

## Determination of SPF and Moisturizing Effects of Liposomal and Conventional Formulations of Octyl Methoxycinnamate as a Sunscreen

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### Abstract

#### Objective

To determine and compare the SPF (Sun Protection Factor) and moisturizing effects of the liposomal and conventional lotion formulations containing octyl methoxycinnamate (OMC) as a sunscreen by *in vivo* methods.

#### Materials and Methods

The multilamellar liposomes (MLVs) containing OMC were prepared by fusion method and o/w emulsion was prepared as FDA standard sunscreen method. The SPFs of the formulations were determined by *in vivo* method according to Australian standard. The exposure area was the back of ten volunteers. Subsites of the backs were exposed to solar simulator as ultraviolet (UV) source. The minimum erythral dose (MED) for unprotected skin was observed in the next day. The sunscreen was spread (2 mg/cm<sup>2</sup>) over the area with a finger stall to achieve a uniform film. Each test subsite in a series was exposed to controlled amounts of simulated sunlight by a constant ratio. In the third day, the MEDs of the formulations were observed. The SPF was determined by the ratio between the time required to produce the minimal erythematous reaction by using sunscreen and the time needed to produce the same reaction without using sunscreen. The moisture content of the skin was determined after 30 min, 2, 3, 6 and 10 hours post-application of the formulations containing OMC and also NaCl 3% in eucerin (as a positive control) using Corneometer by measuring electrical capacitance.

#### Results

The SPF obtained from our *in vivo* results for standard Homosalate reference was almost the same as published SPF for this standard. The SPF of the liposomes containing OMC was a little bit more than lotion at the same concentration of OMC. All the tested formulations significantly increased the moisture content of the skin compared to control (without any treatment), in all the tested point times. After 30 minutes of post-application, the skin moisture content resulted from OMC-lotion was significantly more than liposomal-OMC and NaCl 3% in eucerin; however, 10 hours after post-application there were no significant differences in the skin moisture content of these three treatment groups.

#### Conclusion

MLV liposomes prepared by fusion method is a good vehicle for OMC as a sunscreen since it provides proper SPF and increase the moisture content of the skin.

**Keywords:** Minimum erythral dose, Moisturizing effects, Multilamellar liposomes, Octyl methoxycinnamate, Sun Protection Factor.

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## Introduction

Exposure to sunlight can have both advantageous and harmful effects on the human body (1, 2). Adverse reactions to the sun UV rays include short-term inflammatory responses (erythema, oedema, sunburn) and long-term effects, like cutaneous photoageing (dermatoheliosis), suppression of the immunological system, photosensitivity, hyperpigmentation and skin cancers, which are increasing throughout the world (2- 4).

It has been known for decades that sunscreens are capable of protecting man from harmful effects of solar radiation (2, 5, 6). The evaluation of a sunscreen's efficiency for ultraviolet B (UVB) in humans is based on the determination of the minimum erythematous dose (MED). Sun Protection Factor (SPF) of a sunscreen represents the effectiveness of the sunscreen in protecting against UVR-induced erythema. The sunscreen is spread on the skin which is then exposed to UV light. The SPF is the ratio between the UV dose required or time to produce the minimal erythematous reaction while using sunscreen and the UV dose or time needed to produce the same reaction without the sunscreen, after the application of 2 mg/cm<sup>2</sup> or 2 µl/cm<sup>2</sup> of the sunscreen product (2, 7, 8).

Many factors manage the delivery of the drugs and cosmetics into the skin from topically applied formulations. Liposomes have been used for years to bring active ingredients into the skin (9-13). Several factors; such as, physicochemical properties of the drug and other ingredients present in the liposomal product, lamellarity, lipid composition, charge on the liposomal surface, size of liposomes, vehicle, mode of application and total lipid concentrations have been proven to influence drug deposition into the skin layers (10, 11, 14). Liposomes can provide a drug-delivery system for the skin according to many studies which have produced several fold higher

concentrations in the skin than conventional dosage forms (10, 12, 13, 15). The other advantage of a liposome-based drug product is that less drug needs to be administered. Thus, the probability of systemic absorption and adverse drug reactions is reduced (9).

