Enhanced skin targeting of retinoic acid spanlastics: in vitro characterization and clinical evaluation in acne patients

Rehab Nabil Shamma, Sinar Sayed, Nirmeen Ahmed Sabry & Solwan Ibrahim El-Samanoudy


To link to this article: https://doi.org/10.1080/08982104.2018.1552706

Accepted author version posted online: 02 Dec 2018.
Published online: 05 Feb 2019.
Enhanced skin targeting of retinoic acid spanlastics: in vitro characterization and clinical evaluation in acne patients

Rehab Nabil Shammaa, Sinar Sayeda, Nirmeen Ahmed Sabryb and Solwan Ibrahim El-Samanoudyc

ABSTRACT
Acne vulgaris is the most common dermatological disorder affecting millions of individuals. Acne therapeutic solutions include topical treatment with retinoic acid (RA) which showed a good efficacy in treatment of mild and moderate cases. However, the high prevalence of adverse events, such as skin dryness, shedding and skin irritation affects the patient convenience and obstruct the acne treatment. Thus, the objective of this paper was to produce Span 60 based elastic vesicles enriched with penetration enhancers, and study their influence on the delivery of RA and its skin irritation. RA-loaded nanovesicles, enriched with Transcutol®/Labrasol®, were made using the thin film hydration technique, and assessed for entrapment efficiency, particle size and zeta potential. The optimized RA-loaded nanovesicles (composed of Span 60-Tween 20, and Transcutol®) were morphologically assessed via transmission electron microscopy. Moreover, RA deposition into newborn mice skin was assessed in vitro under non-occlusive conditions, where the optimized RA-loaded nanovesicles showed 2-fold higher RA deposition in the skin compared to the corresponding one lacking Transcutol. The optimized RA-loaded nanovesicles incorporated into 1% carbopol gel was evaluated for in-vivo clinical performance in acne patients, and showed appreciable advantages over the marketed formulation (Acretin®) in the treatment of acne regarding skin tolerability and patient’s compliance.

Introduction
Acne vulgaris is one of the most common dermatological disorder rarely causing a serious threat, however it may cause a decrease in overall performance of millions (Draelos et al. 2007). The prevalence of acne in adolescents can reach up to 80%, and it produces clinical conditions that can continue into adulthood, negatively impacting the quality of life (White 1998). According to the degree of acne severity, acne can be divided into different stages: mild, moderate, and severe acne (Gollnick et al. 2003). Therapeutic treatment selection is based on the type of acne and its degree of severity (Zaenglein et al. 2016). In case of mild and moderate acne, the first line of treatment is using topical treatment, while systemic therapy is used to treat severe cases (Stein and Lebwohl 2001).

Retinoids, natural or synthetic derivatives of vitamin A, are greatly effective in Acne vulgaris treatment, as they can modify abnormal follicular keratinization (Berger et al. 2007). Retinoids exhibit anti-inflammatory, keratolytic, and antiseborrheic effects by altering specific structures like inflammation mediators, keratinocytes and sebocytes (Zouboulis 2001). All trans retinoic acid (RA) has been proven effective in mild to moderate acne treatment (Rathi 2011). Nevertheless, some of these agents usually produce a high incidence of side effects, such as skin dryness, peeling and skin irritation or bacterial resistance (Vyas et al. 2014), thus, affect the patient compliance and obstruct the acne treatment.

