Topical delivery of roxithromycin solid-state forms entrapped in vesicles

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Graphical Abstract

Key:
1 Roxithromycin
2 Roxithromycin: Amorphous form 1
3 Roxithromycin: Amorphous form 2

A Niosomes
B Proniosomes
C Ufosomes
D Pro-ufosomes
Abstract

Recently, considerable interest developed in using newer/improved antibiotics for the treatment of Acne vulgaris. During this study, different roxithromycin solid-state forms (i.e. crystalline and amorphous) were encapsulated into vesicle systems (niosomes, proniosomes, ufosomes and pro-ufosomes) for dermis targeted delivery. Characterization of the vesicles was done with transmission electron microscopy, light microscopy, droplet size, droplet size distribution, pH, zeta-potential and entrapment efficiency percentage. Finally, comparative release and topical diffusion studies were performed, to evaluate if targeted topical delivery was obtained and if the roxithromycin solid-state amorphous forms resulted in improved topical delivery. Vesicle systems containing different roxithromycin (2%) solid-state forms were successfully prepared and characterized. The vesicles showed optimal properties for topical delivery. All carrier systems had topical delivery to the epidermis-dermis, whilst no roxithromycin was found in the receptor compartment or stratum corneum-epidermis. The niosomes were the leading formulation and the two amorphous forms had better topical delivery than the crystalline form. Successful targeted delivery of roxithromycin was obtained in the dermis, where the activity against Propionibacterium acnes is needed. The amorphous forms seemed to have held their solid-state form during formulation and in the vesicles, showing improved topical delivery in comparison to the crystalline form.

Keywords: Vesicles; Pro-vesicles; Amorphous; Roxithromycin; Topical delivery; Targeted delivery
**Abbreviations**

**API**  Active pharmaceutical ingredient

**HPLC**  High performance liquid chromatography

**UV/VIS**  Ultraviolet-visible spectrophotometry

**X-ray powder diffraction**  XRPD

**PBS**  Phosphate buffer solution

**PVDF**  Hydrophilic polyvinylidene fluoride

**Log D**  Octanol-buffer distribution coefficient

**TEM**  Transmission electron microscope

**EE%**  Entrapment efficiency percentage

**ANOVA**  Analysis of variances

**LLOQ**  Lower limit of quantification
Introduction

Acne vulgaris is a chronic inflammatory skin disease, which affects most of the world’s population at some point in their life. The disease develops in the pilosebaceous units of the dermis and is characterized by irregular desquamation in hair follicles, increased sebum production in the sebaceous glands and inflammation caused by infiltration of Propionibacterium acnes to the area [1,2]. The rationale for the use of antibiotics in the treatment of acne is based on the bacteriostatic effect of the inflammatory agent, P. acnes [3]. In recent studies, there has been interest in conducting trials to test the efficiency of newer antibiotics, such as roxithromycin, for acne treatment [3].

Roxithromycin is a semi-synthetic macrolide antibiotic having bacteriostatic, as well as bactericidal, effects on bacterial organisms by terminating their protein synthesis and growth [4-6]. Roxithromycin has a rather broad spectrum of activity, but in this study, the focus is shifted to its activity against P. acnes as this is the micro-organism responsible for causing inflammatory acne [7]. The dermis is the specific area targeted, as it is the region which contains the sebaceous follicles where the accumulation of this P. acnes micro-bacterium occurs [7,8].

One problem which exists with roxithromycin, is its poor aqueous solubility which limits its therapeutic capability. According to Hadgraft & Somers [9], an API (active pharmaceutical ingredient) which is both lipophilic and hydrophilic is ideal for topical delivery as it is able to pass through the lipid layers as well as the aqueous domains of the skin. Roxithromycin has an experimental aqueous solubility of 0.0335 mg/ml at 25 °C, which is poor considering that for an API to optimally penetrate the skin, it requires an aqueous solubility of above 1 mg/ml [10,11]. By using different solid-state amorphous forms of roxithromycin, these insolubility problems may be overcome [12]. Different crystal lattices of the same compound result in altered physico-chemical properties, including that of solubility [13]. Researchers from the Centre of Excellence for Pharmaceutical Sciences (Pharmacen) of the North-West University, South Africa, registered two patents which proved that two amorphous forms of roxithromycin had increased aqueous solubility in comparison to the crystalline form. A 'glassy' amorphous form of roxithromycin
exhibited a 1800% increase in apparent aqueous solubility in water, whilst the chloroform desolvated amorphous form showed a 505.9% increase [14,15].

There has been great interest and growth in developing delivery systems which improve therapeutic effects of drugs. Drug delivery by vesicles has the capability of improving the bioavailability of drugs, particularly those of poor solubility such as roxithromycin [16]. Vesicle systems are formed when amphiphilic components are met with water and arrange themselves into ordered assemblies of one or more lipid bilayers [16]. Vesicles can be made from various lipids including non-ionic surfactants, which result in one of the more popular vesicle systems, niosomes [17]. Niosomes are made from the hydration of non-ionic surfactants and often contain cholesterol [18]. They can act as carriers for lipophilic or hydrophilic drugs with a particle size of 100-1000 nm [16,19]. Roxithromycin is lipophilic with a log P of 2.9, which predicts it will be delivered to the skin in the lipophilic bilayer surrounding the inner aqueous compartment of the vesicles [20]. Ufosomes are another type of liposome prepared from fatty acids. These fatty acid vesicles (ufosomes) are suspensions of fatty acid and ionized (soap) species which form lipid bilayers with an average particle size of 100 nm [16,21].

Pro-vesicular delivery systems are vesicles developed to overcome the instability problems associated with the vesicles suspended in aqueous solutions, as seen with niosomes and ufosomes. Pro-vesicles are systems consisting of a dry, porous powder which is water soluble. The carrier dissolves when an aqueous solvent is added to it before the use of the system [16]. The dry aqueous soluble carriers of pro-niosomes are coated with non-ionic surfactants and niosomes are formed on aqueous hydration and agitation of these systems [16,22]. Pro-ufosomes are vesicle systems for which no information could be found, but it was prepared using the same method as proniosomes.

