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# TRANSDERMAL FORMULATION AND EVALUATION OF KETOTIFEN

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## ABSTRACT

Ketotifen is an anti-histaminic drug ($H_1$-antagonist) used as a long term prevention of bronchial asthma and allergic bronchitis .It is available in the market as oral dosage forms (Tablet , Capsule and Syrup), It is a well-known fact that drugs taken orally pass through the G.I.T. where they experience first pass effect in the liver and 50 % of ketotifen is metabolized in the liver.: The purpose of this study was to formulate and evaluate a new transdermal formulations of ketotifen to avoid the first pass effect .The prepared formulations included; Film, Ointment, Cream and emulsion.: The release studies for the above formulations were done by using semi- permeable cellophane membrane and natural rabbit skin (after removing the hair from the abdominal region), by using diffusion cell operating at 37°C ± 0.5 and 50 r.p.m, the receptor media was a phosphate buffer of pH 7 and the amount of drug released was determined spectrophotometrically at λ max 300nm and at specified period of time. The results obtained showed that in case of using the semi- permeable cellophane membrane, the release of ketotifen from the above mentioned bases can be arranged in the following descending order: Film > Emulsion > Ointment (PEG) > Cream. In-vivo studies using rabbit skin as a membrane showed similar results of release of ketotifen from the above mentioned bases to in-vitro studies. This research included the kinetic studies to determine the correlation coefficient (r), the order of the reaction and the half-life for each base. These results suggest the usefulness of Ketotifen to be administered as a transdermal dosage form which is better than other dosage forms.

**Keywords:** ketotifen, PEG (Polyethylene glycol) , HPMC (Hydroxy propyl methyl cellulose)

## 1-INTRODUCTION

Ketotifen is $H_1$-receptor antagonist, it used in case of recurrent attacks of paroxymal dyspnea, with airway inflammation and wheezing due to spasmodic contraction of the bronchi. Ketotifen is supposed to act by inhibiting the release of inflammatory mediators from mast cells [(1)]. Peak plasma concentration occur 2—4 hours after dose by mouth .It is mainly excreted in the urine as inactive metabolite with a small amount of unchanged drug. The terminal elimination half life is a bout 21 hours. overdoses of ketotifen ranging from 10 to 120 mg were reported in 8 patients. Symptoms included drowsiness, confusion, dyspnoea, bradycardia, disorientation and convulsion [(2)]. Ketotifen has the properties of the anti-histamines in addition to a stabilishing action on mast cells ,lt is used in the prophylactic treatment of asthma and has also been given in the treatment of allergic conditions such as rhinitis and conjunctivitis. ketotifen fumarate is taken by mouth in doses equivalent to l mg of ketotifen twice daily with food [(2)]. Ketotifen fumarate is almost completely absorbed from the gastro intestinal tract following oral administration but bioavailability is reported to be only about 50% due to hepatic first pass metabolism, some previous studies showed good transdermal absorption[(3,4)] . The purpose of this study is to formulate and evaluate ketotifen via the skin as a transdermal formulations such as film, PEG ointment , cream and emulsion to avoid the first pass effect of Kitotifen and increases its bioavailability.

## 2- MATERIALS & EQUIPMENT

A-Materials

- Ketotifen fumarate was given as a gift from the PHARMACARE INT.MFG.CO.Yemen.
- Anhydrous disodium hydrogen orthophosphate ($Na_2HPO_4$)
- Potassium dihydrogen orthophosphate ($KH_2PO_4$)
- Polyethylene glycol 4000 (MERCK-Schuchardt .Germany).
- Polyethylene glycol 400 (MERCK-Schuchardt Germany).
- Tween80 (MERCK-Schuchardt .Germany).
- Chloroform ( CHIRFLY CHEMICAL LTD.SWINDON ENGLAND)
- Stearic acid. sigma (SIGMA CHEMICAL CO. USA).
- Stearyl alcohol sigma (SIGM A CHEMICAL CO. USA).
- Hydroxy propyl methyl cellulose (ALDRICH Steinheim .Germany).
- Cetyl alcohol ( MERCK-Schuchardt .Germany)

- Acetone (BDH).

