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Effects of Transcutol P and Precirol ATO-5 on Percutaneous Absorption of Flutamide from Emulgels

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Abstract:

Background: Flutamide is a non-steroidal anti-androgenic agent which is not only used in treating prostate cancer but also can be effective in some disorders such as androgenic alopecia and male pattern baldness. Several serious side effects for systemic administration of flutamide can be overcome by emulgel dosage form. Absorption enhancers can promote permeation of flutamide through skin. Percutaneous absorption of Flutamide emulgels with different concentrations of Transcutol P and Precirol ATO5 was studied.

Methods: Various emulgel formulations using different concentration of Transcutol P and Precirol ATO5 with 0.5 to 5 % w/w were prepared. Percutaneous absorption was tested with standard Franz Diffusion Cell equipment using full thickness rat skin. Drug concentrations in the samples were analyzed by High-Performance Liquid Chromatography (HPLC) equipped with UV-Vis detector at 305 nm.

Results: The percutaneous absorption of flutamide emulgels formulated with enhancers was higher than control formulations. Enhancement ratio increased from 1.218 to 3.670 and 1.346 to3.900 for Precirol ATO5 and Transcutol P, respectively. Area under the Curve (AUC) increased by increase the enhancers' concentration and a significant upsurge was seen in the concentration 1% for both enhancers.

Conclusion: The flutamide emulgel containing 1% Transcutol P showed more appropriate percutaneous absorption through the skin compared to others.

Keywords: Percutaneous Absorption, Flutamide, Emulgel Formulation, Franz Diffusion Cell, Precirol ATO5, Transcutol P

Introduction

Flutamide is a non-steroidal anti-androgenic agent which competitively binds androgen receptors and inhibits testosterone stimulation.[1] It is used to treat advanced prostate cancer as a hormone treatment to slow the growth of the tumor and in some cases, even shrink it.[2] Several unwanted side effects in systemic administration of the drug have been reported.[3,4] Thus, transdermal drug delivery could be an appropriate administration rout to deal with systemic

side effects. Transdermal drug delivery systems can be used due to some advantages such as bypassing the hepatic first pass metabolism, better patient compliance (non-invasiveness) and less systemic toxicity compared with other administration routs.[5,6] Several anti-cancers and other compounds from different drug classifications such as 5-fluorouracil, methotrexate, mefenamic acid and timolole have been evaluated in order to formulate effective, stable and acceptable transdermal drug delivery system.[7-11] Recent investigations revealed that the topical dosage forms of flutamide could be effective in the treatment of some disorders such as androgenic alopecia, melasma and male pattern baldness.[12,13]

A wide variation of pharmaceutical vehicles such as solids and liquids have been used for topical treatment purposes, but semisolid preparations attract much interests due to their appropriate consistency with biological membranes.[14] Emulgel as a semi-solid formulation is defined as an emulsion (oil/water or water/oil) which is converted to gelly form by adding appropriate gelling agent(s) to the external phase.[15] This type of formulation exhibits satisfying percutaneous absorption in comparison with other semi-solid dosage forms like ointments, creams and gels and serves several favorable properties.[5,6] The most remarkable point about emulgels compared with simple gels, is their ability to carry both hydrophilic and hydrophobic drugs simultaneously and overcome gel limitations. Several researches have illustrated that the amount of drug absorbed from emulgels is significantly higher than simple gels.[16]

Due to physicochemical characteristics of the human skin, it acts as the first barrier against drug permeability.[17,18] Thus, many attempts have been made to circumvent the skin barrier for successful drug permeation. Utilizing skin absorption enhancers is a common approach in topical formulation design to overcome the stratum corneum (SC) as the main barrier of skin. Several groups of compounds such as surfactants, organic solvents, unsaturated fatty acids and some other natural organic materials have been reported to be effective in improving drug permeation through skin.[19,20]

In the current study, the effect of different concentrations of synthetic chemical penetration enhancer Transcutol P (Diethylene glycol monoethyl ether) and Precirol ATO5 (Glyceryl palmitostearate) on flutamide skin permeation were investigated.

