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ORIGINAL ARTICLE

MICROEMULSION: A POTENTIAL APPROACH FOR TRANSDERMAL DELIVERY OF SILDENAFIL CITRATE

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Summary

Sildenafil citrate has low oral bioavailability, systemic adverse effects, and a relatively delayed action. These issues may be addressed through direct transdermal delivery to the penis. This study aims to investigate the microemulsion formulation of the drug for effective transdermal delivery. Sildenafil citrate was formulated as a microemulsion using clove oil, dimethyl sulphoxide, phosphate buffer (pH 7), propylene glycol, Tween®80, and distilled water. Different proportions of these components were used to create six formulations of the microemulsion (F1–F6), which were then characterised by their physical appearance and clarity, pH, viscosity, conductivity, percent transmission, and droplet size. Furthermore, the stability, content analysis, in-vitro drug release, and transdermal permeation of sildenafil citrate from the generated drug-loaded microemulsions were studied. All prepared formulas contained nano-sized oil droplets (less than 20 nm), and the pH values were within the range of skin pH; however, two formulas were not transparent. Additionally, all formulations were thermodynamically stable, passing freeze-thaw, heating-cooling, and centrifugation tests. Next, the formulas demonstrated zero-order release kinetics, indicating that they can provide a sustained release profile for sildenafil citrate. Finally, the microemulsion formulation exhibited a 2.8-fold enhancement in skin permeation compared with that of the sildenafil citrate suspension. The prepared microemulsions demonstrated beneficial physical properties and skin permeation profiles that are promising for the local administration of sildenafil citrate.

Key words: Microemulsion; sildenafil citrate; transdermal delivery; permeation enhancer

Introduction

Sildenafil citrate is the first drug approved for erectile dysfunction to achieve major success, and it acts by selectively inhibiting phosphodiesterase 5. However, the only approved dosage form of the drug is the tablet formulation (1), which has several limitations: up to 71% of an oral dose is metabolised in the first pass, low oral

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bioavailability (40%), adverse reactions from systemic distribution of the drug (hypotension, headaches, nasal congestion, and flushing), and delayed onset and short duration of action (2, 3). Direct application of the drug through transdermal delivery may help to overcome the shortcomings associated with oral use (4).

Nonetheless, skin penetration with sildenafil is an issue because it is amphoteric and has pH-dependent properties, in addition to its poor solubility in both aqueous and nonaqueous media (3). The pH-dependency implies that the drug has different ionisation states based on the pH of the medium, affecting its skin permeation (5). Consequently, to develop topical dosage forms of sildenafil, the parameters responsible for its permeation must be modified (6).

Microemulsions are clear, isotropic liquid combinations of oil, water, and surfactant that are usually combined with a co-surfactant and are thermodynamically stable. They differ from conventional emulsions in that they are formed by simple mixing of the components without the need for the high-shear conditions typically associated with the production of ordinary emulsions (7). Microemulsions are gaining popularity as delivery systems for a multitude of reasons: ease of preparation, thermodynamic stability, transparency and elegance, increased drug loading, improved membrane penetration and bioavailability, and enhanced pharmacokinetic consistency (8).

Clove oil has previously been used in formulations for microemulsions. Notably, it enhances blood flow and nerve stimulation in the genitals, which is effective for treating erectile dysfunction, in addition to delaying premature ejaculation (9, 10). This work aims to combine the therapeutic effects of sildenafil and clove oil in a microemulsion for the treatment of erectile dysfunction while simultaneously exploiting the properties of clove oil and the appropriate solvents to enhance its skin penetration for local application.

Materials and methods

Materials

Sildenafil citrate was donated by Awamedica. Clove oil, dimethyl sulphoxide (DMSO), propylene glycol, and Tween®80 were purchased from Sigma. Dialysis bags (MWCO 3KD) were obtained from Fisher Scientific.

