

Formulation of Melatonin as a Cream and Studying the Release, Diffusion, and Stability of the Cream

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الخلاصة:

تستخدم بعض المستحضرات الصيدلانية ذات الاستعمال الخارجي لأبداء تأثيراتها موضعيا في محل استعمالها بواسطة نفاذ الدواء الى الطبقات المكونة للجلد، الأغشية المخاطية، قرنية العين، الانف، المستقيم، او المهبل. بينما بعض المستحضرات الاخرى ذات الاستعمال الخارجي تصمم لأبداء التأثير العلاجي المرجو عن طريق امتصاص الدواء الى الدورة الدموية.

الميلاتونين هو هرمون متعدد الوظائف، يصنع ويفرز في الانسان بواسطة الغدة الصنوبرية كأستجابة لتغير الأضواء في البيئة المحيطة بالانسان في الليل والنهار، ويعتبر الميلاتونين من اقوى مضادات الأوكسدة المعروفة.

هدف هذه الدراسة هو لتصنيع الميلاتونين على هيئة كريم خارجي ثم دراسة مدى تحرر الميلاتونين من الكريم ومدى قابلية الدواء على النفاذ من خلال الجلد، ومن ثم تقييم تأثيرات الحرارة ومدة الخزن على ثبوتية الدواء في الكريم.

تستخدم طريقة الاندماج أو الانصهار في عملية تحضير الكريم والتي تستخدم عادة لتحضير المراهم والكريمات وقواعد الجل، ثم يتم قياس نسبة الدواء المقرر ونسبة الدواء النافذ من خلال الجلد بأستخدام جلد الفأر المشابه فزيولوجيا لجلد الانسان، ثم يتم قياس ثبوتية الدواء والتي تشمل تحديد تاريخ انتهاء الصلاحية، الخواص الفيزيائية، وقياس درجة حامضية المستحضر.

اظهرت النتائج ان تحرر الميلاتونين من الكريم المستحضر يزداد بمرور الوقت، بعد 6 ساعات كانت كمية الميلاتونين المتحرر يعادل 16.4% من كمية الميلاتونين الاصلية. كانت كمية الميلاتونين النافذ من خلال جلد الفأر 4.1% من كمية الميلاتونين الاصلية.

تم استخدام طريقة أرينوس لتحديد ثابت سرعة تفكك الميلاتونين ومن ثم تحديد تاريخ انتهاء المفعول والذي كان 1.997 سنة. لم تظهر تغيرات على لون او رائحة المستحضر بعد خزنه في وعاء

خاص محكم بعيدا عن الضوء وبدرجات حرارة 50°م, 60°م, و 70°م, وأظهرت النتائج أيضا تغير طفيف في درجة الحمضية حيث كانت 8.4 وأصبحت بعد فترة الخزن (45 يوم) 8.2.

نستخلص من مجمل نتائج الدراسة ان الميلاتونين المستحضر على شكل كريم ممكن ان يبدي تأثيرا موضعيا اكثر من تأثيره عن طريق وصوله الى الدورة الدموية, حيث كانت نسبة الدواء المتحرر أكثر بكثير من نسبة الدواء النافذ من خلال جلد الفأر, مما يعني ان الدواء ممكن ان يكون فعالا في علاج كثير من الامراض الجلدية المختلفة بأستخدامه موضعيا والتي أثبتت فيما بعد في الجزء الثاني من الاطروحة (الدراسة السريرية).

Abstract

The topical preparations are used to give localized effects at the site of their application by penetration of the drug into the underlying layers of the skin, mucous membranes, the cornea of the eye, nasal, rectal, vaginal or urethral mucosa. While some topical preparations are designed for the systemic absorption of drug substances in therapeutic quantities, like the transdermal drug delivery system.

Melatonin is a hormone with multiple functions in human, synthesized and secreted by the pineal gland in response to changes in the darkness and light environment of the human. It is a powerful antioxidant and the most potent free radical scavenger known.

Formulation of melatonin as a cream and studying the release and diffusion of the drug from this formula. Also evaluation the effect of temperature & storage time on the stability of melatonin in the prepared cream. Preparation of the cream base using the general method employed for the preparation of the various ointments, cream and gel bases, the fusion method.

