In-situ gels and nail lacquers as potential delivery systems for treatment of onychomycosis. A comparative study

Noha Ibrahim El-sherif, Rehab N. Shamma*, Ghada Abdelbary

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo, Egypt

**A R T I C L E I N F O**

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**A B S T R A C T**

The aim of this study was to prepare and incorporate TBH-loaded spanastics into different delivery systems for treatment of onychomycosis via the trans-ungual route. Two drug delivery dosage forms; the in-situ gel and the nail lacquer, were developed and evaluated for their ability to deliver TBH encapsulated in spanlastic carriers to the nail plate. In-situ gel formulations were prepared using different Pluronics® in different concentrations using the cold method, and evaluated for sol-gel transition temperature, viscosity, and in-vitro release studies. The nail lacquer formulation were prepared using Eudragit® RLPO as a film forming polymer, and evaluated for drying time, non-volatile content, water resistance and in-vitro release studies. Finally, an ex vivo human cadaver nail permeation study with the optimized formulations was employed in order to assess TBH permeation and retention in the nails compared to the commercially available TBH cream (Lamisil® 1% cream). The optimized in-situ gel formulation G6, showed higher amounts of retained TBH in the nails (2.05 ± 0.008 mg/cm²) compared to the marketed product Lamisil® cream 1% (1.36 ± 0.03 mg/cm²), by an increase of 51%, indicating successful trans-ungual delivery of TBH from the prepared in-situ gels.

**1. Introduction**

In-situ gels are liquid aqueous solution that turns into gel at physiological conditions [1]. In-situ gels have many advantages, such as ease of administration, reduced dose concentration, improved local bioavailability, improved patient compliance and simple manufacturing procedures [2]. Gelation of polymers normally happens due to cross-linkage of the polymer chains. This could be chemical cross-linkage (covalent bond formation) or physical cross-linkage (non-covalent bond formation) [3]. Temperature is the easiest stimulus to manipulate in responsive in-situ gels. The aqueous solutions turn to gel (sol-gel transition) following an increase in temperature, due to the self-assembly of the polymer chains as a result of hydrophobic interactions. Pluronics® or poloxamers are the most common example of polymers that change due to temperature modulations [4].

Nail lacquers have been used for a long time as a cosmetic for decorative purposes and sometimes for protective purposes. Typically a nail lacquer consists of solvents, film forming polymer, resins to increase adhesion of film to nail plate and a plasticizer to increase durability of the film [5]. Medicated nail lacquers have been used for maximal anti-fungal efficacy for trans-ungual drug delivery [6]. They form a film over the nail plate, after the evaporation of the solvent. The drug is released slowly from the film as a result of the concentration gradient [6], and penetrates the nail plate, and the film prevents the tranonychial water loss leading to increased hydration of nail plate [7], providing a sustained diffusion of the drug across the nail [6].

Nail lacquers seems to be commercially favored for lots of reasons, including its high residence time on the nail plate and hardness of wash off or loss. Nail lacquers are also widely acceptable by the patients and easily used, in addition to its ability to prevent the tranonychial water loss and sustained diffusion of the drug by concentration gradient [7].

Joshi et al. [8] formulated matrix based system as nail lacquer, for the enhancement of the trans-ungual delivery of isotretinoin across nail plate. They evaluated the nail lacquer and examined it using an ex-vivo permeation study and confocal laser scanning microscopy. The concluded that using ethylcellulose as a viscosity modifying agent and thiglycolic acid as permeation enhancer resulted in an effective isotretinoin nail lacquer permeation in comparison with the marketed 0.05%w/w retinoin cream. Hafeez et al. [9] also succeeded in formulating ketoconazole in a lacquer formulation for the trans-ungual delivery to treat onychomycosis.

In order to ensure better retention of the colloidal nanocarriers on the nails and enhance patient compliance, suitable delivery systems need to be developed. Thus, the aim of this study was to develop TBH-loaded spanlastic incorporated dosage forms as a potential trans-ungual delivery system.
delivery system for treatment of onychomycosis. Two drug delivery dosage forms; the in-situ gel and the nail lacquer, were developed and evaluated for their ability to deliver TBH encapsulated in spanlastic carriers to the nail plate. Finally, an ex vivo human cadaver nail permeation study with the optimized formulations was employed in order to assess TBH permeation through the nails compared to the commercially available TBH cream (Lamisil® 1% cream).