Material and methods

Material

Flutamide (MYC- China- Batch NO. 20150316), sodium dihydrogen phosphate (Merck- Germany), potassium dihydrogen phosphate (AppliChem- USA), sodium hydroxide (Merck- Germany), Carbopol® 934 (B.F. Goodrich- USA). Transcutol® P and Precirol® ATO5 were gifted from Gattefosse (France), cetyl alcohol (Merck- Germany), stearyl alcohol (Scharlau- France), isopropyl myristate (Merck- Germany), Tween 80 (Merck- Germany), Span 80 (Merck- Germany), cetyl palmitate (Henkel- Germany) and acetonitrile (Merck- Germany).

Methods

Preparation of flutamide emulgel

The organic phase was prepared by mixing 5 g cetyl alcohol, 1 g stearyl alcohol, 1 g cetyl palmitate, 15 ml isopropyl myristate, 2 g Tween 80 and 1 g Span 80. The mixture was heated to 60-65 ºC and 2 g flutamide powder was added while stirring at 500 rpm. The emulsion was formed by adding 60 ml deionized water (60-65 ºC) to organic phase slowly and stirring continuously. Emulgel formulation was formulated by adding emulsion to aqueous Carbopol solution slowly and then neutralizing by addition of triethanolamin solution (1:2) to pH= 5.8. The aqueous Carbopol solution was prepared by dispersing 0.75 g Carbopol® 940 in 20 ml

deionized water (3.75% w/w) while continuously stirring and was kept in the refrigerator for 24 h to form the homogenized gelling solution.

Preparation of flutamide emulgels with enhancers

The emulgels containing Transcutol P and Precirol ATO5 with 0.5, 1, 2 and 5 % w/w were prepared separately. The enhancers were replaced with the same amounts of the organic phase. All formulations were contained 2% w/w of flutamide.

In vitro permeation studies

Evaluating of drug absorption was done by using abdominal skin of male Wistar rats, weighing around 150-180 g. The skin hairs was shaved by electronic clipper (Moser- Germany) after anesthetizing/killing animals by pentobarbital injection (500 mg/kg, i.p.). The experiments were performed in accordance with ethical committee of the Tabriz University of Medical Sciences (TBZMED.REC.1394.723).

The abdominal skin was surgically removed then, adhering subcutaneous fat and connective tissues were carefully excised with surgical scissors. To remove extraneous remnant enzymes and skin hydration, the samples were kept in contact with normal saline solution in refrigerator (4 °C) for 2-4 h. The full thickness skin samples were rinsed by fresh normal saline and attached on the Franz Diffusion Cells (FDC) (Erweka HDT6, Germany) with the dorsal side facing the donor compartment. The available diffusion area of each cell was 3.8 cm^2 and the temperature was fixed at 32 ºC (human skin normal temperature) by water circulation between two layers of cells. The receptor cell volume was 26 cm3 which was fully filled by phosphate buffer (pH= 7.4) and was stirred continuously using a magnetic bar which stirs at 500 rpm. In all experiments, 1 g of the emulgel was placed on the skin surface in the donor compartment. In order to avoid any leaking from the cells, two compartments of FDC were sealed by silicon

grease before clamping and the top of the cells was covered by parafilm to prevent evaporating of the donor phase. Sampling was performed by removing 500 µl of the receptor solution by a micropipette (Transferpette- Germany) at 0, 15, 30, 60, 120 and 240 min and this volume was replaced with the same volume of phosphate buffer solution at 37°C.

Analytical procedure

The amount of flutamide in the receptor phase was assayed with HPLC (KNAUER- Germany) apparatus equipped by UV-Vis detector. A Eurospher100-5 C18 (250×4.6 mm) (KNAUER-Germany) HPLC column was used and the loop volume was 20 µl. The mobile phase consisted of water/ acetonitrile (70:30) mixture and eluted at the flow rate of 1 ml/min. Detection was performed at 305 nm and run time was 10 min. In each sampling time, 30 µl of the sample solution was injected to the column. The retention time of flutamide at this HPLC condition was 4.417 min. Calibration curve with standard concentrations ranging from 0.3125 to 5 µg/ ml of flutamide were prepared using a stock solution (200 µg/ ml). Water/ acetonitrile (70:30) and phosphate buffer (pH= 7.4) were used for preparing stock and standard solutions, respectively.

