



## Preparation and Evaluation of Cyproterone Acetate Liposome for Topical Drug Delivery

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### Abstract

Cyproterone acetate (CA) has been loaded to liposome by solvent evaporation and thin film formation technique. The effects of some formulation variables such as temperature of organic solvent evaporation, rotary evaporator speed, volume of organic solvent, volume of balloon and temperature of hydrating buffer has been evaluated. The data showed that bigger balloon with higher surface area has better capacity for lipidic film formation; also the best temperature for solvent evaporation and film hydration was 40 °C. According to the data with higher drug/lipid ratio, it is possible to load higher amount of drug into liposome but optimum loading could be obtained at phosphatidylcholine (PC): cholesterol/drug ratio of 1:2:0.5 (with maximum 74±6.11% loading efficiency). Finally percutaneous absorption of CA from simple gel and liposomal formulations was assessed. The results showed that liposomal formulation has better penetration potential than conventional CA formulation (simple gel).

*Keywords:* Cyproterone acetate; Liposome; Percutaneous absorption.

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### 1. Introduction

During the past decades, there has been more interest in exploring new techniques to increase drug permeation through the skin. Liposomes, prepared from a variety of natural and synthetic phospholipids, are being considered as drug-carrier structures or vesicles. Liposomes may act as a solubilization matrix [1], as local depot for controlled release of active compound, as permeability enhancer [2-4], or as rate limiting membrane barrier

for the modulation of systemic absorption of drug through the skin [1-7]. Liposomes have become a valuable experimental and commercially important drug delivery system, owing to their biodegradability, biocompatibility, low toxicity and ability to entrap lipophilic and hydrophilic drugs [8-13]. Present applications of the liposomes are in the immunology, dermatology, vaccine adjuvant, eye disorders, brain targeting, infective disease and in tumor therapy [14-16]. Hormonal approaches to treatment of acne have been the subject of much interest since the sebaceous glands were demonstrated to be sensitive to androgens. The use of topical

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antiandrogens has had theoretical appeal since the pilosebaceous unit was found to be an androgen-dependent structure. The reasons for using topical rather than oral therapy include the avoidance of hepatic metabolism, reduction in risk of systemic adverse effects and the possibility of using this treatment in both female and male patients. Unfortunately, this theory has not clinical possibility till now, because of improper skin permeability of antiandrogen drugs such as cyproterone acetate [5, 6]. Several groups showed that liposomes enhance the drug permeation into the skin [8, 17-19]. In this regard, cyproterone acetate was used in liposomal form to overcome low permeability of cyproterone acetate through the intact skin.

## 2. Materials and methods

### 2.1. Materials

Egg phosphatidylcholine (PC) was purchased from Sigma Chemical Co. (USA). Cyproterone acetate (CA) was a generously gift from Iran Hormone Pharmaceutical Co. (Iran). Butylated hydroxy toluene (BHT), cholesterol (C), ethanol, hydroxyl ethylcellulose (HEC) and chloroform were obtained from Merck Company (Germany). All other chemicals and solvents were of analytical grade.

### 2.2. Preparation of liposomes

Multilamellar liposomes were prepared

by thin film hydration technique [20]. Accurately weighed quantities of CA, PC and C were dissolved in chloroform-ethanol mixture in different ratios in a round-bottomed flask. BHT, equivalent to 2% of the total lipids as an antioxidant, was added in the organic phase in the flask. The chloroform-ethanol mixture was evaporated under reduced pressure at different rotating speeds. After complete evaporation of organic solvents, hydration of the thin film was carried out using normal saline solution. The various formulation variables considered in this research have been presented in Table 1 and 2.

### 2.3. Evaluation of the loading efficacy

The separation of unloaded drug was performed by centrifugation method [21]. The liposomal suspension was placed in centrifuge. The centrifugation was carried out at 2000 rpm for 3 min. and elutes containing CA loaded vesicles were collected and observed under microscope for drug particles. The vesicular suspension finally incorporated with chloroform to destroy the vesicle, and the concentration of CA was determined using spectrophotometer (Cecile 9050 UK) at 281 nm. In each formulation mean of three determinations was considered.

### 2.4. Preparation of cyproterone liposomal gel

The HEC gels containing 2 % CA were

**Table 1.** Effect of formulation variables on liposome formation.

Batch No	Solvent volume (ml)	Temperature (°C)	Rotary speed (rpm)	Vacuum level	Rotary speed (rpm)
A1	5	20	60	High	60
A2	5	30	60	High	60
A3	5	40	60	High	60
A4	5	40	100	High	60
A5	5	40	120	High	60
A6	5	40	120	Gradual	60
A7	10	40	120	Gradual	60
A8*	10	40	120	Gradual	60
A9	10	40	120	Gradual	120
A10**	10	40	120	Gradual	60

\*Hydration of lipidic film was performed in a bigger balloon (500 ml vs. 250 ml). \*\*Hydration was performed without glass bead. All of these formulations had the same lipid/drug ratio.