Another obstacle formulators continue to face today, is overcoming the skin’s great barrier, the stratum corneum, as it is this layer of skin which is problematic for the successful penetration of drugs [23]. The skin is a large and convenient area for the administration of drugs and also boasts a number of advantages over oral and parenteral API delivery such as: avoidance of first-pass metabolism, dose dumping, systemic adverse effects and inconvenience of administration for the patient [18,24,25]. It is therefore beneficial to develop products for topical
delivery which successfully penetrate the stratum corneum. There is hope for great improvement in the topical delivery of roxithromycin by using its amorphous forms encapsulated into vesicle systems which overcome the solubility drawbacks of the API and increase stratum corneum penetration and topical delivery.

The aim of this study was to determine if the amorphous forms, with increased solubility encapsulated in vesicles, would have successful topical delivery. The three solid-state forms of roxithromycin (2%), namely, crystalline monohydrate, ‘glassy’ amorphous form and chloroform desolvated amorphous form were each encapsulated into four vesicle systems. The four vesicle systems were niosomes, proniosomes, ufosomes and pro-ufosomes. The topical delivery of all preparations were tested using release and diffusion experiments to determine which of the formulations had the optimal topical penetration into the target area, in this case the dermis.
Materials and methods

Materials

Roxithromycin monohydrate (crystalline form) was purchased from DB Fine Chemicals (South Africa). The ingredients used in the preparations of the vesicle systems were Span® 60, sodium oleate, cholesterol and sorbitol, all purchased from Sigma Aldrich (Germany). Methanol and chloroform were of analytical grade (ACE Chemicals, South Africa) and potassium dihydrogen orthophosphate AR was purchased from LabChem (South Africa). Ultrapure water, with a resistivity of 18.2 MΩ.cm⁻¹, was used in all experiments in this study and was obtained from a Rephile Direct Pure UP water purification system (USA). The acetonitrile used as a component of the mobile phase during high performance liquid chromatography (HPLC) analysis was of chromatography grade (Merck, South Africa).

HPLC analysis method

The use of the HPLC analysis method was to determine the concentration of roxithromycin in all samples obtained during the experiments done in this study. The analysis was performed in a controlled laboratory environment. A Shimadzu (Japan) HPLC (LC-20AD) fitted with a UV/VIS (ultraviolet-visible spectrophotometry) photodiode array detector (SPD-M20A) and a LC-20AD solvent delivery module was used, with LabSolutions, LCsolution Release 1.21 SP1 (Shimadzu, Japan) software, used for data analysis. A Luna C₁₈ 150 × 4.6 mm column (5 μm particle size) was used as a stationary phase, with an injection volume of 10 μl, a flow rate of 1 ml/min with a wavelength set at 205 nm. The mobile phase consisted of 0.06 M phosphate buffer solution (adjusted to pH 7.4 with sodium hydroxide solution) and acetonitrile (50:50 %v/v). Validation of this method resulted in a correlation coefficient of 0.9999 [26].

Preparation of the amorphous forms of roxithromycin

Two amorphous solid-state forms of roxithromycin were prepared. The glassy amorphous form of roxithromycin was prepared by evenly distributing crystalline roxithromycin monohydrate in a Petri dish. The sample was subsequently melted in a laboratory oven (Binder, Germany) at 120 ± 2 °C. The melt was removed from the oven and quench cooled on a cold granite surface to form the amorphous solid [27]. The chloroform desolvated amorphous form was prepared by heating chloroform on a hot plate stirrer to 60 ± 2 °C whilst adding roxithromycin monohydrate
until a saturated solution was obtained. The solution was removed from the hot plate and left for slow evaporation of the chloroform to occur [27]. A dense mass was obtained, which was characterized as the roxithromycin chloroform solvate. The sample was desolvated in a laboratory oven (Binder, Germany), set at 60 ± 2 °C, for a period of 24 h.

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

Powder X-ray diffraction measurements were performed to confirm the crystalline or amorphous habit of the solid-state forms of roxithromycin used in this study. A PANalytical (Almelo, Netherlands) Empyrean X-ray diffractometer with a PIXcel3D detector was used to record XRPD patterns at ambient temperature. Samples were evenly distributed on a zero background sample holder. The measurement conditions for all scans were set as follows: target, Cu; voltage, 40 kV; current, 40 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; scanning speed, 2°/min (step size, 0.02°; step time, 1.0 s).

**Aqueous solubility of solid-state forms of roxithromycin**

The aqueous solubility of roxithromycin monohydrate, glassy roxithromycin and amorphous chloroform desolvate were determined. All three forms were added to 5 ml PBS (phosphate buffer solution; pH 7) in polytops heated to 32 °C (temperature on top of the skin during diffusion studies) and containing magnetic stirrers. Saturated solutions were maintained for 24 h. The solutions were then filtered through 0.45 μm hydrophilic polyvinylidene fluoride (PVDF) filters and analyzed by means of HPLC. The experiment was done in triplicate.

**Octanol-buffer distribution coefficient (log D) of roxithromycin**

The log D of roxithromycin was determined. Roxithromycin (15 mg) was accurately weighed and added to 2 ml pre-saturated n-octanol in a test tube. The test tube was subsequently rotated in a rotating bath at 32 °C for 10 min. Thereafter, 3 ml pre-saturated PBS (pH 7) was added and rotated for 45 min. The test tubes were then centrifuged at 4000 rpm for phase separation of the n-octanol and PBS (pH 7) to occur. The separated phases were placed in polytops where they were then filtered through PVDF filters. The API concentration in each phase was determined using HPLC. The experiment was done in triplicate. The distribution coefficient between the two phases was calculated using the following equation:

\[
\log D = \frac{\text{Concentration in } n\text{-octanol}}{\text{Concentration in PBS (pH 7)}}
\]
Determining formulas for optimal vesicle systems

Table 1: Lipid ratios and sonication times of twelve potential vesicle formulas

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lipid ratio</th>
<th>Sonication time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0 min</td>
</tr>
<tr>
<td>Niosomes</td>
<td>Span® 60:cholesterol (2:1)</td>
<td>(A1)</td>
</tr>
<tr>
<td></td>
<td>Span® 60:cholesterol (1:1)</td>
<td>(A4)</td>
</tr>
<tr>
<td>Ufosomes</td>
<td>Sodium oleate:cholesterol (2:1)</td>
<td>(B1)</td>
</tr>
<tr>
<td></td>
<td>Sodium oleate:cholesterol (1:1)</td>
<td>(B4)</td>
</tr>
</tbody>
</table>