Ramon *et al.* showed that liposomes could be regarded as alternatives to conventional oil/water emulsions in the formulations of lipidic sun filters. When liposomes with a composition and structural organization similar to that of the stratum corneum lipids are used, the skin penetration is retarded (16). First time researchers entrapped some humectants like urea and glycerin in liposomes and they demonstrated that the entrapment of humectants in liposomes improves their skin moisturizing efficacy (17). Some other advantages of liposomes over other cosmetics are as follow: encapsulation of both water-soluble and lipid-soluble substances; higher affinity for cell membrane of the skin and excellent substantivity to keratin, promotion of skin moisturization, improvement of skin roughness, improvement of stability of encapsulated drugs, resistant to washing and removal from the skin especially for entrapped moisturizers, reduction of the toxicity of irritants and lack of toxic effect to the skin (17- 20).

In this study MLV liposomes containing OMC was prepared and characterized, its SPF determined by an *in vivo* method and the moisturizing effect was measured by Corneometer. A standard conventional o/w lotion of OMC was used for comparison and control.

## Materials and Methods

### *Reagents and chemicals*

OMC, cholesterol and vitamin E were purchased from Merck (Darmstadt, Germany). Lanolin, white petrolatum, stearic acid, propylparaben, methylparaben,

disodium EDTA, propylene glycol and triethanolamine were purchased from Sigma (USA). Soya Phosphatidylcholine (Soya PC) was obtained from the Avanti Polar Lipids (Alabaster, Alabama, USA). Phosphate buffered saline (PBS) ingredients were supplied from Sigma (USA). All solvents used in this study were High Performance Liquid Chromatography (HPLC) grade. All chemicals were of the purest grade available.

#### ***Preparation of oil/water emulsion containing OMC***

OMC (7.5%), lanolin (5%), white petrolatum (2.5%), stearic acid (4%) and propylparaben (0.05%), were heated at 77-82°C as oil phase; and methylparaben (0.1%), disodium EDTA (0.05%), propylene glycol (5%) and PBS (pH=7.2) up to 100% were heated as aqueous phase with constant stirring until each part solubilized. The aqueous phase was added to oil phase and the mixture stirred until it cooled down to room temperature (21).

#### ***Preparation of liposomes containing OMC***

Multilamellar liposomes containing OMC were prepared by fusion method (22). Briefly, the lipid components consisted of Soya PC (15%), cholesterol (2%), vitamin E (0.3%), propylparabene (0.05%) were melted in propylene glycol at 77°C (lipid melt). When the temperature of lipid melt was 50°C, OMC (7.5%) was added and mixed completely. PBS (up to 100%) and methylparabene (0.1%) were heated separately at 55°C and was added to previously heated (50°C) lipid melt and vigorously stirred until it cooled down to room temperature.

#### ***Morphology and size analysis of liposomes***

Optical microscope (OLYMPUS, Germany) was used for studying the morphological features of MLV liposomes containing OMC. The mean diameter and particle size

distribution of liposomes were determined by a particle size analyzer (PSA; Klotz, Germany) (23).

#### ***Determination of OMC by HPLC***

A Hitachi L-7100 liquid chromatograph equipped with a 20 µl loop injector and Hitachi L-7420 UV-VIS detector (Hitachi, Tokyo, Japan) were used to carry out OMC determination. The column was a LiChrospher 100 RP-18 (12.5 cm× 4 mm, 5 µm particle size) (Merck, Darmstadt, Germany). OMC was determined at 313 nm. The mobile phase was H<sub>2</sub>O/HAc/EtOH, 29.5:0.5:70 (v/v/v), with a flow rate of 0.5 ml/min (24). The calibration curve was prepared with methanol solutions of OMC at concentrations ranging from 2-18 µg/ml (n=6). The intra-and inter day variation for OMC was performed and there was no significant difference between day-to-day analysis. The validation results were established for three injections per concentration and 6 concentrations. The unknown concentrations were determined by using the standard curve as reference (25).

#### ***Determination of encapsulation efficiency of OMC in liposomes***

In order to determine the encapsulation efficiency, liposomes were separated from untrapped OMC using ultracentrifugation (Beckman Optima 190K, USA) at 100,000 × g for 1h at 4°C and subsequently washed three times with PBS. The supernatant and precipitate were analyzed by HPLC to determine the encapsulation percentage. The entrapment efficiency of liposomes was calculated as follows:

$[(T-C)/T] \times 100$ , where  $T$  is the total amount of drug that is detected both in the supernatant and sediment, and  $C$  is the amount of drug detected only in the supernatant (26).