Methods

1. Construction of pseudoternary phase diagrams

Clove oil was employed as the oil phase for the microemulsion, while Tween®80 and propylene glycol were selected as the surfactant and co-surfactant, respectively. For the aqueous phase, 10% DMSO in phosphate buffer was used. Pseudoternary phase diagrams were constructed using an aqueous titration method to determine the existence zone of the microemulsion. Surfactant and co-surfactant were mixed (S_{mix}) in weight ratios of 1:0, 1:0.5, 1:1, and 1:2.

Clove oil was mixed with the individual S_{mix} mixtures in 16 different ratios (1:9, 1:8, 1:7, 1:6, 1:5,1:4, 1:3.5, 1:3, 1:2.3, 1:2, 1:1.5, 1:1, 6:4, 7:3, 8:2, and 9:1) for the preparation of the separate phase diagrams, where one phase diagram was constructed for each S_{mix}. These combinations were used to determine the maximum ratio that needs to be covered to delineate the boundaries of the specific phase diagram. Visual inspection for a transparent, clear, and easily flowable microemulsion was performed to determine the endpoint for the slow titration using the aqueous phase. Pseudoternary phase diagrams were used to mark the physical state of the combinations, with the three axes representing the oil phase, the aqueous phase, and the surfactant/co-surfactant mixture. These diagrams were constructed using the Ternary Phase Diagram Software (Chemix School Ver.3.50, USA).

2. Preparation of the loaded microemulsion

Tween®80 (surfactant) was dissolved in propylene glycol (co-surfactant) to prepare the oil-in-water (O/W) microemulsion. Clove oil containing sildenafil citrate was added to the mixture, which was then gently mixed with the aqueous phase while being vortexed at room temperature until the resultant system was transparent.

Monophasic formulations formed spontaneously at room temperature. Six formulations (F1–F6) were prepared using different ratios of sildenafil, oil, aqueous phase, and surfactant/co-surfactant, as illustrated in Table 1.

Table 1. Composition (% w/w) of the six formulations of sildenafil citrate microemulsion.

	F1	F2	F3	F4	F5	F6
Sildenafil citrate	2.44	2.44	4.76	2.44	2.44	4.76
Clove oil	9.76	9.76	19.05	9.76	9.76	19.05
Tween®80	9.76	14.63	19.05	13.01	19.51	25.4
Propylene glycol	9.76	14.64	19.05	6.5	9.76	12.7
10% DMSO in phosphate buffer	68.29	58.54	38.1	68.29	58.54	38.1

3. Inspection of the physical properties

Physical properties of the microemulsion, including appearance and clarity, pH, viscosity, conductivity, and % transmission, were evaluated. The appearance and clarity of the drug dissolved in the microemulsion were visually examined.

4. Determination of droplet size in microemulsion

The average size of the droplets in the microemulsion samples was measured at 25°C using a Malvern Zetasizer (Worcestershire, UK) equipped with a 2000 Hydro MU wet dispersion unit. A disposable polystyrene cuvette was used to hold the microemulsion and sildenafil citrate microemulsion-loaded hydrogel (2.74 g), and a plastic syringe or micropipette was used for the transfer. Volume distribution was used for the calculation of the droplet size (11).

5. Testing the stability of the drug-loaded microemulsions

A. Heating-cooling cycles: Six consecutive cycles of heating and cooling were conducted from refrigerator temperature (4°C) to 45°C. Samples were stored for no less than 48 hours at each temperature, and their stability was examined.

B. Centrifugation test: Phase separation was assessed after centrifuging the formulations at 3,500 rpm for 30 minutes.

C. Freeze-thaw cycles: Cycling experiments were conducted in triplicate between −21°C and +25°C, storing the formulations at each temperature for 48 hours.

6. Sildenafil citrate content analysis

For each of the prepared formulations, the content of sildenafil citrate was determined by diluting 100 µL of the microemulsion with phosphate buffer and measuring the content by ultraviolet (UV) spectrophotometry at 280 nm.