Analytical method validation

In order to validate the analytical method, parameters like specificity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) were assessed. Also, based on USP instructions, capacity factor (K'), number of theoretical plates (N) and tailing factor (T_f) were also calculated.

Data treatment

The Fick's second law is used to determine the total amount of drug (Qt) appearing in the receptor solution in time (t) as following Equation 1:

Equation 1
$$Q_t = AKLC_0[(\frac{D_t}{L^2}) - (\frac{1}{6}) - (\frac{2}{\pi^2})\sum\frac{(-1)^n}{\pi^2}]\exp(\frac{D^n 2\pi^2 t}{L^2})$$

Where A, is the effective diffusion area, C_0, represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of the drug between membrane and vehicle. At steady state, it is expressed as follows:

Equation 2
$$\frac{Q_t}{A} = KLC_0[(\frac{D_t}{L^2}) - (\frac{1}{6})]$$

The flux (J) was obtained from the slope of the steady-state portion of the amount of drug permeated divided by A versus time. The lag time value is determined from the x-intercept of the slope at steady-state. The flux is expressed as:

Equation 3
$$J = \frac{C_0 KD}{L} = C_0 K_P$$

Where, K_p is the permeability coefficient.

The Enhancement Ratio (ER_S) for each formulation has been calculated by the following equation in order to present the effect of different concentrations of absorption enhancers on drug permeation:

Equation 4
$$ER_S = \frac{\text{Kp (with pretreatment)}}{\text{Kp (without pretreatment)}}$$

The area under curve (AUC) of permeated drug per unit area per unit time is known as absorption efficacy which was obtained using each formulation chromatogram and used to calculate E_fR (Efficacy Ratio) from the following equation:

Equation 5 $$E_fR = \frac{\text{AUC (with penetration enhancer)}}{\text{AUC (without penetration enhancer)}}$$

All absorption parameters of formulations were evaluated in 3 different times and the average of data was used for calculations.

Results

HPLC is a versatile technique to evaluate the drug concentration after in transdermal preparations. [21-23] . A previously validated HPLC method was used for quantitation analyses.[12] To revalidate the method, specificity and linearity parameters were calculated in the same condition as analyzed samples. In addition accuracy, precision, regression coefficient, LOD, LOQ and system suitability parameters are shown were all in the acceptable range and confirmed the validity of the analytical method. Flutamide LOD and LOQ were as 0.024 and 0.028 µg/ml respectively.

