How lipid nanocarriers improve transdermal delivery of olanzapine

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A B S T R A C T

In the present work, the development of a transdermal nanocarrier drug delivery system with potential for the treatment of psychiatric disorders, such as schizophrenia and bipolar disorder, is described. Lipid nanocarriers, encompassing various solid:liquid lipid compositions were formulated and assessed as potential nanosystems for transdermal delivery of olanzapine. A previously optimised method of hot high pressure homogenization (HPH) was adopted for the production of the lipid nanocarriers, which comprised solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and nanoemulsions (NE).

Precirol® was selected as the solid lipid for progression of studies. SLN exhibited the best performance for transdermal delivery of olanzapine, based on *in vitro* realease and permeation studies, coupled with results from physicochemical characterization of several solid:liquid lipid formulations. Stability tests, performed to give an indication of long-term storage behavior of the formulations, were in good agreement with previous studies for the best choice of solid:liquid lipid ratio.

Overall, these findings highlight the SLN-based formulation as promising for the further inclusion in and production of transdermal patches, representing an innovative therapeutic approach.

Keywords: Lipid nanocarriers; Nanostructured lipid carriers; Solid lipid nanoparticles; Nanoemulsions; Olanzapine; Transdermal drug delivery system

1. Introduction

The skin is the human body's largest organ and a key barrier in transdermal drug delivery (1, 2). The skin consists of three main layers: the epidermis, dermis, and hypodermis. The epidermis, in particular the outermost layer known as the stratum corneum, is the main biological barrier in drug delivery. The stratum corneum is a highly lipophilic membrane comprising tightly packed corneocyte cells embedded within intercellular lipids (3). It acts as an excellent natural barrier, which poses problems for transdermal delivery systems limiting the number of drugs that can cross it, to achieve a therapeutic effect.

Transdermal drug delivery is used in the treatment of a range of conditions, such as smoking cessation (nicotine patches), pain management (fentanyl patches) and angina (glyceryl trinitrate patches). However, other therapeutic areas remains largely unexplored. In the present work, the potential of transdermal delivery of olanzapine, in the treatment of psychiatric disorders such as, schizophrenia and bipolar disorder, was investigated. Olanzapine is currently available as an injection (intramuscular) and in oral dosage forms. It is usually administered as one or two daily doses, with an overall dosage of 5–10 mg/day. Despite being an effective antipsychotic, its low oral bioavailability and low compliance amongst psychiatric patients are serious problems, which modern pharmaceutical technology aims to overcome by developing a transdermal drug delivery system (4). Olanzapine is a leading candidate for transdermal drug delivery, due to its lipophilic nature (log P 2.8), low molecular weight (MW=312.4) and low melting point (195◦C) (5). Apart from the physicochemical properties, its poor oral bioavailability and extensive first-pass metabolism (approximately 40% of the dose is metabolized before reaching the systemic circulation), ideally suit this drug for transdermal administration (6). This route of administration of olanzapine is suitable for patients who cannot tolerate oral dosage forms and does not come with the drawbacks of drug delivery via injection, such as patient compliance, risk of infection and invasive nature, among others (4).

Previous work on transdermal delivery of olanzapine is limited. Nanocarrier-entrapped olanzapine has previously been formulated by Aggarwal et al., who looked into the effects of different permeation enhancers in transdermal drug delivery systems (TDDS) of olanzapine (4). Others have looked at the effect of the lipid matrix, on the entrapment efficiency of olanzapine as well as its release behaviour (7). Research into coencapsulated olanzapine-simvastatin formulations for transdermal delivery, has also been explored (8). Currently, the prospect of olanzapine delivery via transdermal patches, is a relatively new area of research, which requires additional research and optimisation before clinical evaluation and potential commercialization.

This work focuses on the use of solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and nanoemulsions (NE), as potential TDDS of olanzapine. The nature of lipid nanoparticles confers them distinct advantages over conventional nanocarrier systems, such as excellent tolerability, physical stability, and the possibility to modulate drug release (9).

Lipid nanoparticles can be produced using various methods, including high shear homogenization (10), nanoprecipitation (11), spray drying (12), solvent emulsification–evaporation (13) and by high pressure homogenization (HPH) (14). Due to previously optimized preparation strategies, where better performance with respect to drug loading and *in vitro* permeation rate was attained (Vitorino et al. 2014), a hot HPH method was employed in this study. Moreover, this established simple and very cost-effective technique does not have the scale-up problems associated with some other nanoparticle production methods (15).

Physicochemical parameters, such as particle size, zeta potential, polydispersity index and entrapment efficiency, can have a significant effect on the nanocarrier system and its *in vitro* performance in release/permeation studies. Characterisation of the lipid nanoparticles is therefore an essential step, in order to select for the best candidate, for transdermal delivery of olanzapine.

The aim of this study is to identify the best solid lipid candidate for lipid nanocarrier (LN) formulation (i.e. the one which provides the best target product quality profile for administration through the skin) and to systematically assess how the composition of nanoparticles, in terms of solid:liquid lipid ratio, affects different physicochemical parameters. A combination of *in vitro* release and permeation studies, whilst considering previously highlighted parameters, should allow selection of a solid:liquid lipid ratio best suited for transdermal drug delivery of olanzapine.

2. Materials and methods

2.1. Materials

Olanzapine was obtained from Zhejiang Myjoy (Hangzhou, China). Compritol® 888 ATO (glycerylbehenate, melting point: 71–74 ◦C), stearic acid (melting point: 70 ◦C) and Precirol® ATO 5 (glyceryl palmitostearate, melting point: 53–56 ◦C) were provided by Gattefossé (Saint-Priest, Cedex, France). Oleic acid, polysorbate 80 (Tween® 80) and phosphate buffered saline (PBS) pH 7.4, were purchased from Sigma-Aldrich (Sintra, Portugal). All other solvents and reagents were of analytical or high performance liquid chromatography (HPLC) grade.

2.2. Preparation of lipid nanocarriers (LN) by high pressure homogenisation

Hot high pressure homogenisation (HPH) was used to produce the lipid nanoparticles, comprising SLNs, NLCs and nanoemulsions. A concentration of 7.5 % w/V of the lipid phase (solid and/or liquid lipid) was melted at 10 ºC higher than the solid lipid melting point. This was then mixed with 30 mL of 3% (w/V) Tween® 80 in water, at the same temperature (80ºC), and pre-emulsified for 1 min, using an Ultra-Turrax X1020 (Ystral GmbH, Dottingen, Germany) at 25,000 rpm. The pre-emulsion was then put through hot HPH at 1000 bar for 2.5 min using an Emulsiflex®-C3 (Avestin Inc, Ottawa, Canada). The resultant dispersion was then stored at 4 ◦C to form the LN.

