ORIGINAL ARTICLE

Formulation and Evaluation of Tadalafil-Loaded Nano-Ointment and Cream for Raynaud's Phenomenon

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Abstract

Introduction: The research is to prepare and characterize the tadalafil (TDL) loaded cream and nano-ointment for the localized treatment of Raynaud's phenomenon (RP). As far now, there is no marketed topical TDL formulation available for RP, a conventional ointment loaded with TDL was prepared which is used to compare with the TDL cream and nano-ointment formulation. This study compares *in vitro* and *ex vivo* data for TDL-loaded cream and nano-ointment along with the conventional ointment for the RP. Both formulations were assessed for spreadability, as well as *in vitro* and *ex vivo* release studies and drug release kinetics models. The objective was to ascertain which formulation is more effective for treating RP by penetrating deeper into the skin surface. **Materials and Methods:** The TDL cream was prepared with a high-speed homogenizer technique and evaluated for pH, viscosity, spreadability, and drug release studies in both *in vitro* and *ex vivo* through snake shed skin. The nanoparticles were prepared by ionic gelation technique and characterized, then loaded into the simple ointment base and then evaluated for pH, spreadability, and drug release studies in both *in vitro* and *ex vivo* through snake shed skin. **Results and Discussion:** The TDL-loaded nano-ointment was determined to be the superior formulation when compared to TDL-loaded cream. This conclusion stemmed from the ability of nano-ointment to sustain release for more than 120 min, surpassing the performance of the cream. The superiority also attributed to the nano-ointment spreadability, pH, nano-size, and longer contact time with the skin which may exhibit deeper penetration into the skin. **Conclusion:** The TDL-loaded nano-ointment may provide an effective treatment for RP while potentially reducing the systemic side effects associated with oral administration.

Key words: Nano-ointment, Raynaud's phenomenon, snake shed skin and systemic side effects, spreadability, tadalafil

INTRODUCTION

Raynaud's phenomenon (RP) involves intermittent constriction of small arteries due to cold or stress, leading to reduced blood flow, marked by pallor, cyanosis, and erythema. It can be primary or secondary to conditions, such as autoimmune diseases or vibration exposure. The management of RP on alleviating symptoms and preventing episodes, often through vasodilators.[1] The underlying issue is abnormal constriction of small arteries. This can be caused by an imbalance between factors that promote relaxation (vasodilation) and those that cause constriction (vasoconstriction) of blood vessels.[2] Tadalafil (TDL), a PDE5 inhibitor, has emerged as a promising option due to its ability to enhance nitric oxide-mediated vasodilation by preserving current good manufacturing practices. As per the previous study, the authors likely report on the patient's response to TDL treatment, including changes in frequency and severity of Raynaud's episodes, healing of any existing digital ulcers, and overall improvement in symptoms while the patient not responded to sildenafil, a vasodilator.[3] Researchers compared the effects of TDL and placebo on frequency and duration of Raynaud's episodes, Raynaud's condition score (a measure of symptom severity), healing of existing digital ulcers (sores), prevention of new ulcers and overall quality of life, results found that the patients received

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TDL had positive outcomes improved Raynaud's condition score improved, existing digital ulcers showed signs of healing and improved quality of life.[4] Traditional oral administration of TDL might have limitations, such as low bioavailability (absorption) or side effects. Transdermal delivery could offer advantages, such as bypassing the digestive system, potentially leading to higher bioavailability, and fewer side effects.[5] Nanocarriers offer the potential to deliver drugs directly to diseased blood vessels, improving therapeutic efficacy and reducing side effects. Use of nanocarriers in the application of atherosclerosis and other vascular diseases can be used to deliver drugs for lowering cholesterol levels, inhibiting inflammation in blood vessels, and promoting blood vessel repair.[6]

MATERIALS AND METHODS

Materials

TDL was gifted by Sai Mirra Innopharm, Chennai for the preparation of cream and nano-ointment, Soybean oil (Yarrow chem products), lanolin (Loba Chemie Pvt. Ltd, Mumbai), Tween-60 (Loba Chemie Pvt. Ltd, Mumbai), Span 80 (Mohini organics), White soft paraffin (Loba Chemie Pvt. Ltd, Mumbai), cetostearyl alcohol (Loba chemie Pvt. Ltd, Mumbai), hard and soft paraffin (Gattefose Pharmaceuticals), chitosan, sodium tri-polyphosphate (Loba chemie Pvt. Ltd, Mumbai).