The following materials are of pharmaceutical grades:

- White soft paraffin, white bees wax, propylene glycol and potassium hydroxide.

**B-Equipments**

- Ultra-violet spectrophotometer :

SHIMADZU/ Ser. Nr. 10773680120/ made in Australia.

- Dissolution tester : ERWEKA Gmbtt D-63150 Hensenstamm / Ser. Nr. :105416.0 dab /Germany.
- Electronic balance : APX-100/DENVER/ Instrument/Sensitivity: 0.0001g/ Germany.
- Electric heater : Ingenienrbüro CAT,M. Zipper Gmbtt, Ser. Nr. :525020/Germany.
- Magnatic Stirrer : Ingenienrbüro CAT,M. Zipper Gmbtt, Ser. Nr. : 180106/ Germany.

## 3- METHODS

### 3.1-U.V. scanning & λmax determination [5-7]

A solution containing 50 mg of ketotifen /50 ml of phosphate buffer pH 7 was prepared as a stock solution, then 3 ml of the sample was scanning at wave length between 200- 400 nm by using receptor media as a blank, the λ max which obtained was 300 nm.

### 3.2-Calibration curve and determination of (k):

From the prepared stock solution , volumes of ( 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0. 7 ml) were taken and diluted to 10 ml by the phosphate buffer separately, these solutions were equivalent to ( 10, 20, 30, 40, 50, 60 and 70 µg / ml) respectively.

The absorbance of the prepared solutions was measured spectrophotometrically at max 300 nm using phosphate buffer as a blank, the absorbance of each sample was plotted against the corresponding concentrations and the procedural constant K was calculated.

It is worthy to indicate that any material showed an interference with ketotifen at the same wavelength was excluded.

### 3.3 -Preparation of topical formulations

The following formulae were selected in which 2% of ketotifen was incorporated.

#### 3.3.1 Water soluble ointment base

Polyethylene glycol base: (U.S .P .XXII).

PEG 4000 40 gm

PEG 400 60 gm

### 3.3.2 Emulsion base

O/W emulsion base

- White bees wax.....1 gm
- Cetyl alcohol……....15 gm
- Propylene glycol……..10 gm
-Tween 80……...2 gm
- Water……72 gm

### 3.3.3 Vanishing Cream (O/W Cream)

Stearic acid ……13gm

Stearyl alcohol ……..1gm

Cetyl alcohol……..1gm

Glycerin…….10 gm

Potassium hydroxide ……0.9gm

Purified water. qs. ad ……..100gm

### 3.3.4 Preparation of the film [8]

The film was prepared after several trials as the following w/w concentration:-

Table (1): The ingredients of transdermal film

| *Substance* | *Amount.* | *Percentage* |
|---|---|---|
| Ethanol | 18 ml | 69.8% v/v casting solvent |
| Water | 7 ml | 27.2% v/v casting solvent |
| HPMC | 0.4 | 50.1 % w/w of the film |
| PEG 400 | 0.1 | 12.6 % w/w of the film |
| DRUG | 0.298 | 37.3 % w/w of the film |

## 4-Release studies of ketotifen from the prepared formulations [9-11]:

### 4.1 In vitro release studies through the cellophane membrane

In case of ointment, emulsion and cream 1gm of the tested formulation contains 20 mg of ketotifen was accurately weighed in a diffusion cell (basket), while the film was cut into small pieces ( each piece 3.80 $cm^2$ contains 20 mg of ketotifen ) the basket used as diffusion

cell with a diameter of 2.2 cm and the total surface area available for penetration was 3.80 $cm^2$ (which calculated according to the equation $\pi r^2$ , where r is the radius of the basket).

The basket was covered with the cellophane membrane (which was immersed in phosphate buffer for one hour before use) and fixed on the diffusion apparatus with rubber band in which the base of the diffusion cell in touch with the surface of the receptor media, the basket was placed in 1000 ml glass vessel which contains 100 ml of phosphate buffer pH 7, the whole dialysis unit was placed in a thermostatically controlled shaker water bath operating at 37 ±0.5 $^oC$ and 50 r.p.m.