2. Materials

Terbinafine hydrochloride (TBH) (AlAndalus pharmaceutical company, Egypt). Span® 65, Synerponic® F108, Pluronic® F68, and dialysis tubing cellulose membrane 14,000 Mw cutoff (Sigma-Aldrich Co., USA). Sodium Deoxycholate (BASF Co., Florham Park, NJersy, USA). Isopropyl alcohol, chloroform, acetone (Adwic, El- Nasr pharmaceutical company, Egypt). . Lamisil 1% cream (Novartis GmbH, Nürnberg, Germany) was purchased from a local pharmacy and stored according to the package information leaflet.

3. Methodology

3.1. Preparation of TBH-loaded spanlastics

TBH-loaded spanlastics were prepared by the ethanol injection method as prescribed earlier by our group [10]. In brief, accurately weighted amount of TBH was dissolved with a Span® 65 in a mixture of acetone: chloroform (1:2 v/v) and injected quickly into preheated aqueous phase (70 °C) containing sodium deoxycholate as an edge activator, for a total weight of 200 mg to form oil in water emulsion. The emulsion was stirred continuously on a magnetic stirrer at 1000 rotation per minute (rpm) for 15 min at 70 °C. The formed TBH-loaded spanlastics were then left to cool then were sonicated by probe sonicator, and then cooled overnight at 5 °C before further use.

3.2. Preparation of in-situ gel incorporated with TBH-loaded spanlastic

In-situ gels were prepared using the cold method [11]. Accurately weighted amounts of Pluronic® and Synerponic® mixtures were dispersed in 10 mL of the previously prepared TBH-loaded spanlastic dispersion at 4 °C ± 2 °C with continuous stirring using a magnetic stirrer at a speed of 200 rpm for 1 h. The temperature was maintained at 4 °C ± 2 °C using an ice bath throughout the preparation. The formed solution was kept overnight in the refrigerator at 4 °C to ensure complete swelling of the polymers.

4. Characterization of TBH spanlastic-loaded in-situ gels

4.1. Determination of pH of the in-situ gels

The pH of the prepared in-situ gels was determined using a pH meter by placing pH meter in the in-situ gel directly. The pH measurements were carried out in triplicates and an average was determined [11].

4.2. Determination of spreadability of the in-situ gels

Spreadability of the gel was determined by placing 1 mL of gel at the center of a glass plate of size 20 cm × 20 cm. The glass plate was covered with an identical glass plate of the same size. Then a weight of 1 kg was placed carefully on the upper plate for 1 min, forcing the gel to spread between the 2 plates. The diameter of the spread area (cm) was measured. Each measurement was carried out in triplicates [11].

4.3. Determination of viscosity of the in-situ gels

The rheological study was done using the Brookfield digital viscometer LVTD (Model LVTD, Voltage 230 V, Frequency 50 Hz, Brookfield engineering laboratories Inc., USA). The formulation was placed in the sample holder (about 3 mL), maintained at 4 °C and a suitable spindle was lowered vertically into the sample. The speed (in rpm) was changed and the % torque results were recorded [1,11].

4.4. Determination of sol-gel transition temperature

A sample of 1 mL from the formed gel was placed in a test tube, and then the test tube was dipped in a thermostatically controlled water bath (FA90, FALC, Italy) whose temperature was increased by 1 °C by a time starting from 25 °C. The temperature at which the solution was totally converted into gel was considered the transition temperature. This was confirmed when the formulation had no flow when the tube was inverted [12]. This procedure was done in triplicates.

4.5. Drug content

The drug content of the prepared gels was determined spectro-photometrically. One mL of the prepared gel (containing 1 mg of TBH) was dissolved in a mixture of ethanol and chloroform (50:50) and measured at the predetermined λmax.