Then determination the in vitro release and diffusion of melatonin from the cream, also determination the stability of melatonin in the cream formula including the expiration date, Physical Properties, and pH of the cream.

The release was increased with time, and after 6 hours, the amount of melatonin released was approximately 16.4% of the original amount of the drug, the. After 6 hours, the amount of melatonin diffused through mouse skin was 4.1% of the original amount of the drug. Arrhenius plot was utilized to predict the degradation rate constant and then the expiration date was 1.997 years. No change in the color and odor of the cream which stored at 50°C, 60°C and 70°C in a well-closed container and protected from light. Cream pH of the final product was 8.4 and after the storage period (45 days), the pH was around 8.2. The overall results of this study suggested that the prepared melatonin cream exerts its effects locally more than in a systemic way, which may increases its effectiveness in treatment of different skin pathological conditions locally, in the future work.

In the future; it is possible to use different bases and chose the suitable one according to the study results, also enhancers, preservatives, and co-solvents could be used.

Introduction

Topical pharmaceutical preparations are that dosage forms which applied to the epithelium covering one of the body surfaces like the skin, the cornea of the eye, nasal, rectal, vaginal or urethral mucosa. In general, the topical preparations are used to give localized effects at the site of their application by penetration of the drug into the underlying layers of the skin or mucous membranes. Although some unintended systemic drug absorption may occur, it is usually in sub-therapeutic quantities, which is of minor concern ^[1]. However, there are some topical preparations are designed for the systemic absorption of drug substances in therapeutic quantities, like the transdermal drug delivery system ^[2].

According to the therapeutic requirements, the dermatological preparations may achieve local or systemic effect of the active ingredient ^[3].

There is several dosage forms that can be applied topically on the skin, topical semi-solid preparations are intended to be applied to the skin or to certain mucous surfaces for local action or percutaneous penetration of medicaments, or for their emollient or protective action. Creams are multiphase preparations consisting of a lipophilic phase and an aqueous phase ^[4]. Creams are semi-solid emulsions either water in oil (w/o, oily creams) or oil in water (o/w, aqueous creams). They are more acceptable to patients than ointments, because they are less greasy and easier to apply; they interfere less with skin functions ^[5]. The vanishing cream type vehicles are representative of the oil-in-water emulsions, whereas the absorption bases are generally water-in-oil emulsions ^[6].

Other commonly used semi-solid preparations are ointments, gels, pastes, dusting powders, collodions & lotions. While Liniments ^[7], shampoos ^[8], transdermal patches ^[9], topical sprays ^[10] & topical solutions ^[11].

Melatonin (5-methoxy-N-acetyl tryptamine) is a hormone with multiple functions in human. It is a tryptophan derivative synthesized and secreted by the pineal gland as a result of changes in the darkness and light environment of the human ^[12, 13].

Melatonin is a lipophilic hormone, once formed; it is rapidly secreted into the vascular system by passive diffusion. Plasma levels of melatonin seem to reflect closely the amount being synthesized and secreted by pineal gland, Pinelectomy is associated with very low or non-measurable amount of melatonin in plasma ^[14]. Melatonin readily penetrates cell membranes, tissue barriers, and crosses the blood-brain barrier (BBB) in both directions. Melatonin circulates in plasma as free, and it is bound to α_1 -acid glycoprotein and albumin. The protein-bound melatonin is in equilibrium with the free hormone ^[15].

Melatonin is rapidly distributed after intravenous administration (distribution half-life, 0.5-5.6 minutes), and rapidly eliminated (elimination half-life reported between 15-60 minutes with average value between 20-47 minutes) ^[16].

Melatonin is rapidly metabolized in the liver, by hydroxylation to 6-hydroxymelatonin by a microsomal cytochrome-P450, and then followed by sulfate conjugation. This metabolite is more water-soluble, dose not bind to plasma proteins and is rapidly excreted with urine, and to a lesser extent, with bile and feces. A small proportion of intact melatonin is excreted by the kidney ^[17, 18].