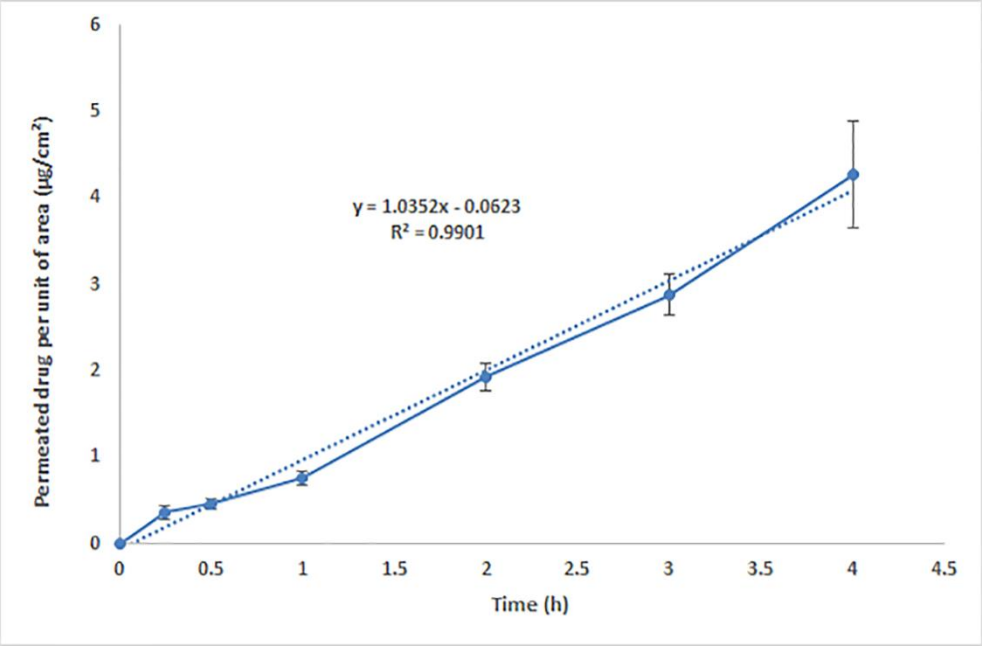

Figure 1. The drug permeation profile for the control formulation

Figure 1 illustrates the permeation profile of 2% flutamide emulgel without any absorption enhancer (control formulation). Similar concentrations of flutamide in transdermal delivery systems have been used in previous studies [12,24].It is clear that the drug permeation diagram follows the classic pattern profile with single steady state portion. Flux is defined as the permeated drug through the skin per unit time and can be obtained from the slop of the linear portion of the graph. The flux of flutamide from emulgel was calculate as 1.035 and the AUC of the control formulation was 7.795 [Figure 1].

The amount of permeated flutamide versus time for formulations with different concentration of Transcutol P are shown in Figure 2. Although the formulation with 0.5 and 1% w/w of Transcutol P showed the classic absorption pattern for lipophilic compounds, emulgels contained 2 and 5 % w/w Transcutol P indicated two-compartment model. Therefore, for first two concentrations (0.5 and 1% w/w) a single flux was defined whereas for the last two fluxes were calculated. In two-compartment model, the first fluxes were greater than second ones and

were used in our evaluations. Comparative absorption parameters including flux values, lag time (t_L), AUC, ER and, E_fR are reported in Table 2. [Figure 2]

