

EFFECT OF TERNARY SOLVENT SYSTEM ON THE PERMEABILITY OF LISINOPRIL ACROSS RAT SKIN *IN VITRO*

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ABSTRACT

Investigation were carried out on the permeation of lisinopril across excised rat skin for selecting a suitable solvent system for the development of a topical gel formulation. The solubility of lisinopril in pure and mixed solvents (propylene glycol: ethanol: water :: 50:30:20 for **S1**; 40:40:20 for **S2** and 30:40:20 for **S3**) was determined and *in vitro* permeation studies for lisinopril from various pure and mixed solvent systems were carried out for enhancing the delivery of lisinopril through skin. The solubility of lisinopril in water, propylene glycol and ethanol was 98.47 mg/ml, 14.2 mg/ml and 1.01 mg/ml respectively, indicating a higher solubility in water than propylene glycol and ethanol. The solubility of the drug in ternary systems **S1**, **S2** and **S3** was 60.56, 50.32 and 33.22 mg/ml, respectively. The highest solubility of drug in a ternary system was in **S1** system, which was approximately 1.2 and 1.8 times more than that in **S2** and **S3**, respectively. The steady state flux of lisinopril across rat skin using water, propylene glycol and ethanol was 10.18 ± 1.03 , 3.21 ± 0.91 and 2.01 ± 0.23 $\mu\text{g}/\text{cm}^2/\text{h}$ respectively. The skin permeation rate of lisinopril from the **S1** mixture was found to be higher as compared to other solvent systems (**S2** and **S3**). The steady state flux of lisinopril across rat skin from **S1**, **S2** and **S3** was 14.32 ± 1.98 , 13.67 ± 1.68 and 9.32 ± 1.76 $\mu\text{g}/\text{cm}^2/\text{h}$ respectively. The result indicated that the ternary solvent system of **S1** is suitable for use as vehicle for developing a topical formulation of lisinopril.

Key words: Ternary solvent system, Lisinopril, Steady state flux, Topical formulation

INTRODUCTION

For several decades, skin as a port of drug entry into the body has been exploited for delivering therapeutic agents into systemic circulation. However, the upper layer of the skin, the *stratum corneum*, poses a barrier to the entry of foreign material including therapeutic agents.¹ Passive transport of drug through skin is often dependent on two major physicochemical properties i.e., solubility and partition coefficient.² The use of solvent system and modifications of the thermodynamic activity of the applied drug are two frequently employed strategies to improve permeation of drug transdermally. The topical delivery of

drug depends on its permeability through skin, which in turn depends on optimal solvent system being used in the formulation. Therefore, selection of solvent for the preparation of topical formulations greatly affects the delivery of drugs. Mostly mixed solvent systems are used in the formulation, which in addition to being utilized for making better formulation may also facilitates the administration of drug through skin at therapeutic level.³ There is hardly any topical formulation prepared by single solvent component; other ingredients such as thickeners, perfumes, gelling agent are included for making its wide applicability onto the skin. Therefore, selections of

solvents for topical formulation development, multitude of mixed solvent system are to be taken into consideration. Understanding of physicochemical properties of drug and solvent could lead to better choice of solvent for topical formulation preparation.

Solvent system (ethanol, propylene glycol, fatty alcohols etc.) are known to enhance drug permeation by several mechanisms, which include disrupting the organized intercellular lipid structure of the *stratum corneum*,⁴ 'fluidizing' the *stratum corneum* lipids,⁵ altering cellular proteins, and in some cases, extracting intercellular lipids.⁶ Solvent systems not only exert a profound influence on the percutaneous delivery of drugs but also facilitate the penetration of drug at desired rate by carefully adjustment of the proportion of the solvents component used. Therefore, optimization of the solvent system for topical formulation of a particular drug is very much important. Keeping in view these facts, the effect of several pure and mixed solvent systems on the *in vitro* skin permeability of lisinopril was investigated for selecting a solvent system for optimal transdermal permeation of drug through skin.

Preparation of mixed solvent systems

One of the primary physicochemical considerations in pharmaceutical preparations is the solubility of the drug in a suitable solvent system. The solubility of a drug in a given solvent is largely a function of the polarity of the solvent. Semi-polar solvents (propylene glycol and alcohols) may induce a certain degree of polarity and thus improve the miscibility of polar and non-polar liquids. The relationship between polarity and solubility may be used in practice to alter the solubility of a drug in a pharmaceutical solution. Thus, by mixing solvents of different polarities a solvent system is produced, which has optimum polarity to dissolve drug. This method is referred as solvent blending or cosolvency and uses the dielectric constant as a guide to develop the solvent system. It is well established that manipulation of solvent dielectric constant could be utilized to bring about dissolution of a solute at desired

concentration and to blend pharmaceutical solvents to a predetermined degree of polarity.⁷

The dielectric constant of a complex solvent mixture may be calculated as:

$$A + B + \dots = f_A e_A + f_B e_B + \dots$$

Where, f_A , f_B , e_A , e_B are volume fraction and dielectric constant of solvent A and B, respectively.

