوم ,علاوة على ذلك فان اضافة الترايبثانولامين5% (وزن\وزن(الى2% (وزن\وزن(من قاعدة الجينات الصوديوم محملة ب 3%(وزن\وزن(من الكلورفنيرامين ماليبيت ,ادى الى زيادة كمية العقار النافذة خلال جلد الجرذ الابرش. وعلى صعيد اخر ,فأن أضافة 2%(وزن\وزن(من مادة بولى أثلين كلايكول) 1000(و 5%(وزن\وزن(من اليوريا بشكل منفصل الى 2% (وزن\وزن(من الجينات الصوديوم ,محملة ب3% (وزن\وزن(من الكلورفنيرامين ماليبيت ,ادى الى الاعاقة بشكل مهم $P<0.05$ كمية العقار النافذة خلال جلد الجرذ للابرش . أن التركيبة المختارة كانت تحتوي على 2% (وزن\وزن(من الجينات الصوديوم كقاعدة هلام محملة ب 3% (وزن\وزن(من الكلورفنيرامين ماليبيت ,اضافة الى صفات فيزياوية جيدة وفترة نفاذية للعقار بحدود3,3 سنة .

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 firs 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 drug [(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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Experimental

Materials and Equipments :

Chlorpheniramin maleate CPM , supplied by Sammraa 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 ,carrywin UV ,Varian , Australia USP dissolution apparatus ,magnetic stirrer ,ultra sonic 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 continous traturation and stirring to obtain homogenous clear drug-gel solution [6].

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 glycirin 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 (6).Different concentration of polymers corresponding to 1% , 2% and 4% (w/w) of Sodium alginate and only 4% (w/w) of NaCMC were used with physical mixture through studying their effect on the release process.

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 [7] . 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 scarified 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 defating procedure [9], 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 [10] .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[8] :

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 mls.were pippeted 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 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 [11]:

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%v/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 [12] .

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 1%w/w in phosphate buffer pH7.4 at 37˚C .

Type of Bases	Amount of CPM released (mg.)/5hr.*	CPM released %	K ($mg.min^{-1/2}$)
4%w/w SA	9.06 ±0.09	90.6	0.427
4%w/w NaCMC	6.77 ±0.11	67.7	0.401

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

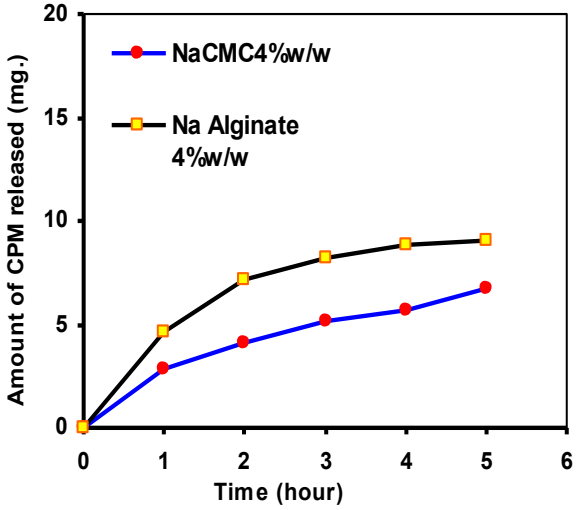

Figure (1) . The effect of different bases on the release of CPM 1%w/w at pH 7.4 and 37˚C

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 up take by polymer is not affected by the concentrations of the polymer it self , 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 percutanous absorption . these results were in agreement with the results obtained from the permeation of carvedilol transdermal patches [11] .

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

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Sodium alginate Concentratin	Amount of CPM (mg./5hr.)*	CPM released %	Rate constant (K) ($mg.min^{-1/2}$)
1%w/w	8.934±0.17	89.3	0.4983
2%w/w	9.435 ±0.14	94.3	0.5713
4%w/w	9.060 ±0.04	90.6	0.4273

