Enhanced Skin Penetration of Ascorbyl Palmitate Achieved via Liposomal Encapsulation: Tape Stripping Evaluation

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Article

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Abstract: L-ascorbic acid represents one of the most potent antioxidant, photoprotective, anti-aging and anti-pigmentary cosmeceutical agents, with a good safety profile. However, the challenge remained to design the stable topical formulation, with such a permeability of L-ascorbic acid as active that the optimal effects on skin could be achieved. The aim of our research was to evaluate the difference between the penetration ability of ascorbyl palmitate incorporated in the same percentage (2%) within the creams and emulgels as carriers, as well as to determine the impact of its incorporation into liposome on the kinetics of penetration. Tape stripping was applied for studying the penetration of the ascorbyl palmitate into the stratum corneum. In addition, the sensory and textural properties of examined formulations were determined. In both cases (liposomal and non-liposomal formulations), creams exhibited a better penetration profile of active substance compared to the use of emulgel. Encapsulation of ascorbyl palmitate into liposomes led to an increase in the adhesiveness and density of the prepared cream and emulgel samples. The best spreadability and absorption during application were detected in liposomal samples. The obtained results confirmed improved dermal penetration of ascorbyl palmitate, either cream or emulgel bases were employed, which further indicates the advantages of its liposomal encapsulation.

Keywords: ascorbyl palmitate; liposomes; cream; emulgel; tape stripping

1. Introduction

L-ascorbic acid and its derivatives (ascorbyl palmitate, magnesium ascorbyl phosphate, tetraisopalmitoyl ascorbic acid), are and the basic ingredients in anti-ageing products, due to their potent antioxidant activities, photoprotective properties and involvement in collagen biosynthesis [1–6]. By interfering with tyrosinase, L-ascorbic acid can treat hyperpigmentation, melasma and sunspots [7,8]. Also, it was successfully applied for reducing the post-laser resurfacing erythema and decreasing the acne scars [9]. The clinical studies suggested ascorbyl palmitate anti-inflammatory activity and possible beneficial effect in treating some inflammatory dermatoses [8,10].

The topical application of L-ascorbic acid brings different challenges (instability, low skin penetration, easy oxidation, etc.) [11]. Thus, there is a need for the development of more stable

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derivatives and safer new delivery systems for achieving the desired efficiency in different skin conditions [12–15].

Emulsion-based formulations are commonly utilized for the topical administration of active ingredients due to their ability to effectively interact with both lipids and water in the outermost layer of the skin, known as the stratum corneum [16]. Consequently, creams formulated as emulsions, particularly oil-in-water (O/W) creams, have gained significant popularity. They are easily applied, spread smoothly, and impart a light sensation on the skin. These sensory properties are often critical for consumer acceptance, and patient compliance, and significantly influence the sales potential of the products [17].

The emulgel expansion in cosmetics and pharmaceutical preparations is due to their favorable properties such as better loading capacity, selectivity to a specific site, and suitability for medications with short biological half-life and narrow therapeutic window [18,19]. Advantages in terms of spreadability, adhesion, viscosity, and extrusion contribute to their better efficiency. These transparent, attractive, and non-greasy novel vehicles for drug delivery are obtained by conversion of classic emulsions by adding a gelling agent in the water phase [20,21].

In the present work, we focused on amphiphilic L-ascorbic acid derivative, ascorbyl palmitate that compared to L-ascorbic acid has better stability and ability to penetrate the skin [22,23]. Its instability is a result of its oxidative degradation process, catalyzed by metal ions and/or by light, and significantly influenced by its initial concentration [24]. Therefore, an appropriate carrier system, initial concentration and storage conditions are crucial when it comes to stability of ascorbyl palmitate in topical formulations [23].

Nowadays, phospholipids are very attractive and intensively investigated components in dermal products used as emulsifiers, liposome-forming agents, or wetting agents [25]. Liposomes as a small vesicles with unique structure, have a long list of advantages that include biodegradability and nontoxicity, moisturizing and restoring action of the constitutive lipids, the easiness of preparation and continuous supply of active ingredients over a sustained period [26,27]. Not only do they act as “drug transporters” but also as “drug localizers” that can avoid systemic absorption and consequent side effects [15].

Tape stripping is simple, minimally invasive and the most widely used technique for measuring the input kinetics and elimination of the drug from the stratum corneum [28]. In this manner, the influence of the type of formulation on the delivery and concentration of different active entities in the viable epidermis can be determined [29,30].

The aim of our research was to examine dermal penetration efficacy of ascorbyl palmitate from liposomal and nonliposomal creams and emulgels, incorporated in the same percentage (2%), theirs textural characteristics and stability.

2. Materials and Methods

2.1. Materials

Following oil phase components were used for preparation of the creams and emulgels: Caprylic/capric triglycerides (Myritol® 318) from BASF (Ludwigshafen, Germany), olive oil (Paryol 165 OL/R) from A&A FratelliParodi (Campomorone, Italy) and isopropyl myristate from Merck Schuchardt OHG (Hohenbrunn, Germany). MontanovTM82 and MontanovTM14 from Seppic (La Garenne-Colombes, France) were used as emulsifiers. As a gelling agent, hydroxyethylcellulose from Chem Point (Kraków, Poland) was used, while propylene glycol from Fagron (Rotterdam, Netherlands) was used as a humectant and Euxyl PE 9010 (phenoxyethanol (and) ethylhexylglycerin (90:10), from Schulke&Mayr (Germany) as preservative. The active component of tested samples was ascorbyl palmitate (Chemkart, India). For obtaining liposome dispersion, Phosal 40IP (Lipoid, Switzerland) was used. Purified water originated from the Faculty of Medicine (University of Niš, Serbia). L-Ascorbic acid and meta-phosphoric acid (MPA) was purchased from Sigma–Aldrich.