Five ml was withdrawn at specific time (60 min. intervals for 6 hrs), and replaced with an equal volume of receptor media at the same temperature to keep the volume constant during the experiment study.

The amount of drug released from each formulation to the receptor media was assayed spectrophotometrically at 300 nm using the receptor media as a blank.

Each experiment was done in triplicate and their average was calculated. The amount of the drug released in mg from each formula was calculated from the slope of ketotifen and the absorbance at each time interval multiplied by the volume at the receptor media.

**4.2 In -vivo release studies through the natural rabbit skin**

**4.2.1 Preparation of the rabbit skin [(12)]**

The abdomen rabbit skin was shaved by an electrical hair clipper, the rabbit was scarified, the skin was then excised surgically, without injury. The skin was washed with distilled water, dried between two filter papers and stored in the refrigerator for not more than three days [(13)] the frozen skin was thawed before cutting into pieces which were rehydrated by immersing in distilled water for one hour before being placed on the diffusion cell.

**4.2.2 The method of release study**

The extent of Ketotifen released from different topical formulations (ointment ,cream , emulsion and film ) were determined by using a diffusion apparatus as mentioned above in releasing study of ketotifen through cellophane membrane , in which rabbit skin was immersed in phosphate buffer for one hour before use. and the dermal side was directed toward the receptor compartment and the stratum corneum facing the donor compartment .

**5-Pharmacokinetic Studies of Ketotifen cross the rabbit skin [13]**

The amount of drug (mg) in the receptor media was assayed spectrophotometrically and the released drug was determined and computed, then regression analysis linearity and parameters of the permeation for each formula, were treated in which, the correlation coefficient (r) was calculated for each formula by each kinetic equation, to determine whether the penetration of the drug through the skin follows zero order, first order or diffusion release model., then the analytical permeation parameters such as steady state flux (J) and permeability coefficient (Kp) were calculated by plotting the amount of drug permeated (µg /cm $^2$) versus time.

All of these calculation were carried according to the following kinetics equations

**5.1- Zero order kinetic**

$A = K_o t + A_o$ Where:

$A_o$ = The amount of drug at t =0

Ko = Zero - order release rate constant

Plotting of A (amount of drug released in mg) versus time (t) would yield a straight line with correlation coefficient (r) and intercept (y) equal To $A_o$ and the slope of the line would be equal to $K_o$.

**5.2- First order kinetic**

$\text{Log } A = K t / 2.3 + \text{Log } A_o$

Where:-

$A_o$ = amount of drug at time (t)

T = time interval.

K = the first order constant.

Plotting of log A (amount of drug released in mg) versus time (t) would yield a straight line, with correlation coefficient(r) and the intercept (y), which is equal to log $A_o$, while the slope and half-life are as follows:-

Slope = K / 2.303 $t_{1/2} = 0.693 / k$

$$Kp = \frac{J}{C_0}$$

### 5.3- Higuchi-diffusion model [14]

Fick's second law of diffusion states that drug molecules diffuse from a region of higher concentration to a region of lower concentration. The equation for the release rate of drugs from an ointment base derived by T. Higuchi and W.Higuchi simplified this equation to:

$$Q = 2\,Co\left(\frac{Dt}{\pi}\right)^{\frac{1}{2}}$$

Where:-

Q = the amount of drug released to the membrane in (mg) at time (t) in minutes.

Co = the initial concentration of drug in the vehicle.

D = the diffusion coefficient of drug in the vehicle.