4.6. In-vitro release studies

The same procedure mentioned previously by Elsherif et al. [10] was followed, where an amount of gel containing 1 mg TBH was placed in the dialysis bag instead of the TBH-loaded spanlastic nanovesicular formulations, using the dialysis bag diffusion technique in a thermostatically controlled water bath shaker with the dissolution media maintained at 32 ± 0.5 °C. The dialysis tube was immersed in a beaker containing 50 mL mixture of PBS (pH = 7.4) and ethanol (50:50) and shaken at 100 strokes per minute.

5. Study of the effect of different formulation parameters on properties of the prepared in-situ gels using full factorial design

A 2³ full factorial experimental design was employed in order to investigate the effect of different formulation parameters on the properties of the prepared in-situ gel using Expert-Design® 7 software. In this design, three factors are evaluated, each at 2 levels and the experimental trials were performed at all 9 possible combinations. As shown in Table 1, the independent variables were the concentration of Pluronic® F127 (X1), the type of co-polymer (Pluronic® F68 or Synerponic® F108) (X2), and the total polymer concentration in the gel preparation (X3). The Sol-Gel temperature (Y1), the %TBH release after 2 h (Y2) and the %TBH release after 8 h (Y3) were selected as dependent responses. Table 2 shows the composition of the prepared gels.

6. Preparation of nail lacquer containing TBH-loaded spanlastic

Different solvents including methanol, ethanol, isopropanol, acetone, methylene chloride and chloroform were tried as solvents for the preparation of nail lacquer, dissolving Eudragit® RLPO while
keeping the integrity of the spanlastic nanovesicles.

Different concentrations of Eudragit® RLPO (10, 15, and 20%) were dissolved in the organic solvent of choice, and then amount of TBH-loaded spanlastics nanovesicles formulation equivalent to (1% w/v) was dispersed in the polymer solution [8].

Characterization of TBH-spanlastic nail lacquer

7.1. Drying time

On a glass plate, an area of 4 × 4.5 cm² was marked and 1 mL of the film was applied on with the help of a brush. The time needed for the film to achieve complete dryness was recorded using a stop watch [8].

7.2. Non-volatile content

On a silicon plate, 1 g of each sample was poured and spread evenly. The dish was accurately weighted and then placed in an oven (Genlab, MINO/6, UK) at a temperature of 100 °C for 1 h. The plate was then removed, allowed to cool and re-weighed. The non-volatile content was calculated using the following equation: [8].

\[
\text{Non volatile content} = \frac{\text{Final weight of film}}{\text{Original weight of film}} \times 100
\]

7.3. Water resistance

This test was done on silicone plates of diameter (2 × 2 cm²), where an amount of 0.1 mL of the preparation was spread evenly and left to dry. The plate was then weighted and immersed in 500 mL of water maintained at 37 °C for 24 h. The plate was then removed, dried, re-weighted and the difference in weight was calculated using the following equation [8]:

\[
\text{Original weight of plate} - \frac{\text{Final weight of plate}}{\text{Original weight of plate}} \times 100
\]

7.4. Blush test

On a silicon plate, on an area of 2 × 2 cm², an amount of 0.1 mL of the preparation was spread evenly. The plate was then weighted and left to achieve complete dryness was recorded using a stop watch [8].

7.5. Drug content

One milliliter of the prepared gel was dissolved in 10 mL of a mixture of ethanol and chloroform (50:50), and the amount of TBH present was measured spectrophotometrically at the predetermined \( \lambda_{\text{max}} \).

7.6. In-vitro release studies

The same procedure mentioned previously by Elsherif et al. [10] was followed, where an amount of nail lacquer equivalent to 1 mg TBH was placed in the dialysis bag instead of the TBH-loaded spanlastic nanovesicular formulations, and samples were withdrawn at predetermined time intervals and measured spectrophotometrically.

8. Ex-vivo nail permeation studies

Human cadaver nail samples were collected from human corpses at Kasr-Eleiny hospital, after approval of the ethical medical committee of the hospital. The nails of 1 or 2 years old corpses, which have been used in anatomy courses, were collected [13,14]. Nail samples were kept at -20 °C till further use.