Skin is considered an ideal route of drug administration; however, cutaneous drug delivery route may be limited due to poor permeation due to the stratum corneum which joiners absorption. Skin represents an ideal route of drug administration. However cutaneous drug delivery is limited by the poor penetration of drugs through the stratum corneum barrier (Abd et al. 2016). To overcome this problem, vesicular systems have been much tested as carriers for skin delivery of drugs in order to increase skin permeation of drugs (Desai et al. 2010). Several studies investigated the ability of colloidal carriers as cutaneous drug delivery routes in order to achieve better permeation (Souto and Muller 2006), such as transfersomes and spanlastics (Elsherif et al. 2017). PEVs also known as Penetration enhancer-containing vesicles are vesicles produced from phospholipids and penetration enhancers such as oleic acid, Labrasol® and diethylene glycol monomethyl ether (Transcutol®) or propylene glycol. The permeation enhancer offers a dual function in transdermal/cutaneous delivery of drugs; it increases vesicular bilayer fluidity and also reduces the SC barrier function temporarily (Dragicevic and Maibach 2017). Surfactant-based
nanovesicular carriers consisted of Span 60 as a non-ionic surfactant together with an edge activator have recently been developed with the aim of for increasing the transdermal/cutaneous permeation of drugs is (Kakkar and Kaur 2011, Basha et al. 2013). These elastic vesicles were especially able to squeeze themselves through the stratum corneum (Zaenglein et al. 2016), thus enhance transdermal/cutaneous drug delivery and improve the ex vivo cutaneous drug deposition compared to the conventional vesicles (Bsieso et al. 2015).

The objective of this paper was to assess the ability of the different penetration enhancers to produce Span 60-based elastic vesicles and to research the influence of the PEVs on cutaneous delivery of RA and on its skin irritation effect. The potential of using the PEVs or elastic vesicles in the clinical treatment of acne in human volunteers were trialled in the current work.

Materials

RA was supplied by ChemFine International Co. Ltd. (China); Span 60, and Tween 20 were acquired from Acros Organics, Fair Lawn, NJ. Triethanolamine and ethanol were purchased from ADWIC, Egypt. Carbopol 934 was acquired from Goodrich Chemical Company (Charlotte, NC). Labrasol® (caprylocaproyl macrogol 8-glycerides) and Transcutol® P (2–2-ethoxyethoxy ethanol) were kindly offered by Gattefosse Co. (France). Acretin 0.05% TRT was purchased from Jamjoom Pharma, Saudi Arabia. Methanol and glacial acetic acid HPLC grade were acquired from Sigma Aldrich Chemical Co. (St. Louis, USA). All other materials were of analytical grade and used as received without any further refining.

Methods

Preparation of retinoic acid (RA) nanovesicular carriers
RA loaded nanovesicles were fabricated by thin film hydration technique. Briefly, precise weight of RA is added to a blend of Span® 60 and Tween® 20 in three different ratios and dissolved in 10 ml ethanol in a round-bottom (1000 ml) flask immersed in water bath at 60°C. The solvent was slowly vaporized at 60°C under negative pressure using a rotary evaporator (Buchi R-110 Rotavapor, Flawil, Switzerland), turning at 120 rpm till a thin dry film was obtained inside the flask. In order to ensure complete hydration of the film, the deposited dried film was hydrated with distilled water (~9 ml) and the film was kept to rotate for 30 min at a constant hydration temperature of 60°C under normal pressure. The final volume was completed into 10 ml at room temperature (25°C) to attain nanovesicular dispersions. The acquire dispersion was kept at refrigerator overnight at 4°C for growth.

In an attempt to study the influence of using skin penetration enhancer namely, Labrasol® and Transcutol®, four PEVs of RA and the selected formulation ratio were prepared by the same previously mentioned thin film hydration technique. Table 1 shows the composition of RA nanovesicles.

Characterization of RA-loaded nanovesicles

Mean particle size, polydispersity index (PI) and zeta potential (ZP)
The average particle size (PS), polydispersity index (PI) and zeta potential (ZP) were detected by dynamic light scattering using Nano ZS Zetasizer at an angle of 90° in 10 mm diameter cells at 25°C (Malvern Instrument Ltd., Worcestershire, United Kingdom). Prior to PS measurement, all designs were suitably diluted with double distilled water to have a proper scattering intensity. The PDI was taken as a sign for width of the nanovesicles size distribution. A tiny value of PI declares a homogenous size scattering, whilst a large PI reveals a higher heterogeneity. Three size measurements for each sample was determined and the mean ± SD was calculated.

