

Research Article



The Relationship between Solubility and Transdermal Absorption of Tadalafil

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Abstract

Purpose: The aim of this study was to find a relationship between drug solubility and its transdermal permeation and find the best vehicle composition to improve transdermal permeation of Tadalafil.

Methods: Pure or binary mixtures of commonly used solvents in pharmaceutical sciences including ethanol, glycerin, N-methyl pyrrolidone (NMP), polyethylene glycol (PEG) 400 and propylene glycol (PG) were evaluated for drug solubility and transdermal delivery through the exercised rat skin employing Franz diffusion cells.

Results: Tadalafil showed higher solubility in NMP compared to the other solvents. The amount of Tadalafil permeation from the pure vehicles was ranked as follow: Ethanol >glycerin >NMP>PEG 400 >PG. Furthermore, the solubility and transdermal delivery from binary mixtures of NMP and PG were higher than that obtained from pure PG, and accordingly, both increased with increasing NMP concentration in the binary solvent mixtures. The Flux values were determined as following order for Ethanol>NMP>glycerin>PG>PEG 400.

Conclusion: Generally, increase in Tadalafil solubility resulted in a decrease in its skin penetration rate and amount. However, NMP exhibited substantial drug skin penetration rate and amount accompanying with appropriate drug solvency. In conclusion, the results of this study introduced NMP as a solvent suitable for application in the formulation of topically applied drug delivery systems.

Introduction

Transdermal and dermal delivery systems represent an attractive alternative route of administration to oral delivery systems, which are available for systemic and local effect of drugs. Dermal and transdermal delivery systems have proven to be effective in reducing dose frequency, long duration of activity, achieving target delivery, easily administrating and avoiding hepatic first pass metabolism.¹⁻⁴ The stratum corneum, or horny layer, is the outermost layer of the skin and has been identified as the main barrier of the most drug permeation. Various strategies have been employed in attempts to improve the efficacy of topical delivery of drugs, including iontophoresis, electroporation, occlusion and ultrasound.⁵⁻⁹ Furthermore, an alternative approach is to use chemicals, known as permeation enhancers, which are materials that can partition into, and interact with, skin constituents to induce a temporary and reversible decrease in the skin barrier properties.^{10,11} The therapeutic effects of transdermal formulations depend on the drug action and also on other structural factors of the vehicle. Therefore, when a drug is formulated to be administered by transdermal route, it is essential to

investigate the excipients and vehicles properties.^{3,12-16} There are a few solvents which have been used frequently in the formulation of TDDSs as carrier and penetration enhancer, as well, such as ethanol, glycerin, propylene glycol (PG) and polyethylene glycols (PEGs).¹⁷⁻²⁰ Pyrrolidones, such as N-Methyl-2-pyrrolidone (NMP), have been proposed as penetration enhancers. As with many other penetration enhancers, pyrrolidones are able to promote both the penetration of hydrophilic drugs and the penetration of lipophilic drug substances. NMP is also a cosolvent and very strong solubilizing agent that has important applications in different fields of industry.²¹⁻²⁴ NMP is a biodegradable FDA approved solvent listed in GRAS (generally recognized as safe), therefore, environmental contamination considerations are fewer in its applications.^{22,25}

Tadalafil is a potent, reversible and competitive inhibitor of phosphodiesterase 5 which inactivates cyclic guanosine monophosphate used in the treatment of erectile dysfunction by facilitating relaxation of smooth muscle resulting to penile erection. Tadalafil clearance is

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mainly via hepatic metabolism by CYP3A to a catechol that undergoes methylation and is extensively conjugated to form a methyl catechol glucuronide which is a major circulating metabolite.²⁶⁻²⁸

The administration of Tadalafil through the skin could provide many benefits compared with the oral administration, where the first pass metabolism as well as gastro intestinal side effects will be avoided. Moreover, side effects like headache, stomach upset, back pain, muscle pain, stuffy nose, flushing, or dizziness were reported as well. Therefore, it is valuable local therapy application in male genital system.²⁹⁻³¹ However, it is difficult to reach therapeutic levels because of the difficulty for the drug to penetrate through the skin barrier. It was shown that increase in drug solubility in the carrier causes decrease in skin penetration ability of the topically designed dosage form. On the other hand, drug solubility guarantees content uniformity of dosage form which is important for topical delivery that intrinsically suffers from erratic and unpredictable drug absorption.³²⁻³⁴ Therefore, investigation on the selection of proper solvent for formulation of TDDSs which simultaneously provides adequate drug solubility and appropriate skin penetration would be invaluable. Therefore, the aim of the present study was to investigate the effects of different vehicles on the solubility and transdermal delivery of Tadalafil.