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

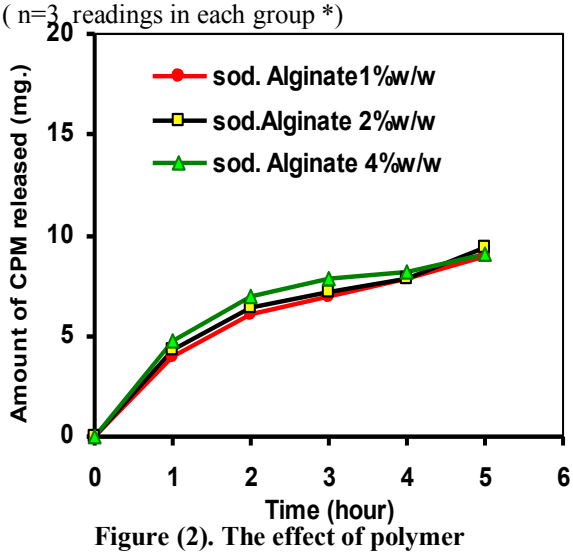

Figure (2). The effect of polymer concentration on the release of CPM 1% w/w through rat skin at pH 7.4 and 37˚C

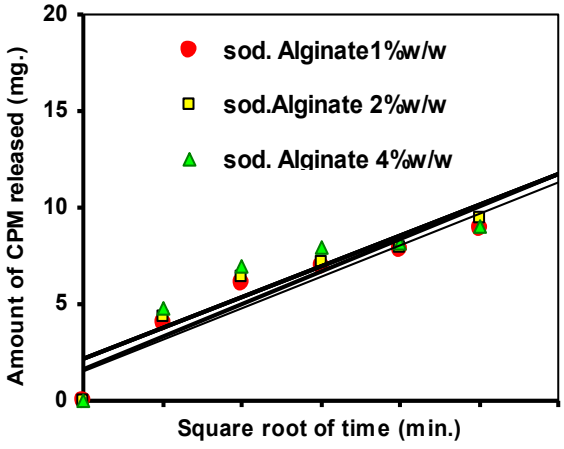

Figure (3) . The kinetic analysis of CPM 1% w/w release from different polymer concentration at pH 7.14 and 37˚C

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 (5hours) , 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 S0dium Alginate (SA) gel .

CPM concentration %	CPM amount diffused mg./5hr. *	Rate constant (mg.min $^{-1}$/2)
1%w/w	7.54±0.09	0.582
3%w/w	11.765±0.21	0.8217

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

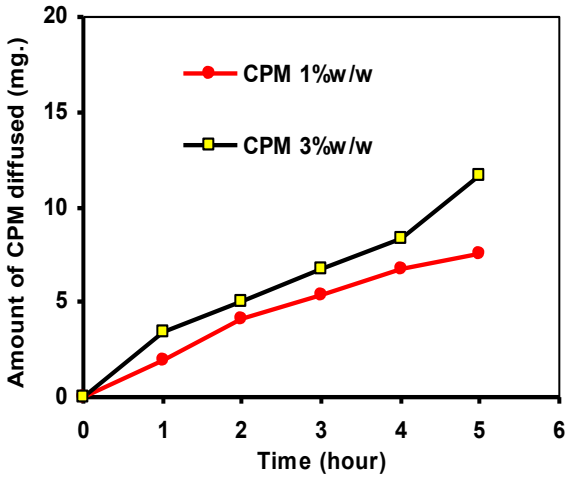

Figure (4) . The effect of CPM concentration on the diffusion process of through rat skin from sodium alginate 2%w/w gel at pH 7.4 and 37°C

Effect of different enhancers and their concentrations on the diffusion of CPM 3%w/w through rat skin:

The enhancers which they are used in this study are water soluble types , they include urea 5% w/w , PEG 1000 2 % w/w , and TEA 2.5% w/w Table 4 . and figure 5 , illustrate the effect of incorporation the above enhancers separately on the diffusion of 3% w/w CPM loaded in 2% w/w SA gels through rat skin . It was seen that both urea 5% w/w and PEG 1000 2% w/w significantly decrease $P < 0.05$ the amount of the drug diffused through rat skin. Since incorporation of urea in hydro gel may activate the hydrolysis process of urea to form ammonia and carbon dioxide , which lead to elevate the pH of the medium of environment , which in turn dissociate the CPM into maleate anion and chlorpheniramine cation , these ionized species may hinder the diffusion process of the drug [15] . Moreover , the alkyl amine group , of the drug may complex ethylene oxide (CH2-Ö-CH) group of PEG1000 that decrease the amount of the drug available for permeation, this result is in a consistent with that result obtained , when lidocaine was formulated as a topical gel [16] . On the other hand , incorporation of 2.5%w/w of TEA which counter irritant to the skin as an enhancer , showed a slight increase of CPM permeation through the rat skin , this effect may be attributed to both effects of TEA as a basic tertiary amine enhancer once that is compatible with alkyl amine anti histamine (CPM) , and second may be referred to the effect of emulsification of TEA with malic acid to form water soluble salt that easily allow the drug penetration through the rat skin [17].