For olanzapine-loaded formulations, 80 mg of olanzapine was incorporated into the melted lipid phase. This amount was used following preliminary solubility studies. NLC formulations of varying solid lipid, were prepared according to the ratios shown in **Table 1**.

Table 1. Composition of NLCs prepared from different solid lipids; Precirol®, Compritol® and stearic acid.

Formulation (50:50)	Amount of solid lipid (g)	Amount of liquid lipid (g)
Precirol®:oleic acid	1.125	1.125
Compritol®: oleic acid	1.125	1.125
Stearic acid:oleic acid	1.125	1.125

The amounts of Precirol® and oleic acid used to carry out investigations, after the selection of solid lipid, are shown in **Table 2**. The term lipid nanocarrier/s (LN) encompasses all physical forms.

Table 2. Ratios of Precirol®:oleic acid used in formulations. The physical form of nanocarrier produced by this methodology resulted in the production of SLNs, NLCs or NE, depending on the ratio of Precirol:oleid acid.

Formulation (Precirol®:oleic acid ratio)	Amount of solid lipid (g)	Amount of liquid lipid (g)	Type of nanocarrier
100:0	2.250	0.000	SLN
75:25	1.688	0.563	NLC
50:50	1.125	1.125	NLC
25:75	0.563	1.688	NLC
0:100	0.000	2.250	NE

Note that although NLCs are generally obtained from blends of solid with liquid lipids, preferably in a ratio of 70:30 up to a ratio of 99.9:0.1 (16), recent studies have reported the use of lower solid to liquid lipid ratio (e.g. 50:50 ratio (17), or even lower (18, 19)) without compromise of their solid nature.

2.3. Particle size analysis

Dynamic light scattering (DLS) was used to determine the mean hydrodynamic diameter and polydispersity index (PI). The PI measures the width of the size distribution. These were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) set at a detection angle of 173◦ at 25◦C. Samples were appropriately diluted in ultra-purified water (pH ≈ 5.5) and analysed three times each. Cumulants method was used for data analysis. Results are shown as mean ± standard deviation.

2.4. Zeta potential

Zeta potential (ZP) reflects the charge on the particle surface, giving an indication of physical stability of the system. ZP was determined using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) set at a temperature of 25 ◦C. Samples were diluted suitably in ultra-purified water and analysed three times each. The Helmholtz-Smoluchowsky equation was used for ZP calculation.

2.5. Occlusive effect studies

To study the effect of the LN formulations on skin occlusivity, *in vitro* occlusion tests, modified from de Vringer and de Ronde were performed (20, 21). Beakers (50 mL) were filled with 30 g of water and covered with filter paper (cellulose acetate filter, 90 mm, Whatman nº6, cutoff size 3 μm), and sealed. 600 μL of LN formulation was spread homogeneously across the filter paper, covering the entire surface area of 15.9 cm^2. These were then stored at 32 ºC for 48 h to simulate skin surface temperature. The weight of water remaining in the beakers, was weighed at time intervals of 24 and 48 h. Beakers in the

same conditions, but covered with 600 μL of water were used as a reference. The tests were conducted in triplicate for each formulation. The occlusion factor (F) was calculated according to Eq. 1;

$$F = (A\text{-}B / A) \times 100 \quad \text{Eq. 1}$$

where A is the water loss without formulation applied (reference) and B is the water loss with LN formulation applied. A value of 0 indicates no occlusive effect compared to the reference, while a value of 100 corresponds to maximum occlusiveness.

2.6. Entrapment efficiency and drug loading

Entrapment efficiency, relates to the amount of drug that can be incorporated in LN, either within the nanoparticle or adsorbed onto its surface. Olanzapine entrapment efficiency was determined indirectly, by measuring the concentration of free drug within the aqueous phase of the LN dispersion. An ultrafiltration-centrifugation method was performed, using centrifugal filters (Amicon® Ultra-4, Millipore, Germany) with a molecular weight cutoff 1000 kDa. 500 μL of the formulations were pipette into the top chamber of the centrifuge filter and centrifuged for 45 min at 3000 x g at 4 ◦C. 400 μL of the aqueous dispersion phase was collected from the outer centrifugal filter after separation and combined with 400 μL of mobile phase for drug quantification. The mobile phase comprised ammonium acetate (0.02 M in water), methanol and acetonitrile in the volume ratio 30:35:35. The total amount of drug present in the system was also quantified. For that, 50 μL of the nanoparticle formulation was diluted into 1950 μL of the mobile phase and heated at 60 ◦C for 15 min. The formulation was then centrifuged for 15 min at 12600 x g and the supernatant filtered using a 0.22 μm membrane for drug quantification. All drug quantification was carried out by high performance liquid chromatography (HPLC) as described in Section 2.10. Entrapment efficiency (EE) and drug loading (DL) were calculated using Eq. 2 and Eq. 3;

$$\% EE = (W_{total\ drug} - W_{free\ drug} / W_{total\ drug}) \times 100 \quad \text{Eq. 2}$$

$$\% DL = (W_{total\ drug} - W_{free\ drug} / W_{lipid}) \times 100 \quad \text{Eq. 3}$$

where $W_{total\ drug}$ is the amount of olanzapine determined in the whole system, $W_{free\ drug}$ is the amount of free drug determined in the aqueous phase after separation of the nanoparticles and W_{lipid} is the weight of the lipid phase.

2.7. *In vitro* release studies

In vitro release studies were conducted using static Franz diffusion cells (PermeGear, Inc., PA, USA) with a diffusion area 0.636 cm^2 and a receptor compartment of 5 mL, as described by Vitorino et al. (8). Tests were performed using a dialysis cellulose membrane (MWC0 ~12,000, avg. flat width 33 mm, D9652, Sigma-Aldrich, Sintra, Portugal), which was clamped between the donor and receptor compartments. The receptor compartment contained a medium of 70% PBS (pH=7.4) and 30% ethanol to ensure sink conditions. The receptor medium was stirred at 600 rpm and maintained at 37 ◦C, ensuring a temperature of 32 ◦C at the skin surface in order to mimic skin conditions. 400 μL of the formulations were applied to the donor compartment and to prevent evaporation of formulation, covered with

Parafilm®. Samples of the receptor medium were collected at set time intervals over a period of 48 h and volumes extracted were replenished with the same volume of fresh medium. Samples were stored at 4◦C until analysis by HPLC (Section 2.10.).