Materials and Methods

Materials

Tadalafil was kindly donated from OSVE Pharmaceutical Company (Tehran, Iran). Acetonitrile, glycerin, propylene glycol, polyethylene glycol 400 (PEG 400) and N-methyl pyrrolidone (NMP) were purchased from Merck Company (Darmstadt, Germany).

Solubility study

Solubility of Tadalafil in ethanol, acetonitrile, glycerin, propylene glycol, PEG 400 and N-methyl pyrrolidone and different combinations of mixture of NMP-PG was checked. Excess amounts of Tadalafil were added to sealed vials containing solvents or mixtures. All dispersions were shaken for 24 h at room temperature (25 °C). The dispersions were then filtered using hydrophilic Durapore filters (0.45 µm, Milipore, Ireland). To determine the amount of drug dissolved, HPLC method was employed. Aliquots were examined and the solubility of drug was identified in each sample. Experiments were carried out in triplicates.

In vitro skin permeation studies

The abdominal skin of Wistar male rats, weighing 140-180 g, was shaved using an electric razor after scarifying animals by excess chloroform anesthesia (24 h before the treatment). The experiments were performed in accordance with ethical committee and the guide lines of the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran (National

Institutes of Health Publication No 85-23, revised 1985). The abdominal skin was surgically excised and excess subcutaneous fat was carefully removed. To remove extraneous debris and leachable enzymes, the dermal side of the skin was kept in contact with a normal saline solution up to 12 h before using for permeation studies. The skins were mounted on the Franz diffusion cells (Erweka HDT6, Germany) (with an available diffusion area of 3.14 cm²) with the stratum corneum facing the donor compartment and the dermis facing the receptor. Each set of experiments was performed with three diffusion cells. Equal amounts of Tadalafil was dissolved in solvents. Twenty four milliliter of hydroethanolic solution (50:50, v:v) was used as the receptor medium and 2 mL of the each solution was placed on the skin surface in the donor compartment. The temperature of the receptor medium was maintained at 37±2 °C by circulating of warm water between two layers of the diffusion cells and contents of receptor medium were stirred magnetically at a constant rate of 750 rpm. Samples (0.1 mL) were withdrawn from the receptor compartment at different time intervals (30, 60, 120, 240, 360, 480 and 720 min) and replaced with the same volume of hydroethanolic solution at 37±2 °C to maintain a constant volume. The amount of Tadalafil in the receptor phase was assayed with an HPLC method. The cumulative amount of the permeated Tadalafil per unit area of skin was plotted versus time. The permeability test was performed in triplicate.

Analytical procedure

The HPLC apparatus (Knauer, Germany) equipped with UV detector and an ODS C18 (250×4.6 mm, 5 µm) column was used to assay the amount of Tadalafil. The mobile phase consisting of phosphate buffered saline (PBS): acetonitrile (30:70) mixture eluted the column at the flow rate of 0.7 mL/min and the effluent was monitored at 290 nm using a UV detector. 20 µl of sample was injected into the HPLC column and the retention time of Tadalafil at this HPLC condition was 5.1 min. Calibration curve with standard concentrations ranging from 0.125 to 5 µg/mL of Tadalafil in mobile phase was constructed to measure the drug concentration in the samples.

Data treatment

The cumulative amount of the permeated drug was plotted against time. Slope of the linear portion of the cumulative drug permeated vs. time plot and the intercept on the time axis are considered as the steady-state Flux (J) value.