Methods

Construction of pseudoternary phase diagram

A pseudoternary phase diagram was constructed using the water titration method to determine the microemulsion region. Tween-60 and Span-80 were blended in a 1:1 weight ratio and mixed with soya bean oil at various weight ratios (1.0:9.0–9.0:1.0). Ultrapure water was added dropwise until the mixture turned turbid, indicating the transition from micro emulsion to coarse emulsion. The amount of water required to induce slight cloudiness was recorded, and the monophasic regions were identified using CHEMIX SOFTWARE (Figure 1).[7]

Calculation of HLB value by Griffin method and selection of emulsifiers

The data obtained from the pseudoternary plot, the amount of oil, surfactant, and water to be taken for preparation of TDL cream was determined. Quantify emulsifying agents were calculated based on the formula based on which emulsifier was selected.[8] HLB was found as 7 and 8 for soybean oil and lanolin, respectively.

Since TDL is dissolved in 10 g of soybean oil. The soybean oil with the drug 0.188 g, lanolin 10 g, and the emulsifiers (Tween-60 of 1.8 g and Span-80 of 4.2 g) are taken in one beaker and melted at 75°C and in another beaker of water; 13.8 g was taken and boiled at 75°C. The oil phase was slowly added into the water by continuous stirring with the help of a high-speed homogenizer until a homogenous creamy mixture texture appeared. When the temperature of the cream gets cooled, perfuming agents and preservatives are added. In this preparation, the water phase will be less and the oil phase will be more as represented in Table 1.

Characterization of TDL cream

Evaluation of type of emulsion or cream

A dye test, dilution test, and creaming of emulsion were done as per the standard procedure to determine the type of emulsion or cream.

Viscosity

In a 50 mL beaker, half of the beaker was filled with TDL cream, using spindle 64, the viscosity of the cream was determined through a Brookfield viscometer.

Acid value

10 g of the substance (n) were dissolved in a measured 50 mL mixture of alcohol and solvent ether. The solution was heated under reflux until complete dissolution, followed by the addition of 1 mL of phenolphthalein and titration with 0.1 N NaOH (w) until a faint pink color appeared after shaking for 30 s.

Acid value = n × 5.61/w.

Saponification value

2 g of the substance (w) underwent reflux with 25 mL of 0.5 N alcoholic potassium hydroxide (KOH) for 30 min. Then, 1 mL of phenolphthalein was added, and the mixture was titrated against with 0.5 N HCl, recording the reading as "a." The process was repeated without the substance, noting the reading as "b."[9]

Saponification value = (b–a) × 28.05/w.

Preparation of nanoparticles

a. Chitosan was dissolved in water with acetic acid overnight to produce a product where the drug dissolved

Table 1: Formulation of TDL nano-ointment[15]

Formulation (CHI: TPP: DRUG)	Particle size (nm)	PDI	Zeta potential (mv)	Entrapment efficiency (%)
F1 (1:1:1)	252	1.000	1.56	75.92
F2 (3:1:1)	386.9	0.776	20.4	70.86
F3 (5:1:1)	208	0.404	31.0	88.75

TPP: Tripolyphosphate, PDI: Polydispersity index

with chitosan in the ratios of (F-1 [1:1:1]; F-2 [3:1:1]; F-3 [5:1:1]) Chitosan: Tripolyphosphate: Drug.

b. Tri polyphosphate was mixed with water and drug solution for 30 min to obtain product B.
c. TDL nanoparticles were formulated by adding the drug solution dropwise into continuously stirred chitosan solution. The nano-formulation was subjected to lyophilization at −80°C for 24 h, resulting in the formation of a dry, free-flowing powder devoid of any aggregates, which was utilized for subsequent characterization.

Characterization of nanoparticles

Particle size, zeta potential, and poly-dispersity index of nanoparticles were analyzed by Malvern Zetasizer.

Entrapment efficiency

The entrapment efficiency of the drug (TDL) in the nanoparticles was determined by ultraviolet (UV)-Visible spectrophotometer analysis of supernatant liquid after centrifugation. The amount of drug entrapped in the nanoparticles was calculated by subtracting the drug amount in the supernatant from the total drug added during preparation, yielding the entrapment efficacy.[10]

$$\%DRUG\ ENTRAPMENT = \frac{W - w}{W} \times 100$$

Preparation of TDL ointment

Ointment was prepared by loading, lyophilized TDL nanoparticles into the simple ointment using the trituration technique. Incorporate 800 mg of TDL nanoparticles into a portion of the base on a slab, mixing thoroughly in a geometric pattern. Gradually add the remaining base and ensure thorough mixing.[11]

Characterization of TDL nanoparticles loaded ointment and TDL cream

Organoleptic characteristics

The prepared nano-ointment and cream were evaluated for physical appearance such as color, odor, grittiness, homogeneity, and roughness.

pH

5 g of the nano-ointment and 5 g of the cream were weighed and dissolved in 50 mL of distilled water, 3 readings of the pH was determined using a pH meter.