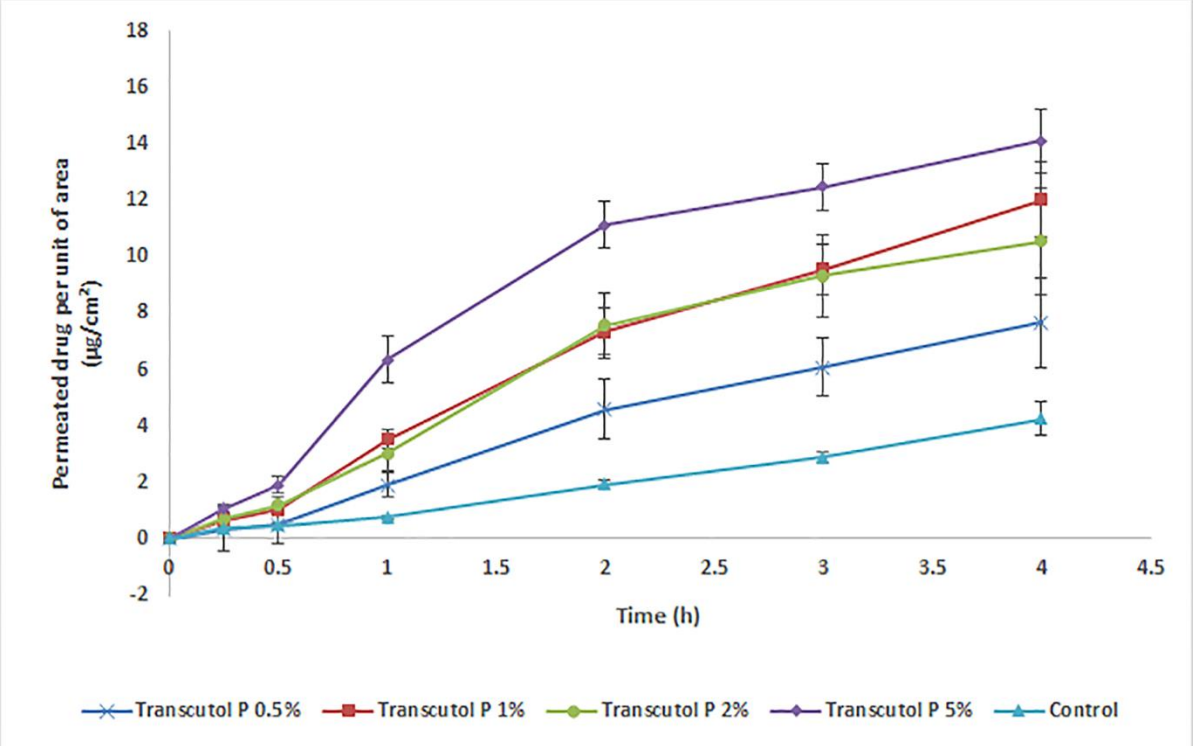

Figure 2. Permeation profile of flutamide from emulgel formulations containing different concentration of Transcutol P as absorption enhancer

Table 2. Absorption parameters for formulations contained different ratio of Transcutol P

	CONC[1]. (% W/W)	FLUX (µG/CM²/MIN)	ER[2]	T_L[3] (H)	AUC[4]	E_FR[5]
TRANSCUTOL P	0	1.035	-	0.206	7.795	-
	0.5	2.024	1.975	0.208	16.299	2.091
	1	3.132	3.026	0.207	26.154	3.355
	2	3.826	3.696	0.159	25.085	3.218
	5	5.799	5.601	0.073	36.450	4.676
PRECIROL ATO5	0	1.035	-	0.206	7.795	-
	0.5	1.261	1.218	0.421	10.635	1.364

	1	2.647	2.557	0.815	20.174	2.588
	2	2.756	2.662	0.050	23.178	2.973
	5	3.799	3.670	0.073	30.403	3.900

[1] Conc: Concentration
[2] ER: Enhancement Ratio
[3] t_L: Lag time
[4] AUC: Area under Curve
[5] E_fR: Efficacy Ratio

Flutamide transdermal permeation from emulgels containing various concentration of Precirol ATO5 are presented in Figure 3. These diagrams have classic pattern with one steady state zone similar to the control. Permeation kinetic parameters such as AUC and flux reveal that the increase in the enhancer concentration can lead to higher rate and amount of permeability through the skin (Table 2). [Figure 3]