This equation describe drug release as-being linear with the square root of time

$$Q = K\sqrt{T}$$

### 5.4- The flux (J) and permeability coefficient ($K_p$) [14-15]

The flux (J) of Ketotifen through the rabbit skin was determined from the slope of the steady-state portion of the amount of drug permeated (µg/cm$^2$) versus time. Which was expressed by Santoyo et al, 1995, according to the following equation:

**$J = C_o K_p$**

Where: J= The flux µg/cm$^2$. hour $C_o$ = The applied dose $K_P$ = The permeability coefficient, then, the permeability coefficient $K_P$ was determined from dividing the permeation flux by the concentration of the donor phase

## 6- RESULTS

**Table (2):Release of ketotifen from different formulations via cellophane membrane**.

| % of drug released after 6 hrs | Base | Amount of drug release in Amount in mg/3.8 cm$^2$ after the following time interval (minutes). | | | | | |
|---|---|---|---|---|---|---|---|
| | | 60 | 120 | 180 | 240 | 300 | 360 |
| *46.45* | *Film* | 4.588 | 7.564 | 8.319 | 8.696 | 8.984 | 9.297 |
| *43* | *Emulsion* | 3.582 | 6.718 | 7.591 | 7.972 | 8.279 | 8.605 |
| *40* | *PEG* | 3 | 5.5 | 5.6 | 6.0 | 7.0 | 8.06 |
| *1.8* | *Cream* | 0.053 | 0.106 | 0.172 | 0.250 | 0.326 | 0.361 |

**Table (3):Kinetic data of ketotifen released from different formulations by using cellophane membrane**

| Formula | Correlation coefficient (r) | | | The observed order | Slope |
|---|---|---|---|---|---|
| | Zero | First | Diffusion | | |
| Film | 0.81889 | 0.99793 | 0.88181 | First | 0.00079 |
| Emulsion | 0.87037 | 0.821 | 0.92189 | Diffusion | 0.410 |
| PEG | 0.946 | 0.93955 | 0.959 | Diffusion | 0.392 |
| Cream | 0.99583 | 0.96769 | 0.98854 | Zero | 0.00108 |

**Table (4): Pharmacokinetic parameters of ketotifen released from different formulations by using cellophane membrane**

| Formula | Order | Slope | K | $T_{1/2}$ (min.) |
|---|---|---|---|---|
| Film | First | 0.00079 | 0.00182 | 381 |
| Emulsion | Diffusion | 0.410 | 0.410 | 592 |
| PEG | Diffusion | 0.392 | 0.392 | 650 |
| Cream | Zero | 0.00108 | 0.00108 | 9259 |

**Table (5) In-vivo: release of ketotifen from different formulations through the natural rabbit skin**

| % of drug released after 6 hrs | Base | Amount of drug release in mg after the following time interval (minutes) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 60 | 120 | 180 | 240 | 300 | 360 |
| *19.85* | *Film* | 0.774 | 1.422 | 2.172 | 2.839 | 3.484 | 3.969 |
| *12.5* | *Emulsion* | 0.441 | 0.959 | 1.307 | 1.653 | 1.8976 | 2.5 |
| *10.6* | *PEG ointment* | 0.315 | 0.623 | 1.029 | 1.373 | 1.806 | 2.123 |

**Table (6): Kinetic data of ketotifen released from different formulations by using natural rabbit skin**

| Formula | Correlation coefficient (r) | | | The observed order | Slope |
|---|---|---|---|---|---|
| | Zero order | First order | Diffusion order | | |
| Film | 0.99827 | 0.97463 | 0.99573 | Zero | 0.01087 |
| Emulsion | 0.993 | 0.97178 | 0.988 | Zero | 0.0.0064 |
| PEG ointment | 0.99911 | 0.9657 | 0.98935 | Zero | 0.00616 |

**Table (7): Pharmacokinetic parameters of ketotifen released from different formulations by using natural skin**

| Formula | Order | Slope | K | $T_{1/2}$ (min.) | J µg/ $cm^2$ $hr^{-1}$ | Kp $cm^2$ $hr^{-1}$ |
|---|---|---|---|---|---|---|
| Film | Zero | 0.01087 | 0.01087 | 920 | 0.171 | $8.6x10^{-6}$ |
| Emulsion | Zero | 0.0064 | 0.0064 | 1562 | 0.101 | $5.0x10^{-6}$ |
| PEG ointment | Zero | 0.00616 | 0.00616 | 1623 | 0.097 | $4.8x10^{-6}$ |