2.8. *In vitro* permeation studies; skin preparation and integrity tests

In vitro permeation studies were carried out in the same manner as *in vitro* release studies (Section 2.7.), except new-born pig epidermis was used as the skin model. The new-born pig (3 weeks,~ 6 kg) was obtained from the local slaughter house. Using the heat separation technique, the subcutaneous fat was sectioned off and the epidermis separated from the underlying dermis. The skin was immersed in 60 ◦C hot water for 1 min and then the epidermal layer was gradually removed from the dermis. The epidermis sheets were cut into 2.5 cm x 2.5 cm pieces, wrapped in aluminium foil and stored at 20 ◦C until used. One day prior to conducting the studies, frozen skin pieces were thawed and placed in PBS (pH 7.4) to be hydrated overnight in the refrigerator (~ 4 ◦C). Before starting the studies, skin integrity was checked using a VapoMeter® (Delfin Technologies Ltd, Kuopio, Finland) to measure the transepidermal water loss (TEWL). Only skin samples with TEWL values of less than 15 g/m^2.h were used in studies. Skin and sample preparation techniques used, complied with European guidelines (22).

2.9. Calculations

The cumulative amount of olanzapine diffused per unit area of skin (Q_n) is given by;

$$Qn = \left(C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_I \right) / A \qquad \text{Eq. 4}$$

and is expressed in μg/cm^2.This was plotted as a function of time (t, h). In Eq. (4), C_n corresponds to the drug concentration in the receptor medium at each sampling time, C_i is the drug concentration of the i^{th} sample, A the effective diffusion area, V_0 and V_i represent the volumes of the receptor compartment and the sample, respectively. The total quantities of the drugs obtained after 24 h (Q24) and 48 h (Q48) were used for comparison among formulations. The slope of the linear region of the plot; amount of drug permeated by unit area *versus* time, was used to calculate the flux at steady state. According to Fick's first law of diffusion, the flux (μg/cm^2.h) can be expressed by;

$$Jss = C_0KD/L = C_0K_p \qquad \text{Eq.5}$$

where C_0 represents the drug concentration in the donor compartment, D is the diffusion coefficient, L the thickness of the membrane and K the partition coefficient of drug between membrane and vehicle. K_p stands for the permeability coefficient. The lag time (t_{lag}) which relates to the amount of time required to achieve steady state flux of a drug through the skin, was estimated by extrapolation of the linear portion of the plot to the x-axis.

For release studies, only the cumulative percentage of drug was considered and plotted, using the same method as described above for permeation studies.

2.10. HPLC determination of olanzapine

HPLC analysis was performed using a Shimadzu apparatus (Shimadzu Co., Kyoto, Japan) equipped with a quaternary pump (LC-20AD), a degasser unit (DGU-20A5), an auto-sampler (SIL-20HT), an oven (CTO-10AS) and an UV/VIS photodiode array detector (SPD-M2OA). A Luna Phenyl-Hexyl (5 µm; 150 mm x 3 mm) Phenomenex (USA) analytical column was used for analysis. The mobile phase consisted of ammonium acetate (0.02M in water): methanol: acetonitrile in the ratio 30:35:35 (V/V/V) and was set at a constant flow rate of 0.8 mL/min. A run time of 4 min was established and detection was carried out at 230 nm. A10 µL injection volume was used for each sample and calibration curve standards. Under these conditions, olanzapine was eluted at around 2 min. This method was previously validated (23).

2.11. Stability studies

The physical stability of the LN formulations was accessed using the LUMiFuge® (L.U.M. GmbH, Germany) stability analyser. This comprised an analytical centrifugation system, which measures the intensity of transmitted near infrared (NIR) light, over the total length of the cell, while the sample is being centrifuged. LUMiFuge® analysis allows attainment of an instability index value as well as transmission profiles, which enables comparison of phase separation and destabilization of formulations. The instability index is quantified by dividing the clarification of the sample at a given separation time, by the maximum sample clarification. Clarification quantifies the resultant increase in transmission, due to phase separation by creaming and flocculation. The instability index is a dimensionless number between 0-1, where “0” means no changes in particle concentration (stable formulation) and “1” means the dispersion has completely phase separated (unstable formulation). Thus, low values indicate greater physical stability (24, 25).

Transmission profiles, unlike instability índex values, are displayed in real time as a function of radial position, allowing study of the formulation characteristics with respect to time. Analysis of changes in light transmission and movements in phase boundaries, allows comparisons in separation behaviour of formulations. Formulation stability was analysed after 210 min centrifugation, at a speed of 2300 x g at 25 ◦C.

2.12. Statistical analysis

Statistical significances were evaluated using student t-test. A value of $p<0.05$ was considered significant. This analysis was performed using Microsoft Excel® (Microsoft®Excel®, version 14.5.1)

3. Results & discussion

The development of a suitable nanocarrier system as a strategy to promote the transdermal delivery of olanzapine is described. This included the selection of an appropriate nanoparticle lipid matrix, the characterization and assessment of the nanosystem in terms of physicochemical, performance and stability. Preliminary screening solubility studies were performed for the selection of the solid lipid.

3.1. Selection of solid lipid

From **Table 3,** it can be seen that Precirol®-based nanoparticles exhibited the smallest size, followed by Compritol® and lastly stearic acid. NLC particle sizes usually range between 40-1,000 nm, where smaller particle sizes are generally most desirable (8). It should be noted that a smaller particle size leads to greater surface area contact between the NLC and skin surface, allowing for a higher drug absorption rate through the skin and, in turn, greater efficacy as a transdermal drug delivery system (TDDS) (17). A low polydispersity index value is desirable, as it means that the formulation is more homogenous. PI values range from 0 (monodisperse) to 0.5 (very broad distribution), where values less than 0.1 indicate high homogeneity and a narrow size distribution, making them optimal for TDDS (26). From **Table 3**, it can be seen that PI values were very similar. In general, PI values of less than 0.3 are considered optimal for the particle dispersion and homogeneity of such nanoparticles (27).