All vesicle systems were prepared by the hand-shaking method adapted from New [28] with slight modifications to suit this study. To determine the optimal lipid ratio for niosomes and ufosomes, two different lipid ratios for each system were made and each were sonicated at three different sonication lengths. This resulted in six formulations of each vesicle systems as seen in Table 1. The parameters used to determine the optimal lipid ratio was Transmission Electron Microscopy (TEM), light microscopy and droplet size and distribution. Only the vesicles with no sonication were viewed with the TEM (FEI Tecnai G2, FEI, Holland) to determine if vesicles had indeed formed. Thereafter, all vesicles were viewed under the Nikon Eclipse E4000 microscope (Nikon, Japan) to determine if vesicles still existed after sonication. Next the droplet size and distribution was tested using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) to show which formulation had better distribution and therefore, better stability [22]. The optimal lipid ratios were then decided by choosing one of the lipid ratios for niosomes and ufosomes from Table 1.

The optimal concentration of entrapped roxithromycin monohydrate was determined by loading each of the optimal lipid vesicles (niosomes and ufosomes) with four different concentrations of drug. The entrapment efficiencies of all ratios were tested in duplicate using the mini-column centrifugation method of New [28]. The formulation with the better entrapment efficiency, as well as physical appearance, was chosen as the final optimal formula for each vesicle system.
Preparation of optimal vesicle systems

The preparation of the twelve optimal vesicle systems were based on the results obtained from determining the optimal formulas of the niosomes and ufosomes containing roxithromycin monohydrate. The pro-vesicular systems’ (proniosomes and pro-ufosomes) ratios were exactly the same as the optimal ratios of the vesicle systems (niosomes and ufosomes) and all three solid-state forms of roxithromycin (2%) were entrapped in the same way. This was decided in order to minimize possible variation amongst vesicles, which made it easier for comparison and explanation of experiments that followed. Table 2 shows a summary of the twelve vesicle carriers in this study.

Table 2: Summary of vesicle preparations in this study

<table>
<thead>
<tr>
<th>Roxithromycin forms encapsulated</th>
<th>Vesicle systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Niosomes</td>
</tr>
<tr>
<td>Roxithromycin monohydrate</td>
<td>(1)</td>
</tr>
<tr>
<td>Glassy amorphous form</td>
<td>(5)</td>
</tr>
<tr>
<td>Chloroform desolvated amorphous form</td>
<td>(9)</td>
</tr>
</tbody>
</table>

As mentioned above, the niosomes and the ufosomes were prepared according to the hand-shaking method of New [28], where the lipid components of the vesicles and the API were dissolved in chloroform/methanol solution and left to evaporate. Upon appearance of a thin film at the bottom of the beaker, PBS (pH 7.4) and a magnetic stirrer were added to completely remove the film in the formation of vesicles. Niosomes were then sonicated for 2.0 min and ufosomes for 3.5 min and left to stand for 2 h before use.

The slurry method was used for the preparation of the proniosomes and pro-ufosomes [28]. This method consisted of dissolving the lipid layer components and API in chloroform/methanol and adding it drop-wise to sorbitol powder in order for the vesicles to coat the powder. The chloroform/methanol evaporated leaving a dry powder which was constituted with 5 ml PBS (pH
7.4) just before it was needed for further experiments. Proniosomes were sonicated for 2 min and pro-ufosomes for 3.5 min and left to swell for 2 h before use.

Table 3 shows the ingredients of each optimal vesicle system prepared. The niosomes and proniosomes had a Span® 60:cholesterol:roxithromycin ratio of 2:1:1; the ufosomes and pro-ufosomes also had a sodium oleate:cholesterol:roxithromycin ratio of 2:1:1. The preparation of each vesicle system was repeated in triplicate using each solid-state form of roxithromycin.

Table 3: Ingredients used for optimal vesicles systems

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Niosomes</td>
</tr>
<tr>
<td>Span® 60</td>
<td>200 mg</td>
</tr>
<tr>
<td>Sodium oleate</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>100 mg</td>
</tr>
<tr>
<td>Chloroform/methanol (2:1)</td>
<td>5 ml</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>1000 mg</td>
</tr>
<tr>
<td>PBS (0.06 M at pH 7.4)</td>
<td>5 ml</td>
</tr>
</tbody>
</table>

**Characterization of vesicles**

Although all vesicles were made according to the optimized formulas discussed earlier, it was still necessary to characterize all twelve vesicle systems of different vesicles containing different solid-state forms of roxithromycin.

**TEM**

The morphology of the vesicles was investigated using a FEI Tecnai G2 TEM (FEI, Holland) [28]. Only the four optimal vesicle systems containing no API were viewed using the TEM as there was a risk of crystallization of API with subsequent damage to the TEM. Each vesicle was freshly prepared, diluted 10x with distilled water and one drop was placed on the carbon-coated copper grid which was left to dry. A drop of osmium was used to stain the lipid components. Once dry, it was loaded into the TEM and viewed at 5000-20000x magnifications.
Light microscopy

Light microscopy, using a Nikon Eclipse E4000 microscope (Nikon, Japan), was performed in order to determine if all systems containing API consisted of vesicles. The shape and lamellarity of larger vesicles could also be seen [30]. Samples were prepared and diluted 20x with distilled water. A drop was placed on a microscope cover slip and viewed at a magnification of 50x (max. for the instrument). The experiment was done in triplicate for each carrier system.

Zeta-potential

The zeta-potential was tested in order to determine the surface charge of each vesicle system, as surface charges can have an influence on the stability of the droplets [22]. Highly charged droplets prevent aggregation and fusion of vesicles [30]. A Malvern Zetasizer Nano ZS (Malvern Instruments, UK) was used to test the zeta-potential. Fresh samples were diluted (20x with distilled water) and a small amount was placed in a cuvette for triplicate readings. The experiment was repeated twice more.

Droplet size and distribution

The droplet size and distribution of each vesicle system was tested using the Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The particle size and distribution is important as it has a great influence on the stability as well as entrapment efficiency of a system [22]. The twelve vesicular systems were prepared in triplicate and diluted 20x, where 2 ml was injected into a plastic cuvette. The reading of each sample was taken three times for an accurate average.

pH

The pH of the lipid layers of vesicles in solution was determined using a Mettler Toledo pH meter (Mettler Toledo, Switzerland). The twelve 5 ml vesicle solutions, undiluted, were freshly prepared and the pH readings taken three times. The entire experiment was repeated in triplicate with fresh samples each time.