Spreadability

TDL nano-ointment and cream were determined by Texture Analyzer (TA.XT plus). Calibration was done followed by placing the sample on the cone sample holder. The force required to spread the nano-ointment and cream was measured.

Drug content

100 mg of the nano-ointment and cream was diluted with methanol and analyzed at 283 nm using a UV-spectrophotometer.

In vitro drug release studies

The *in vitro* drug release study for the prepared TDL-loaded nano-ointment, conventional ointment, and TDL-loaded cream was conducted over a 2-h period using the open-end cylinder method in 200 mL of phosphate saline buffer solution at pH 7.2 at 37 ± 0.5°C. Samples were collected at intervals of 5, 15, 30, 45, 60, 75, 90, 105, 120 min. For nano ointment 135 min and 165 min sampling for conventional ointment was also taken based on the release of the drug to 100%. To maintain sink conditions throughout the *in vitro* release studies, the samples were replaced with the freshly prepared phosphate buffer pH 7.2. The withdrawn samples were partitioned with methanol (combining 1 mL of the sample with 1 mL of methanol) and centrifuged at 5000 rpm for approximately 20 min. During centrifugation, the drug completely dissolved in methanol. Subsequently, 1 mL was extracted from the centrifuge tube, by discarding the supernatant liquid, diluted, and made up to a volume of 10 mL with methanol. The amount of TDL drug released was determined by a UV spectrophotometer at 283 nm.

Ex vivo drug release studies

An *ex vivo* drug release study for TDL-loaded nano-ointment and TDL-loaded cream was conducted over 2 h using the open-end cylinder method. A freshly shed skin from Naja naja was washed twice with distilled water to remove any dirt and then soaked in phosphate-buffered saline to smoothen its surface. After 24 h, the skin shed was cut into squares of 3 cm^2, with the epidermis facing up. One gram of the sample was evenly distributed over the skin's surface, and it was secured onto the open-ended cylinder with a rubber band, ensuring the cylinder's tip was slightly immersed in a beaker containing 200 mL of phosphate-buffered saline at pH 7.2. This setup was placed on a magnetic stirrer rotating at 300 rpm for 2 h. At specific time intervals (5, 15, 30, 45, 60, 75, 90, 105, and 120 min. 5 mL aliquots were withdrawn and replaced with freshly prepared phosphate buffer at pH 7.2 to maintain sink conditions. Each withdrawn sample (1 mL) was mixed with 1 mL of methanol and then centrifuged at 5,000 rpm for 20 min. The supernatant liquid was discarded, and the remaining sample was diluted with methanol in a 10 mL standard flask. The amount of TDL released was determined by UV spectrophotometry at 283 nm.[12,13]

Comparative study

The comparison of results between the TDL cream and nano-ointment along with the conventional ointment aimed

to ascertain the superior topical formulation for RP. The comparison was done for the evaluation of spreadability, *in vitro* and *ex vivo* release as well as for the drug release kinetics profile for the TDL-loaded cream and nano-ointment.

RESULTS AND DISCUSSION

Construction of pseudoternary phase diagram

The Smix (Span 80 and Tween-60) ratios were fixed at 1:1, and oil was added to the surfactant mix at a 1:1 ratio. The mixture was placed in a 20 mL beaker on a magnetic stirrer. Water was titrated dropwise using a 1 mL micro-pipette until the solution became clear. The amount of water added was then calculated, and a ternary plot was generated using CHEMIX Software to identify the emulsion region represented in Table 2.[14]

Characterization of TDL cream

Evaluation of type of emulsion

Dye test

The dye test for the cream confirms that the W/O type of emulsion is formed under the optical microscope using amaranth dye (water-soluble). Continuous phase- oil (colorless) and dispersed phase - water (red).

Dilution test

The dilution test confirms that a W/O type of emulsion was formed, by diluting a small amount of cream in 10 mL of distilled water. Immiscible with each other (water and oil).

Creaming

A creaming test for the cream was determined, it confirms that downward creaming of oil was formed by diluting a small amount of cream with 10 mL of distilled water under heating. So oil is less dense than water.