7. *In-vitro* drug release

This was performed using the analysis bag method. Each of the prepared formulations (1 mL) was transferred to dialysis bags (MWCO 3KD). The bags were then fixed to a dissolution paddle with 200 mL of phosphate buffer as the dissolution medium. Samples (3 mL) were taken from the medium at specified intervals and their sildenafil citrate content was determined by UV spectrophotometry at 280 nm. Fresh buffer solution was added at each time point to compensate for the volume sampled. The spectrophotometric measurements were performed in triplicate.

8. Transdermal permeation study

Permeation of sildenafil citrate from the microemulsion formulations through the skin was examined and compared with that of the suspension (25 mg/mL of sildenafil citrate). This experiment was performed using an automated Franz diffusion cell apparatus (MicroettePlus; Hanson Research, Chatsworth, CA, USA).

The process started with the excision of full-thickness skin from the abdomen of rats. After removing the hair with a clipper and surgically removing the subcutaneous tissue, the remaining skin was placed between the chambers of the Franz apparatus with an effective diffusion area of 1.76 cm^2 and a cell volume of 12 mL. Phosphate buffer (pH 7) was used as the receptor fluid and placed in the corresponding chamber of the Franz diffusion cell apparatus. The microemulsion (1 mL) was applied to the donor chamber in direct contact with the animal skin. Samples (3 mL) were collected from the receptor chamber at 0.5, 1, 1.5, 2.5, 3.5, 6.5, 10.5, and 24 hours. After each collection, the volume in the acceptor chamber was replenished with 3 mL of fresh buffer solution. Then, the amount of sildenafil in each of the collected samples was determined spectrophotometrically at 280 nm. In addition to the prepared microemulsion formulations, a suspension of sildenafil citrate was used in the permeation study for comparison.

A plot was generated for the accumulated sildenafil citrate that permeated the skin, per unit area of the membrane, over time. The slope of the linear portion was used in the calculation of the steady-state transdermal flux [J_{ss} ($g/cm^2/h$)]. The permeability coefficient [P_c (cm/h)] was computed by dividing the steady-state flux by the initial drug concentration in the donor compartment (C_d), and the diffusion coefficient (D) was obtained using the following equation: $\boldsymbol{D = (Slope/2C_d)^2 * \pi}$. Finally, the enhancement ratio (ER) was calculated as the ratio of J_{ss} for the optimised microemulsion to that of the suspension (4).

9. Adherence to ethical standards

This work did not involve experiments on living organisms. Therefore, no ethical approval was needed.

Results and discussion

According to the results, the formulation of sildenafil citrate as a microemulsion, utilising clove oil as a carrier, Tween®80 as a surfactant, propylene glycol as a co-surfactant, DMSO as a co-solvent and a phosphate buffer aqueous phase, exhibits high drug solubility, skin penetration, and overall bioavailability and action.

Microemulsions, in general, have a penetration-enhancing ability owing to their oily nature and small droplet size (8, 10). For the formulation in this study, there is an additional enhancement of sexual activity brought about by clove oil, which can help to delay premature ejaculation (9, 12).

Sildenafil citrate solubility and penetration profiles are pH-dependent. Whereas the maximum drug solubility is achieved at acidic pH levels, maximum penetration requires a higher pH value (in the alkaline range, between 8 and 11) (10, 13). To achieve the balance between solubility and penetration in this study, phosphate buffer at pH 7 was used as the aqueous phase, along with DMSO as a co-solvent. This combination was considered to provide acceptable solubility of sildenafil citrate, enhanced by the co-solvent, which also served to increase permeation through its recognised permeability augmenting effect. It has been reported that the sildenafil citrate solubility in Tween®80 and propylene glycol is high relative to other surfactants and co-surfactants (14); therefore, their selection in this work aids solubility further.