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2.2. Methods

2.2.1. Preparation of Liposome Dispersion

The primary liposome dispersion (Table 1) was made using T18 basic Ultra-Turrax® Homogenizer (IKA Werke, Germany). The final liposome dispersion was made using LiposoFast® LF-50 Avestin extruder. The dispersion was extruded twice through a 0.2 μm poly-carbon filter membrane (Whatman, USA) and later twice through a 0.1 μm poly-carbon filter membrane (Whatman, USA). The liposome dispersion obtained in this way was stored in a refrigerator at a temperature of 2 – 8°C and used to prepare liposomal formulations [31].

Table 1. Qualitative and quantitative compositions (%, (w/w)) of ascorbyl palmitate dispersion.

Ingredients (INCI name)	%, (w/w)
Phosal 40IP	10.00
Ascorbyl palmitate	5.00
Propylene glycol	10.00
Phenoxyethanol (and) Ethylhexylglycerin	1.00
Aqua (Water)	ad 100.00

2.2.2. Preparation of the Creams and Emulgels

For the purposes of this study, two creams and two emulgels (Table 2) were made, one of each type containing liposomal dispersion (Table 1). The creams were prepared by a standard emulsion preparation procedure, while the principle of preparation of emulgels was given in previous [11]. The active substance was added at the same concentration to all formulations, either as a solution of ascorbyl palmitate in isopropyl myristate (cream and emulgel) or as a ascorbyl palmitate liposomal dispersion in Phosal phospholipids (lipocream and lipoemulgel).

Table 2. Qualitative and quantitative compositions (%, (w/w)) of investigated formulations.

Ingredients (INCI name)	LipoEmulgel (LE)	Emulgel (E)	LipoCream (LC)	Cream (C)	Function in the formulation
Oil phase					
Caprylic/capric triglycerides	11.00	11,00	11.00	11.00	Emollient
Isopropyl myristate	7.50	7.50	7.50	7.50	Emollient
Olive oil	3.00	3.00	3.00	3.00	Emollient
Cetearyl alcohol (and) Coco-glucoside	7.00	7.00	7.00	7.00	O/W emulsifier
Myristyl alcohol (and) Myristyl glucoside	1.50	1.50	1.50	1.50	O/W emulsifier
Ascorbylpalmitate	-	2.00	-	2.00	Active substance
Ascorbylpalmitatedispersion	40.00	-	40.00	-	Active substance
Water phase					
Hydroxyethyl cellulose (HEC)	1.00	1.00	-	-	Thickener/ Gelling agent
Propylene glycol	10.00	10.00	10.00	10.00	Humectant
Phenoxyethanol (and) Ethylhexylglycerin	1.00	1.00	1.00	1.00	Preservative
Aqua (Water)	ad 100.00	ad 100.00	ad 100.00	ad 100.00	Solvent

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2.2.3. Physico-Chemical Characterization of the Liposome Dispersion

The size of liposomes in primary liposome dispersion was determined by measuring the turbidity of 0.025% of liposome dispersion diluted with phosphate buffer (pH=7.2) using equation developed by Ohsawa et al. [32].

Analysis of liposome size in final liposome dispersion was carried out by using Zetasizer (Nano series) ZS 90, Malvern Instruments whose working principle is based on Dynamic Light Scattering (DLS) and Photon Correlation Spectroscopy (PCS) [33].

2.2.3.1. Evaluation of Encapsulation Efficiency

The ascorbyl palmitate encapsulation efficiency was determined by using the previously established protamine aggregation method [34]. HPLC method was used to determine ascorbyl palmitate both in supernatant and in the sediment (dissolved in 10 ml MeOH), to make sure that the entire quantity of ascorbyl palmitate used in the formulation (entrapped into liposomes and free form) was detected.

The encapsulation efficacy was calculated as the ratio between ascorbyl palmitate detected in the formulation over the initial concentration used to make the formulation.

2.2.4. Physico-Chemical Characterization of Creams and Emulgels

All formulations were analyzed organoleptically (color, smell, appearance) in order to detect potential aspects of emulsion instability. Afterward, the pH of 10% solution of prepared formulations was measured at room temperature (pH 211 Microprocessor pH Meter, Hanna Instruments, USA). The electrical conductivity of the formulations was measured, by directly immersing the conductometer electrode CDM 230 (Radiometer, Denmark). The same measurements were performed after centrifuge assay (at 3000 rpm for 15 min, at the room temperature) and after three cycles of accelerated aging test conducted on three different temperatures for 24 hours (room temperature, temperature of 5 ± 2°C, temperature of 45 ± 2°C). Finally, a real-time aging stability test was conducted after 1 month of storage of the samples at room temperature.

2.2.5. Texture Profile Analysis (TPA) of Creams and Emulgels

The CT3 Texture Analyzer (Brookfield, AMETEK Inc., USA) was used for performing the texture profile analysis (TPA). The experimental conditions were set as presented in Table 3.

Table 3. The conditions for texture profile analysis (TPA).

Test Speed	2 mm/s
Target Value	2 mm
Trigger load	10g
Probe	Cone probe, TA-STF
Measured parameters	Hardness cycle 1 Hardness cycle 2 Cohesiveness Adhesiveness

The TPA was performed in triplicate.