Entrapment efficiency

The entrapment efficiency (EE%) determined the difference between the total amount of free drug and the unentrapped drug to determine the amount of drug encapsulated in the vesicles [31]. The entrapment efficiency of the twelve vesicle systems were tested using the ultra-
centrifugation method [32]. This method was discovered later in the study and proved to be faster and more convenient than the mini-column centrifugation method used for optimizing formulas. A Beckman Coulter Optima L-100 XP ultracentrifuge (Beckman Coulter, South Africa) with a 50.2Ti fixed rotor was used. Samples were diluted 25x, placed in the centrifugation tubes and centrifuged at 25000 rpm for 30 min. The clear supernatant was then removed and analyzed on the HPLC, where the entrapment was calculated afterwards using the following equation of Kurakula et al. [33]:

\[
EE\% = \frac{C_t - C_f}{C_t} \times 100
\]  

(2)

Where \( C_t \) is the total amount API and \( C_f \) the amount of free unencapsulated drug (x value calculated from HPLC analysis equation; \( y = mx + c \)).

**Membrane release studies**

Membrane release studies were done on all twelve vesicle systems to determine if the API was released from the vesicles. The donor phases during the release studies were samples of vesicles containing the different solid-state forms of roxithromycin and the receptor phase consisted of 0.06 M PBS dissolved in distilled water with a pH of 7.4. Each experiment had 12 Franz cells, with 10 being test cells and two being controls. The donor phase and the receptor phase were prepared and the donor phase was placed in a 32 °C water bath (to correlate with the skin surface’s temperature) and the receptor phase was placed in a 37 °C water bath (temperature of human blood). Vacuum grease was placed on either side of the Franz cells, a magnetic stirrer was placed in the receptor compartment and a PVDF filter was cut circularly and placed between the compartments. The Franz cells were sealed with vacuum grease and a horseshoe clamp. 2 ml PBS (pH 7.4) was placed in the receptor compartment and 1 ml of the vesicles containing 2% roxithromycin (donor phase) was placed in the donor compartment and sealed with Parafilm®. The cells were placed on a Variomag® magnetic stirrer plate (Variomag, USA) in a Grant® water bath (Grant Instruments, UK), where the temperature was maintained at 37 °C. Extractions and replacements of the PBS (pH 7.4) took place at extraction times of 1, 2, 3, 4, 5 and 6 h. The extractions were analyzed using HPLC to determine the concentration of API which permeated through the membrane and in turn, the amount of API released from each formula.
Preparation of skin for diffusion studies

The skin for the diffusion studies was female Caucasian skin, obtained after surgery of the abdominal area, with informed consent having first been obtained from patients before such use. Ethical approval was granted by the Research Ethics Committee of the North-West University (Reference: NWU-00114-11-A4). Skin was received within 24 h after the abdominal surgery and was transported directly to the North-West University in an ice box. Full-thickness skin was cut (thickness of 400 µm) using a Dermatome™ (Zimmer LTD, United Kingdom). The dermatomed skin was placed on Whatman® filter paper, closed with foil and stored in a freezer at -2 to -5 °C until needed. Skin over six months old was discarded in order to prevent using skin that might have an impaired barrier [34].

Skin diffusion studies

Skin diffusion studies were conducted to determine if the API (2% roxithromycin) penetrated the skin to reach the systemic circulation (receptor phase). The procedure followed for the diffusion studies was exactly the same as the membrane studies discussed earlier, except that circles of dermatomed skin on Whatman® filter paper was cut and placed between the donor and receptor compartments, with the stratum corneum facing upwards instead of the PVDF membranes. The extraction times also differed. The diffusion studies had only one extraction after 12 h based on pilot studies conducted beforehand.

Tape stripping

The tape stripping method as explained by Pellet et al. [35] and Fox et al. [36] was undertaken to determine if there was API in the stratum corneum-epidermis and epidermis-dermis and to see if the roxithromycin amorphous forms had better topical delivery than the roxithromycin monohydrate. The skin samples were removed from the Franz cells and pinned to a board so that the diffusion area of the skin could be seen. It was dabbed with a paper towel and 16 strips of 3M Scotch® Magic™ tape were used to remove the stratum corneum-epidermis from the skin (first strip was discarded). All 15 tape strips were placed into a polytop and 5 ml ethanol was added to dissolve the API. The remaining epidermis-dermis skin was cut into tiny pieces and also placed into a polytop with 5 ml ethanol. The samples were left to stand for 8 h at 2-5 °C for the API to be extracted from both the stratum corneum-epidermis and epidermis-dermis. The
API concentration in each of the samples was determined using HPLC analysis. The process was repeated after all twelve diffusion studies.

Statistical analysis

Data analysis was performed using the epidermis-dermis results obtained from HPLC analysis as this was the only group of samples in which API concentration was found. Statistics were used to compare which vesicle systems, as well as which solid-state form, of roxithromycin had the better topical delivery. Simple non-parametric tests were performed. The Kruskal-Wallis analysis of variance (ANOVA) compared the different formulations and the multiple comparisons test was used to compare among pairs of formulations. Formulations had a significant difference if their $p$-value was smaller than 0.05.
Results

Preparation of amorphous forms

The preparation of the glassy form of roxithromycin produced a compound which had the appearance of tiny glass flakes. The chloroform desolvate of roxithromycin also resulted in an amorphous form with a rough granular appearance. The amorphous habit of both the glassy roxithromycin and the chloroform desolvate was confirmed by an amorphous halo diffraction pattern obtained from XRPD analyses. The aqueous solubility of the three solid-state forms in PBS (pH 7) at 32 °C were in very close range of one another with a value of 1.98 mg/ml for roxithromycin monohydrate, 1.88 mg/ml for the glassy roxithromycin amorphous form and 1.92 mg/ml for the chloroform desolvated amorphous form of roxithromycin. The log D for roxithromycin was found to be 1.52 which was optimal for topical delivery [18].