Table 3. Characterisation of 50:50 ratio, solid: liquid lipid NLC with varying solid lipid. Key: PI: Polydispersity index, F: Occlusion factor; where F24h and F48 are the occlusion factors at 24 and 48 hours, respectively. Results are expressed as mean± SD (n=9)

Formulation	Hydrodynamic diameter (nm)	PI	Zeta potential (mV)	F 24h	F 48h
Precirol®	209.17 ± 2.93[b]	0.295 ± 0.008	-29.58± 2.10	63.54±1.18	63.62±2.68
Compritol®	290.34± 28.84[a]	0.299 ± 0.013	-28.91±0.89	59.99±1.84	55.97±3.81
Stearic acid	396.52± 7.23[a,b]	0.330± 0.016	-32.89±1.18[b]	61.59±1.77	59.59±1.80

[a] $p<0.05$ vs. Precirol®, [b] $p<0.05$ *vs*. Compritol®

The F values at 24 h and 48 h did not vary significantly. A high occlusive effect is desirable, as this will result in lower transepidermal water loss, where the resulting hydration effect, leads to disorganisation of corneocyte packing within the stratum corneum (28). This has a direct effect on percutaneous absorption and improves drug permeation through the skin, once released from the drug dosage form (29). The results (**Table 3)** are in agreement with Müller et al. (30) study, where it was found that NLCs with smaller particle size, tend to have greater occlusive effect due to their tight packing and

greater particle surface – skin contact, forming a film which inhibits evaporation from the skin surface (31).

Zeta potential was also used as a selection parameter; in general, zeta potential values of -30 mV or less are desirable in nanoparticle production, resulting in high physical stability (17). All formulations had very similar zeta potential values, approximating -30 mV; thus, making them suitable for nanoparticle preparation, as the repulsion between the charged particles prevents aggregation (32).

Taking all these physicochemical parameters into consideration, it was concluded that Precirol® was the most suitable solid lipid to progress with further investigations, as it exhibited the most promising properties to ensure the drug penetration through the skin: it allowed for production of small nanoparticles, which had a relatively tight size distribution and were physically stable. Additionally, olanzapine incorporated in nanoparticles using Precirol®, would be subjected to a lower termal stress due to its lower melting point (53–56 ◦C), compared to Compritol® and stearic acid.

3.2. Evaluation of the influence of solid:liquid lipid ratio

In general, as the ratio of solid:liquid lipid decreased, the mean particle size of the lipid nanocarriers tended to decrease (**Table 4).** This behaviour was more evident for unloaded nanoparticles. These findings are in agreement with the majority of existing literature and can be attributed to a lower viscosity of the dispersed phase (8). The same trend was observed for PI values, indicating greater formulation homogeneity as the proportion of liquid lipid is increased.

With regards to zeta potential, as the solid:liquid lipid ratio decreased, more negative zeta potential values were obtained. Irrespective of the presence of olanzapine, all the formulations except LN100:0 can be considered stable, since they usually tend to be close to or lower than -30 mV. Such behaviour could be attributed to the inclusion of oleic acid. This is contradictory to Arts et al. (33) findings, where the addition of oleic acid resulted in a slight increase in zeta potential values. It should be noted that incorporation of olanzapine within the LN 100:0 formulation, produced stable OL-LN 100:0 nanoparticles, with a zeta potential value of approximately -30 mV (17).

From **Table 4,** it can be seen that for the different solid: liquid lipid ratios, as the amount of liquid lipid was increased the occlusive effect decreased. This is in agreement with Souto et al. (34), who when comparing NLC with varying liquid lipid content concluded that an increase in liquid lipid content led to a decrease of the occlusive factor. Thus, as mean particle size of LNs decreased so did the respective occlusive factor; this is in contradiction with the findings in **Table 3**. An explanation for this could be related to the degree of crystallinity of the lipid nanoparticles (35). The greater crystallinity would result in the formation of a tightly packed dense film, which would intrinsically prevent transepidermal water loss to a greater degree. Such a behaviour could be attributed to the direct influence of the different solid lipid content of the formulations.

Table 4. Characterisation of olanzapine-loaded and unloaded lipid nanocarrier formulations of varying Precirol®:oleic acid composition. Key: PI: Polydispersity index, EE: Entrapment efficiency, DL: Drug loading, F: Occlusion factor; where F 24h and F48h are the occlusion factors at 24 and 48 hours, respectively; OL = Olanzapine, LN = Lipid nanocarriers, OL-LN = Olanzapine loaded lipid nanocarrier formulation. Results are expressed as mean± SD (n = 9).

Formulation solid: liquid lipid ratio	Hydrodynamic diameter (nm)	PI	Zeta potential (mV)	EE (%)	DL (%)	F 24h	F 48h
UNLOADED FORMULATIONS							
LN 100:0	653.60±72.73	0.351±0.023	-20.95±0.42		-	-	-
LN75:25	347.45±39.30[a]	0.297 ± 0.014	-29.40±1.96[a]	-	-	-	-
LN 50:50	209.17±2.93[a]	0.295±0.008	-29.58±2.10[a]	-	-	-	-
LN25:75	157.27±3.20[a]	0.249± 0.006	-36.45±2.36[a]	-	-	-	-
LN 0:100	155.35±4.14[a]	0.207 ± 0.014	-40.75±3.35[a]	-	-	-	-
OLANZAPINE-LOADED FORMULATIONS							
OL-LN 100:0	509.19±78.56[c]	0.316±0.041	-28.77±1.73[c]	94.56±3.58	2.60±0.17	59.60±2.87	64.91±0.34
OL-LN 75:25	454.91±35.08[b,c]	0.281±0.036	-31.34±1.62[b,c]	97.25±1.60	2.72±0.81	59.25±1.08	64.88±0.43
OL-LN 50:50	194.30±14.38[b,c]	0.290±0.020	-30.51±3.31	98.28±1.16	2.86±0.25	54.81±1.10	59.04±2.10
OL-LN 25:75	174.00±6.92[b,c]	0.239±0.012	-32.88±1.55[b,c]	98.52±1.04	2.77±0.22	43.31±2.85	43.21±2.35
OL-LN 0:100	165.24±4.32[b,c]	0.214±0.033	-39.68±2.57[b]	97.89±0.40	3.01±0.40	40.80±2.89	35.22±2.27

[a] $p<0.05$ *vs*. LN 100:0, [b] $p<0.05$ *vs*. OL-LN 100:0, [c] $p<0.05$ *vs*. corresponding LN

3.2.1. Entrapment efficiency and drug loading

The solubility of olanzapine is known to be higher in oleic acid than in Precirol®, so it is likely, that as the amount of liquid lipid incorporated is increased so would the entrapment efficiency and consequent drug loading (8).