It is important for microemulsion preparation to establish the appropriate range of component concentrations essential for its existence and stability, for example, by developing the ternary phase diagram. Figure 1 shows the three essential elements of the microemulsion (oil, aqueous phase, and surfactant/co-surfactant mixture), represented as a ternary diagram. The curve represents the line that separates the area of translucent microemulsion (above the curve) and the area of turbid emulsion (below the curve), based on visual observation. The diagram shows that when the co-surfactant ratio is increased, the microemulsion area increases. This effect of the co-surfactant may be attributed to its ability to lower the surface tension, bringing it close to zero by fluidising the film

of molecules at the interface and aiding the surfactant, Tween®80, in lowering the surface tension, as well as its ability to exist in both phases of the microemulsion, thus enhancing the drug solubility (11, 15).

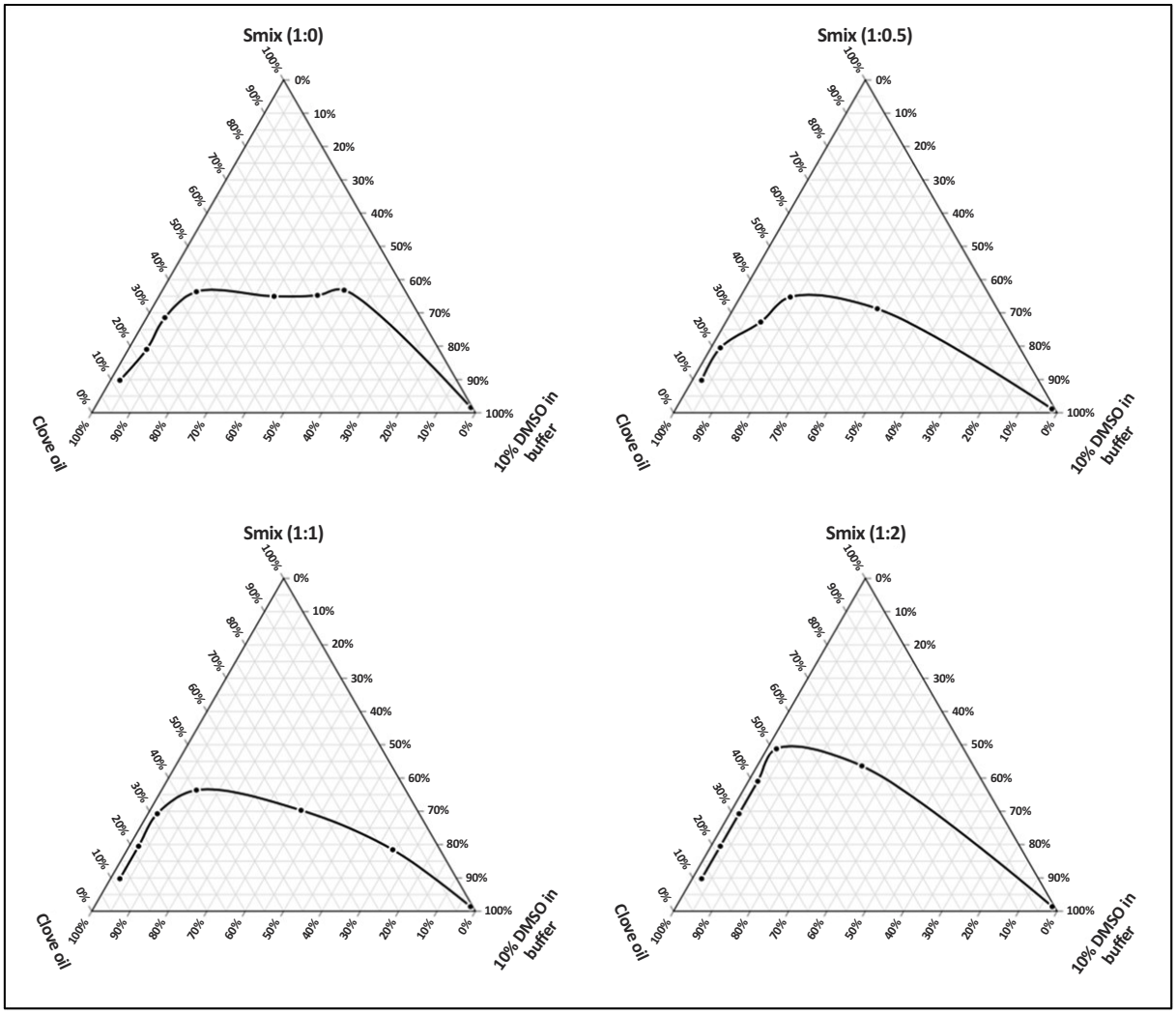

Figure 1. The pseudoternary diagram of sildenafil citrate microemulsion, composed of clove oil, 10% DMSO in phosphate buffer (pH 7), and Tween®80/propylene glycol as the oil phase, aqueous phase, and S_{mix}, respectively. The areas above the curves represent the areas of the microemulsion.

The balance in the surfactant/co-surfactant ratio was achieved by maximising the microemulsion area, which occurred at the ratio of 1:1 (surfactant:co-surfactant). Further increasing the co-surfactant content (1:2) reduced the microemulsion area within the ternary phase diagram. Theoretically, the interaction between the surfactant and the co-surfactant occurs at the surfactant monolayer of the interface, thereby affecting the packing of the two components and providing additional fluidity to the interfacial film.

Based on the results of the ternary diagram, S_{mix} ratios of 1:0.5 and 1:1 were selected to formulate sildenafil citrate-loaded microemulsions, and three formulas based on each S_{mix} ratio were prepared (F1–F6), as shown in Table 1. For the preparation of drug-loaded microemulsions, the required amount of sildenafil citrate was dissolved in the oil phase and mixed with the specified amount of the surfactant and co-surfactant mixture. The aqueous phase (10% DMSO in phosphate buffer at pH 7) was then added dropwise until a clear and transparent liquid was obtained.