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

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

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

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 catoionic irritant enhancers [18] .

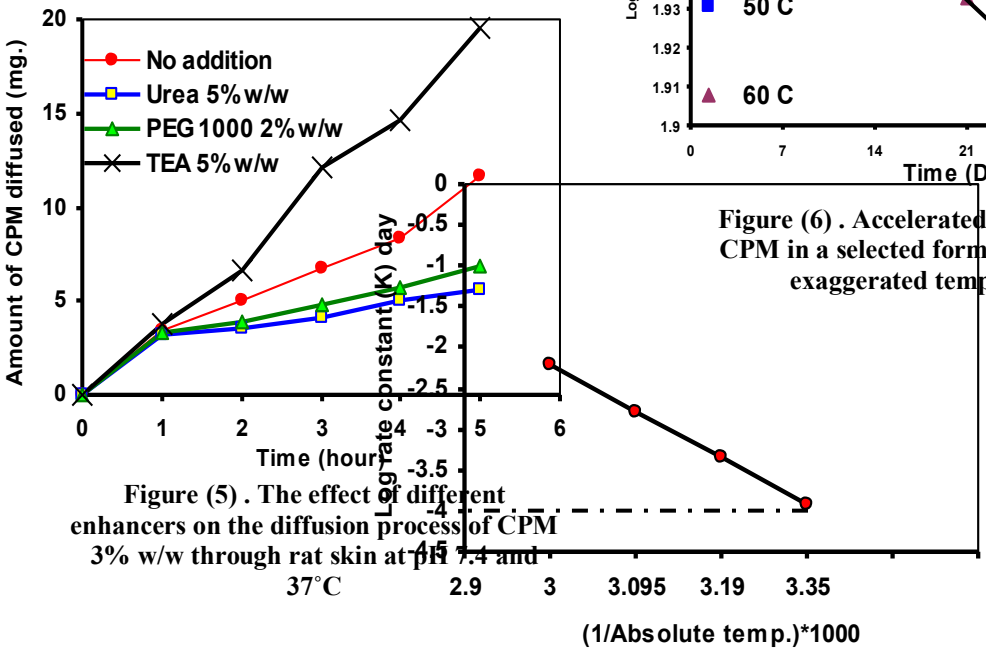

Figure (5) . The effect of different enhancers on the diffusion process of CPM 3% w/w through rat skin at pH 7.4 and 37˚C

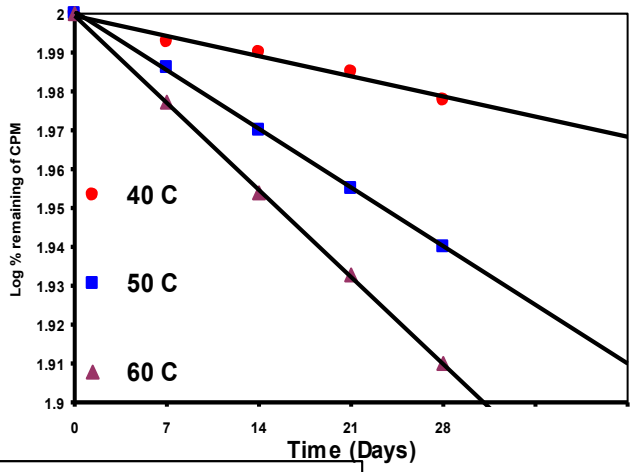

Figure (6) . Accelerated degradation of CPM in a selected formula at different exaggerated temperatures

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 a 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^{-} x 10 $.day^{-1}$, 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 .

$t\ 90\% = 0.105/0.872^{-}day = 1204$ 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 time 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 of the results obtained , one can conclude , the followings :

1. Maximum CPM release was achieved, when 4% w/w of SA was introduce 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/w 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/w 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 .

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