Melatonin is soluble in lipid (lipophilic) and slightly soluble in water, therefore it is able to enter all cellular body compartments and move from one to other. Study by Kandimalla and coworkers (1999), have shown that melatonin is more soluble in ethanol and propylenglycole than water ^[19]. Melatonin is a white to creamish crystalline powder with melting point between 116 °C to 118 °C, and its molecular weight is 232.28 ^[20].

Materials and Methods

All chemicals and reagents were of the highest available purity. Specific chemicals used in this study are shown in the following table with their suppliers.

Chemicals	Suppliers
Disodium hydrogen phosphate (Na ₂ HPO ₄)	Fluka-Garantie, Switzerland
Ethanol 95%	BDH, Chemicals, UK
Ether	BDH, Chemicals, UK
Glycerol	BDH, Chemicals, UK
Liquid paraffin	BDH, Chemicals, UK
Potassium dihydrogen phosphate (KH ₂ PO ₄)	Merck, Chemicals, W.Germany
Sodium borate	Fluka-Garantie, Switzerland
White beeswax	May and Bake. LTD, England

Table 1: Chemicals and their suppliers.

Preparation of Cream Base:

The general method employed for the preparation of the various ointment, cream and gel bases was the fusion method ⁽²¹⁾. The drug was then incorporated

by spatula and slab; the quantities of ingredients were taken on weight/weight (w/w) bases. The following base was used:

Oil in water emulsion base (cream base):

Mineral oil	50.0
Beeswax	14.0
Borax	0.7
Purified water	35.3

The oil and beeswax were heated to approximately 75°C and the borax was dissolved in water and heated to the same temperature in another container. Then the water phase is added to the oil phase slowly and with continuous but gentle stirring until congealing. The temperature should be maintained for about 10 minutes and allowed to decrease slowly^[22].

In Vitro Melatonin Release Test:

Five grams of cream base containing 5% w/w of melatonin was prepared in order to fill a small funnel with a diameter of 2.3 cm. The mouth of the funnel was covered with filter paper, which was tightly fixed on the funnel wall. The funnel (dialysis cell) was inverted and immersed within 0.5 cm below the upper surface of 500 mls of phosphate buffer pH 7.4 contained in a flask of the dissolution apparatus (collecting medium). The flask was partially immersed in a large water bath at a constant temperature of 37°C within the dissolution apparatus. The stirrer was immersed too in the collecting medium to reach below the filter paper surface of the dialysis cell and the rate of stirring was maintained at 100r.p.m.^[12]. The net release of melatonin was followed by monitoring the receiver medium concentration for 6 hours. After 0.25, 0.5, 1, 2, 3, 4, 5 and 6 hours, five mls samples were withdrawn by a pipette from the collecting medium and replaced with an equal volume of fresh phosphate buffer of pH 7.4 at 37°C, the samples then analyzed spectrophotometrically at melatonin λ max (223 nm)^[23].

According to the method of Skelly and coworkers^[13], the preparation of mouse skin was carried out; the skin was either used immediately or frozen until ready for use^[13, 24].

In Vitro Melatonin Diffusion Test:

A small test tube with a diameter of 1.4 cm (diffusion cell) was used in this study. Five grams of cream base containing 5% w/w of melatonin was applied on the epidermal surface of mouse skin. The skin was stretched over the mouth of the test tube. The base should be in contact and spread on the epidermal surface of the diffusion membrane. Then, the skin was ligated with a cotton thread in order to be fixed on the surface of the diffusion cell. The diffusion cell was inverted and immersed within 0.5 cm below the upper surface

of 500 ml phosphate buffer at pH 7.4 contained in a flask of the dissolution apparatus (collecting medium). The flask was also immersed in a large water bath at a constant temperature of 37°C inside the dissolution apparatus. The stirrer was immersed too in the collecting medium to reach below the skin surface at the diffusion cell and stirring rate was maintained at 100 r.p.m. [25].

The net diffusion of melatonin was measured by monitoring the receiver medium concentration for 6 hours. Five mls samples were withdrawn by a pipette from the collecting medium after 0.25, 0.5, 1, 2, 3, 4, 5 and 6 hours and replaced with an equal volume of fresh phosphate buffer pH 7.4 at 37°C. The samples were then analyzed spectrophotometrically at a wavelength of melatonin λ max (223 nm).