Evaluation of TDL cream

Viscosity

The viscosity of the TDL cream decreases with the increase in shear rate which indicates that the cream follows non-Newtonian flow.

Acid value

The acid value for the TDL cream was found to be 6.439 mg of KOH/g of sample.

Saponification value

The saponification value for the TDL cream was found to be 192.5 mg of KOH required to saponify per gram of the sample.

Characterization of nanoparticles

Particle size, zeta potential, and polydispersity index (PDI)

The F3 of TDL nanoparticles exhibited a particle size of 208 nm, confirming their nanometer scale. In adition, the PDI of 0.404 indicated the mono-disperse nature of the nanoparticles as shown in Figure 2. The stability of the F3 nanoparticles was determined by the surface charge and it was found to be 31.0 mV.

Table 2: Ternary plots obtained by water titration method

Smix (1:1)	Oil	Water
60.38	3.17	36.45
54.54	6.09	39.37
47.06	8.30	44.64
38.18	9.54	52.28
32.80	10.93	56.27
29.90	11.96	58.14

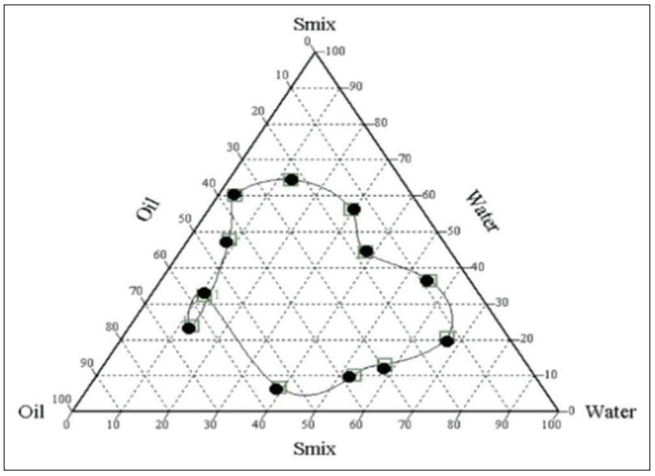

Figure 1: Pseudo ternary plot of microemulsion

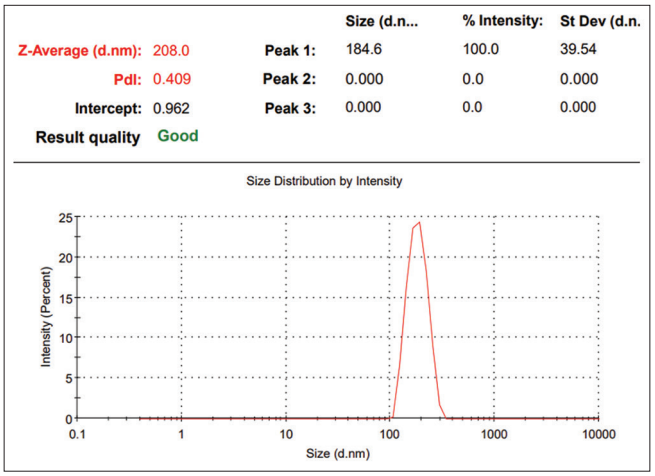

Figure 2: Particle size and poly dispersity index of formulation F3

Characterization of TDL nanoparticle loaded ointment F3 and TDL cream

Organoleptic characteristics

The prepared nano-ointment was half white in color, thick and oily in appearance also free from grittiness whereas cream was pale yellow in color, smooth and opaque in appearance also free from grittiness.

pH measurement

pH of the nano-ointment (or) cream was determined using a pH meter and the average was found to be pH 6.1 ± 0.16 for nano-ointment and pH 5.3 ± 0.11 for cream.

Spreadability

The maximum positive peak observed in Figure 3 indicated firmness and the corresponding maximum positive area indicated work of shear to spread the nano-ointment (or) cream. The maximum negative peak observed indicated the stickiness of the sample and the maximum negative area indicated the work of adhesion. The firmness and work of shear were found to be 1882.73 g and 4459.626 g/s for nano-ointment and 339.3 g and 337.28 g/s for cream.[16]

Drug content

The drug content for the nano-ointment was found to be 89% and the cream was found to be 91.1% by analyzing under a UV spectrophotometer.

In vitro drug release studies

TDL-loaded nano-ointment (or) TDL cream drug release was performed for 2 h using open-ended cylinder method. For the first 5 min, the release was found to be 9.15% and 16.08%, at the end of 120 min, the release was found to be 84.14% and 96.00% for TDL-loaded nano-ointment and cream, respectively.