Decision for optimal vesicle formulations

The TEM images in Fig.1 reveal that all four samples of non-sonicated vesicles without API did indeed form vesicles.
Fig. 1: TEM photomicrographs of formation of vesicles. a) Niosome with Span® 60:cholesterol (2:1), b) niosome with Span® 60:cholesterol (1:1), c) ufosome with sodium oleate:cholesterol (2:1) and d) ufosome with sodium oleate:cholesterol (1:1).
<table>
<thead>
<tr>
<th>Ratio</th>
<th>No sonication</th>
<th>2.0 min sonication</th>
<th>3.5 min sonication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niosome 2:1</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Niosome 1:1</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Ufosome 2:1</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>Ufosome 1:1</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

It can be seen from Table 4 that all sonicated samples still contained vesicles. The micrographs indicate the non-sonicated samples of all formulations had larger droplets. The niosomes sonicated for 2.0 min and 3.5 min had very similar micrographs while for ufosomes, the samples sonicated for 3.5 min appeared to have smaller and more uniform droplets than those sonicated for only 2.0 min.
Fig. 2: Droplet size distribution of lipid ratios of niosomes and ufosomes. a) Droplet distribution of niosomes (ratio 2:1) sonicated for 2.0 min, b) droplet distribution of niosomes (ratio 1:1) sonicated for 2.0 min, c) size distribution of niosomes (ratio 2:1) sonicated for 3.5 min, d) size distribution of niosomes (ratio 1:1) sonicated for 3.5 min, (e) droplet size distribution of ufosomes (2:1) sonicated for 2.0 min, (f) droplet distribution of ufosomes (1:1) sonicated for 2.0 min, (g) size distribution of ufosomes (2:1) with a sonication time of 3.5 min and (h) size distribution of ufosomes (1:1) with a sonication of 3.5 min.
The droplet size of the non-sonicated samples could not be measured as the data quality was too poor for accurate readings and this ruled them out as potential formulas. The remaining eight sonicated formulas were tested. From Fig. 2 it can be seen that both niosomes with a lipid ratio of 2:1 (a) and 1:1 (b) sonicated at 2.0 min displayed very good droplet size distribution with droplet sizes of 123 and 160 nm, respectively. Niosomes sonicated for 3.5 min for ratios 2:1 (c) and 1:1 (d) had poor droplet size distribution. It was clearly evident that ufosomes 2:1 sonicated for 3.5 min (g) had the best droplet size distribution, with an average droplet size of 83 nm. Ufoosome ratios of 2:1 (e) and 1:1 (f) sonicated for 2.0 min had poor distribution and ufosome ratio 1:1 (h) sonicated for 3.5 min had poor repeatability of results.

Table 5 contains the EE% of the potential niosome and ufosome formulations as well as their physical appearance after the drug loading.

### Table 5: EE% and physical appearance of potential niosome and ufosome formulas

<table>
<thead>
<tr>
<th>Niosomes</th>
<th>Span® 60:cholesterol:API</th>
<th>Average%</th>
<th>Physical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0:1.0:0.5</td>
<td>51.68</td>
<td>Good suspension</td>
</tr>
<tr>
<td></td>
<td>2.0:1.0:1.0</td>
<td>59.81</td>
<td>Good suspension</td>
</tr>
<tr>
<td></td>
<td>2.0:1.0:2.0</td>
<td>52.16</td>
<td>Good suspension</td>
</tr>
<tr>
<td></td>
<td>2.0:1.0:4.0</td>
<td>63.84</td>
<td>Good suspension</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ufosomes</th>
<th>Sodium oleate:cholesterol:API</th>
<th>Average%</th>
<th>Physical appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0:1.0:0.5</td>
<td>38.69</td>
<td>Good suspension</td>
</tr>
<tr>
<td></td>
<td>2.0:1.0:1.0</td>
<td>84.36</td>
<td>Good suspension</td>
</tr>
<tr>
<td></td>
<td>2.0:1.0:2.0</td>
<td>92.99</td>
<td>Thickish gel</td>
</tr>
<tr>
<td></td>
<td>2.0:1.0:4.0</td>
<td>94.31</td>
<td>Thick gel</td>
</tr>
</tbody>
</table>

**Characterization of vesicles**

Fig. 3 illustrates the morphology of all four optimal vesicle systems containing no API as investigated by TEM analysis.
**Fig. 3:** TEM images of optimized vesicles containing no API. (a) Niosomes sonicated for 2.0 min, (b) proniosomes sonicated for 2.0 min, (c) ufosomes with a sonication time of 3.5 min and (d) pro-ufosomes sonicated for 3.5 min.

All the niosome and proniosome images taken using light microscopy looked similar to the niosomes (2:1) sonicated for 2.0 min photomicrographs taken during the decision-making process for optimized formulas and all the ufosome and pro-ufosome formulations appeared the same as ufosomes (2:1) sonicated for 3.5 min images, as seen in Table 4.
Table 6: Summary of characterization results of twelve optimal vesicle systems

<table>
<thead>
<tr>
<th>Vesicles</th>
<th>API solid-state form</th>
<th>Average zeta-potential</th>
<th>Average droplet size</th>
<th>Average pH</th>
<th>Average %EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Monohydrate</td>
<td>-50.06±17.81</td>
<td>164.08±51.17</td>
<td>6.96±0.06</td>
<td>77.78±3.27</td>
</tr>
<tr>
<td></td>
<td>Glass</td>
<td>-42.28±13.70</td>
<td>160.56±52.52</td>
<td>7.16±0.11</td>
<td>83.42±4.37</td>
</tr>
<tr>
<td></td>
<td>Desolvate</td>
<td>-53.80±17.26</td>
<td>127.49±27.14</td>
<td>7.21±0.22</td>
<td>82.74±3.15</td>
</tr>
<tr>
<td>2</td>
<td>Monohydrate</td>
<td>-68.42±7.07</td>
<td>125.57±49.68</td>
<td>7.27±0.04</td>
<td>66.64±8.99</td>
</tr>
<tr>
<td></td>
<td>Glass</td>
<td>-65.24±2.92</td>
<td>77.68±49.96</td>
<td>7.26±0.06</td>
<td>50.88±7.61</td>
</tr>
<tr>
<td></td>
<td>Desolvate</td>
<td>-56.22±5.07</td>
<td>121.92±18.14</td>
<td>7.23±0.01</td>
<td>63.83±5.67</td>
</tr>
<tr>
<td>3</td>
<td>Monohydrate</td>
<td>-73.16±11.11</td>
<td>156.66±14.69</td>
<td>8.73±0.11</td>
<td>96.27±1.24</td>
</tr>
<tr>
<td></td>
<td>Glass</td>
<td>-99.62±24.37</td>
<td>140.33±10.34</td>
<td>8.52±0.23</td>
<td>96.51±0.77</td>
</tr>
<tr>
<td></td>
<td>Desolvate</td>
<td>-100.3±21.99</td>
<td>112.94±14.42</td>
<td>8.78±0.04</td>
<td>94.58±2.06</td>
</tr>
<tr>
<td>4</td>
<td>Monohydrate</td>
<td>-80.27±17.99</td>
<td>102.50±10.27</td>
<td>8.56±0.05</td>
<td>93.41±2.59</td>
</tr>
<tr>
<td></td>
<td>Glass</td>
<td>-87.92±13.93</td>
<td>70.48±8.07</td>
<td>8.46±0.39</td>
<td>92.81±1.70</td>
</tr>
<tr>
<td></td>
<td>Desolvate</td>
<td>-72.04±4.75</td>
<td>100.09±26.77</td>
<td>8.51±0.08</td>
<td>94.73±1.91</td>
</tr>
</tbody>
</table>