Stability Studies:

The stability studies were carried out using the cream base containing 5% w/w melatonin, including:

Determination of Expiration Date of Melatonin in the Cream Formula: The prepared cream was divided into three groups to carry out the study, each group consist of three collapsible tubes were filled with 5 gm of 5% w/w melatonin cream and kept in ovens at 50°C, 60°C, and 70°C [26].

Melatonin concentration in the stored cream was checked at day 1, 15, 30, and 45 days. One gram of the cream was dissolved in a suitable solvent (90% distilled water & 10% ethanol), shake, and filtered; the filtrate was taken and diluted, then analyzed spectrophotometrically at melatonin λ max.

The concentration of melatonin was measured by preparing a standard solution of melatonin powder dissolved in the same solvent (90% distilled water & 10% ethanol), diluted and analyzed spectrophotometrically at melatonin λ max.

Physical Properties:

Over the storage period (45 days), physical properties (color, odor) of the stored cream were observed.

pH of the Cream:

At first day, 15, 30 and 45 days, 2 gm of the stored cream were shaken with 10 ml of distilled water; the pH was then measured using a pH meter.

Results

Release of Melatonin from Cream Base:

One of the main functions of semisolid dosage form base is the control which it exerts over the release and hence the therapeutic activity of the drug [21].

Figure (1) shows the release of melatonin from a cream base containing 5% w/w of melatonin. The release was increased with time, and after 6 hours, the amount of melatonin released was approximately 16.4% of the original amount of the drug, as shown in table (2).

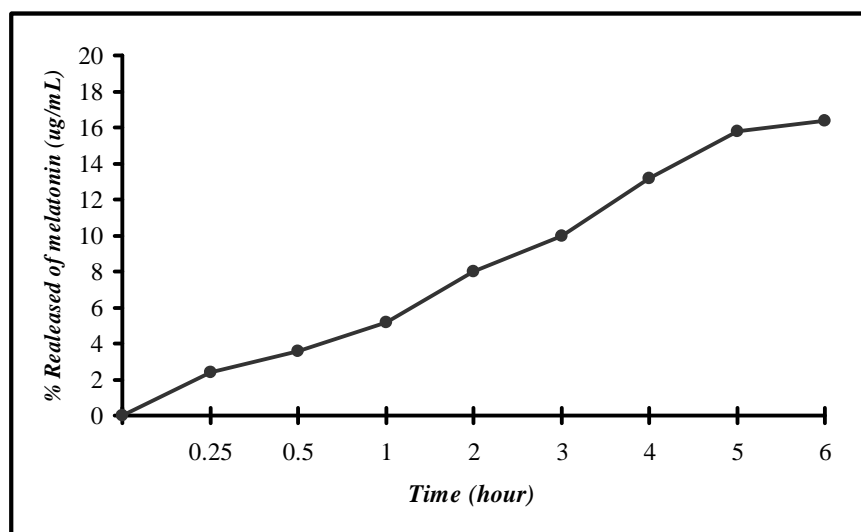


Fig. 1: Release of melatonin 5% w/w from cream base.

Time (hour)	0.25	0.5	1	2	3	4	5	6
% Release	2.4	3.6	5.2	8	10	13.2	15.8	16.4

Table 2: Percentage of melatonin released from cream base vs. time.

Diffusion Process of Melatonin through Mouse Skin:

Figure (2) shows the diffusion of melatonin 5% w/w cream base through mouse skin with time. After 6 hours, the amount of melatonin diffused was 4.1% of the original amount of the drug as shown in table (3).