2.2.6. Examination of Sensory Properties

Twenty volunteers were involved in the sensory study where they were asked to fill in the questionnaire regarding the sensory characteristics of the formulations prior to, during, and after the application. For each sensory characteristic, a list of descriptive terms was provided. A similar questionnaire was used in our previous studies [11,35,36]. All participants were in a well-lit room, where the temperature was set at 21 ± 2 °C and humidity at 45 ± 3%.

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2.2.7. Tape Stripping

Six volunteers (all women, 23 to 30 years old), with no history of dermatological disease, took part in the study. They were asked not to apply or use any products on the left forearm one day prior to the study. The frame with square-shaped holes, whose dimensions were 2.5 cm × 2.5 cm was used to mark the place for applying 0.2 mL of each of the formulations (LE, E, LC, C). Before application of the formulations, transepidermal water loss (TEWL) was measured on each site using Tewameter®TM 300, probe attached to Multi Probe Adapter MPA®9 (Courage&Khazaka Electronic GmbH, Germany). The formulations were applied to four sites while taking care that no preparation was applied 5 cm above the wrist and 1 cm below the elbow. Two hours after the application of the samples to the intended area, by using a laboratory glove, the excess sample that was not absorbed into the skin was wiped with gauze, and tape stripping was conducted. For this procedure, Transpore 3 M adhesive tapes were cut in the square dimensions 2.5 cm x 2.5 cm (Figure 1), carefully adhered to each site, using a roller for providing uniform pressure, and removed. For each site, 16 pieces of adhesive tape were removed and collected. TEWL was measured at each site after 4, after 8, after 12, and after 16 strips with the aim of detecting the removal of stratum corneum. The literature data suggests that an eightfold increase in TEWL indicates that stratum corneum was completely removed [30]. For analyzing the content of ascorbyl palmitate in each strip, HPLC analysis was performed.

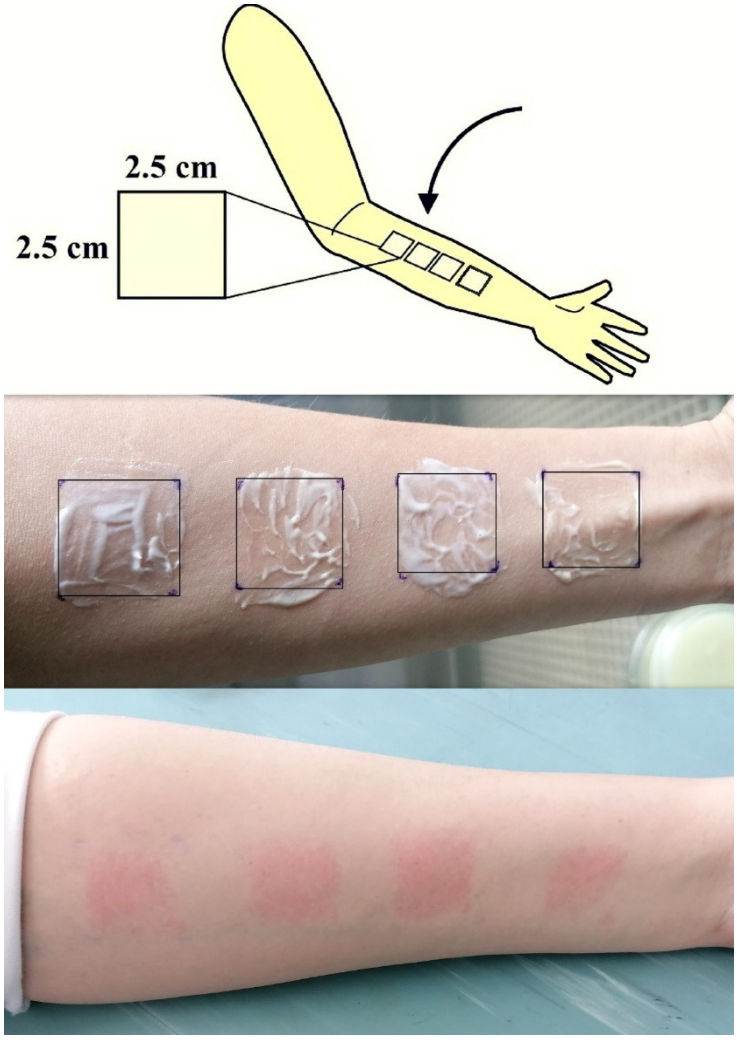

Figure 1. Tape stripping procedure.

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2.2.8. HPLC Analysis

2.2.8.1. The chromatographic Conditions

All analyses were performed on Thermo Scientific (DionexUltiMate 3000) HPLC system with a binary pump and electrochemical detector with the glassy carbon as working electrode. The Acclaim Polar Advantage II C18 column 5µm (150 x 4.6 mm) was used as the stationary phase with 20% m-phosphoric acid adjusted at pH 2.00 as the mobile phase. Instrument control and data acquisition are carried out by the Chromeleon7 Chromatography Data System (Thermo Scientific).

The correlation coefficient of the calibration curve was 0.9994 with a relative standard deviation of 1.4%. The obtained limit of detection was 0.0032 ppm whereas the limit of quantification was 0.0099. The recovery of determination was determined for all matrices and the recovery range was from 93–107%. The quality control mixture used for monitoring instrument performance has a concentration of 10 ppm.