Using the same instrument, the ZP of the charged nanovesicles was carried out by measuring particles electrophoretic mobility in an electrical field. The ZP measurements were determined in double distilled water kept conductivity to 50 mS/cm using sodium chloride solution (0.9%, w/v). Average ZP for three replicates for each formulation were deduced.

Determination of RA entrapment efficiency (EE)
To detect the amount of encapsulated RA in the formed nanovesicles, the RA-loaded nanovesicles were separated from the free drug by ultra-centrifugation at 18 000 rpm and temperature 4°C for 1 h (Beckman cooling centrifuge, Fullerton, Canada). The loaded nanovesicles were detached from the supernatant, disrupted and sonicated for 15 min in ethanol so as to achieve a clear solution. The amount of entrapped RA was detected by assessing the concentration of RA spectrophotometrically at the predetermined λmax of 346 nm in ethanol (Shimadzu UV spectrophotometer, 2401/PC, Japan). The RA EE % was repeated three times.

### Table 1. Composition and evaluation results of RA-loaded nanovesicles.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Span® 60 (mg)</th>
<th>Tween® 20 (mg)</th>
<th>Labrasol® (mg)</th>
<th>Transcutol®</th>
<th>EE (%)</th>
<th>PS (nm)</th>
<th>PDI</th>
<th>ZP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>100</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>76.7 ± 1.72</td>
<td>120.6 ± 41.8</td>
<td>0.769 ± 0.07</td>
<td>–26.7 ± 0.40</td>
</tr>
<tr>
<td>F2</td>
<td>140</td>
<td>60</td>
<td>–</td>
<td>–</td>
<td>83.62 ± 3.08</td>
<td>688.4 ± 184.8</td>
<td>0.637 ± 0.08</td>
<td>–30.6 ± 2.12</td>
</tr>
<tr>
<td>F3</td>
<td>180</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>89.74 ± 0.07</td>
<td>1486.6 ± 188.1</td>
<td>0.906 ± 0.06</td>
<td>–38.12 ± 2.12</td>
</tr>
<tr>
<td>F2-L25</td>
<td>140</td>
<td>60</td>
<td>25</td>
<td>–</td>
<td>86.9 ± 0.70</td>
<td>406.95 ± 12.1</td>
<td>0.139 ± 0.19</td>
<td>–33.7 ± 0.28</td>
</tr>
<tr>
<td>F2-L50</td>
<td>140</td>
<td>60</td>
<td>50</td>
<td>–</td>
<td>80.75 ± 0.91</td>
<td>203.75 ± 33.25</td>
<td>1.00 ± 0.00</td>
<td>–36.8 ± 2.33</td>
</tr>
<tr>
<td>F2-T25</td>
<td>140</td>
<td>60</td>
<td>–</td>
<td>25</td>
<td>82.9 ± 0.84</td>
<td>378.2 ± 8.2</td>
<td>0.826 ± 0.07</td>
<td>–31.2 ± 0.84</td>
</tr>
<tr>
<td>F2-T50</td>
<td>140</td>
<td>60</td>
<td>–</td>
<td>50</td>
<td>82.7 ± 0.70</td>
<td>429.8 ± 37.0</td>
<td>0.669 ± 0.17</td>
<td>–31.0 ± 0.96</td>
</tr>
</tbody>
</table>

All formulations contained RA (0.5 mg/mL).
times and the mean ± SD was determined from the following equation:

\[
EE\% = \frac{\text{amount RA entrapped}}{\text{total amount of RA}} \times 100
\]

**Morphology**

The optimized RA-loaded nanovesicular formulation was pronounced using transmission electron microscopy (TEM) at an acceleration voltage of 80 kV (model JEM-1230, Jeol, Tokyo, Japan). Where one drop of the diluted nanovesicular dispersion was dehydrated on the surface of a carbon coated copper grid and then stained with 2% phosphotungstic acid then permitted to dehydrated at room temperature for 10 min for TEM inspection.