Before beginning of ex-vivo permeation study, nail samples were left over night for equilibration at 25 °C. The nails were placed in permeation media for 1 h, in order to achieve maximum hydration [13]. The nails were then attached to a plastic cylinder of a diameter of 0.6 cm and length of 5 cm using cyanacrylate adhesive. The cylinder was placed in the receptor compartment containing 50 mL of PBS pH 7.4 with the nail just touching the permeation media. The whole apparatus was covered totally and placed in a thermostatically controlled water bath adjusted at 32 ± 0.5 °C at 100 strokes per minute. Formulations containing 1 mg TBH were placed in the plastic cylinder. At predetermined time intervals, samples were withdrawn from the receptor compartment and replaced with fresh media to maintain constant volume. Samples were withdrawn from the receptor compartment at 0.5, 1,2,3,4,5,6,24,48 and 72 h times. The amount of TBH permeated was measured spectrophotometrically at the predetermined \( \lambda_{\text{max}} \) 282.4 nm [8,13].

8.1. Pulverization of nails

At the end of the permeation study, the nails were removed; washed with distilled water to remove any adhered drug and then cooled at -25 °C for 24 h. The nails were then pulverized manually by striking with a hammer to convert the nails into fine pieces. In order to extract TBH retained in the nail pieces, the pulverized nail pieces were
suspended in ethanol: chloroform (1:1) and shaken for 14 h followed by centrifugation for 15 min at 15000 rpm. Supernatants were measured spectrophotometrically at \( \lambda_{\text{max}} = 282.4 \) nm. The amount of TBH retained in nail plate was presented as mass per unit area from the cadaver nail (mg/cm²).

9. Results and discussion

In-situ gel preparation using cold method is an easy, industrially scalable and economic method with low investment and manufacturing costs [15]. It is more advantageous compared to other techniques employing high temperatures, as it facilitates polymer dissolution and limits the possible alterations [16]. Pluronic® and Synperonic® are Poloxamers, which are ideally suited for in-situ gel preparation using the cold method as they are more soluble in cold water than in hot water, due to extensive hydrogen bonding between water molecules and the ethereal oxygen atoms of the polymer [17].

9.1. Characterization of the in-situ gel

Table 2 presents the sol-gel transition temperatures (the temperature at which the solution is transferred to gel state), pH values, and spreadability results of all the prepared formulations. The pH values of all the prepared formulations were in the physiologically accepted range (6.84 ± 0.065 to 7.13 ± 0.095). This ensures no irritation on all the prepared formulations were in the physiologically accepted spreadability results of all the prepared formulations. The pH values of 9.1. Characterization of the in-situ gel

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9.2. Characterization of TBH spanlastic-loaded in-situ gel

9.2.1. Drug content

The average drug content measurements varied from 91.7 ± 4.23% to 108.47 ± 0.9% (Data not shown). The results were of the accepted range of 90% -110%, which could indicate that the method of preparation is adequate to produce formulas of accepted drug content [11].

9.2.2. In-vitro drug release from in-situ gels containing TBH-loaded spanlastics

The release was conducted at 32 °C, thus only trials G6 and G8 were in the gel state, while all the other trials were in the solution state. Figure 2a and b present the release profiles of in-situ gels containing TBH-loaded spanlastics prepared using Pluronic® F68, and Synperonic® F108, respectively, in comparison with TBH-loaded spanlastics. Similarity factor (\( f_2 \)) was calculated for the preparations using FDA equation in comparison with TBH-loaded spanlastics and all results were between 50 and 100 (Data not shown), proving that there was no significant difference in the release profile of TBH-loaded spanlastics upon incorporation in in-situ gels.

10. Statistical analysis of the factorial design

10.1. Effect of formulation variables on the sol-gel transition temperature

The sol-gel transition temperatures varied from 27.73 ± 1.55 °C to more than 75.00 ± 0.00 °C. All the tested variables had a significant impact on the sol-gel transition temperatures as shown in Table 2. The concentration of Pluronic® F127 had a significant impact on the sol-gel transition temperatures of the prepared in-situ gels (\( p = 0.0124 \)), where the formulations containing higher concentration of Pluronic® F127 showed a lower transition temperature as shown in Figure 3a. This is in accordance with the previous literature [16, 21-24]. Poloxamers are formed of polyoxyethylene and polyoxypropylene units. At the lower temperature, hydrogen bonding between polyoxypropylene chains and water keeps hydrophobic portions of poloxamers separate. At higher temperature, hydrogen bonding is disrupted and the hydrophobic interactions cause a gel to be formed. Thus, gelling properties of poloxamers are dependent on proportion of hydrophobic portion. With the increase in the concentration of Pluronic® F127, a decrease in the gelation temperature was observed, due to the increase in the hydrophobic portion of Pluronic® F127 [17, 24-26].