2.2.8.2. Sample Extraction

The test strips were mixed with 2 mL of mobile phase and sonicated in an ice-cold ultrasonic bath at 0°C for 15 minutes. The solution was filtered through a 0.22 µm nylon syringe filter and kept in a freezer until analysis.

2.2.9. Ethical Standards

All participants who were involved in the sensory study and tape stripping were well-informed about the course of the study and signed informed consent. The entire study was conducted in accordance with the Helsinki Declaration, and the guidelines and published recommendations while permission was provided by the Ethics Committee of the Medical Faculty in Niš (Serbia), protocol code 12-11272/2-1 from the 25 September 2023.

2.2.10. Statistical Analysis

The obtained results of the textural analysis for each parameter were presented as mean ± standard error. A statistical analysis was performed using One-way analysis of variance ANOVA with a post-hoc Tukey's test by using IBM SPSS Statistics 20 (IBM Corporation, Japan). Differences at $p < 0.05$ were considered statistically significant. The results of the sensory analysis were presented using an ordinary scale from 1 to 10.

3. Results and Discussion

L-ascorbic acid is present in the skin in relatively low amounts (~41 ng/mg (dry weight) for the entire skin). As the stratum corneum contains only 7 ng/mg (dry weight) of L-ascorbic acid, the topical application is justified in order to increase its cutaneous levels [37]. As a hydrophilic and charged molecule (pKa 4.2), one of the L-ascorbic acid disadvantages is poor skin penetration [38]. Modification of L-ascorbic acid hydroxyl groups has an important influence on its therapeutic properties leading to improvement of antioxidant potential, as well as the antitumor and antiviral activities [39]. The introduction of the lipophilic moieties into the structure of L-ascorbic acid increases the thermal and oxidative stability of obtained derivatives [40], at the same time affecting their mobility and distribution through the phospholipid bilayer membrane [41].

In recent years, there has been intensive research regarding the delivery of active components into different layers of the skin using specific types of liposomes. It has been shown that cellular L-ascorbic acid intake increased significantly when the yeast-based liposomes were used as a carrier system [42,43]. Moreover, according to literature data, the encapsulation of L-ascorbic acid into a lipospheric form resulted in better transport into the deeper layers of the skin [44,45]. Serrano et al., showed in their study that a new ascorbate-phosphatidylcholine liposome formulation as a carrier system improved the topical ascorbic acid treatment of skin [46]. The literature data pointed out that the formulation type affected the release rate of ascorbyl palmitate from topical preparations, while

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several papers dealt with stability studies of L-ascorbic acid when it was encapsulated either into chitosan-coated or pectin-coated liposomes [47–49]. In addition, it was demonstrated that the liposomal formulations for topical application significantly increased the rate and extent of L-ascorbic acid ester absorption into the epidermis [50].

In our paper, we examined four types of formulations (Table 2) with incorporated ascorbyl palmitate: cream (C), emulgel (E), lipocream (LC) and lipoemulgel (LE). In LC and LE, ascorbyl palmitate was primarily incorporated in liposome dispersion. The assessment of physico-chemical properties of tested samples was presented in Table 4.

Table 4. pH, electrical conductivity (μS/cm) values and organoleptic properties of LE, E, LC and C samples before and after **c**entrifuge assay, as well as after accelerated and long-term stability test.

	pH			
	Before centrifuge assay	**After centrifuge assay**	**After accelerated stability test**	**After 30 days ((21 ± 2°C)**
LE	4.50	4.55	4.51	4.53
E	4.66	4.65	4.59	4.56
LC	4.39	4.31	4.35	4.35
C	4.90	4.93	4.91	4.87
	Electrical conductivity (μS/cm)			
	Before **c**entrifuge assay	After **c**entrifuge assay	After accelerated stability test	After 30 days ((21 ± 2°C)
LE	50.20	51.10	51.44	51.41
E	52.90	50.40	52.95	52.47
LC	39.60	37.80	40.11	39.87
C	59.10	57.10	56.15	58.65
	Organoleptic properties (color, smell, appearance)			
LE	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy
E	white, no odor, glossy	white, no odor, glossy	white, no odor, glossy	white, no odor, glossy
LC	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy
C	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy	yellowish-white, no odor, glossy

The emulgels and creams were shown to be stable according to the results of analyzes before and after centrifugation.

The diameter of particles of primary liposome dispersion calculated spectrophotometrically was 0.863 μm, while the diameter of particles of final liposome dispersion was 0.783 μm. The pH of the final liposome dispersion was 4.13 and the zeta potential value was -63.67 ± 0.81 mV, while the polydispersity index (PdI) was 0.67±0.01. If a PdI value is greater than 0.3, it indicates that diameters of liposomes are within wide range [51]. Zeta potential (surface charge) is an important parameter that determines the stability of liposomal dispersion. Particles with a zeta potential of greater value than +30 mV and less than -30 mV are considered stable [52]. According to the obtained zeta potential value, the prepared liposomal dispersion was stable. Hence, in spite of the wide range of particles size, our result was in the line with our goal to encapsulate ascorbyl palmitate and form stable liposomes. The entrapment efficiency of ascorbyl palmitate into formulated liposomes was also evaluated. The average (of three measurements) entrapment efficiency of ascorbyl palmitate-loaded liposomes was 92.02%, indicated the successful encapsulation of active substance into liposomes.