***In vitro stability studies***

All formulations were stored in light proof glasses in 4°C for 3 months. The encapsulation efficiency of each formulation was analyzed at the day of preparation (starting point). Samples were then analyzed monthly for 3 months. The encapsulation efficiency, microscopic observations and particle size of the liposomes were monitored within three months (20, 23).

***Preparation of Homosalate reference as standard sunscreen***

A standard sunscreen formulation is needed for ensuring reproducible results in SPF determinations. This standard was prepared according to FDA and Australian standards. This standard contains two different parts which are presented in Table 1. In this study Homosalate reference 8% was used as standard sunscreen. According to FDA the SPF of this standard is  $4.47 \pm 1.279$  (21, 27).

Table 1. The compositions of the Homosalate standard sunscreen formulation according to FDA and Australian Standards

	Ingredients	% (w/w)
<b>PART A</b>	Homomenthyl Salicylate (Homosalate)	8.0
	White Petrolatum	2.5
	Stearic Acid	4.0
	Lanolin	5.0
	Propylparaben	0.05
<b>PART B</b>	Methylparaben	0.1
	Disodium EDTA	0.05
	Triethanolamine	1.0
	Propylene Glycol	5.0
	Water purified or phosphate buffer	74.3

***SPF determination of Homosalate reference, COLIPA standard, o/w emulsion and liposomes containing OMC by in vivo method***

In this study 10 subjects, female and male, were used for SPF determination. Informed consents were obtained from each subject. Their skin types were type I – III according to Australian standard (27). Volunteers with a history of abnormal response to medications, UV radiations or allergies to topically applied cosmetics were excluded. The back of these subjects were used for UV exposure.

Ultraviolet Solar Simulator Model 16S was used. The radiation is produced by a xenon arc lamp and unwanted spectral regions are removed by filter systems. The spectral irradiance is shown in Figure 1.

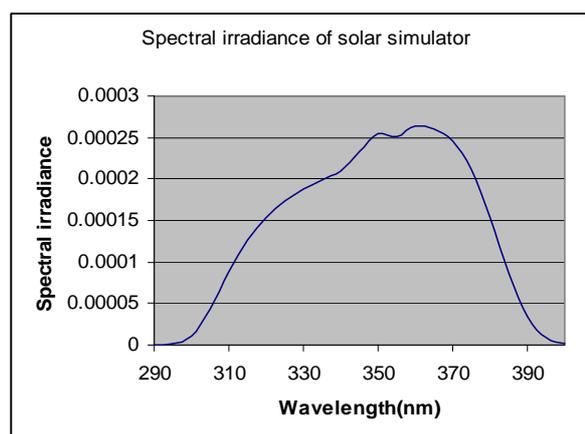


Figure 1. The spectral irradiance ( $\text{Wcm}^{-2}\text{nm}^{-1}$ ) distribution of solar simulator UV source as *in vivo* method of UVR from 290 nm to 400 nm.

For SPF determination by *in vivo* method, 3 days was needed. In first day, the back of the subjects were exposed to UV

simulator. According to the skin type, the times of exposure were different. For example for subjects with estimated MED of 8 seconds, the exposure time started with 4 seconds to 10 seconds. After about 20 hours, the MED of the subjects were observed. UV exposure sites are known as subsites. These subsites are the areas of skin where the test products or the reference products are applied and tested. To determine the MEDs, the row of test subsites from the low exposure to the end was observed and then the point at which the first minimal redness perceptible to the eye was determined and selected. There should be at least one lower exposure subsite showing no erythema adjacent to the selected subsite. The precision of the test can be improved if there is also a subsite with a higher degree of redness adjacent to the selected subsite. The MED of a subject is shown in Figure 2. Therefore, in the second day the MEDs without sunscreen were observed and then the tested sunscreen was applied. The test site should be divided into at least five subsites to avoid overlap of radiation. The application amount of tested sunscreen samples and standard formulations were  $2 \text{ mg/cm}^2$ . Residual amounts of products remaining on the finger stall were taken into account. The sunscreen was spread over the area with a finger stall, to achieve a uniform film. After application, the product was allowed to dry for 15 min before irradiation. Air conditioned premises at  $20^\circ\text{C}$  to  $25^\circ\text{C}$  was used during the waiting period. Each test subsite in a series was exposed to controlled amounts of simulated sunlight using the solar simulator. From one area to the next, the exposure time should be increased by a constant ratio. According to Australian standard, as the estimated SPFs were less than 25, the increments between subsequent exposures were 1.25 times. For example, in the person with MED 8 and Homosalate reference sunscreen formulation with an estimated SPF of 4.47, the exposure