Statistical analysis

The data was demonstrated as mean ± SD. Analysis of variance (ANOVA) was performed for multiple comparisons using SPSS software (version 15). A level of significance of P < 0.05 was set to determine any significant difference between the formulations.

Results and Discussion

Solubility of Tadalafil in different vehicles

Table 1 shows the solubility of Tadalafil in different vehicles used for transdermal drug delivery. Results demonstrated that Tadalafil showed the highest solubility in NMP compared to the other used pure or binary vehicles. Results also indicated that the solubility of Tadalafil was increased significantly via increasing the ratio of NMP in the binary solvent mixtures with PG. Although the studied solvents are routinely used in the formulation of TDDSs, few disadvantages are reported

such as skin dryness using ethanol³⁵ as well as sticky and greasy characteristics for glycerin³⁶ which cause patient incompletion and even stop the treatment procedure. PG have shown some merits among these solvents for application in TDDSs such as lack of above mentioned drawbacks and skin permeation enhancement ability.^{3,37} Therefore, the binary mixtures of PG and NMP in different ratios were prepared to provide the benefits of skin permeation enhancement and high drug loading and solubility providing content uniformity of dosage form, simultaneously.

Table 1. Permeated Tadalafil from the rat skin during 12 h ($\mu\text{g}/\text{cm}^2$), Flux values ($\mu\text{g}/\text{cm}^2/\text{h}$) and solubility (mg/mL) of Tadalafil in different vehicles. Data are presented as mean \pm standard deviation (n=3).

Vehicles	Glycerin	Ethanol	PG	PEG 400	NMP	PG: NMP (75: 25)	PG: NMP (50: 50)	PG: NMP (25: 75)
Permeated drug after 12h	5.37 \pm 0.44	6.01 \pm 0.10	1.88 \pm 0.12	2.41 \pm 0.16	4.55 \pm 0.31	3.21 \pm 0.05	2.30 \pm 0.19	1.57 \pm 0.55
Flux	0.26 \pm 0.02	0.41 \pm 0.03	0.14 \pm 0.01	0.10 \pm 0.02	0.34 \pm 0.02	0.23 \pm 0.01	0.11 \pm 0.02	0.06 \pm 0.02
Solubility	0.17 \pm 0.05	1.98 \pm 0.30	1.99 \pm 0.08	14.7 \pm 2.86	344.99 \pm 13.10	13.71 \pm 0.56	26.77 \pm 3.80	220.87 \pm 10.12

Skin permeation study of Tadalafil from different vehicles

Figure 1 illustrates the skin permeation of Tadalafil from different vehicles. As it is shown, Tadalafil which dissolved in glycerin, ethanol or NMP showed higher skin permeation over 12 h than that of dissolved in PG or PEG 400. Accordingly, calculated Flux values of Tadalafil from different formulations also indicated that formulations containing ethanol, NMP and glycerin possess the higher Flux values compared with those possess PG and PEG 400. Furthermore, incorporation of PG and NMP as Tadalafil vehicle increased the Flux value and amount of permeated drug over 12 h. On the

other hand, as shown in Figure 1, Glycerin not only increased the total amount of permeated drug over 12 h, but also enhanced the rate of permeated drug, where highest amount of Tadalafil was permeated during 1 h compared to the other vehicles.³⁸ This could be advantageous in the reducing the time needed for the onset of action especially for drugs such as Tadalafil.

Although it was shown that improving the drug solubility by addition of NMP to PG decreased drug penetration rate and amount through skin, but NMP with almost more than 20 times higher capacity for drug solubility illustrated better drug transdermal potential and homogenous distribution for NMP than PG and PEG400.