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In the present study, we provided new evidence on topical bioavailability of novel ascorbyl palmitate liposomes in the form of emulgel and cream. Tape stripping, as a minimally invasive technique, was applied to investigate in vivo skin permeation of examined formulations (LE, E, LC, and C) containing ascorbyl palmitate. This method provided the evaluation of the penetration profile and quantification of the amount of ascorbyl palmitate accumulated in the stratum corneum.

The overall percentages of ascorbyl palmitate extracted from all 16 tapes, compared to the starting amount of ascorbyl palmitate incorporated into the investigated formulations were presented in Figure 2.

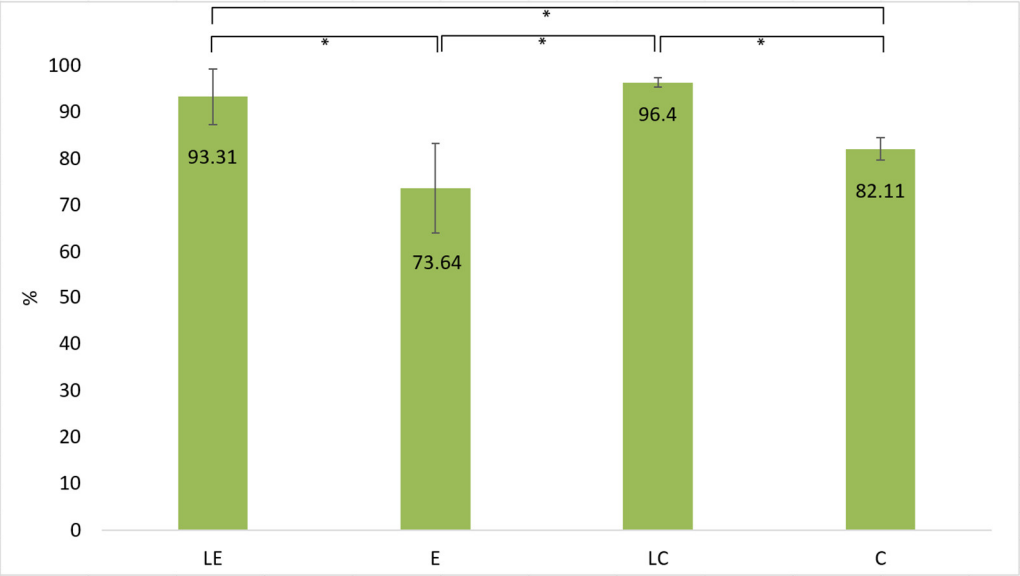

Figure 2. Total amount percentage of ascorbyl palmitate recovered in the *stratum corneum* for formulations LE, E, LC and C after 2h.Significant differences are marked with * ($p < 0.05$).

The penetration profiles of ascorbyl palmitate through human stratum corneum from lipoemulgel (LE), emulgel (E), lipocream (LC), and cream (C) were shown in Figure 3. The results represented the total extracted ascorbyl palmitate after each strip underwent the quantification by HPLC method, expressed as a percentage in relation to the total amount of incorporated ascorbyl palmitate in different types of formulations – LE, E, LC and C.

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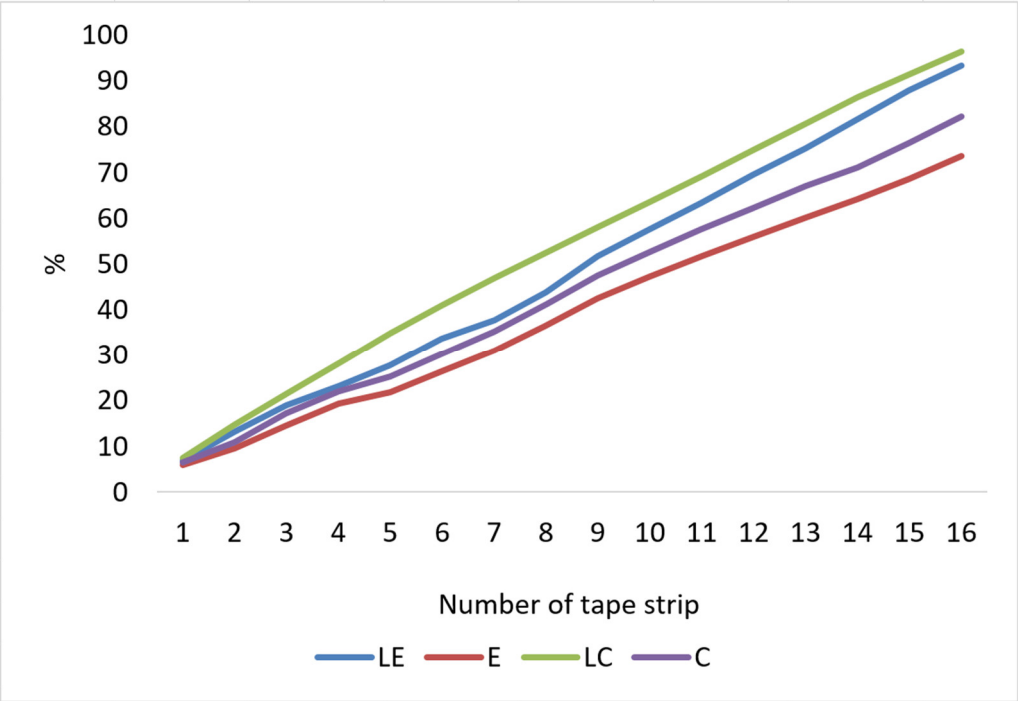

Figure 3. Penetration profiles through human *stratum corneum* of ascorbyl palmitate formulated in LE, E, LC and C (cumulative amount of ascorbyl palmitate (%) in relation to the amount applied, at every application site).