The use of Synperonic® F108 as a co-polymer had a significant negative impact (\( p = 0.0026 \)) on the transition temperature as shown in Figure 3b. Synperonic® F108 has more polyoxyethylene and polyoxypropylene units than Pluronic® F68 [26], thus it has a higher hydrophobic portion, leading to a decrease in gelation temperature compared to in-situ gel containing Pluronic® F68 as a co-polymer [17]. Increasing the whole polymer concentration in the formulation resulted in a significant decrease (\( p = 0.0001 \)) in the transition temperature of the prepared in-situ gels, as shown in Figure 3c. These findings were shared by many researchers [3, 16, 22]. This also can be explained by the increase of the total hydrophobic portion in the preparation, thus occurrence of micellar entanglement at lower temperatures and formation of gel [17].

10.2. Effect of formulation variables on the %TBH released from the prepared in-situ gels after 2 h

Table 2 shows the %TBH released from the prepared in-situ gels after 2 h. The release results ranged from 24.92 ± 4.34% to 34.29 ± 2.60%. Only the total concentration of the polymers in the formulation (\( X_3 \)) showed significant effect on this response as represented by line chart in Figure 3d. As the total concentration of polymers increased, the in-vitro release of TBH was significantly decreased (\( p = 0.0013 \)) as in case of TBH-loaded spanlastic in-situ gels after 2 h, owing to the increase in viscosity of the gel, thereby reducing the release rate of the drug, and the drug diffusion through the gel matrix was prolonged. Similar results were observed by Inal & Yapper [27] in their study on the effect of mechanical properties on the release of meloxicam from poloxamer gel bases. These results are also in accordance with the results obtained by Ricci et al.
Fig. 1. The viscosity readings of in-situ gel formulations at: a) sol state, b) gel state.

Fig. 2. The release profile of: (a) TBH-loaded spanlastic in-situ gels containing Pluronic® F68 as co-polymer, (b) TBH-loaded spanlastic in situ gels containing Synperonic® F108 as co-polymer, (c) TBH-loaded spanlastic nail lacquer containing Eudragit® RLPO; in comparison to the corresponding TBH-loaded spanlastic nanovesicular formulations.
in their study of the release of lidocaine from poloxamer based gels.

10.3. Effect of formulation variables on the %TBH released from the prepared in-situ gels after 8 h

The % TBH released from the prepared in-situ gels after 8 h are represented in Table 2. None of the formulations factors and parameters showed any significant effect (p > 0.05).

11. Optimization

The optimized formula for both in-situ gel formulations was obtained using Design-Expert® software by the graphical and numerical analysis. In-situ gel formulation G6 composed of 30% Pluronic® F127: Synperonic® F108 (1:1) with a desirability of 0.703. This formulation was selected for further investigation using ex – vivo permeation studies.

12. Evaluation of TBH-loaded spanlastics nail lacquer

The prepared TBH-loaded spanlastics only retained its shape when using isopropanol and did not cause vesicular dissolution, thus it was the organic solvent of choice. Drying time is of profound importance in discussing nail lacquer, as prolonged drying will lead to uneven and streaky application of the film, whereas the fast drying time of the lacquer could lead to hardening of the nail lacquer on the brush and poor pick of nail film on brush may occur [8]. The drying time of the prepared nail lacquer formulations was found to be in range of $63.2 \pm 2.0, 65.4 \pm 4.0,$
70.4 ± 5.0 s for formulations prepared using 10%, 15%, and 20% Eudragit® RLPO, respectively as shown in Table 3. It is clear that by increasing the concentration of the polymer Eudragit® RLPO, the drying time increased. This could be explained that as the solute concentration increases, the vapor pressure decreases and boiling point increases, thus increasing of drying time. Same results were also observed by Joshi et al. [8] in their study of matrix based systems of isotretinoin as nail lacquer. They found that the drying time decreased with the increasing of Eudragit® RS100 concentration.