**Differential scanning calorimetry (DSC)**

The thermograms of RA pure powder, Span 60, Caropol 934, and the optimized lyophilized RA-loaded nanovesicular formulation were identified using Shimadzu differential scanning calorimeter (DSC-50, Kyoto, Japan). Nearly 2 mg of each specimen were exposed to heat in standard aluminum pots in a temperature range of 10 to 300°C at a constant heating rate of 10°C/min under inert nitrogen flow of 25 ml/min.

**Powder X-ray diffraction (XRD)**

Diffraction patterns of RA pure powder, Span 60, Caropol 934, and optimized lyophilized RA-loaded nanovesicular formulation were obtained in a Scintag X-ray diffractometer (USA) with Cu- radiation via a nickel filter working at 45 kV and 40 mA with scanning speed of 0.02°/sec. The reflection peaks between 2θ = 2° and 60°, the relevant spacing (d, Å) and the related intensities (I/I0) were determined.

**Formulation of RA-PEV hydrogel**

A selected RA-loaded nanovesicular formulation was incorporated into 1% (w/w) carbopol 934 hydrogel using magnetic stirring at 800 rpm till complete dispersion. Triethanolamine solution was used in order to neutralize the nanovesicular dispersions.

**Skin deposition study**

Permeation of RA through hairless skin of freshly born mice were utilized. Each formulation was prepared in triplicate. Prior to experiment the whole skin was soaked in phosphate buffer saline (PBS 7.4) for 24 h before the experiment. The donor compartment was filled with 1 g of RA-loaded nanovesicular formulation containing Transcutol (F2-T25), RA-loaded nanovesicular formulation without Transcutol (F2) or market product containing 0.05% RA. The tissue surface area available for diffusion was 7.065 cm². The receptor compartment was filled with the PBS maintained at 37 ± 0.5°C, and constantly stirred at 300 rpm for 24 h. At the end of the study, skin was removed and washed for 10 s. with distilled water to eradicate any adsorbed drug then divided into tiny segments and sonicated for 30 min in 5 ml methanol to liberate any deposited drug. The segments were centrifuged and the clear supernatant was filtered (0.45 μm membrane filter) and HPLC analyzed using a validated HPLC method using methanol: water: glacial acetic acid (85:15:0.5 V:V:V) as mobile phase at 1.5 ml/min using a reversed-phase column (Nucleosil C18, PS, 10 ml, 250 mm 4.6 mm, Germany) with an ultraviolet detector (Model SPD-10 A; Shimadzu) with a detection wavelength adjusted at 353 nm. All analysis was accomplished at ambient conditions.

**Skin irritation test**

In this section, the study protocol and the clinical evaluation section were authorized by the institutional review board of Faculty of Pharmacy, Cairo University (PT 1998). This study was performed according to the Declaration of Helsinki (Declaration of Helsinki–Current, 2013). Fifteen healthy subjects (aging between 15 and 33 years) contributing in this study. The study procedures were thoroughly explained to the participating subjects and a written informed consent was acquired from all contributors earlier to the beginning of the study procedure. Each participant received once application of the tested formulation (the optimized formulation F2-T25, and the market product, Acretin®) on a surface area of 5 cm² on forearm at a dose of 0.3 g. The test specimen was removed by washing with tap water after 6 h, and examined for any observable change such as erythema. The mean erythema scores were identified (ranging from 0 to 4), where 0 means no erythema while four represents severe erythema.