The results presented at Figures 2 and 3 pointed out that the highest amount of ascorbyl palmitate was extracted from tape strips in the sites where formulations with liposome dispersions were applied. A statistical comparison between the presented data revealed that there was a significant increase in the level of ascorbyl palmitate in the stratum corneum after LE (93.31%) and LC (96.4%) were applied in comparison to both E (73.64%) and C (82.11%). The recovered amount of ascorbyl palmitate from LE and LC application sites after 120 min was significantly higher when compared with E and C application sites. The results showed that although there was an increase in the amount of penetrated L-ascorbyl palmitate in cream samples compared to emulgel samples (C compared to E, and LC compared to LE), this increase was not statistically significant. Therefore, it could be concluded that the incorporation into liposomes led to statistically significant difference in the amount of penetrated active substance, while the type of carrier itself did not cause the statistically significant changes.

The obtained results clearly indicated that encapsulation of ascorbyl palmitate promoted its penetration through the stratum corneum. The ability of liposomes to enhance the delivery of active ingredients from topical formulations has been attributed to their specific structure. Liposomes represent concentric bilayer vesicles that can fuse with other bilayers (cell membrane), releasing the content in this way [27]. Also, it is important to notify that the liposomes are loaded with active agent both inside and outside of their phospholipid membrane [46]. The presented results were in accordance with the data from the literature that topical application of liposomal formulations, led to a significant increase in the rate and extent of drug absorption [50]. According to previous research, the hydrophobic liposome structure is responsible for the interaction with corneocytes to the extent that seems to be highly dependent on the lipid composition of liposomes. This interaction between lipid vesicles and skin is also important to improve access to the epidermis of encapsulated active substances [53]. Contreras et al. have also studied hydroalcoholic gels that contain all-trans retinoic acid in free form or encapsulated in stratum corneum lipid liposomes, using the tape stripping method to establish the accumulation of the active substances in the surface and skin layers [54]. This method contributed to the overall conclusion that encapsulation of retinoic acid not only prolonged drug release but also promoted drug retention by the viable skin. Doi et al. investigated a serial tape

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stripping technique to detect the content of the 3-O-cetyl ascorbic acid, one of the lipophilic L-ascorbic acid derivatives, in the stratum corneum, uptaken from examined cream [55].

During the tape stripping procedure, TEWL measurements were carried out before the application and detachment of the first adhesive tape, and then after 4, 8, 12 and 16 adhesive tapes were applied and removed successively from the same treated skin area. The purpose of the TEWL measuring was to detect the removal of stratum corneum. The literature data suggests that an eightfold increase in TEWL indicates that stratum corneum was completely removed [30]. Based on the difference between basal TEWL values and measured values after 16 tape strips, it can be considered that stratum corneum was removed completely from the application sites during our study (Figure 4).

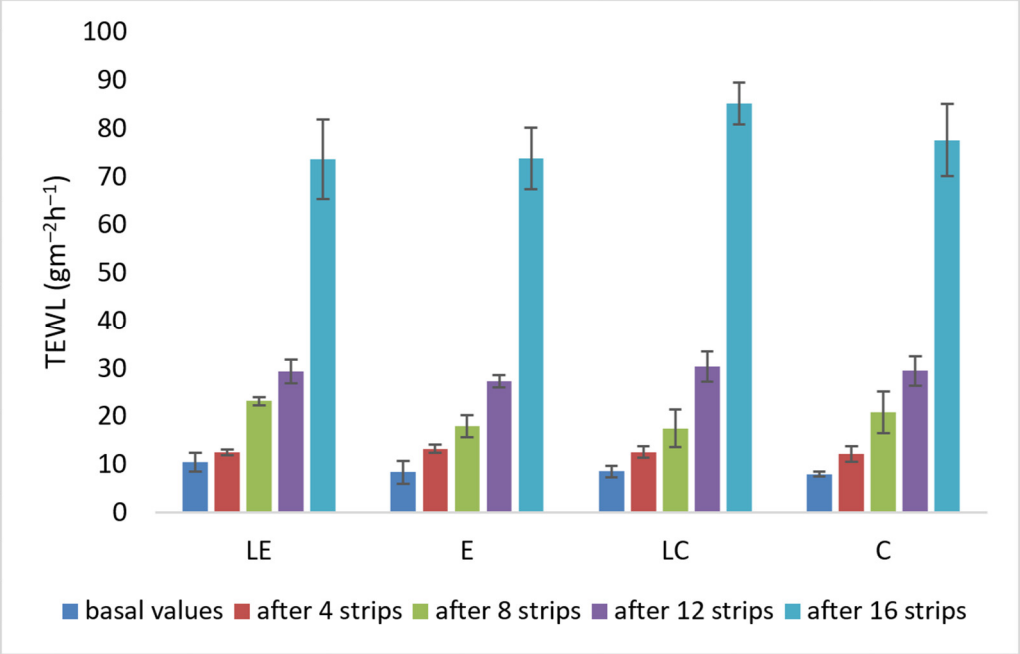

Figure 4. TEWL changes depending on the number of strips removed.

After 12 tape strips in a uniform manner, more than 70% of the applied ascorbyl palmitate was found on the adhesive tapes from liposomal formulations (LE and LC), and about 60% from emulgels and creams that do not contain encapsulated L-ascorbic acid in liposome dispersion (E and C) (Figure 5). The results presented at Figure 5 pointed out that the penetration of ascorbyl palmitate had different kinetics depending on the type of formulation. The increase of TEWL corresponded to the depth of the penetration in the epidermis.