Table 2 presents the physical properties of the prepared formulas, showing that all of them contained nano-sized oil droplets (less than 20 nm). The viscosity and conductivity change with formula composition; the viscosity increases and the conductivity decreases with the increasing ratio of oil to S_{mix}. The pH values of the formulas depended on the concentration of clove oil, but they were still in the range of skin pH. The entrapment efficiency exceeded 90%, except for F3 and F6, which had higher oil ratios, reflecting the effect of the aqueous phase containing DMSO on sildenafil citrate solubility.

Table 2. Physical properties of the prepared microemulsion formulas of sildenafil citrate.

	Size (nm)	pH	Conductivity	Viscosity (mPas)	Transmittance %	Entrapment %
F1	16 ± 6.3	6.5	1400	9.75	92.4	90.5
F2	13.6 ± 5.4	6.5	840	43.7	97.4	92.0
F3	14.8 ± 6.3	5.5	193	124.6	95.9	78.1
F4	12.6 ± 5.7	6.5	1200	14.3	63.5	90.5
F5	11.7 ± 3.5	6.5	1200	54.4	98.8	95.1
F6	12.7 ± 4.7	5.5	425	140.6	98.1	64.9

All formulas were transparent by visual inspection, except for F1 and F4, which had hazy and opaque appearances, respectively, despite their small droplet size. This observation is reinforced by the transmittance results. The appearances of F1 and F4 may be attributed to the low solubility of sildenafil citrate, either because of the high concentration of the drug or the low amount of propylene glycol in the formulations.

The freeze-thaw cycling of the sildenafil citrate microemulsion formulations showed that the emulsions were thermodynamically stable, without any signs of turbidity or phase separation. In addition, centrifugation was performed to ensure the stability of the formulations under high-shear conditions. Successful microemulsion formulations do not show any phase separation, turbidity, colour change, or drug precipitation, and all of our prepared sildenafil citrate formulations passed this assessment.