Table 4 however, shows no significant difference in entrapment efficiency between the different nanoparticle formulations, suggesting that the inclusion of liquid lipid allows no significant increase in entrapment efficiency. A possible explanation for this is that the amount of olanzapine used was below the saturation limit, in that the lipid matrix had not reached its maximum drug loading capacity for EE differences to become apparent (7).

The overall entrapment efficiencies of all formulations were between 97 and 98%. This confirmed that the drug dissolved in lipid matrix, remained associated with the matrix and that there was no drug diffusion (36).

3.3. *In vitro* release studies

Release studies give a preliminary indication to release behaviour of the drug from the formulation, in the absence of the main biological barrier, the stratum corneum. Release from the LNs depends on the partitioning of olanzapine within the lipid matrix and the subsequent rate of diffusion from the dialysis membrane.

Fig.1 shows that in general, as the liquid lipid ratio is increased the release of olanzapine is slower. The solid lipid nanoparticle formulation is an exception to this; such behaviour could be attributed to its greater particle size (**Table 4**), that may have decreased drug diffusion from the nanoparticle surface.

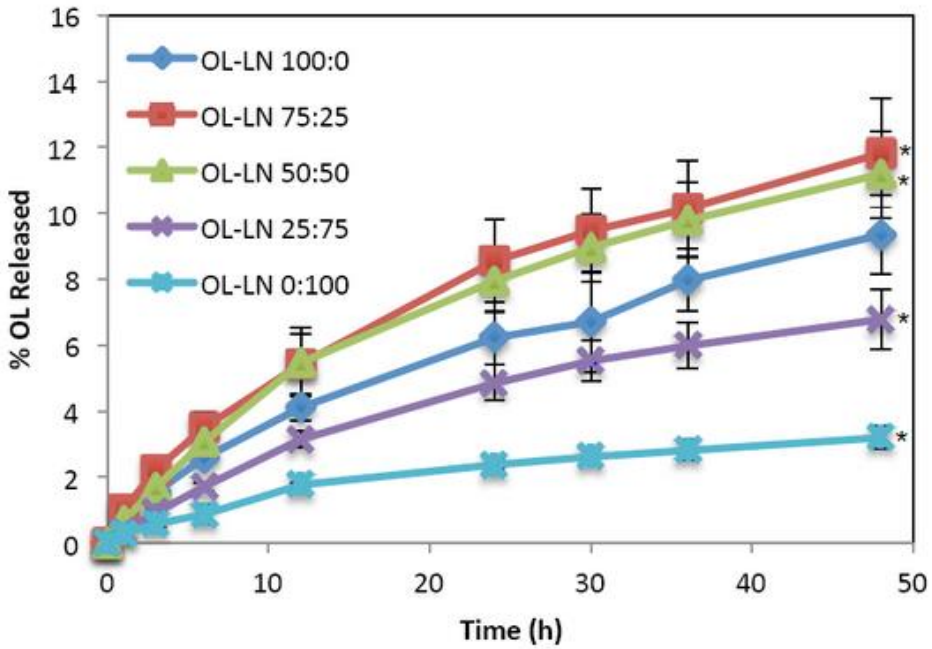


Fig. 1. *In vitro* release profiles of olanzapine-loaded LNs (OL-LN), of varying solid:liquid lipid composition. Release medium composed of 70% PBS (pH=7.4) and 30% ethanol. The results are expressed as mean ± SD (n = 6); * $p<0.05$ *vs*. OL-LN 100:0.

It should be noted that although SLN formulations did not produce the fastest release profile, results are significantly faster than previous release studies (34). Such behaviour could be attributed to the

production of SLN type II nanocarriers, which are referred to as the drug enriched shell model (15, 29). This model is additionally supported by the more negative zeta potential values obtained after olanzapine incorporation into 100:0 LN formulations (**Table 4**), indicating drug disposition at the surface of the nanoparticle (9).

Despite OL-LN 0:100 and OL-LN 25:75 formulations having a greater proportion of liquid lipid and a resultant smaller particle size, they produced the slowest release profiles. This may be due to olanzapine having a greater solubility in oleic acid; the favourable lipophilic conditions within the nanoparticle may have impeded partitioning of olanzapine, from the lipophilic environment within the nanolipid matrix, to the external aqueous medium (28). In this instance, the solubility of olanzapine into oleic acid, dominates over the effects of particle size.

Although the diffusion of a drug is usually more limited in a solid matrix in comparison to a liquid matrix, the dominant effect is the high solubility of olanzapine in oleic acid, which imparts a low partition between the oil and aqueous phase.

All formulations exhibited initial burst release followed by a sustained release. The initial burst release is likely to be due to drug adsorbed on the nanoparticle surface and sustained release due to diffusion of drug from the lipid matrix (7).

3.4. *In vitro* permeation studies

In vitro permeation studies allow mimicking of physiological and anatomical conditions, to measure drug permeation across a membrane; being an important step in the TDDS development. Permeation studies consider the release of the drug from the lipid nanocarriers followed by penetration of the permeant into the stratum corneum. Release behaviour cannot, however, be directly related with permeation, as this considers the additional barrier of the stratum corneum (8). This is illustrated when comparing **Fig. 1** and **Fig. 2**.

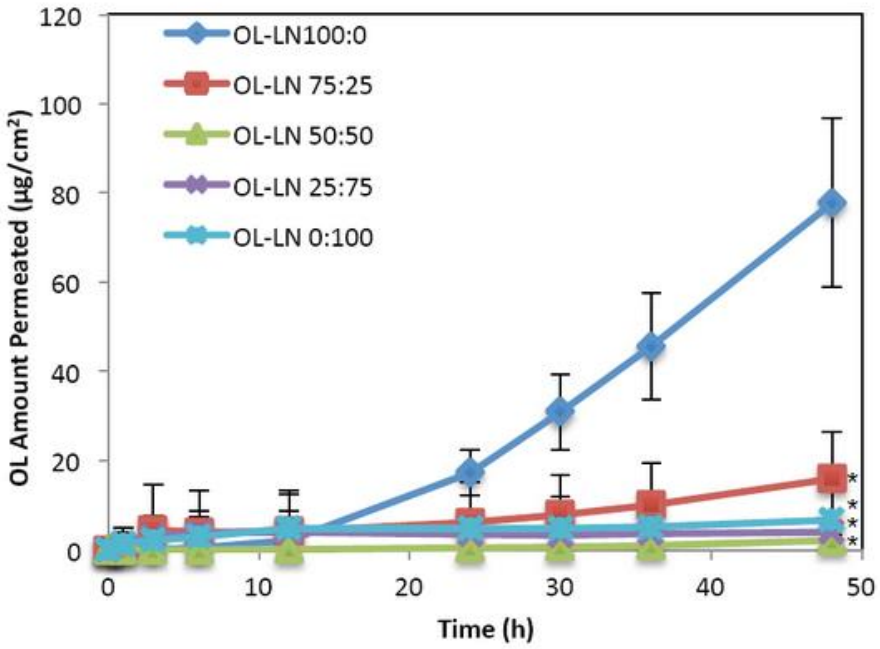


Fig. 2. *In vitro* permeation profiles of olanzapine-loaded LN (OL-LN), of varying solid:liquid lipid composition. Permaetion medium composed of 70% PBS (pH=7.4) and 30% ethanol. The results are expressed as mean ± SD (n = 8), * $p<0.05$ *vs.* OL-LN 100:0.