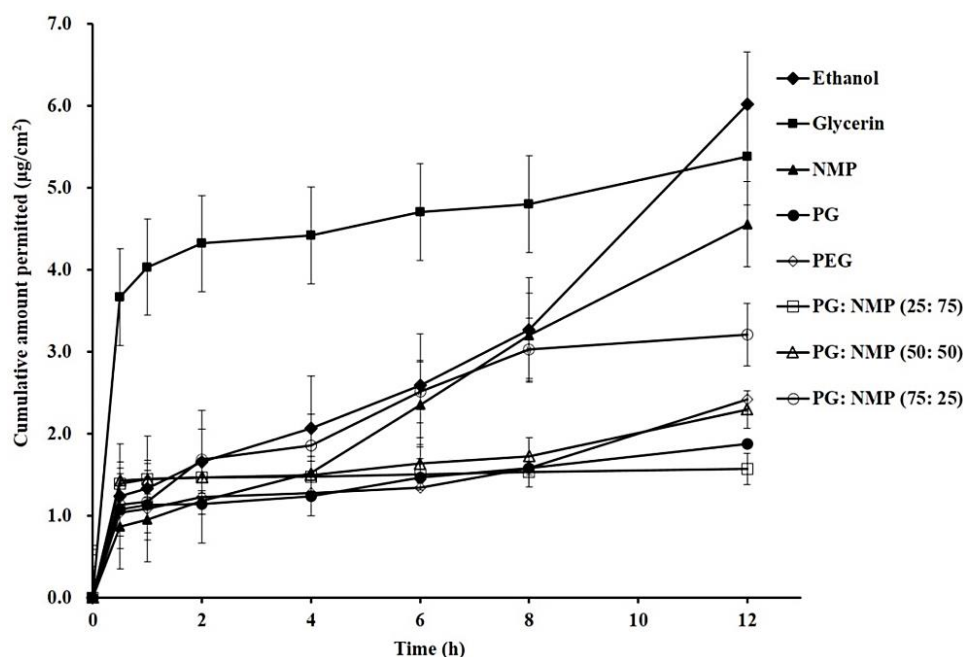


Figure 1. Tadalafil skin permeation from different vehicles. [N-Methylpyrrolidone: NMP, propylene glycol (PG) and poly ethylene glycol (PEG) 400].

Figure 2 compares the solubility and total permeated Tadalafil during 12 h ($\text{AUC}_{0-12\text{h}}$) by using different

vehicles. Although glycerin and ethanol showed higher permeability, Tadalafil exhibited lowest solubility in

these solvents. On the other hand, Tadalafil exhibited the highest solubility and AUC_{0-12h} value in the case of NMP compared with other solvents. Figure 2 also indicates that by using the binary solvent mixtures,

composed of PG and NMP, and increasing the ratio of NMP, both of the solubility and AUC_{0-12h} values were increased simultaneously.

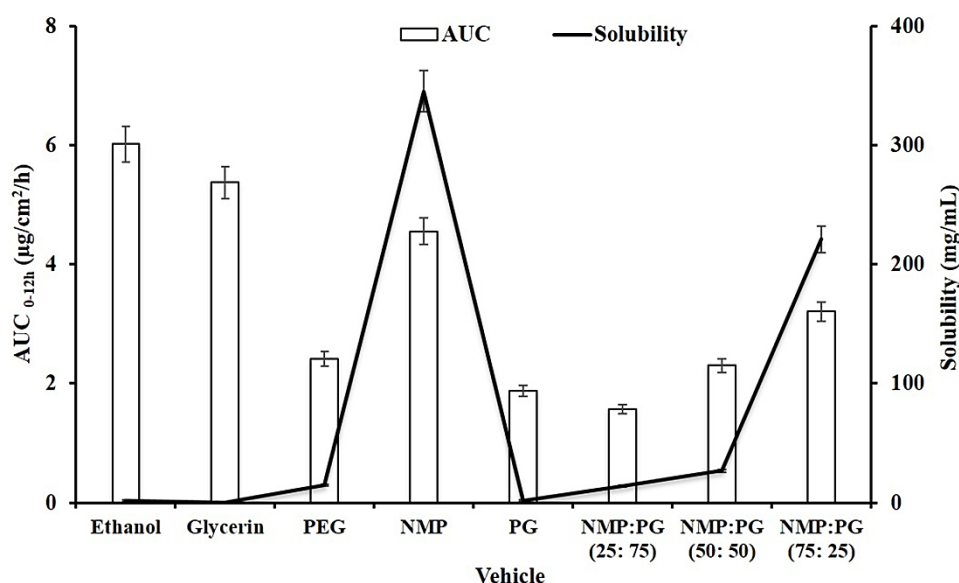


Figure 2. Solubility and Permeated Tadalafil from the rat skin (AUC_{0-12h}) using different vehicles