times were 23, 29, 36, 45 and 56 seconds. If the MED is unknown, a wider range of exposures might be needed. In the third day the MEDs of sunscreens were observed. The dose required to elicit MED varies from individual to individual, depending on several factors, including, epidermal melanin fraction volume, stratum corneum thickness and nutrition (27, 28).



Figure 2. The MED observed 20 hours after UV exposure from UV source of solar simulator to a subject with the skin type II. The fourth erythema from right is MED.

***Moisture content measurement of the skin following application of o/w emulsion, liposomes containing OMC and NaCl 3% in eucerin using Corneometer***

The moisture content of the skin was measured by Corneometer (Courage & Khazaka, Cologne, Germany) following application of OMC lotion, OMC MLV liposomes and NaCl cream (3% in eucerin) as a positive control. In practice, the technique is used to measure the difference in stratum corneum hydration before and after the application of a cosmetic or other skin treatment. The test was carried out on 6 volunteers with normal skin at room temperature. Their ages were between 20 and 35 years. Before the measurements, subjects were given time to adapt to room conditions

without covering the measuring sites with clothes. On the day of examination, the skin was not washed and nothing was applied to the skin surface. Subjects were instructed not to apply any preparation to the site to be examined one week before investigation. The Corneometer CM 820 was used to determine the humidity level of the stratum corneum by measuring electrical capacitance. In all subjects, the measured positions were free of eczematous involvement. All measured values were expressed as the median of three recordings. The measurements were carried out on exactly the same sites. There is a location-dependent change in the SC hydration state; therefore the site of measurement was always in the same location. The measuring place was in the middle of the forearm. In the first instant, the moisture content of the skin without any application of the product was measured, and then the measurement was carried out after 30 min, 2, 3, 6 and 10 hours after application of the sunscreens (29- 31).

### **Statistical Analysis**

One-way ANOVA and t student statistical tests were used to assess the significance of the differences. In case of significant F value multiple comparison Tukey-Kramer tests was used to compare the means of different treatment groups. Results with  $p < 0.05$  were considered to be statistically significant.

## **Results**

### **Assay of OMC by HPLC method**

Analysis of variance on data obtained by HPLC method resulted in a linear regression between optical densities (OD) and concentration of 2 - 12  $\mu\text{g/ml}$  of OMC in methanol. Low percentages of coefficient of variations (%CV) indicate reproducibility of this method and the recovery percentage (%R) shows the reliability of the method. Chi-square ( $X^2$ ) test showed no significant

difference between intra and inter-day determinations ( $p > 0.05$ ).

### **Characterization of the Liposomes**

The liposomes made by fusion method were morphologically homogenous multilamellar vesicles, as observed under optical microscope (Figure 3). Mean diameters of MLVs liposomes containing OMC determined by PSA were  $2.21 \pm 0.06 \mu\text{m}$  ( $n = 3$ ). The size of liposomes was  $2.43 \pm 0.022$ ,  $2.58 \pm 0.21$  and  $2.86 \pm 0.33 \mu\text{m}$  after 1, 2 and 3 months storage at  $4^\circ\text{C}$ , respectively. These liposomes exhibited slight increase in their size but this was not significant. In this study, liposomes with high encapsulation efficiency were prepared. Encapsulation efficiency of MLVs containing OMC was  $89.66\% \pm 2.08$  ( $n=3$ ). These liposomes exhibited no changes in encapsulation efficiency when stored at room temperature for over 3 months. The encapsulation efficiencies were  $89.50\% \pm 2.45$ ,  $88.45\% \pm 2.12$  and  $87.9\% \pm 1.89$  after 1, 2 and 3 months storage at  $4^\circ\text{C}$ , respectively. These liposomes exhibited no changes in encapsulation efficiency when stored at  $4^\circ\text{C}$  for 3 months. The difference between encapsulation efficiency for 0, 1, 2 and 3 months were not significant ( $p > 0.05$ ).