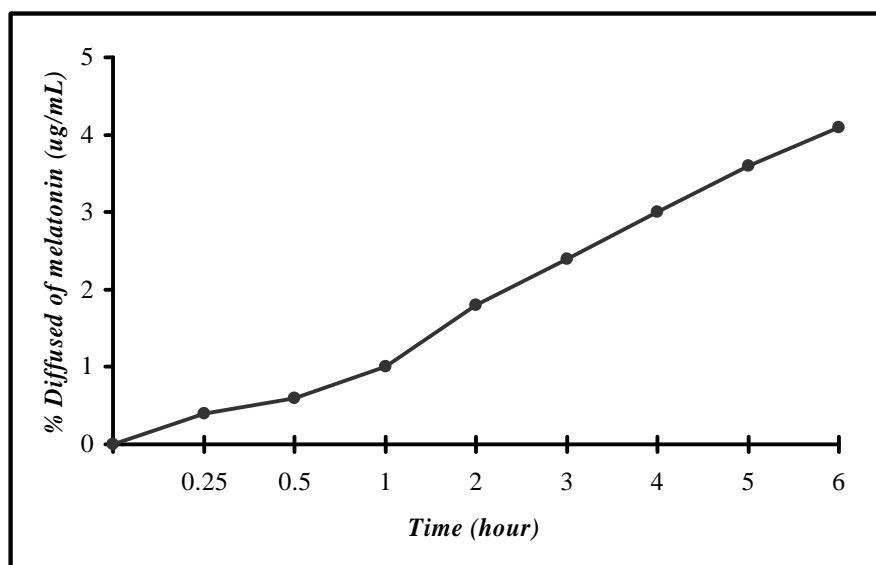


Fig. 2: Diffusion of melatonin 5% w/w from cream base.

Time (hour)	0.25	0.5	1	2	3	4	5	6
% Diffused	0.4	0.6	1	1.8	2.4	3	3.6	4.1

Table 3: Percentage of melatonin diffused from cream base vs. time.

Stability Studies:

Effect of Storage Time and Temperature on the Degradation of Melatonin and Determination of Expiration Date of the Cream Base: The Stability of melatonin in o/w cream base was studied at various temperatures 50°C, 60°C and 70°C for 45 days.

The degradation of melatonin follows first order kinetics, since straight lines were obtained when the logarithm of % remaining of melatonin was plotted versus time as shown in figure (3).

The degradation rate constant (K) at 50°C, 60°C and 70°C were calculated from the slopes of the lines as shown in table (4).

To determine the expiration date ($t_{10\%}$), Arrhenius plot was utilized to predict the degradation rate constant by plotting $\log K$ versus $1/T$ as shown in (Fig. 4), on which the degradation rate constant at 25°C was obtained from the extrapolation of the resulted straight line to 25°C. The slope of the line, T. being the absolute temperature ^[26].

The calculated K at 25°C was equal to (0.144×10^{-3}) . Since the degradation of the drug follows first order kinetics, therefore, the expiration date can be calculated using the following equation [27]:

$$t_{10\%} = \frac{0.105}{K_{25\text{ }^\circ\text{C}}} = 1.997 \text{ years}$$

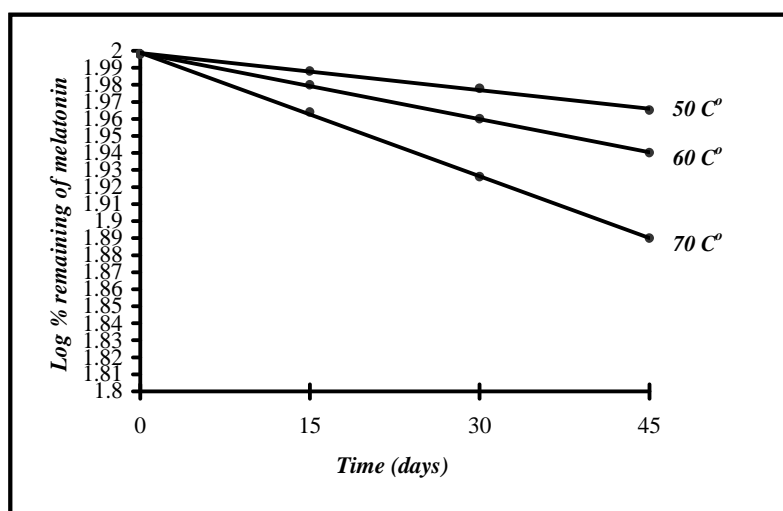


Fig. 3: Percentage of melatonin remaining vs. time at different temperatures.