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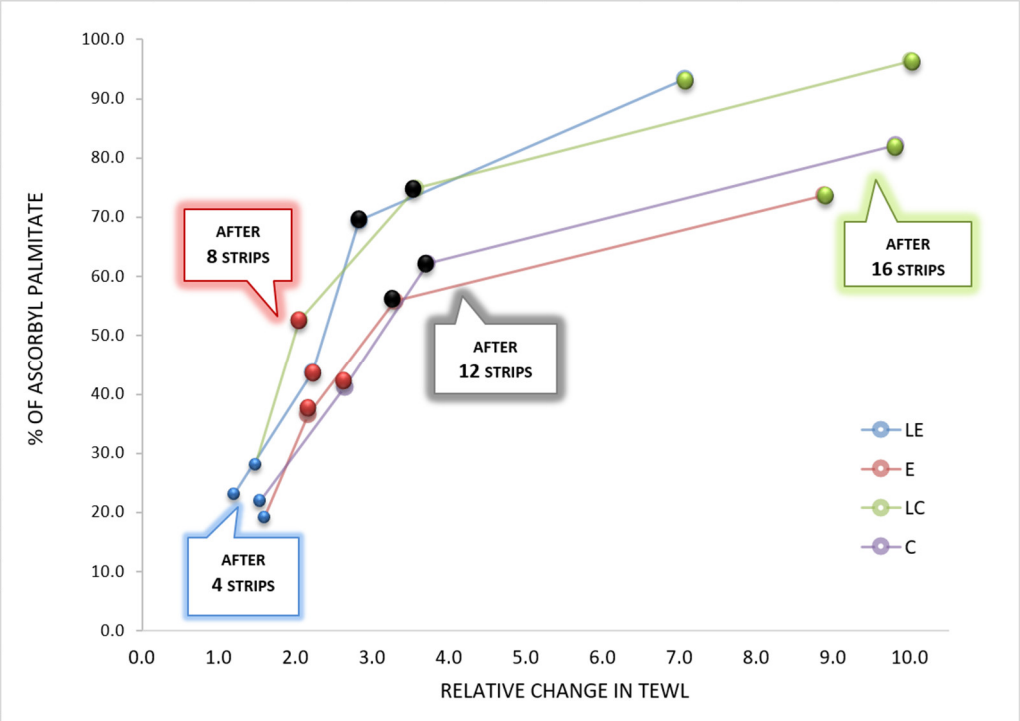

Figure 5. Percentage of ascorbyl palmitate in the strip of the total amount of ascorbyl palmitate applied with each formulation (LE, E, LC, C) compared to the relative changes in TEWL after 4, 8, 12,and 16 tape strips were removed from the skin.

Texture and sensory analysis is an important weapon in the hands of formulators in pharmaceutical and cosmetic industry. As indicators of mechanical and applicative properties of formulations, texture analysis serves as an objective method, while sensory analysis offers subjective insight. Together, they offer information that is important for both activity of the formulations and the compliance of the consumers of the drug/cosmetics [56].

The results of the TPA (Table 5) pointed out that the presence of liposome dispersion did not influence the adhesiveness of the emulgel. However, that was not the case with the cream. LC was the formulation with the highest adhesiveness – the stickiness of the product to other surfaces. On the other hand, the LC had the lowest cohesiveness of all. It indicates the strength of the internal bonds of the products. The results have shown that the presence of dispersion caused the lowering of the cohesiveness since E and C had the greatest values of cohesiveness. The Hardness describes the force required to rub a product between fingers and is inversely proportional to the spreadability of the preparation. The highest Hardness values were recorded for the LE sample, while the lowest values were for the sample C. The values of Hardness cycle 2 followed the values of the first cycle. The lack of significant difference between Hardness Cycle 1 and Hardness Cycle 2 values indicated that the structure of the preparation did not weaken after the first compression cycle.

Table 5. Results of the Texture Profile Analysis of the formulations LE, E, LC and C.

	Adhesiveness (mJ)	Cohesiveness	Hardness Cycle 1 (g)	Hardness Cycle 2 (g)
LE	0.43±0.06	1.54±0.18	27.67±3.79	25.67±4.04
E	0.43±0.06	1.78±0.15	25.33±1.53	24.33±1.53
LC	0.50±0.20	1.48±0.28	25.67±4.51	24.33±5.03
C	0.33±0.06	1.74±0.24	23.33±2.52	22.00±2.65

The results of the sensory analysis were in accordance with the results of the texture analysis regarding consistency (Table 6). The sample LE was characterized with the highest consistency which

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was expected based on the obtained results for the parameter Hardness. The participants marked LE as the sample with the highest density during application exhibiting the slowest absorption rate (Figure 6) and retaining the most expressed residual film (Figure 7). In addition, the participants marked sample LE as the one with the best spreadability, followed by LC and C, while E was characterized as the most difficult to spread.

Table 6. Results of the Sensor Analysis of the formulations LE, E, LC, and C before the application.