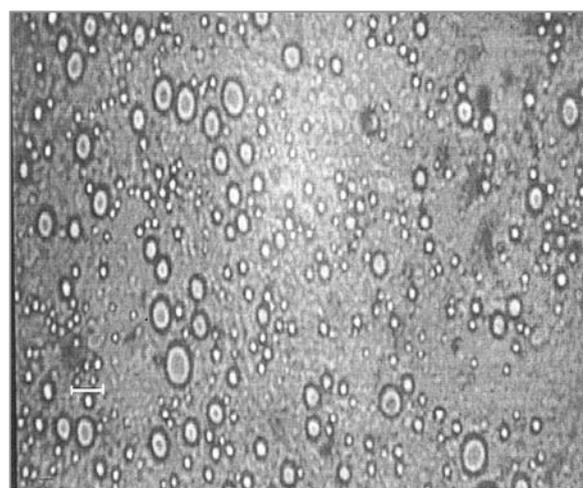


Figure 3. Optical microscopic pictures of liposomes prepared by fusion method containing OMC (magnification of image is  $\times 1000$ ).

**SPF determination of Homosalate reference by *in vivo* method**

In this study 10 subjects, female and male, were tested for SPF determination of Homosalate standard product. The SPFs of the reference in these subjects were determined by Australian standard *in vivo* method. According to this method, the SPF of Homosalate standard product was calculated as  $4.35 \pm 0.64$ , and there were no

significant difference between our *in vivo* study value ( $4.35 \pm 0.64$ ) and claimed SPF ( $4.47 \pm 1.279$ ) with  $p > 0.05$ .

Table 2 shows the SPFs of Homosalate reference in ten subjects. The data obtained from our study show that the difference between SPFs obtained from our *in vivo* results and published SPFs for Homosalate reference is not significant.

Table 2. The SPF values of Homosalate standard determined by *in vivo* method in ten subjects with different skin types and MEDs

Number	Sex	Skin type	M. E. D.	SPF
1	F	II	11	4
2	M	III	13	4.46
3	F	I	7	3.56
4	F	III	11	4.90
5	F	I	7	4
6	M	III	13	4.92
7	F	I	8	3.62
8	F	I	9	4.44
9	M	II	11	5.63
10	M	III	18	4.05

**Determination of the SPF of COLIPA reference, o/w emulsion and liposomes containing OMC by *in vivo* method**

As in the Australian standard, Homosalate reference product and COLIPA high reference product are both used to validate the results. In this study, ten subjects were tested and the SPF of Homosalate standard

and COLIPA standard were determined as references. The SPFs of the prepared samples, OMC lotion and MLV liposomes containing OMC were determined by Australian standard *in vivo* method using the same volunteers. Their skin types were I - III and their MEDs were from 7 to 18. The SPF results are shown in Table 3.

Table 3. The SPF values of COLIPA, OMC lotion and OMC-MLV liposomes determined by *in vivo* method in ten subjects (S) with different skin types and MEDs.

Sunscreen	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	SPF(Mean±SD)
Homosalate reference	4	4.46	4.56	4.90	4	4.92	3.62	4.44	5.63	4.05	$4.35 \pm 0.64$
COLIPA	12.4	15.5	19.4	14.5	17.1	12.4	15.5	15.5	12.4	19.4	$15.2 \pm 3.4$
OMC lotion	14.2	15.5	12.4	12.3	15.5	17.1	12.3	12.4	15.5	12.3	$13.95 \pm 1.83$
OMC liposomes	17.1	19.3	19.4	12.4	15.5	14.5	19.4	17.1	14.5	15.5	$16.85 \pm 1.40$

**Measurement of the moisture content of the stratum corneum following application of o/w emulsion, liposomes containing OMC and NaCl 3% in eucerin**

The liposomal formulation containing OMC, o/w lotion of OMC and NaCl 3% in eucerin vehicle, as positive control, were used in our experiments and the water capacitances were measured using Corneometer. NaCl 3% in eucerin is a clinically used material for the treatment of skin dryness; therefore, this cream was used as positive control.