Zero-order kinetic profiles were observed for sildenafil citrate release from the formulations (Figure 2), which may be explained by the partial solubility of the drug within the external phase and the action of DMSO and the surfactant/co-surfactant, resulting in the subsequent release from the internal phase of the microemulsion. Nevertheless, these formulations can provide a sustained release profile of sildenafil citrate.

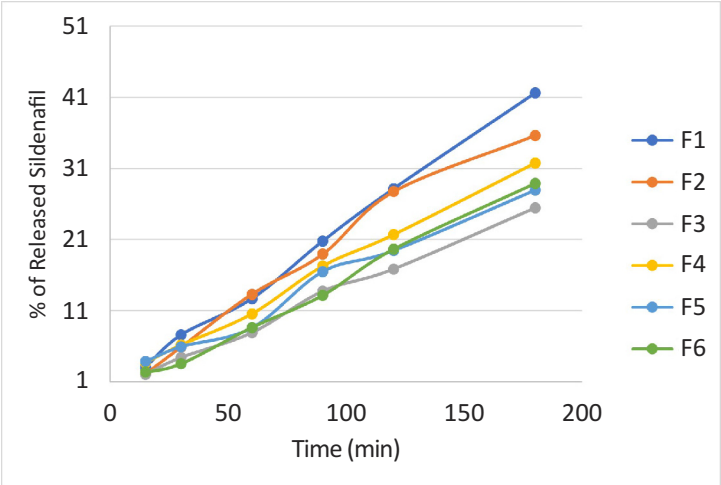

Figure 2. The dissolution profile of the prepared sildenafil citrate formulations in phosphate buffer (pH 7) using dialysis bags (MWCO 3KD).

The *in-vitro* skin permeation study demonstrates the permeation ability of the selected microemulsion formulas and their advantage over the sildenafil citrate suspension in terms of the cumulative amount of drug that permeated through the skin, in addition to the transdermal flux and permeability coefficient, as shown in Table 3 and Figure 3. Further analysis of the results reveals that the permeation parameters were higher for formulas with an S_{mix} ratio of 1:1 (F1–F3) compared with the ratio of 1:0.5 (F4–F6), which indicates the effect of propylene glycol on skin penetration. Moreover, F1, which contains a larger portion of the aqueous phase (10% DMSO in phosphate buffer), showed the highest transdermal flux and permeability coefficient, most likely related to the permeation-enhancing effect of DMSO.

Table 3. Results of the *in-vitro* skin permeation study of the prepared sildenafil citrate formulas.

Formula	J_{ss} (μg/cm^2/h)	P_c (cm/h)	D (cm^2/h)	ER
F1	283.4	9.6	3596.5	2.4
F2	275.3	9.3	3386.1	2.3
F3	239.2	5.7	2524.2	2.8
F4	232.1	6.0	1418.1	1.5
F5	193.9	7.8	2363.3	1.9
F6	150.2	5.5	2382.0	2.8
SC suspension	100.4	4.0	633.6	---