Particles with a higher proportion of solid lipid (OL-LN 100:0 and OL-LN 75:25), presented the fastest flux, in particular the SLNs (**Fig. 2**). This is in concordance with occlusive factor results (**Table 4**) and release studies (**Fig. 1**). Additionally, this corroborates the previous model suggested for the structure of SLN type II. This has already been observed by Teeranachaideekul et al. (35), where SLNs were found to provide a greater occlusive effect due to their greater crystallinity, which allows formation of a thin occlusive film that prevents transepidermal water loss. The greater water retention, increases inter-corneocyte gaps, providing a more disorganised structure of the stratum corneum, enhancing drug permeation.

Permeation parameters derived from the permeation studies are shown in **Table 5**. SLN formulation had the highest flux and permeability coefficient, which gave rise to greater Q24 and Q48 values.

Table 5. Formulations and respective permeation parameters of olanzapine-loaded LNs (OL-LN). Key: J_{SS} = Flux at steady-state, Kp = Permeability coefficient, t_{lag} = lag time, Q24 and Q48 = Cumulative amount of olanzapine permeated after 24 h and 48 h, respectively. Data are expressed as mean ± SD (n = 8).

Formulation	J_{ss}(µg/cm².h)	Kp (cm/h)(x 10^{-3})	t_{lag} (hr)	Q24 (µg/cm²)	Q48 (µg/cm²)
OL-LN100:0	2.47 ± 0.885	1.17 ± 0.40	17.89± 2.12	17.01± 7.00	73.67 ± 25.60
OL-LN 75:25	0.49 ± 0.23[a]	0.22 ± 0.10[a]	18.76 ± 2.54	3.24 ± 2.32[a]	14.83 ± 6.29[a]

OL-LN 50:50	0.18±0.12[a]	0.087 ± 0.06[a]	-	1.35 ± 1.24[a]	1.89 ± 1.40[a]
OL-LN 25:75	0.58±0.93[a]	0.294 ± 0.01[a]	-	5.78 ± 5.65[a]	6.26 ± 5.07[a]
OL-LN 0:100	0.02 ± 0.01[a]	0.011± 0.01[a]	-	6.98 ± 2.17[a]	8.63 ± 3.35[a]

[a] $p<0.05$ *vs*. OL-LN 100:0

Oleic acid is thought to disrupt packing within the stratum corneum and act as a chemical penetration enhancer (CPE) (37). The long chained CPE interacts with lipid chains, forming pools in the skin known as fluidization, which allows the drug to diffuse faster between inter-corneocyte gaps (38). Despite having no oleic acid, SLN formulations had the fastest permeation rate (**Fig. 2**). This could be due to its greater occlusive effects (**Table 4**), which result in greater percutaneous absorption.

According to current olanzapine dosing regimen, a daily dose of 5-10 mg is required to achieve therapeutic effect. Since oral bioavailability of olanzapine is ca. 60%, the estimated transdermal dose to obtain therapeutic effects is 3-6 mg per day (8). Thus, the target transdermal flux based on *in vitro* permeation studies should be between 3.1-6.2 μg/cm^2.h, considering a 40 cm^2 patch size. This yields an amount permeated per day of 75-150 μg/cm^2. The *in vitro* results obtained are all below transdermal target values. Considering the SLN formulation with the highest flux, a fine-tuning of formulation could allow achievement of target therapeutic doses. For example, by the increase in lipid concentration, or by the incorporation of chemical enhancers in the aqueous phase of SLN (39).

Sustained release formulations are desirable for olanzapine delivery, to allow a constant therapeutic delivery. A doubling in Q24 value for Q48 value, would indicate sustained release. Comparing Q24 and Q48 values in **Table 5** however, it can be seen that this was not the case and that sustained release formulations were not produced.

Considering t_{lag} values, although they were generally high they do not represent an issue as we are dealing clinically with chronic conditions, where therapeutic doses are maintained between new doses.

3.5. Stability studies

3.5.1. Instability index

Instability values are a good indicator of long-term storage stability. According to **Fig. 3**, formulations with low oleic acid content would be best in long-term storage.

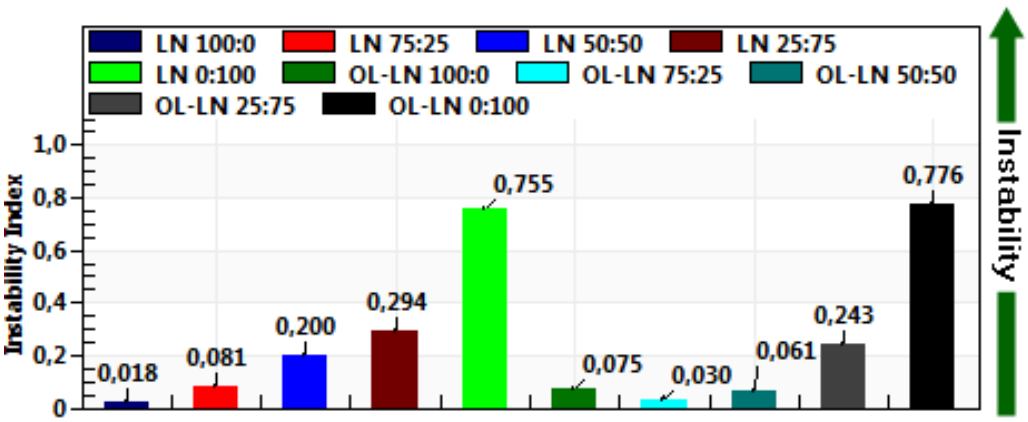


Fig. 3. Instability index values for various solid:liquid lipid formulations, generated via LUMiFuge analysis. Lower values are indicative of formulations having greater stability.