Upon the application of the prepared film, the solvent evaporates leaving behind Eudragit® film of TBH-loaded spanlastics that should provide coverage to the whole nail plate. All the prepared formulations complied with the standards stated by the Bureau of Indian standards [8,29] and the Draft East African Standard [30], where a non-volatile content was not less than 20% (90.57 ± 0.31%, 95.48 ± 0.22%, and 96.05 ± 0.58% for formulations prepared using 10%, 15%, and 20% Eudragit® RLPO, respectively as shown in Table 3).

Water resistant test was done to determine the resistance of the nail lacquer to water, as uptake of water, followed by surface erosion, may lead to loss of film and hence, loss of the treatment. All the prepared nail lacquer formulations were found to be water resistant, showing weight loss < 1% (0.006%, 0.080, and 0.790% for formulations prepared using 10%, 15%, and 20% Eudragit® RLPO, respectively).

Blush test was done to check the physical changes that might occur in the nail lacquer when exposed to water and external environment [8]. The nail lacquer is considered to pass if it did not show slight whitishness, and should not show any blistering or peeling off after dryness [30]. This test was carried out to ensure the absence of blistering and peeling of the nail lacquer when in contact with water. All the prepared formulations succeeded to pass the blush test.

The drug content of the nail lacquer was done to ensure that TBH-loaded spanlastic nanovesicles were equally distributed throughout nail lacquer preparation. The three formulations showed a uniform drug content of 99.80 ± 0.26, 96.27 ± 0.68, and 92.30 ± 0.35, for formulations prepared using 10%, 15%, and 20% Eudragit® RLPO, respectively.

12.1. In-vitro release studies

Figure 2c presents the release profiles of TBH from the prepared nail lacquers in comparison with TBH-loaded spanlastics. Similarity factor (f2) was calculated for the prepared preparations using FDA equations in comparison with TBH-loaded spanlastics and all results were ≥ 50, proving that there was no significant difference in the release profile of TBH-loaded spanlastics upon incorporation in nail lacquer.

13. Ex-vivo permeation studies from in-situ gel formulations containing the optimized TBH-loaded spanlastic nanovesicles

Figure 4 shows the amount of TBH permeated through the nails after incorporation of TBH-loaded spanlastics in in-situ gel, and nail lacquer formulations in comparison with the market product, Lamisil® cream. Incorporation of TBH-loaded spanlastic nanovesicles in the in-
in situ gel, and the nail lacquer resulted in higher permeation amounts compared to the Lamisil® cream, by 16%, where the permeated amount of TBH through the nail after 72 h from the optimized in-situ gel formulation G6, the optimized nail lacquer formulation, and Lamisil® were 3.44 ± 0.125, 3.44 ± 0.047 and 2.97 ± 0.027 mg/cm², respectively.

Figure 5 shows the amount of drug retained in the nail plates after application of the optimized in-situ gel formulation G6 and nail lacquer formulations in comparison with the market product, Lamisil® cream. The in-situ gel formulation G6, showed higher amounts of retained TBH in the nails (2.05 ± 0.008 mg/cm²) compared to the marketed product Lamisil® cream 1% (1.36 ± 0.03 mg/cm²), by an increase of 51%. This could be an indication for a successful trans-ungual delivery of TBH from the prepared in-situ gels. The optimized nail lacquer formulation showed higher amount of retained TBH in the nails (1.67 ± 0.084 mg/cm²) compared to the marketed product Lamisil® cream 1% (1.36 ± 0.03 mg/cm²), although it was lower compared to the in-situ gel formulation, probably due to the solid nature of the nail lacquer.

14. Conclusion

The optimized TBH-loaded spanlastic nanovesicular formulations were successfully incorporated in two drug delivery forms: in-situ gel and nail lacquer.

References


[23] X. Xu, et al., Preparation and in vitro characterization of thermosensitive and