## 7- DISCUSSION

Ketotifen fumarate has low bioavailability approximately 50% even though it almost completely absorbed from the gastrointestinal tract. This due to the fact that it is so rapidly undergoes first pass metabolism. Transdermal drug delivery is an alternative route for the delivery of systemically acting drugs. This route has advantages of avoidance of first pass metabolism, predictable and extended duration of activity, minimizing under able side effects, utility of short half- life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter and intra patient valuations, and most importantly, it provides patient compliance [16,17]

Therefore, the propose of the present study was to formulate ketotifen in a formulae suitable for transdermal application in an attempt to avoid the hepatic effect thus achieving higher systemic bioavailability of drug and to develop a sustained release formulation with extended clinical effect.

Traditional classes of transdermal formulations were prepared such as emulsion, ointment, cream and film, it is obvious that the release of the drug from different bases depends on various factors such as: the physicochemical properties of the drug and the nature of the drug carrier matrix [18] . Therefore, the greater release of drug is expected when there is less affinity of drug for the base.

The amount of the drug released from different bases (emulsion, PEG ointment, cream and film) by using cellophane membrane is illustrated in table (2) while by using natural rabbit skin illustrated by the table (5) .

It is clear that, the amount of ketotifen fumarate released from different bases by using cellophane membrane and skin of rabbit can be arranged according to the following descending order:

Film > Emulsion > PEG > Cream.

This similarity between both in-vitro and in-vivo results illustrates the value of administering ketotifen fumarate as a transdermal dosage form.

As shown in tables (2,3,and 4), the release of drug from film via cellophane membrane was 46.45% after 6 hours with a half-life of 381 minutes, and follows first order kinetic The high release may be attributed to the lipophilic property of the drug, which was incorporated in a hydrophilic film that well enhance the drug release or it may be due to ease of penetration of the drug through the pores of the film (as it is a thin layer that contains pores which helps the release of the drug from the base and penetration to the skin), .Also it may be due to the presence of PEG as a plasticizer that increases the elasticity of the film.

The release of drug from o/w emulsion base was 43% after 6 hours with a half-life of 592 minutes and follow Higuchi diffusion model, the higher drug release level may be due to the presence of Tween 80 and propylene glycol as components of the base which may affect the character of the skin by decreasing skin water evaporation and increasing it's hydration or may be due to that Tween 80 and propylene glycol act as a penetration enhancers, also the presence of water in a high amount helps in dissolving the drug and increase skin hydration.

PEG ointment is a water soluble base in which the drug is easily dissolved ,therefore the diffusion of the drug from the base via cellophane membrane will be enhanced that it's penetration was 40% after 6 hours with a half-life of 650 minutes and follow Higuchi diffusion model.

The release of the drug from the vanishing cream via cellophane membrane was 1.8% after 6 hours with a half-life of 9259 minutes and follows zero order kinetics. The slow release of the drug may be due to the high affinity of the drug to the base and therefore decreasing the portioning of release of the drug . Furthermore this slow release may be due to the absence of the oily phase in this formula.

As shown in tables (5,6,7) the drug released from the film and penetrated the rabbit skin was by zero order kinetic, and the concentration observed after 6 hours was 19.85% with a half-life of 920 minutes, and the flux (J) of the drug through the skin was 0.171 $\mu g/cm^2.hr^{-1}$.

The drug released from the o/w emulsion base follow zero order kinetic, and the concentration observed after 6 hours was 12.5% with a half-life of 1562 minutes, and the flux (J) of the drug through the skin was 0.101 µg/cm$^2$.hr$^{-1}$.

The drug released from the PEG base through rabbit skin follow zero order kinetic, and the concentration observed after 6 hours was 10.6% with a half life of 1623 minutes, and the flux (J) of the drug through the skin was 0.097 µg/cm$^2$.hr$^{-1}$, while the drug released from vanishing cream was not detected.

## 8- CONCLUSION

These results suggest the usefulness of Ketotifen to be administered as transdermal dosage form. Furthermore this study reveals that Ketotifen may be administered for the treatment or prevention of allergic asthma.

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