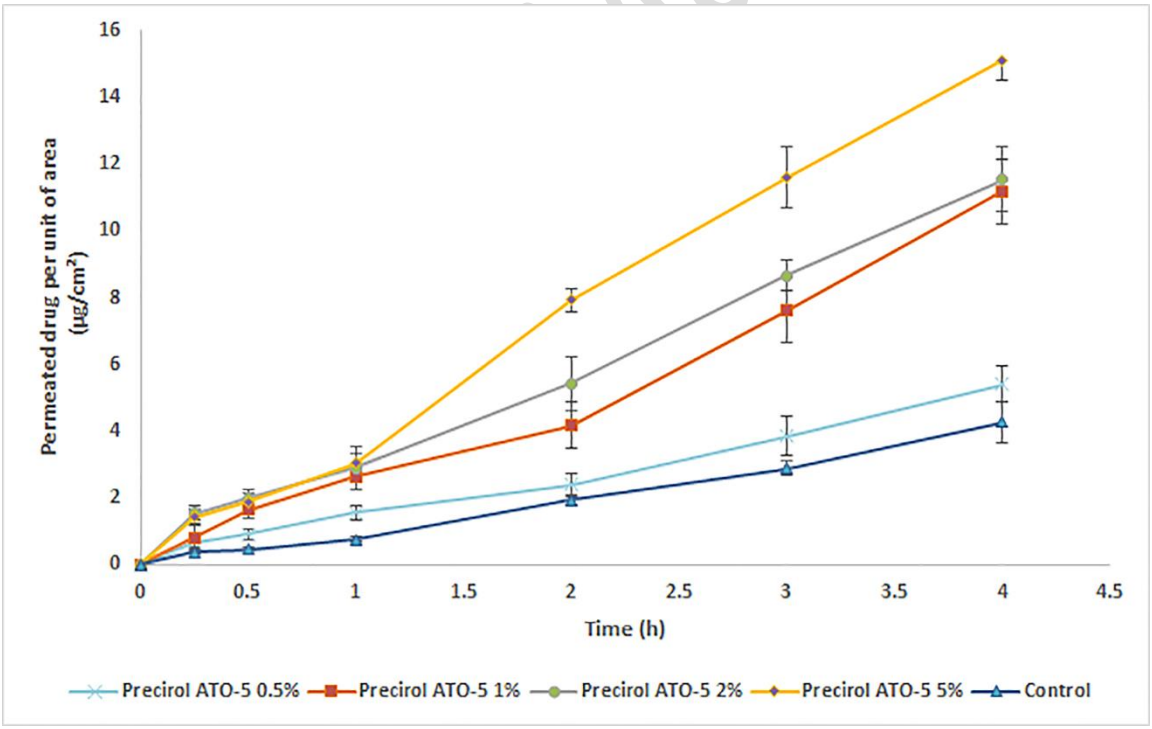

Figure 3. Flutamide skin penetration from emulgels containing different concentration of Precirol ATO5 as absorption enhancer

Increasing the enhancers' concentrations lead to elevated AUC values, significantly. Meanwhile, in all concentration the higher AUC values resulted from Transcutol P compared to the control.

As results demonstrate both enhancers can be effective in improving the skin permeability at this concentration but emulgel base with Transcutol P is capable of transporting more flutamide through the skin.

Discussion

Flutamide is categorized as a lipophilic compound (Log P = 3.35) thus, it is expected to be highly soluble in organic phase and slightly soluble in water.[25] Therefore, the hydro-gel formulation cannot be an appropriate vehicle for the drug whilst, the emulgels could be a suitable dosage form for transdermal delivery purposes.[26-28] Furthermore, several previous studies have revealed that emulgels indicate not only higher flux and permeation of drug compared with gels but they also offer more skin compatible formulations because they are alcohol free and have biphasic (aqueous and organic phases) structure, which allow the incorporation several permeation enhancers.[9,21,29]

Intact stratum corneum (SC) or horny layer is the major barrier against skin permeation that can significantly affect drug absorption thus drug diffusion through skin is assumed as a rate-limiting step.[30,31] The rate of absorption is another important parameter for topical vehicles, especially for aqueous formulations such as solutions, lotions and gels as their fewer resistance time on the skin.[22] Therefore, flux and lag time are two important absorption parameters in transdermal delivery evaluations to ensure that the formulation has acceptable properties in transmitting adequate amount of drug through the skin. To clarify, an appropriate topical preparation need to have a good flux value and an acceptable lag time, simultaneously. Therefore, there is a great interest to develop enhancers to improve percutaneous absorption of

therapeutic agents. These chemicals in topical vehicles could change flux, lag time and total amount of the permeated drug. Enhancers can increase skin permeability by different mechanisms like temporary destruction or changing in physicochemical characteristics of the SC to decrease its resistance.[32,33]

Transcutol P is used in topical products as a powerful solubilizing agent and penetration enhancer.[34] The results of this study shows that Transcutol P in all concentrations (0.5 to 5% w/w) increase flutamide permeation rate (flux) compared with the simple emulgel. The permeation profiles of formulations with 0.5 and 1 % w/w Transcutol P follow the classic permeation pattern but, their flux (2.02 and 3.13 µg/cm^2/h for 0.5 and 1 % respectively) are higher than the simple emulgel (1.035 µg/cm2/min). Meanwhile, two- compartment model of absorption profile of emulgs containing 2 and 5 % w/w of Transcutol P, indicate fluxes almost 4 to 5 times greater than the blank emulgel. The similar observations have been reported by previous investigations about Transcutol P effects on drugs transdermal permeation.[34-36] Additionally, ERs are getting higher in consequence of increasing the enhancer concentration (1.975 and 5.601 for 0.5 and 5 % w/w respectively). Also according to the results, the increase in enhancer concentration lead to greater E_fR values (2.091 and 4.676 for 0.5 and 5 % w/w respectively). Consequently, according to the results, any increase in the enhancer concentration leads to improve all absorption parameters compared with the blank emulgel. This issue can be attributed to Transcutol P promoting role in the flutamide solubility in emulgel biphasic base with limited organic phase that could provide higher driving force to move drug molecules through the skin with higher rate of diffusion. Transcutol P capability to solve lipophilic compounds could alter the skin double layers lipid structure. Due to its physicochemical properties, Transcutol P could penetrate into skin rapidly which leads to co-transportation of the significant amounts of the drug through the sample. The high concentration of Transcutol P as a solvent could cause irreversible destructive changes in skin structure which