Ex vivo drug release studies

TDL-loaded nano-ointment (or) cream of *ex vivo* study was performed with the snake shed skin for a period of 2 h using open-ended cylinder method. For the first 5 min, no release but at the end of 120 min, release was found to be 90.49% and 90.80% for nano-ointment and cream, respectively.

Comparative study

Spreadability comparison for TDL-loaded cream and formulation F3 nano-ointment

From Figure 3, it is concluded that the spreadability index for the nano-ointment is higher, indicating that the nano-ointment is likely to spread more easily compared to the cream.

In vitro drug release study

The TDL conventional ointment exhibited the slowest drug release rate among the formulations, TDL-loaded cream and nano-ointment displayed similar release profiles, with the cream showing a slightly faster release rate, reaching 96% release by 120 min compared to 84.14% for the nano-ointment. The order of release was found to be conventional ointment <nano-ointment <creams, possibly due to the extended contact time of the ointment with the skin. However, for RP, nano-ointment emerges as the preferred formulation due to its nano-size range.

Ex vivo release studies of TDL-loaded Formulation F3 nano-ointment and cream

At the time of 5 min, drug release was not found for both TDL-loaded nano-ointment and cream. However, after 10 min, TDL-loaded cream and nano-ointment were found to be about 14.97% and 9.86% of the drug through snake shed skin and at the end of 120 min, it was found to be 97.8% and 81.80%, respectively.

Drug release kinetic modeling

TDL cream follows first-order release kinetics *in vitro* model and all other study evidence that release kinetics follows zero-order including *ex vivo* studies for cream, conventional ointment, and nano-ointment. Korsmeyer Peppas model of n value was found to be 0.5–1 except TDL conventional ointment, (n = 1.239). Hence, the prepared nano-ointment formulation follows an anomalous non-fickian transport mechanism with drug release of both diffusion and erosion. Higuchi model with r^2 value of <1, indicates release of fairly linear to linear diffusion. Weibull distribution model b value of 1.452 (b > 1) indicates the shape of the curve gets sigmoidal with timing point follows both diffusion and erosion mechanism.

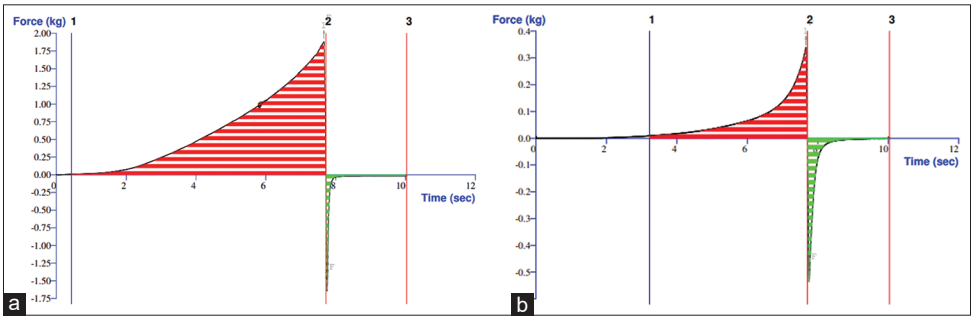

Figure 3: Spreadability (a) tadalafil (TDL) nanoparticles loaded ointment F3 (b) TDL cream

CONCLUSION

In conclusion, the study found that TDL nano-ointment has prepared with a particle size of 208 nm and PDI of 0.404 to evidence mono-dispersion and zeta potential of 31.0 mV indicates the formulation is stable. The pH of the nano-ointment also confirms the suitability of the formulation for human use (pH = 6.1 ± 0.16). The nano-ointment is free from grittiness and further spreadability value ensures formulation suitability for large-scale production. When compared to TDL-cream, nano-ointment demonstrated permeation of the drug in a controlled release by zero-order rate in *in vitro* and *ex vivo* evaluations with better *in vitro* and *ex vivo* correlation. The release mechanism also confirms the *n*-value 0.928 (*n* of > 0.5–< 1.0) to prove erosion and diffusion release of the drug by the Korsmeyer Peppas model. A regression value of 0.8964 ($r^2 < 1$) by Higuchi confirms the release as fairly linear to linear drug release. Nanoparticles utilized in topical delivery have the potential to achieve deeper penetration into blood vessels compared to intravenous administration when given without ligands as per the previous studies.[17] This entails conducting evaluations with cell line studies using the specific cells of RP individuals, known as Human umbilical cord endothelial vein cells. Further studies and research are necessary to advance the developed TDL-loaded nano-ointment formulation in animal and human models.

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