The Corneometer was used and calibrated to the base line value for each subject before applying the formulations on the skin. During the first 30 minutes after application, the level of water content is usually higher than normal. Measurement of water content of skin at this time may result in erroneous data (31). Therefore, the first measured time was 30 minute after application.

Figure 4 shows the normalized (RCU, Relative Corneometer Units) hydration values for the readings of the reference site measured with Corneometer for the three formulations at 0.5, 2, 3, 6 and 10 hours post-application versus baseline without treatment (control). All the tested formulations significantly increased the moisture content of the skin compared to control, in all the tested point times ( $p < 0.01$ ). The trend of the curves in all the treatment groups was nearly the same. At 30 minutes application, the highest water content was observed for OMC lotion; however, there were no significant differences between NaCl 3% and liposomes containing OMC. After 2 hours the moisture contents were decreased in all of the formulations. After 10 hours of application, the moisture content of the skin for all the preparations was almost the same, they reached nearly to the same degree of hydration and there were no significant differences among them. The moisture

content at this time point for all the preparations was significantly higher than control ( $p < 0.01$ ).

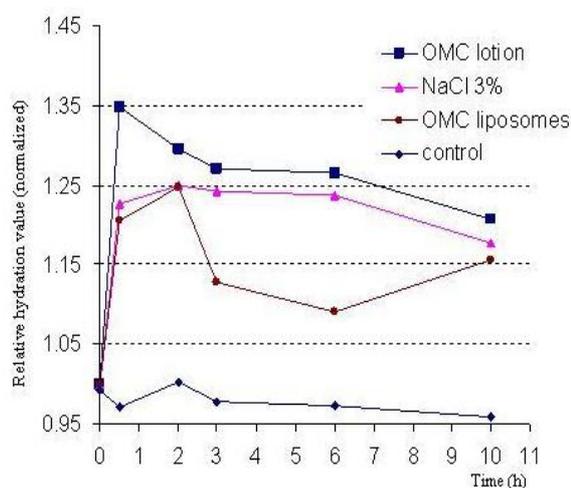


Figure 4. Normalized relative hydration values after application of OMC lotion, OMC liposomes and NaCl 3% vs. control without application of the product (n = 6).

## Discussion

Sunscreens have been widely recommended as a protective film against sunburn, photo-ageing and skin cancer (32). To date, only the *in vivo* methods have been employed as SPF determination for SPF labeling (33). It was demonstrated that the vehicle of UV filters can affect on skin penetration (34, 35). Results of several studies indicate that liposomes have been reported as carrier for active cosmetic ingredients such as humectants (9, 17) and also pharmaceutical drugs (10, 12, 13, 15).

In this study, the SPF and moisturizing effects of the liposomes and conventional formulations containing OMC were investigated. The study indicated that, the SPF of the liposomes containing OMC was greater than OMC lotion at the same concentrations; however, this was considered not quite significant by unpaired t-test. Fusion method was used to prepare MLV liposomes containing OMC. This method is one of the most suitable methods for the

preparation of topical liposomes that provides homogenous MLV liposomes. This was confirmed by microscopic studies and particle size analyzer. This method is simple, efficient, reproducible, does not need organic solvents like chloroform and yields homogenous liposomes with high encapsulation efficiency (22).

The main phospholipid, which was used in liposome preparations, was soybean lecithin which contains phospholipids present in physiological membranes, so that they are incorporated in the normal reactions of the lipid metabolism. It contains polyunsaturated fatty acids like linoleic acid that are designated as essential for healthy skin (36). In the study of Ghyczy *et al.* it was reported that there is an acute and significant increase in skin humidity in formulations containing soybean PC as phospholipids (37). Cholesterol in liposomes formulations can stabilize the lipid bilayer and decrease the leakage of encapsulated drugs and vesicle aggregation (14, 23).

In the current study, liposomes with high encapsulation efficiency were prepared. Encapsulation efficiency of MLVs liposomes containing OMC was  $89.66\% \pm 2.0.8$  ( $n=3$ ). Most likely the location of OMC in liposome, due to its hydrophobic character is membrane interior of bilayers. This could be another reason for high encapsulation efficiency of OMC, since hydrophobic material in proper formulation could have encapsulation efficiency near to 100 percent (22, 23).