It can be noticed that conversely to zeta potential values, in general as the amount of liquid lipid incorporated into the nanolipid system increased, so did nanolipid instability. Unloaded and loaded 0:100 formulations were the most unstable. Such a trend has been reported previously (40).

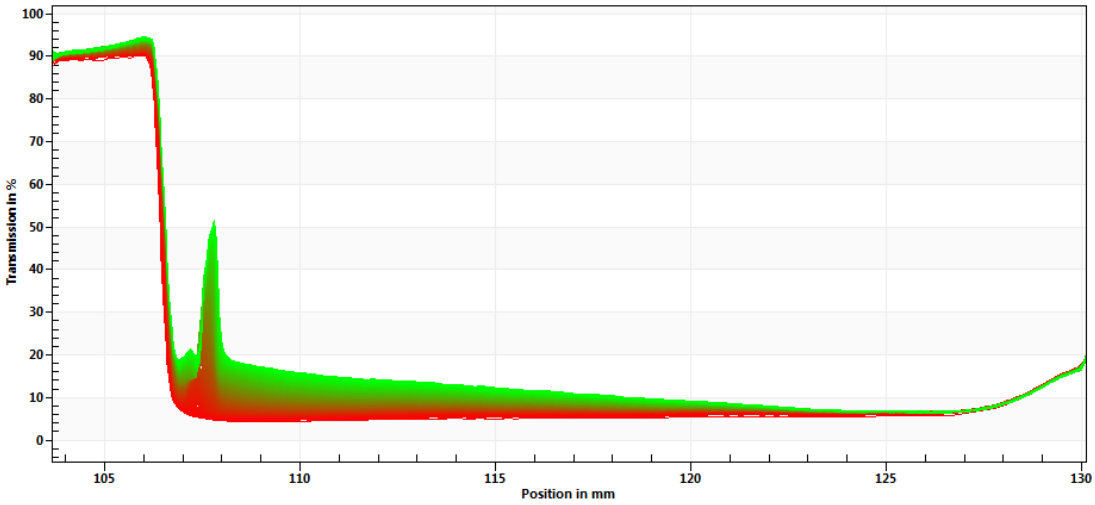
Incorporation of olanzapine did not promote a significant change in stability of the formulations. This is generally in agreement with particle size results (**Table 4**).

Particle size, surface charge and the percentage lipid ratio, are thought to affect the overall stability of the nanocarrier system. Generally, a higher particle size is found to correlate with formulation viscosity and an increase in the rate of particle aggregation. Surface charge is considered a key factor contributing to stability of the nanocarrier (32).

3.5.2. Transmission profiles

Transmission profiles in **Fig. 4** are taken over time, throughout the length of the sample and represent potential phase separation within the different formulations. Differences in transmission throughout a sample, allow estimation of the extent of flocculation or creaming present, if at all. Red profiles represent earlier profiles and green profiles represent the last profiles. Transmission profiles are in agreement with instability data (**Fig. 3**), formulations with a high liquid lipid content are less stable. Comparing **Fig. 4(a)** with **Fig. 4(e)**, a pronounced difference was observed. **Fig.4 (e)** shows creaming and flocculation within the OL-LN 0:100 formulation over time, whilst subjected to centrifugation. This is indicated by the greater transmission values, towards the bottom of the sample (right hand side of figure) and relatively lower transmission values at the top of the sample. The relatively lower transmission values indicate regions of lower clarity, as less NIR light is able to pass through and be detected, these regions indicate; aggregation of particles resulting in flocculation and creaming at the top. In contrast, SLN formulation (**Fig. 4(a)**) remained intact and showed no significant signs of separation, demonstrating higher physical stability.

(a)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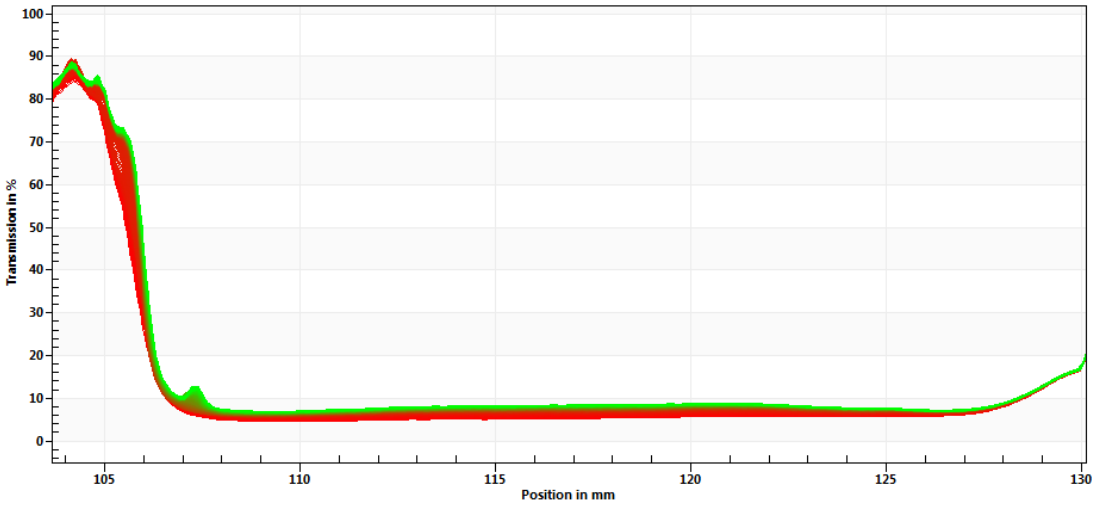
(b)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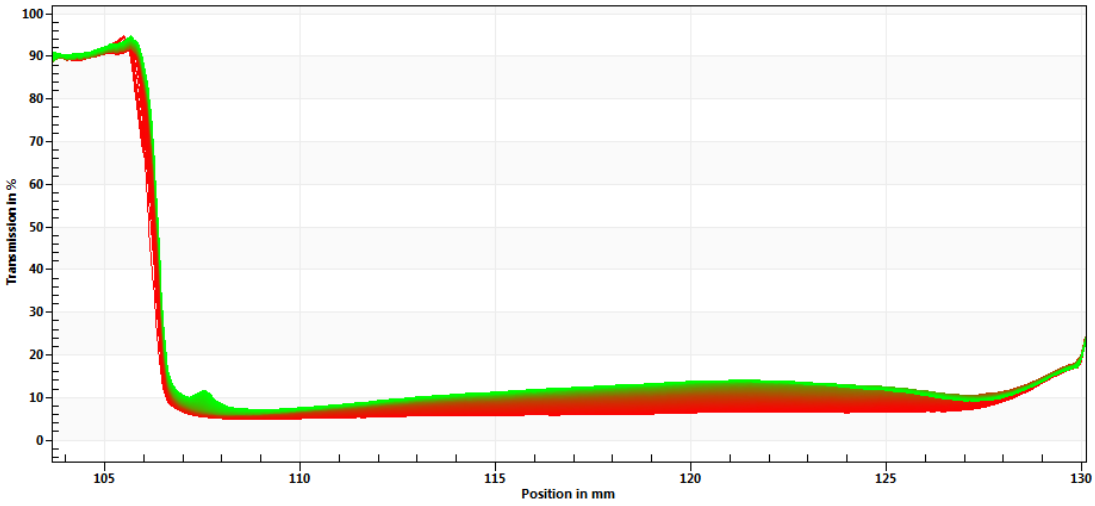
(c)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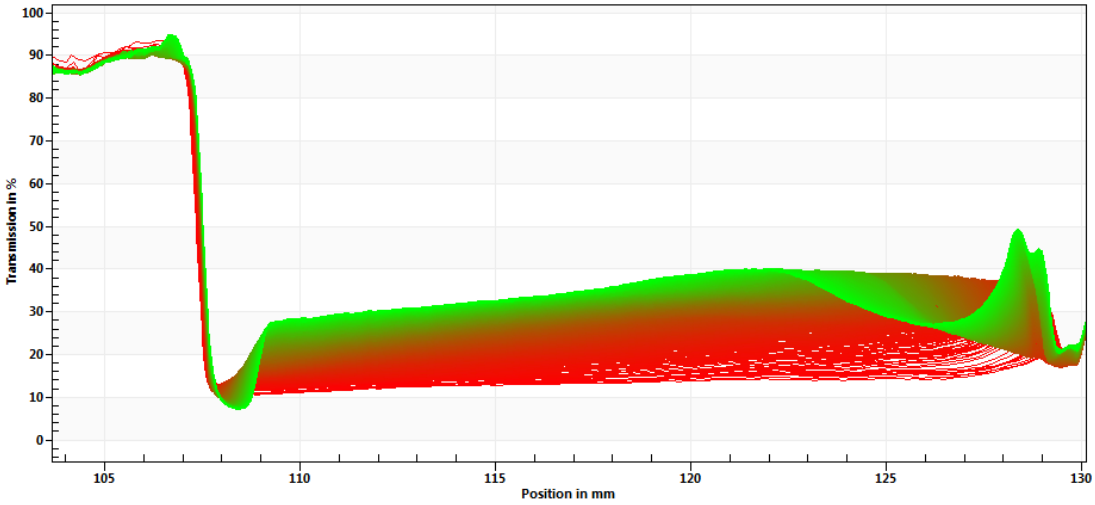
(d)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(e)