can be harmful, so low concentration may be a preferable formulation. Furthermore, almost in all parameters the significant surge in the presence of 1 % of the enhancer can be seen without any major difference (P value> 0.05). Additionally, the formulation showed fewer Lag time in comparison with simple emulgel in an acceptable range. Thus, emulgel with 1 % w/w Transcutol P has been selected as the most appropriate one due to less skin destruction probability.

In the case of Precirol ATO5 as a lipid matrix, it is detectable that all permeation profiles follow the classic pattern of lipophilic compounds. A direct relationship was observed between the total amount of flutamide penetrated through the skin (AUC) and the concentrations of the enhancer. Therefore, E_fR values gradually increased when higher concentration of Precirol ATO5 used in the formulations. Generally, different concentration of the enhancer show greater flux than the simple emulgel (ER>1) but not in the same way as Transcutol P does. The highest ERs (2.662 and 3.670) are devoted to emulgels with 2 and 5 % w/w Precirol ATO5, respectively. Precirol ATO5 (glyceryl palmitostearate) has lipophilic innate and is highly soluble in organic phases. Moreover, the similarity between Precirol ATO5 chemical structure and ceramides is remarkable. Ceramides are one of the essential compounds of epidermis layer of skin. They exhibit the excessive molecular variation with lipophilic nature such as sphingoid and fatty acid compositions.[37,38] Thus, Precirol ATO5 can be easily solved in epidermis lipophilic base and penetrate through the skin and facilitate the drug permeation. It seems that in initial time the skin was saturated and afterward drug permeation reaches steady state with a constant diffusion rate. Although lag time for emulgels with Precirol ATO5 were acceptable, the enhancer addition up to 1 % illustrated greater lag time compared with enhancer free emulgel. A significant lag time decrease for emulgels containing 2 and 5 % w/w of the enhancer were observed that could be caused by enhancer effects on skin barriers function. The selection of an optimum enhancer percentage should be based not only on its efficacy in improvement of

skin permeation but also on its compatibility with skin and possible side effects.[39,40] Hence, whilst, emulgel with 5% w/w Precirol ATO5 proves better kinetic permeation parameters over other concentrations and the base emulgel, it seems as a consequence of higher damage on the skin structure. Although the stability of the formulations kept in the refrigerator during the study period (2 months) was acceptable, detailed stability evaluation should be assessed in the future studies.

As there was no difference between the high and low enhancer concentrations, thus the formulation containing Precirol ATO5 in a 1% concentration is introduced as the optimum formulation.

Conclusion

According to the findings, the optimum concentration of both enhancers were find to be 1 percent. The enhancers were capable of transferring flutamide more efficiently through the rat skin compared to the blank emulgel. Based on the absorption parameters, Transcutol P was a better enhancer for flutamide emulgel preparation compared to Precirol ATO5. The results of this study reveal that the enhancer type and concentration can significantly affect the kinetic of the drug permeation through the skin, and need to be considered and optimized as essential formulation variables.

Author contribution:

FM: Main idea, study design, Data interpretation, editing the manuscript, JS: Skin studies and Formulation design, Data interpretation, PK: Drafting and editing the manuscript, SA: Data acquisition, drafting the work. All authors have read and agreed to the published version of the manuscript.

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Declaration of interest statement

Authors declare no conflict of interests

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