1 niosome; 2 proniosomes, 3 ufosomes and 4 pro-ufosomes

The zeta-potential, droplet size, pH and EE% of all twelve formulations are summarized in Table 6. The droplet size distribution of the vesicles all looked very similar to the droplet distribution curves seen earlier in Fig. 2, with niosomes’ and proniosomes’ droplet distribution resembling that of Fig. 2.a and ufosomes and pro-ufosomes resembling Fig. 2.g. The pH of the lipid surface of niosomes and proniosomes was about 7.0 for each of the formulas, whilst ufosomes and pro-ufosomes had a pH of about 8.5.

Membrane release studies

The average flux and average %released of the different roxithromycin solid-state forms for each vesicle system that was released through the membrane after 6 h can be seen in Table 7.
Table 7: Average flux and average %API released of the different roxithromycin solid-state forms for all formulations after a 6 h membrane study

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average flux (µg/cm².h)</th>
<th>Average %API diffused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niosome monohydrate</td>
<td>184.54±19.32</td>
<td>3.11±0.31</td>
</tr>
<tr>
<td>Niosome glass</td>
<td>169.86±18.29</td>
<td>2.79±0.32</td>
</tr>
<tr>
<td>Niosome desolvate</td>
<td>183.50±18.45</td>
<td>3.22±0.30</td>
</tr>
<tr>
<td>Proniosome monohydrate</td>
<td>90.14±8.47</td>
<td>1.62±0.15</td>
</tr>
<tr>
<td>Proniosome glass</td>
<td>112.34±10.12</td>
<td>1.80±0.16</td>
</tr>
<tr>
<td>Proniosome desolvate</td>
<td>75.62±10.42</td>
<td>1.26±0.15</td>
</tr>
<tr>
<td>Ufosome monohydrate</td>
<td>20.75±3.87</td>
<td>0.36±0.07</td>
</tr>
<tr>
<td>Ufosome glass</td>
<td>21.29±2.36</td>
<td>0.36±0.04</td>
</tr>
<tr>
<td>Ufosome desolvate</td>
<td>17.58±2.89</td>
<td>0.28±0.05</td>
</tr>
<tr>
<td>Pro-ufosome monohydrate</td>
<td>56.29±10.64</td>
<td>0.81±0.13</td>
</tr>
<tr>
<td>Pro-ufosome glass</td>
<td>25.72±1.39</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>Pro-ufosome desolvate</td>
<td>29.22±4.12</td>
<td>0.55±0.08</td>
</tr>
</tbody>
</table>

**Skin diffusion studies**

During these studies no roxithromycin was detected in the receptor phase of most samples except for four of the formulations, namely niosomes glass (0.0053±0.0003%; n = 7), niosomes desolvate (0.0031±0.0007%; n = 10), proniosomes monohydrate (0.0133±0.0035%; n = 10) and pro-ufosomes monohydrate (0.0104±0.0051%; n = 10). No API was found in the stratum corneum-epidermis layer of the skin.
Fig. 4: Concentration (µg/ml) of the sum of three forms of the API per vehicle system delivered topically in the epidermis-dermis after the 12 h skin diffusion study.  N represents the niosomes, PN represents the proniosomes, U represents the ufosomes and PU represents the pro-ufosomes.

Fig. 4 is a graph derived from the ANOVA statistical analysis of the concentrations of API found in the epidermis-dermis skin layer after the 12 h diffusion study. In order from highest to lowest, API delivery was niosomes glass (18.016±5.36 µg/ml), niosomes desolvate (7.497±3.57 µg/ml), ufosomes monohydrate (5.445±3.23 µg/ml), ufosomes glass (2.303±1.45 µg/ml), niosomes monohydrate (2.273±2.58 µg/ml), ufosomes desolvate (1.432±1.12 µg/ml), proniosomes monohydrate (1.304±0.73 µg/ml), proniosomes glass (0.968±0.66 µg/ml), proniosomes desolvate (0.816±0.73 µg/ml), pro-ufosomes desolvate (0.719±0.33 µg/ml), pro-ufosomes monohydrate (0.569±0.22 µg/ml) and lastly, pro-ufosome glass (0.400±0.16 µg/ml) with the least amount of API. The results of the comparison of the three solid-state forms of roxithromycin, regardless of the vesicle type it was encapsulated in, is illustrated in Fig. 5.
Fig. 5: Total sum of the epidermis-dermis concentrations (µg/ml) of each solid-state form of roxithromycin after the 12 h skin diffusion studies regardless of what vesicle system it was encapsulated in.

**Statistical analysis**

Results obtained from the Kruskal-Wallis ANOVA by ranks analysis of API concentrations found in the epidermis-dermis, illustrated the statistical significance between formulation pairs. There was only statistical significance for the niosome (p = 0.0001) and the ufosome (p = 0.0128) vesicle systems. The multiple comparisons tests performed on niosomes indicated showed that there was a statistical difference between roxithromycin monohydrate and the glass (p = 0.00005) and for ufosomes there was a difference between the roxithromycin monohydrate and the desolvate (p = 0.01035). The remaining pairs had no statistical significances (p > 0.05).