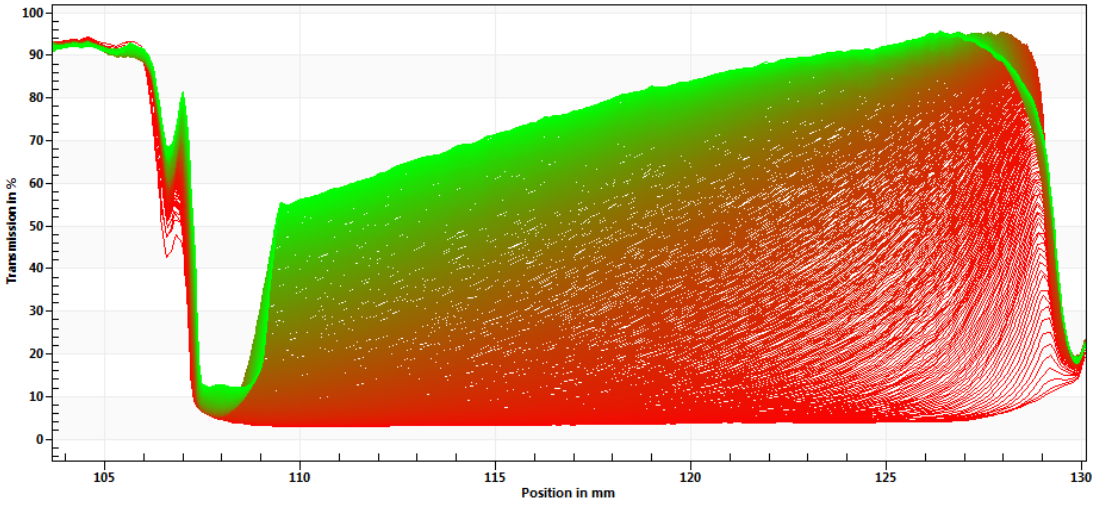

Fig.4. Transmission profiles for various solid:liquid lipid formulations generated via LUMiFuge analysis. (a) OL-LN 100:0, (b) OL-LN 75:25, (c) OL-LN 50:50, (d) OL-LN 25:75 and (e) OL-LN 0:100. The sequence of profiles is shown from the bottom (red), for the first profiles, to the top (green) for the last profiles.

Regarding the transmission profiles, greater phase separation was exhibited with formulations of greater liquid lipid content. This in in agreement with Vitorino et al. findings, where it is expected that with an increase of the liquid lipid a more unstable system would be obtained (coalescence tends to occur with a lower solid content) (9).

4. Conclusion

The study has demonstrated successful production of various solid:liquid lipid ratio nanocarriers, where Precirol® was found to be the most efficient solid lipid of those tested. This was based on the low particle size attained for the NLCs, combined with the analysis of other selection parameters. *In vitro* performance of formulations along with their characterisation, allowed selection of SLNs as the most appropriate choice for transdermal drug delivery of olanzapine. SLN formulations performed well in the majority of physicochemical parameters. They exhibited good physical stability, greatest occlusive effect, a relatively fast release rate and the highest flux. The present work shows that SLNs are a promising nanocarrier system for transdermal delivery of olanzapine. Optimisation of SLNs is however required such as obtaining a lower PI, which would allow greater control on TDDS in terms of release. Fine-tuning

of the formulation, to reach a target flux would also be required, so that an effective therapeutic dose of olanzapine is administered. Although the SLNs performed well in stability studies, long-term storage studies over a period of six months would also be needed. Future prospects include formulation of the SLN into reservoir system-transdermal patches, for effective olanzapine delivery.

Declaration of Interest

The authors report no declarations of interest.

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