For cosmetic treatment, the target is only the horny layer of the skin. It was demonstrated that a concentration of up to  $1 \text{ mg/cm}^2$  PC on skin surface, seems to be the best compromise between efficacy and safety (37). In this study the PC concentration in the liposomal formulations was 15 %, which indicates that 100 mg total liposomes contain 15 mg PC. Since the sunscreen liposomal formulations is applied at  $2 \text{ mg/cm}^2$

concentration; for  $50 \text{ cm}^2$  area, 100 mg total liposomes is needed. Therefore, 15 mg was applied to  $50 \text{ cm}^2$ . This equals to  $0.3 \text{ mg/cm}^2$  of PC which is less than  $1 \text{ mg/cm}^2$  PC and is suitable to prepare the liposomes containing OMC as a sunscreen agent.

Ramon *et al.* showed that liposomes could be regarded as alternatives to conventional oil/water emulsions in the formulations of lipidic sun filters. When liposomes with a composition and structural organization similar to that of the stratum corneum lipids are used the skin penetration is retarded (16). As the intercellular lipids are important in controlling the percutaneous absorption, MLVs may mix with the intercellular lipids and cause the swelling of them without altering the multiple bilayers structure of the stratum corneum and produce a sustained release carrier system that act as a reservoir for OMC; therefore, the sunscreen remains longer on the outermost layers of the skin (11). This property is important for sunscreen agents because the amount remained inside the stratum corneum maybe directly related to its sun protection value (6, 34).

*In vivo* studies have been shown that liposomal encapsulation of triamcinolone (13), hydrocortisone (10), lidocaine (12), and minoxidil (10) produced several-fold higher concentrations in the epidermis and dermis at the same time compared with the conventional products (gel, lotion, ointment). The results support the possibility of developing liposomal products that are superior to existing topical dosage forms (38).

Topical application of OMC liposomes, OMC lotion and NaCl 3% in eucerin produced a significant increase in the water content of the human stratum corneum. Some moisturizers are designed to promote water retention by their hygroscopic nature while others are designed to prevent water loss from the skin surface by providing an

occlusive film or by supplying SC-like lipids (39). In NaCl 3% in eucerin, the occlusive property of this vehicle showed less decrease in water content. The protective effect of eucerin is due to the occlusive film on the skin rather than to improve the SC regeneration. The liposomes due to their lipophilic structure and their similarity to SC lipids can improve the moisture content but not as well as OMC lotion and NaCl 3%. Liposomes provide their own water content and share the water with the skin to which they are applied. The problems of loss of water migrating from the underlying tissues are resolved by the use of liposomes. Application of humectants to the skin alone is unsatisfactory since they are not substantive to the skin; they are water soluble and are readily rinsed off (40).

In a patent filed by Unilever Brothers, they use humectants entrapping liposomes in cosmetic creams. These humectants were glycerin, urea and sodium pyroglutamate. Their results showed that the humectant-entrapped liposomes absorb great quantities of water. Oleniacz, in his patent has disclosed the use of liposomes as skin moisturizers (41). Sone *et al.* reported that monoglycerides from vesicles and that the unilamellar vesicles formed by monoglycerides are transformed into multilamellar vesicles with the addition of  $\text{Ca}^{+2}$ , and these vesicles produced a significant increase in the water content of the stratum corneum. However the stability of these vesicles was poor. They increased the stability of vesicles with addition of PVP

(Poly vinylpyrrolidone) to these monoglycerides vesicles. They demonstrated that monoglycerides with a non-lamellar structure did not increase the water content of the human SC (42). It has been shown that the prevention of water loss from the stratum corneum and subsequent increase in water content of the SC apparently enhances the penetration of the drugs in conventional formulations (43).

Various methods have been summarized by Fluhr *et al.* (44) for measuring the hydration state of the SC. Among these tests Corneometer has been used more widely (45). The results obtained from Dykes study (31) clearly showed an increase in capacitance readings is indicative of increased water within the stratum corneum. It was also claimed by Ghyczy and Gareiss that liposomes promote the moisturizing factor of the skin (37).

In brief, the results of study showed that MLV liposomes prepared by fusion method is a good vehicle for OMC as a sunscreen since it provides proper SPF and increase the moisture content of the skin.

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