**Clinical evaluation of the selected formulae**

**Study subjects**

The study was operated in the dermatology outpatient clinic in Kasr Al-Aini Teaching Hospital, Cairo, Egypt. The subject informed consent and study protocol for this section and the clinical evaluation section were authorized by the institutional review board of Faculty of Pharmacy, Cairo University (PT1998). A total of 15 clinically confirmed acne patients were selected for study inclusion. A signed informed consent about the purpose of the study was obtained from each participating subject and also approval be photographed before and after treatment. Subjects were included/excluded from participating in the study according to the following criteria:

**Inclusion criteria**

Subjects participating in this study were adult patients (15–33 years old) diagnosed with acne. Two weeks prior to the study, the use of any acne products was prohibited, and throughout the entire study period.

**Exclusion criteria**

Subjects administering any acne treatment such as oral antibiotics, benzoyl peroxide, or oral retinoids, and those using
contraceptives (either oral, or implantable) were excluded from the study. Moreover, subjects with diabetes mellitus, or any endocrine disease, or physical illnesses were also excluded from the study. In addition, systemic medications known to alter (either by flaring or by healing it up) such as oral phenytoin, flutamide, testosterone, finasteride, spironolactone, and corticosteroid intake via the topical, systemic, inhaled, or intraocular route were prevented for at least 4 weeks earlier to the study.

### Study design

In this study design, each subject acted as his/her own control. The face is shared into two areas; one side for applying of the optimized formulation (F2-25T) once daily while the other side for the usage of marketed RA product (Acretin 0.05%) once daily. During the baseline evaluation visit (Day 0), each patient was instructed how to spread on the formulae above the affected areas on the selected face side. Every patient was given two identical tubes one for each formula and one of them was labelled “to be applied on the right side of the face” and the other has the labelled with “to be applied on the left side of the face” in Arabic.

During a period of 4 weeks, the safety, tolerability, and the efficiency of the applied medications were evaluated by examining the patients at the follow-up visit. Subjects were questioned concerning any sign/symptom of peeling, dryness, erythema, burning sensation, or pruritus using uniform structured questions at each visit. Lesions counting, was carried out by a blinded and trained dermatologist in order to assess the efficacy of the applied medication. The total lesion count before treatment beginning was considered as 100% and any reduction in the count of lesions was determined and considered as percentage reduction. An enhancement was documented when the count of papules, open and closed comedons was reduced at the follow-up visit. Every week, the mean percentage reduction and standard deviation were recorded for each group of subjects and were analysed statistically for each type of lesions and for the total lesion count.

### Statistical analysis

Results of EE%, PS, ZP of the prepared RA-loaded nanovesicles are showed as the means of three replicates ± SD and statistical analysis was accomplished by means of the one-way analysis of variance (ANOVA). A value of $p < 0.05$ was reflected statistically significant.

Results of the clinical assessment were statistically evaluated, and the significant change between percent change in the total count of lesions after treatment with the marketed product and the optimized formulation (F2-T25) were established by using student t-test at $p < 0.05$. SPSS version 22 (SPSS Inc., Chicago, USA) was used for data analysis.

### Results and discussion

#### Vesicle formation and characterization

In this work, new type of nanovesicles enriched with different penetration enhancers together with Span® 60 and Tween® 20 as the chief constituents of the vesicular bilayer. These novel vesicular systems were prepared and evaluated for the capability of the permeation enhancers to increase the cutaneous delivery of RA. To this objective, two hydrophilic penetration enhancers which are commonly used in topical formulations, Labrasol®, and Transcutol®, were used in the structure of the new nanovesicular systems.

Labrasol® is a nontoxic, nonionic hydrophilic surfactant (HLB = 14) fabricated by Gattefossé Corp. (Saint-Priest, France) (Koga et al. 2006, Manconi et al. 2011). Transcutol® (diethylene glycol monoethyl ether) is a nontoxic, biocompatible with skin, penetration enhancer that works via the swelling of stratum corneum intercellular lipids without modifying their numerous bilayer structure (Godwin et al. 2002), and has been examined in PEV formulations (Manconi et al. 2011, Mura et al. 2009).