Although total permeated Tadalafil during 12 h from formulations containing ethanol and glycerin were higher than that contains NMP, higher solubility of Tadalafil in NMP allows using minimum amount of NMP with ideal homogeneity in the formulation. This resulted in lower probable harmful effects of NMP on the skin. Results of using binary solvent mixtures also indicated that by increasing the ratio of NMP, solubility and skin permeation values of Tadalafil were increased concurrently.

Pyrrolidone is a component of natural moisturizing factor, therefore its derivatives and structurally related compounds are studied as penetration enhancers.^{21,23,25} These enhancers are known to improve transport of drugs of varied lipophilic (betamethasone-17-benzoate, hydrocortisone and progesterone)/hydrophilic (mannitol, 5-fluorouracil and sulphaguanidine) properties. Mechanism of action of the pyrrolidones is partition into human stratum corneum. NMP is a polar and clear solvent, liquid at room temperature, miscible with most common solvents and was used to extract aromatic moieties from oils, olefins and animal feeds.^{22,24,39,40} The mechanism of solubilization of drugs by NMP is ambiguous, and there are various theories for the same, including its action as a cosolvent, complexing agent and surfactant. The NMP molecule has nonpolar carbons, which can weaken the hydrogen-bonded structure of water, thus enabling it to act as a cosolvent. In addition, the presence of a large planar nonpolar region can lead to hydrophobic interactions between NMP and drugs. NMP exerts its direct influence on the aqueous regions of skin between the polar lipid head groups of the bilayer. It penetrates into this region of skin in such amounts that

they alter the solubilizing ability of this site, thereby promoting drug partition into skin, which subsequently results in increased flux of the penetrant.⁴¹ Comparison of the Tadalafil penetration patterns of different solvents demonstrated that NMP caused a sustained penetration for Tadalafil (Figure 2). It was reported that, inside the tissue, NMP alters the solvent surrounding the membrane and creates reservoirs in skin membranes, giving potential for sustained release of a drug from the stratum corneum during prolonged time periods.^{23,25,37,40}

In Lee et al.'s investigation, NMP was found to be an effective enhancer for transdermal lidocaine from a hydrophobic formulation and also mixtures of NMP with isopropyl myristate resulted in synergistic improvement in lidocaine transdermal delivery.¹⁴ Our findings showed that binary mixture of NMP and PG did not enhance drug permeation synergically, but our goal was to improve enhancement efficacy of PG with NMP which was successfully carried out. Further investigations indicated that combination of NMP with lauryl-2pyrrolidone and isopropyl myristate significantly increased the permeation of phenol red and 5-fluorouracil. Furthermore, NMP in combination with Isopropyl myristate increased metronidazole penetration through human skin.^{22,23,42}

NMP has been also used to accelerate the permeation of mefanamic acid across rabbit skin in vivo.⁴³ Other study showed that NMP in combination with propylene glycol enhanced the transport of naloxone through human skin.⁴⁴

Although NMP in the present study and previous reports exhibited substantial results in transdermal delivery of various classes of drugs, however, the clinical use of

pyrrolidones as skin penetration enhancers is limited because of adverse reactions to these compounds which are associated with local adverse reactions, including irritant dermatitis and erythema.^{22,41} Therefore, the acute and chronic toxic effect of NMP on skin is open to be studied.

Conclusion

Skin permeation enhancement technology is a rapidly developing field which would significantly increase the number of drugs suitable for transdermal drug delivery. The results of this study showed that the type and concentration of solvent are very important variables for solubility, rate and extent of transdermal delivery of Tadalafil. The results indicated that using NMP as Tadalafil solvent resulted in the substantial improvement in skin penetration of Tadalafil comparable with routinely used solvents such as ethanol and glycerin. NMP showed the fast penetration rate of Tadalafil, as well. Further investigation in this area will be needed to determine the safety of chronic administration of NMP in TDDS.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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