Temp. °C	1/T x 10 ⁻³	Log K
50	3.09	-3.11
60	3.00	-2.865
70	2.91	-2.61
25	3.35	-3.84

Table 4: Effect of temperature on the rate of degradation of melatonin cream.

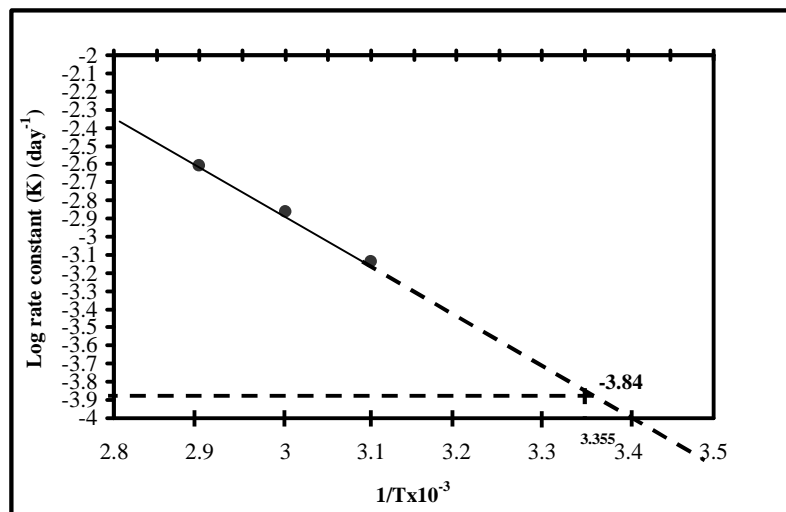


Fig. 4: Arrhenius plot for expiration date estimation of melatonin.

Effect of Storage Time and Temperature on the Physical Properties of the Cream:

The physical properties of the cream formula was examined; however, no change in the color and odor of the cream which stored at 50°C, 60°C and 70°C in a well-closed container and protected from light.

Effect of Storage Time and Temperature on the pH of the Cream:

The possible pH changes as a result of the indicated storage time of melatonin 5% cream were also followed. Cream pH of the final product was 8.4 and after the storage period, the pH was around 8.2.

Discussion

Release and diffusion of melatonin from a cream base:

Figures (1) and (2) shows the release and diffusion of melatonin from a cream base containing 5% w/w melatonin, respectively. The results demonstrated a good release and poor diffusion percents which are in agreement with the finding of Al-Tawil study concerning the release and diffusion of glycyrrhizic acid from o/w emulsion base ^[21]. The poor diffusion observed in this study may be due to the affinity of the drug to the oily composition of the base. So, according to Kikwai and coworkers, vehicles with high melatonin solubility showed low permeability coefficient values and the flux had no correlation to the solubility data, suggesting that high solubility values do not translated to high drug permeation ^[14].

The higher percentage of melatonin released than the percentage diffused through mouse skin may be because the skin acts as a barrier which decreases

the amount of drug diffused, so the prepared melatonin cream exerts its effects locally more than in systemic way, which lead to increase its effectiveness in treatment of skin pathological conditions locally. A study by Bangha and coworkers indicated that melatonin, after topical application onto the human skin, might accumulate in the stratum corneum with prolonged release into the blood system from this depot ^[15].

Stability Study:

Effect of Storage Time and Temperature on the Stability of the Drug and Determination of Expiration Date of the cream base: The results showed that the expiration date of melatonin in a cream base was 1.997 years. The emulsifying agent is the sodium soap formed by the reaction between the alkaline sodium borate and free fatty acids in the beeswax to give more stable creams, the soap contributed to the stability of the cream. The amount of borax usually recommended to give a stable cream is 5-7% of beeswax weight. In addition, because the greater stability of mineral oil, the problem of preservation was eliminated, this is always a complication in use of vegetable oils.

Recommendation for Future Work:

Since this novel preliminary study aimed to formulate melatonin as a cream and evaluate the possible release & diffusion of melatonin from the cream base, and stability of formulated melatonin. So; I would like to recommend the followings for the future work:

- Use of different bases in the formulation of melatonin cream and comparing the release, diffusion, and stability study of the drug.
- Formulation of melatonin cream in a base containing new components including suitable enhancers, preservatives, and co-solvents.

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