#### EE%

The ability of spanlastics to encapsulate substantial quantity of RA is critical for its targeted use for treatment of acne. All formulations were able to incorporate a good amount of RA, with the EE% of spanlastics ranging from 76.7±1.71 to 89.7±0.07 (Table 1). Results show that the concentration of EA significantly affected the EE% of RA-loaded nanovesicles. Increasing the concentration of Tween® 20 from 20 mg to 100 mg resulted in a significant reduction in RA EE% in the prepared nanovesicles ($p < 0.01$). This could be attributed to the fact that Tween® 20 has induced micellar solubilization of RA in the hydration water (El Zaafarany et al. 2010, Goindi et al. 2013, Wavikar and Vavia 2013), thus decreasing the % RA entrapped.

Statistical analysis using Dunnett test revealed that Labrasol® and Transcutol® containing formulations gave EE% statistically comparable to that of the control F2 ($p \geq 0.05$).

Interestingly, increasing Transcutol® amount in the nanovesicles significantly lowered RA EE% in the nanovesicles ($p = 0.000$). On the other hand, increasing the Labrasol® amount has no significant outcome on the drug EE% ($p = 0.834$). This could be endorsed to the hydrophilic nature of Transcutol® compared to Labrasol®, which enhanced the drug escape out of the nanovesicles, and hence lower EE%.

#### PS and PDI

In the development of cutaneous delivery systems, PS is of a profound importance, as it influences vesicles’ penetration through the skin. Thus, preparing nanovesicles with reduced PS to guarantee deeper penetration was an important goal for this study. PS of the prepared RA-loaded nanovesicles are represented in Table 1. The PS fluctuated from 120.6±40.8 to 1486.67 ± 188.1 nm.

Results revealed that increasing the concentration of EA resulted in significant reduction in the PS ($p < 0.005$).
This could be attributed to the reduction of interfacial tension with increasing the surfactant concentration. Similar results were obtained by Dora et al. in a study on the preparation of glibenclamide nanoparticles (Dora et al. 2010). They reported that higher EA concentrations reduce the surface tension, facilitating particle partition and formation of smaller nanovesicles. Salama et al. (Salama et al. 2012) also shared the same results in their study on the preparation of olanzapine transferosomal vesicles. Another study reported that increasing the concentration of sodium deoxycholate as an EA resulted in the formation of significantly smaller spanlastics vesicles (Elsherif et al. 2017).

Addition of the PE to the spanlastics formulation F2, resulted in significant reduction in the particle size of F2 ($p < 0.05$). Moreover, increasing the amount of the PE from 25 mg to 50 mg resulted in a significantly lower particle size ($p < 0.05$). This could be attributed to the surfactant nature of the used PEs, Labrasol® and Transcutol®, which facilitated the formation of smaller nanovesicles.

**Polydispersity index and zeta potential**

PDI is a measure of the width of size distribution of the formulation. Their values always vary from 0 to 1. Low PDI values represent a monodisperse population and homogeneity in the particle size distribution (Esquerdo et al. 2015), while a larger PDI reflects a higher heterogeneity. The higher the polydispersity, the lower the uniformity of the vesicle size in the formulation (ElMeshad and Mohsen 2016). PDI values of the prepared nanovesicles ranged between 0.13 and 1.

In general, the system is considered stable when the ZP value is around ±30 mV due to electrical repulsion between particles (Muller et al. 2001). ZP results of our formulations were found to range from $-26.7 ± 0.40$ to $-38.12 ± 2.12$. From these high negative results, we can predict stability of RA-loaded spanlastic nanovesicles and avoidance of aggregation of the nanovesicles (Abdelbary and AbouGhaly 2015).