**Table 2.** The composition of various formulations prepared for optimization of cyproterone acetate loading.

Batch No	PC (mg)*	Cholesterol (mg)	CA (mg)**
1	37.5	12.5	25
2	75.0	25.0	25
3	150.0	50.0	25
4	150.0	100.0	25
5	25.0	25.0	25
6	75.0	100.0	25
7	225.0	25.0	25

\*PC: Phosphatidylcholine; \*\*CA: Cyproterone acetate.

formulated as simple gel and liposomal gel. HEC has been dispersed in water (2 %) and then CA in adequate quantity has been added to the gel to make simple gel or as liposomal suspension.

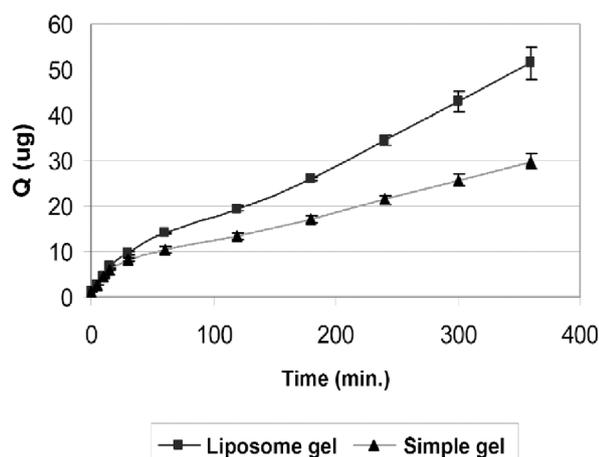
### 2.5. *In vitro* permeation study

Permeation studies were carried out through abdominal skin of guinea pig using the modified Franz diffusion cell. The animals were sacrificed by an overdose of chloroform inhalation. The hairs of the dorsal side of animal were removed with the help of scissors. The shaven part of the skin was separated from the animal and mouthed to diffusion cells and water:ethanol (50:50) solution was used as receptor medium. In each experiment, 200 mg of gel was applied onto the prepared guinea pig skin facing to the

diffusion cell and each experiment was done in triplicate. The samples were quantified by UV spectrophotometer (Cecile 9050 UK) at 281 nm. After each sampling, aliquot volume of receiver phase was replaced and dilution factor finally was introduced in concentration calculations.

### 3. Results and discussion

The data showed that the optimum temperature for organic solvent evaporation is 40 °C. Also, data showed that hydration of lipidic film in 40 °C is better and when the rate of rotary evaporator revolution was increased a better lipidic film was performed. The results showed when bigger balloon with larger surface area were used, the better lipid film was formed. The formation of lipidic film in presence of glass bead also was better and



**Figure 1.** The percutaneous penetration profile of CA from simple and liposomal gel via excised guinea pig skin in *in vitro* condition.

**Table 3.** The loading efficacy of cyproterone acetate in various liposomal formulations.

Batch No.	Initial lipid/drug ratio PC: C/ CA	% of CA incorporation	Final lipid /drug ratio in liposomes (%)	% of CA loading
1	2:1/2	44.0±4.93	3:0.88	29.3
2	2:1/1	51.0±4.36	3:0.51	17.0
3	2:1/0.5	55.3±5.03	3:0.28	9.3
4	2:2/0.5	63.0±7.21	4:0.32	8.0
5	2:0.5/0.5	52.2±3.46	2.5:0.26	10.4
6	1:2/0.5	74.0±6.11	3:0.37	12.3
7	2.5:0.5/0.5	49.0±4.58	3:0.25	8.3

PC: phosphatidylcholine; C: Cholesterol; CA: Cyproterone acetate.

gradual increment in vacuum had the same effect. Increment in organic solvent also produced more uniform lipidic film and hydration of this film was better. All of the formulations in Table 1 have the same formulation in respect to PC, C and CA. The results showed that changing the lipid ratio and changing the lipid/drug ratio, it can change the loading efficiency of liposomal vesicles. Maximum loading was observed in PC: C: CA ratio of 2:1:2 (Table 3). Although according to the data presented on Table 3, the loaded amount of CA based on the added amount of CA to lipidic mixture is higher in the ratio of 1:2:0.5. Loading efficiency has been calculated base on the Equation 1.

$$\text{Loading Efficiency} = \frac{\text{Amount of added CA to lipidic layer}}{\text{Amount of trapped CA into the vesicle}} \times 100 \quad (\text{Equation 1})$$

The percutaneous absorption of CA as a simple gel and liposomal gel has been shown in Figure 1. According to this Figure liposomal gel of CA had better absorption capability. The same data have been published with Valenta and Janisch [22]. Although they directly applied hydrated lipidic mixture to guinea pig skin without any further formulation but comparable results is published in their report. The percutaneous absorption data revealed that the kinetic of CA penetration from liposomal gel well followed by the zero order model ( $R^2=0.9921$ ), but the simple gel absorption data of CA was better fitted to the Higuchi model ( $R^2=0.9801$ ).

#### 4. Conclusion

According to results of this study, the lipid/drug ratio had considerable effects on total loading efficiency. Also, increase in cholesterol ratio decreased the loading efficiency. According to this research it is possible to increase the percutaneous permeability of CA from topical formulations as liposomal dosage form. Also liposomal form of CA would be a suitable means to produce a more uniform release profile.

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