	Before Application			
	LE	**E**	**LC**	**C**
Consistency	10.00	9.71	9.71	9.71
Gloss level	6.95	6.12	6.59	6.12

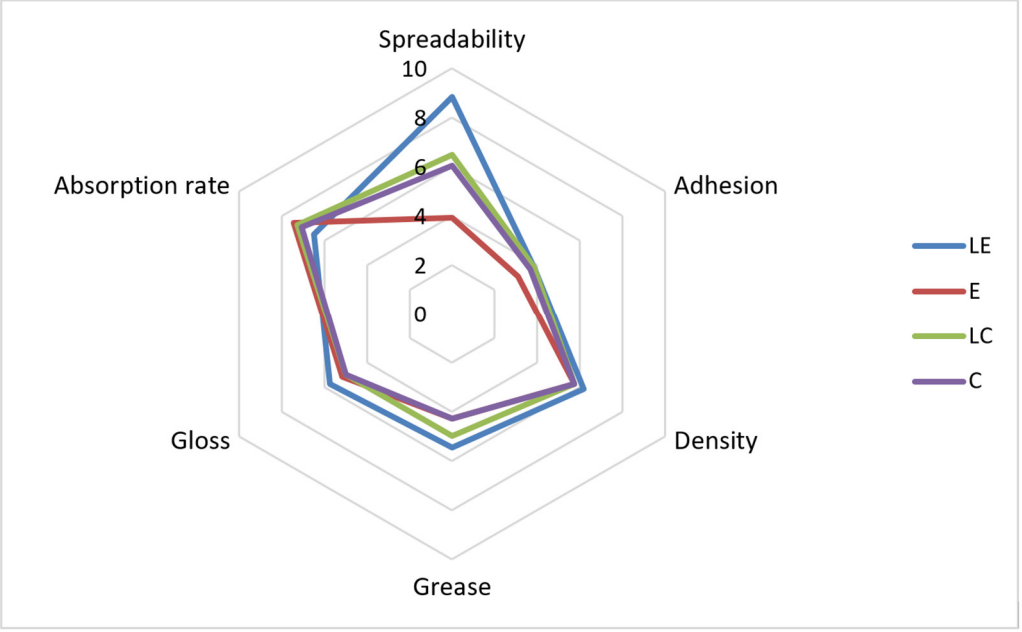

Figure 6. The results of sensory analysis of the investigated samples during application (L, LE, LC, C).

Figure 7. The results of sensory analysis of the investigated samples after application (L, LE, LC, C).

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Organoleptic properties of formulations summarized according to the results of sensory analysis favored the sample LE, since LE was described as the one with the highest gloss level prior to application and the one leaving the most gloss on the skin during the application.

Concerning the sample LC, as another formulation with liposome dispersion, the texture analysis indicated that of all, the LC has the closest textural characteristics to LE. The same observation was made based on the sensorial analysis.

By comparing the results of tape stripping, texture, and sensory analysis (Table 7), it was assumed that the penetration of active substance – ascorbyl palmitate in the skin from the formulation LE, which was over 90% (93.31%) represented the consequence of its textural characteristics, being the best consistency and spreadability, accompanied with the most prominent residual film among tested formulations. The tape stripping results revealed that the amount of penetrated ascorbyl palmitate was even higher for LC (96.4%) (Figure 2). However, by comparing the TEWL increase in relation to the initial values after 16 strips were detached from the sites where formulations LC and LE were applied, the results pointed out the values for TEWL being 10.01 times compared to 7.07 times, respectively (Figure 5). This indicated that less amount of stratum corneum was removed from the site where formulation LE was applied, explaining why the total amount of penetrated ascorbyl palmitate was lower for formulation LE in comparison to the LC.

Table 7. Comparison of the characteristics of the samples LE, E, LC, C. The sample with the most pronounced characteristic is marked with "+".

		LE	**E**	**LC**	**C**
Physico-chemical characteristics)	Organoleptic properties	Acceptable	Acceptable	Acceptable	Acceptable
	pH	Within the range suggested for topical preparations	Within the range suggested for topical preparations	Within the range suggested for topical preparations	Within the range suggested for topical preparations
Tape stripping	Total percentage of penetrated ascorbylpalmitate	>90%	<90%	>90%	<90%
				+	
Sensory analysis	Consistency	+			
	Gloss	+			
	Spreadability	+			
	Residual film	+			
	Fast absorption		+	+	+
	Slow absorption	+			
	The least sticky		+		
	The least greasy feeling on the skin		+		+
Texture analysis	Hardness	+			
	Consistency	+			
	Cohesivenes s		+		+

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	Adhesiveness			+	
	Spreadability				+
	Deformity after pressure	Stable structure	Stable structure	Stable structure	Stable structure

4. Conclusions

By examining dermal penetration of liposomal and non-liposomal creams and emulgels with 2% ascorbyl palmitate using the tape stripping method and their sensory and applicative characteristics, the superiority of liposomal compared to non-liposomal samples was shown. Namely, liposomal encapsulation increased the dermal penetration of ascorbyl palmitate when both emulgel LE (93.31%) and cream LC (96.4%) were used as a base in comparison when conventional emulgel E (73.64%) and cream C (82.11%) were employed. At the same time, it was shown that use of cream as a base led to a better penetration profile compared to the use of emulgel (in the case of both liposomal and non-liposomal formulations).

Comparing the non-liposomal and liposomal formulations (cream and emulgel), liposomal cream and emulgel had good skin applicability (primarily spreadability) and sensory properties (the film formed on the surface of the skin creates conditions for a good moisturizing effect), while the non-liposomal formulation had a weaker ability to penetrate, worse spreadability and did not leave a film on the surface of the skin (which can potentially reduce TEWL).

According to obtained results, the liposomal emulgel exhibited the most efficient penetration kinetics through the *stratum corneum*.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are contained in the article

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