**Transmission electron microscopy (TEM)**

The TEM micrographs of formulation F2-T25 are illustrated in Figure 1. Dark vesicular structures were observed, almost spherical in shape, with the possibility to notice the outermost bilayer. Dark vesicles were observed probably due to the strong interaction between the PE and the phosphotungstic acid used in staining the particles (Mura et al. 2009). The particle size observed from the TEM micrographs are close to that obtained from Malvern zetasizer measurement.

**Differential scanning calorimetry (DSC)**

Figure (2a) represents the thermograms of pure RA, Span® 60, Carbopol and lyophilized optimized RA-loaded nanovesicular formulation. The DSC thermogram of pure RA exhibited a melting endotherm at 184.9°C, corresponding to its melting point. Similarly, Span 60 showed endothermic peak at 54.44°C corresponding to its melting temperature (Basha et al. 2013). The endothermic peak of RA disappeared in the lyophilized optimized formulation, suggesting homogeneous dispersion of the drug throughout the formulation in an amorphous form (Basha et al. 2013).

**Powder X-ray diffraction (XRD)**

The physical state of pure RA, Span 60, Carbopol and lyophilized optimized RA-loaded nanovesicular formulation was evaluated by XRD analysis (Figure 2(b)). The X-ray powder diffractogram of pure RA exhibited a series of intense sharp peaks $5.19^\circ$, $13.52^\circ$, $14.71^\circ$, $15.56^\circ$, $20^\circ$, $20.8^\circ$, $22.81^\circ$, $24.98^\circ$, $24.98^\circ$, $26.15^\circ$, $27.23^\circ$ 20 values, indicating its crystalline character. However, the diffraction pattern of PEVS indicates an amorphous nature of the structure by the absence of intense sharp peaks, suggesting that RA is dispersed within the nanovesicular formulation in an amorphous form.

On contrary, Span 60, and Carbopol are completely amorphous and showed no distinct diffraction peaks. The incorporation of RA in the spanlastic nanovesicles reduced its crystallinity. Thus, the disappearance of certain drug peaks in the XRD of the RA-loaded spanlastic nanovesicles could indicate the dispersion of the drug within these spanlastics in a micro-crystalline or a semi-crystalline form (Mandal 2010, Tayel et al. 2013).

**Skin deposition study**

RA deposition into newborn mice skin was evaluated in vitro under non-occlusive conditions. Figure (3a) illustrates the skin deposition of RA from two selected formulations (F2-T25, and F2) in comparison with that from the market product (Error bars represent standard deviation). Both selected formulations succeeded to enhance the drug deposition in the skin compared to the market product (Acretin®). RA deposition in the skin provided by RA-loaded nanovesicular formulation containing Transcutol (F2-T25) and the
corresponding formulation without Transcutol (F2) was significantly higher compared to the market product (33.65 and 16.68 fold, respectively). Moreover, the presence of Transcutol, as a penetration enhancer, significantly enhanced the skin deposition of RA. The formulation containing Transcutol (F2-T25) achieved 2-fold higher RA deposition in the skin compared to the corresponding one lacking Transcutol (F2). Interestingly, no permeation of RA across the whole skin thickness was detected in the present study from any of the tested formulations and the market product.

**Skin irritation test**

In order to ensure the safety of a drug delivery system on the skin, its tolerability should be tested. Therefore, the topical irritancy of our optimized formulation as well as the market product, Acretin<sup>V</sup>R was studied. Results showed that over the study period (6h), the tested formulations showed only slight degree of redness, with no symptoms of inflammation, proving the safety of the tested formulations to be applied topically on the skin.

**Clinical study**

A total of 15 adult patients were recruited from the outpatient dermatology clinic, Kasr al-Aini teaching hospital, Cairo University. Patients were followed up for a period of 4 weeks.