The Kruskal-Wallis ANOVA by ranks was also tested on all three solid-state forms which proved to have significant differences with roxithromycin monohydrate (p = 0.002), roxithromycin glass (p = 0.000) and roxithromycin desolvate (p = 0.0000). For the multiple comparisons tests on
roxithromycin, there was only a significant difference for ufosomes and pro-ufosomes ($p = 0.000966$), while the glassy solid-state form group had a difference between the niosomes and proniosomes ($p = 0.003279$), niosomes and pro-ufosomes ($p = 0.000027$) and ufosomes and pro-ufosomes ($p = 0.024711$). The niosomes and proniosomes ($p = 0.000056$) and niosomes and pro-ufosomes ($p = 0.000193$) had statistical differences in the desolvate amorphous group. The remaining groups displayed no significant differences.
Discussion

**Preparation of amorphous forms**

The two amorphous forms were successfully prepared and showed to be true amorphous solid-state forms due to the halo-like diffraction patterns obtained during XRPD analyses. These results correlated very well with that reported in literature [14,15,27]. The experimental results mentioned in the patents of Liebenberg *et al.* [14] and Liebenberg & Aucamp [15], indicated that the roxithromycin amorphous forms exhibited increased aqueous solubility, but in this study the values were all similar to one another. It is suggested that during this 24 h solubility experiment, performed in PBS (pH 7) at 32 °C, the amorphous forms converted back to the stable crystalline form which resulted in the similar solubility values [13]. The log D value of roxithromycin showed to be within the optimal range (log D of 1-3) for topical delivery [18]. The value obtained in this study correlated well with the results found in literature (log D of 1.7) [20].

**Decision for optimal vesicle formulations**

The TEM images indicated that niosomes with a lipid ratio of 2:1 displayed better membrane formation than the niosomes ratio 1:1. Both formulations formed almost perfect spheres. The ufosomes formed perfect spheres with thick uniform membranes, but ufosomes ratio 2:1 appeared to have a stronger lipid membrane than ufosomes 1:1.

TEM analyses revealed all four formulations did produce vesicles, but proof that vesicles still existed after different sonication times was obtained by using light microscopy on the same non-sonicated and sonicated samples. With a magnification of 50x, only the larger droplets could be seen and it must be kept in mind that there were many other smaller droplets present in each formulation.

A better indication of droplet size was measuring the droplet size and droplet size distribution. For a stable formulation, a narrow uniform droplet size distribution is optimal [21], therefore sodium oleate:cholesterol lipid ratio of 2:1, sonicated for 3.5 min, was chosen as the optimal lipid formula for ufosomes. The ufosomes 2:1 also displayed a stronger lipid membrane from TEM analysis than the 1:1 ratio. The niosomes ratio 2:1 also appeared to have a better formed membrane than the 1:1 ratio. Niosomes had two formulas which revealed optimal droplet distribution, but the Span® 60:cholesterol 2:1 sonicated for 2.0 min was chosen as the optimal
niosome lipid preparation based on the ufosome decision. By having the same lipid ratios for both niosomes and ufosomes, unnecessary variations were minimized in order to make comparisons between the two vesicles easier.

The last step in the decision for an optimal formula was loading an appropriate concentration of the API into the chosen lipid ratios. The EE% of four possible concentrations of roxithromycin in niosomes and ufosomes was tested. All the niosome ratios had fairly close EE% with good physical appearances. The ratio of 2:1:1 had fairly good entrapment and increasing the amount of drug had little or no increase in EE%. The ufosomes’ EE% increased as the concentration of drug increased, but the physical appearance of the ratios 2:1:2 and 2:1:4 were poor with the formation of thickened gels. As a result, the ratio of 2:1:1 for both niosomes (59.81 EE%) and ufosomes (84.36 EE%) were chosen as the final optimal formulas for each vesicle system and the preparation of proniosomes and pro-ufosomes were also based on these ratios.

**Characterization of vesicles**

TEM analyses showed that all final formulations prepared had indeed formed vesicles and the images compared well to those obtained by Ammar *et al.* [29]. From the photomicrographs, niosomes displayed a dense arrangement of vesicles with various sizes; the cracks seen in the membranes may be due to the fusion of smaller vesicles. The pro-niosomes also consisted of various spherical vesicles. The ufosomes had a large amount of very densely packed vesicles mostly in the 50 nm size range, with a few larger particles in between. The pro-ufosomes appeared to be more spaced out with a rougher membrane appearance than the rest of the carriers.

The results obtained from the light microscopy of all the vesicles encapsulating the three solid-state forms of roxithromycin was similar to the photomicrographs taken of the optimal formulas during the process of deciding on the optimized formulas, as discussed above. The droplet size of the sonicated samples was very small, so the particles seen using light microscopy with magnification of 50x were not very clear.

The zeta-potential values of all carrier systems were well into the negative axis, which concluded that the chances of aggregation and fusion of any of the vesicles was rather limited and that all the vesicle membranes were stable [22,30]. The ufosomes and pro-ufosomes were
slightly more charged than the niosomes and proniosomes and as a result, the particles had a stronger repelling force on one another.

The droplet size distribution of the vesicles revealed narrow distribution curves, which had a positive influence on the stability of the vesicles [22]. Niosomes and proniosomes sonicated for 2.0 min had a fairly larger average particle size than the ufosomes and pro-ufosomes sonicated for 3.5 min, which was attributed to the different sonication times needed for good size distribution curves.

The determined pH values of all the systems illustrated that niosomes and proniosomes had a slightly lower pH than ufosomes and pro-ufosomes, probably due to the latter consisting of sodium oleate rather than Span® 60. Nevertheless, all systems had a pH very close to that of the buffer (pH 7.4) used in experiments which guaranteed no crystallization or salt formation.

Lastly, the entrapment efficiency of all systems illustrated that ufosomes and pro-ufosomes had the best entrapment efficiency, followed by niosomes and proniosomes. The better entrapment efficiency can be explained by referring to a similar study conducted by Agarwal et al., [30]. The difference seen was attributed to the API having greater affinity to the sodium oleate of ufosomes and pro-ufosomes than to the Span® 60 of niosomes and proniosomes, which resulted in more API being entrapped in the aforementioned. It should also be mentioned that not one solid-state form had the best overall entrapment as it varied for each type of vesicle.