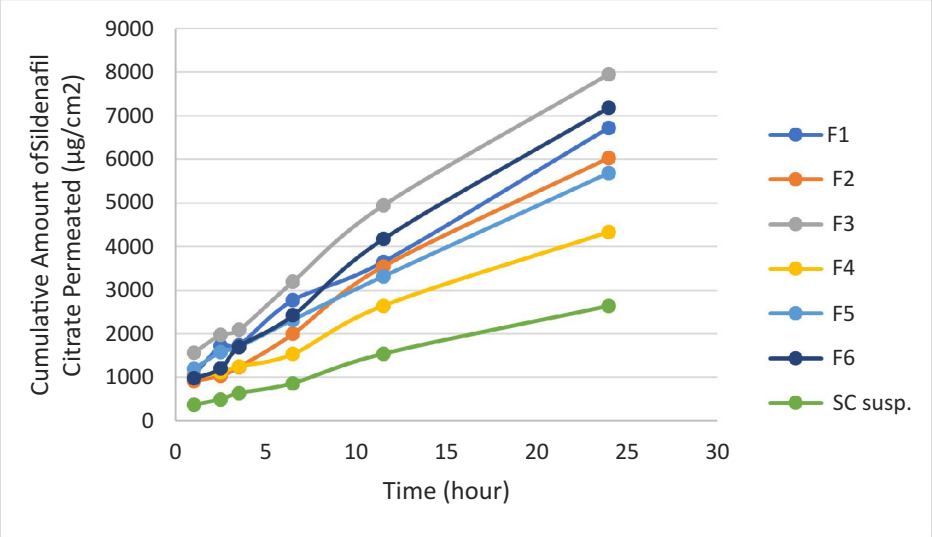

Figure 3. *In-vitro* permeation study of sildenafil citrate-loaded microemulsion formulas against sildenafil citrate suspensions through rat skin.

Figure 3 shows that the amount of sildenafil citrate permeation by microemulsion formulations was significantly higher than that from the suspension. The factors associated with the permeation can be related to the type of motion of the active substances within the formulation and their ability to release from the formulation and pass through the skin, especially the outer surface (stratum corneum). These factors affect the thermodynamic behaviour of the drug within the formulation, ultimately influencing the release and permeation through the skin.

The formulation of sildenafil citrate as a microemulsion imparts several advantages to drug permeation, as shown in Figure 3. This can be explained by the increased contact area between the drug and the skin, partly due to the particle size reduction provided by the formulation itself and also owing to the film-forming ability.

These factors maximise the amount of drug penetrating the skin surface. Additionally, the clove oil, surfactant, and DMSO all act by slackening the skin lipoid membrane to potentially boost sildenafil citrate solubility in the skin. The partition coefficient is another parameter that was improved by the microemulsion formulation and the choice of its components, particularly the hydrogen-bonding solvent, DMSO. This may cause membrane expansion and an increase in diffusivity and drug flux through the skin membrane, resulting in smooth drug partitioning from the formulation into the "universal solvent" within the tissue (11).

Most of the adverse reactions associated with the use of DMSO as a co-solvent and penetration enhancer are related to its concentration in the formulation and are most prominent at concentrations equal to or exceeding 60%. Fortunately, many studies report that DMSO is a safe pharmaceutical additive when used within a 10% limit, which is the case in this study (11, 16, 17). Moreover, the solubility of sildenafil citrate in Tween®80 and propylene glycol is high relative to other surfactants and co-surfactants (14); therefore, their selection for use in the formulations in this work imparts further aid in augmenting the solubility of sildenafil citrate.

Conclusions

Transdermal application is a promising route for the effective and selective administration of sildenafil citrate. However, the physicochemical characteristics of this drug, and their effects on skin penetration, present difficulties in the development of a successful transdermal formulation. Microemulsions, like other lipid-based systems, typically exhibit high skin penetration. Therefore, we investigated a unique microemulsion composition (clove oil, 10% DMSO in phosphate buffer, and Tween®80/propylene glycol) for transdermal administration of sildenafil citrate. This combination leverages different permeation enhancers (the oil, DMSO and S_{mix}) to offer high solubility of sildenafil citrate at high pH, with the additional sexual benefits imparted by clove oil for treating premature ejaculation. The results indicated that S_{mix} ratios of 1:1 and 1:0.5 provided the highest performance. All prepared microemulsions contained nano-sized oil droplets and were stable under stress conditions. In addition, the microemulsions demonstrated higher skin permeation compared with conventional drug suspensions. The formulas with an S_{mix} ratio of 1:1 showed the highest permeation parameters, particularly the formula with a high percentage of the aqueous phase and DMSO. In future work, it is necessary to examine the skin penetration and therapeutic effects of the formulations in animals.

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Conflict of interests

The authors have no conflicts of interest to declare.

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