All the patients were asked about any adverse event they experienced while applying the medication. Only one patient out of the 15 tested patients confirmed not experiencing any adverse event with the any of the two tried products. The rest of the patients experienced at least one manifestation (peeling, burning sensation, dryness or pruritus). Table 2 showed superiority for the optimized formulation (F2-T25) compared to the marketed product (Acretin<sup>V</sup>) performance over the whole study period with respect to the improvement of the individual lesions. Comparing the efficacy, both groups started to show a significant improvement from acne symptoms starting one week after the application of the two products (Acretin<sup>V</sup> p < 0.00001 and the optimized
formulation \( p < 0.00001 \), with more than 70% improvement after 4 weeks in both formulations (Table 2; Figure 3(b)). These results indicate that the small size of vesicles enhanced penetration of the RA through the stratum corneum. Similar results have been previously obtained in the literature (Jaafari et al. 2009, Duangjit et al. 2011).

Previously studies suggest that the maximum comedolytic activity of tretinoin is achieved at 5–10 fold lower concentration upon incorporation into liposomes, compared to the conventional alcoholic gels (Nikouei et al. 2011). Figure 4(a,b) shows clinical evidence of the efficacy of the optimized formulation (F2-T25) represented by an acne case. Representation of the left cheek before treatment (a), and after 4 weeks of treatment (b) show almost complete resolution of acne comedones.

When the safety of the two formulations were compared, it was noticed that the formulate was clinically safer during the whole study period with a significantly less side effect after the first week (\( p = 0.0266 \)) and the 4th week (\( p = 0.0219 \)) compared to the market product (Acretin), as shown in Table 3.

**Conclusion**

The present studies highlight the importance of formulating a new type of nanovesicles prepared by using different PE molecules with Span 60 and Tween 20 as the major constituents of the vesicular bilayer to achieve the desired attributes of safety and efficacy. The optimized RA-loaded nanovesicular formulation composed of RA, Span 60-Tween 20 (70:30 W/W), in addition to Transcutol and included into 1% carbopol gel showed appreciable advantages over the marketed formulation in the treatment of deep-seated skin disorder like acne regarding skin safety and patient’s compliance.

**Disclosure statement**

The authors hereby report that there are no conflicts of interest. The authors alone are accountable for the content and writing of this article.

---

**Table 2.** The effect of the two tested formulae (Acretin versus the optimized formulation) on the different types of lesions (comedons, postules and papules) during 4 weeks study period.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Acretin (Average improvement % (SD) (N = 15))</th>
<th>Optimized formulation (Average improvement % (SD) (N = 15))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comedons</td>
<td>After 1 week: 12.234 (14.22)</td>
<td>After 1 week: 8.741 (14.49)</td>
</tr>
<tr>
<td>Postules</td>
<td>After 2 weeks: 30.817 (28.98)</td>
<td>After 2 weeks: 29.66 (26.6)</td>
</tr>
<tr>
<td>Papules</td>
<td>After 3 weeks: 39.47 (32.04)</td>
<td>After 3 weeks: 44.283 (32.607)</td>
</tr>
<tr>
<td>Total</td>
<td>After 4 weeks: 49.798 (38.09)</td>
<td>After 4 weeks: 54.38 (37.42)</td>
</tr>
</tbody>
</table>

**Table 3.** Average number of side effects in both groups during the study period.

<table>
<thead>
<tr>
<th>Week</th>
<th>Average number of lesions (SD)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>4.973 (2.55)</td>
<td>0.0266(^a)</td>
</tr>
<tr>
<td>Week 2</td>
<td>3.666 (2.968)</td>
<td>0.0688</td>
</tr>
<tr>
<td>Week 3</td>
<td>1.866 (2.325)</td>
<td>0.070</td>
</tr>
<tr>
<td>Week 4</td>
<td>0.733 (1.032)</td>
<td>0.0219(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Comparison was done using \( t \)-independent test with a level of significant as at \( P \) less than 0.05.
References


Mandal, B., 2010. Preparation and physicochemical characterization of Eudragit® RL100 Nanosuspension with potential for Ocular Delivery of Sulfacetamide, University of Toledo.