**Membrane release studies**

The membrane release studies revealed API was released from all twelve formulations and this ruled out any problems related to the release from vesicles if no API was found in the skin during topical diffusion studies. The release results illustrated that the API was better released from the niosomes and proniosomes, which again was due to the API having lower affinity to the Span® 60 than for sodium oleate (ufosomes and pro-ufosomes) [37]. In general, a trend according to the type of vesicle system can be seen with niosomes having by far the best release, followed by proniosomes, then pro-ufosomes and ufosomes having the least amount of API release of all vesicles. The release trend was grouped according to vesicle types, whose results were very close, rather than the solid-state form of API as no form had a leading release concentration. It was important to remember that the samples with the best release were not
necessarily expected to have the best topical diffusion as there were other factors which also needed to be taken into consideration.

**Skin diffusion studies**

The topical diffusion study was able to distinguish the concentration of roxithromycin in the receptor phase (representing the bloodstream), the stratum corneum-epidermis and the epidermis-dermis. No quantifiable amounts (only traces) of API were found in the stratum corneum-epidermis, which shows that the API successfully penetrated through this tough layer and only four vesicles showed small API concentrations in the receptor phase, which may have been because of differences in the hydration, damage and age of skin used from different donors causing differences in API delivery [18]. The percentages diffused were very small but worth mentioning as they are above the lower limit of quantification (LLOQ; 0.5 µg/ml) obtained during HPLC validation of this study. The topical diffusion studies revealed targeted delivery to the epidermis-dermis only, which proved very successful as this is the target area for roxithromycin to have activity against acne causing micro-organisms [7,8].

Once again, as with the membrane studies, there was a noticeable trend which was grouped together by vesicle type rather than by the type of solid-state form of roxithromycin encapsulated. The niosomes were the vesicle system which had the best overall topical delivery regardless of which solid-state form it contained, the ufosomes had the second best API diffusion, followed by the proniosomes and lastly, the pro-ufosomes. When the delivery of the four vesicle systems is compared; roxithromycin monohydrate had the highest delivery from the ufosomes, while the glass and chloroform desolvate were best delivered from the niosomes.

There are many factors to take in to consideration when discussing the reason for this result. Firstly, niosomes may have had better API delivery than the ufosomes, because of the better API release from the formulas; which resulted in a larger concentration for diffusion from the surface of the stratum corneum to the epidermis-dermis [18]. The same can be said about the proniosomes and their better topical delivery in comparison to pro-ufosomes. As mentioned above, the affinity of the API for the sodium oleate (ufosomes and pro-ufosomes) was much greater than for the Span® 60 (niosomes and proniosomes), which illustrated that even though the ufosomes and pro-ufosomes had the best entrapment efficiencies (which usually results in
better delivery), the API was poorly released because of its great affinity to these vesicles [37]. Ufosomes had the second best API topical delivery despite their poor release from vesicles, which shows that the API from these vesicles had exceptionally good diffusion through the skin, as many more droplets had to release API for a fair amount to diffuse to the target area compared to niosomes and proniosomes which had excellent release. This good diffusion may be attributed to the many very small particles being densely packed, as illustrated on the TEM images, compared to the other vesicles which made it possible for many droplets to line the stratum corneum; therefore increasing the available surface area for API release [37]. The morphology of the vesicles also revealed the “cracks” in the niosomes' membranes, which may have contributed to the easier release of API from these vesicles. The properties of the ingredients of the vesicles also played a major role in the topical delivery. The Span® 60 (niosomes and proniosomes) had a lower melting point and as a result, a better solubility than the sodium oleate (ufosomes and pro-ufosomes) [38,39]. Compounds with lower melting points, along with better solubility, tend to have better skin penetration and topical delivery and this explains why the API from niosomes had better delivery than ufosomes and proniosomes had better delivery than pro-ufosomes [18]. Components of the Span® 60 membranes may have also diffused after the release of the API due to its good solubility and its penetration enhancing effect. The Span® 60 and sodium oleate are also known as penetration enhancers for lipophilic drugs and each ingredient may have had different degrees of penetration enhancement [37]. Span® 60 and sodium oleate are also known as gelling agents which have the ability to form gels when added to other components [40,41]. The reason for the proniosomes and pro-ufosomes having the lowest topical delivery may be due to the thickening of both formulas after the 12 h diffusion studies. The thick gel prevented optimal release and delivery, which may only have occurred with these two formulas because of the extra sorbitol powder which contributed to the total mass of the systems.

When a comparison was done between the better topical deliveries of the three solid-state forms of roxithromycin, regardless of which vesicle it was in, the two amorphous forms of roxithromycin had higher topical delivery than the crystalline monohydrate. The roxithromycin
glass was by far the form which had the highest dermal concentration, followed by roxithromycin
chloroform desolvate and lastly, the crystalline roxithromycin monohydrate.

The results correlated well with the aim of this study for improving the topical delivery of the
roxithromycin crystalline form by using its two amorphous forms. The increased topical delivery
results suggested that the amorphous forms retained their theoretical improved solubilities,
determined by Liebenberg & Aucamp [15] and Liebenberg et al., [14], during the 12 h diffusion
studies while being encapsulated in the vesicles. The use of amorphous forms and vesicles
proved successful in increasing topical delivery of roxithromycin.
Conclusions

This study illustrated that roxithromycin and its two solid-state amorphous forms were capable of being entrapped into selected vesicles (niosomes, proniosomes, ufosomes and pro-ufosomes). These vesicles were all successful in releasing the drug for diffusion into the skin and all systems had targeted delivery specifically into the epidermis-dermis where the therapeutic effect of roxithromycin against *P. acnes* is needed. The niosomes, in total, delivered the highest amount of API followed by ufosomes, proniosomes and lastly, pro-ufosomes. Higher roxithromycin concentrations were found in the dermis with the amorphous forms in comparison to the crystalline form, which suggests that the amorphous forms increase the topical delivery of roxithromycin.

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Disclaimer

Any opinion, findings and conclusions, or recommendations expressed in this material are those of the authors and therefore the NRF does not accept any liability in regard thereto. The authors declare no conflict of interest.
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