This method of calculating dielectric constants of complex mixture is based on a simplification of Onsager-Kirkwood equation⁸ and is useful for precise adjustment of solvent polarity in pharmaceutical formulation work and in addition, would be of potential interest to various types of physicochemical investigations of the behavior of these solvent systems.⁸ With the help of this formalism a solvent system of appropriate polarity can be developed. However, calculated solubility values are only approximate and may increase or decrease depending upon the interactions between multiple solutes and solvents. Yet the use of the dielectric constant in formulating solvent systems gives us a simple and scientific approach for estimating our requirements. It is, therefore, a useful tool that can be applied to develop new solvent system to formulate gel formulation for topical use. Hence, various theoretically calculated ratios of propylene glycol, ethanol and water were blended to find combined solvents having appropriate polarity to dissolve drug. Three ternary solvent system, **S1** (50% propylene glycol, 30% ethanol, 20% water), **S2** (40% propylene glycol, 40% ethanol and 20% water) and **S3** (30% propylene glycol, 40% ethanol and 20% water) were prepared by simple mixing. Since calculated value of dielectric constant for **S1** is 55, resultant combination would be polar in nature. At this polarity, it was expected that the drug would be soluble. Combined dielectric constant of other proportions of solvent **S2** and **S3** would be less than 55, indicating that the drug would be less soluble. However, ultimate selection of solvent blend for the preparation of gel was to be based on experimental results. The effects of different ratios of solvent system

were evaluated on excised rat skin's for skin lisinopril permeation.

MATERIALS AND METHODS

Lisinopril was procured from Ranbaxy Research Laboratory, Gurgaon and analytical grade potassium dihydrogen phosphate and ninhydrin were obtained from Qualigens fine chemicals, Mumbai. The other chemicals such as propylene glycol, ethanol and dimethylformamide (DMF) were purchased from S.d.fine Chem., Ltd, Mumbai, Thomas Baker, Mumbai and Loba Chemie (P) Ltd, Mumbai, respectively.

Quantitative estimation of lisinopril

Sensitive spectrophotometric method as described by Nafisur Rahaman *et al.*,⁹ was adopted for the determination of drug in solution and for estimating skin permeates.

Preparation of stock solution

100 mg of lisinopril was dissolved in 10 ml of distilled water to make 0.1% solution. This solution was added to 90 ml DMF, resulting mixture of DMF and 0.1% drug solution in a ratio of 9:1. Further subsequent dilution were made by mixture of DMF and water (9:1).

Aliquots of gel corresponding to 50-250 mg lisinopril were transferred into a series of 10 ml standard volumetric flask. To each flask, 1.7 ml of 2.0 % ninhydrin solution was added and diluted to volume with DMF. The contents of the mixture were mixed well and sample was analysed at 595 nm (λ_{max}) using UV-Vis spectrophotometer (Hitachi- U-2800, USA) as a function of concentration against the reagent blank at room temperature. The calibration curve was constructed by plotting log absorbance Vs log molar concentration.

Analysis of lisinopril in skin permeate

Skin permeate (1.0 ml) was diluted with 5.0 ml methanol and was mixed well. It was then filtered through 0.4 μ m membrane filter. The filtrate was evaporated to dryness. The residue was dissolved with 1 ml of distilled water and

diluted to 9ml DMF. It was then analysed by spectrophotometric method as described earlier.

Determination of the solubility of lisinopril

In order to find out appropriate ratio of solvents with good solubilizing capacity for lisinopril, the solubility of lisinopril was investigated in pure solvents and mixtures of solvents. An excess amount of drug was added to 5.0 ml of pure or mixed solvent system and vortexed. The mixture was immersed in a water bath at 37°C and allowed to equilibrate. The suspension was filtered through a membrane filter (0.45 μ m), and the filtrate was suitably diluted and the concentrations of lisinopril was estimated as described earlier.

Collection and preparation of skin sample¹⁰

The excised male Wistar rat skin was used as the model membrane because the flux of drug through rat skin was more close to that through human skin than any other animal skin types.¹¹ Wistar male rats (8-12 week old) were scarified by cervical dislocation. The hair of the rat was removed with electric clippers and the abdominal skin was excised after careful shaving and whole thickness of skin was removed and divided along the sagittal plane into two pieces (left and right sides); excess adipose tissue was removed by gentle scraping. The skin pieces were soaked in the receptor buffer solution for approximately 45 min prior to being placed in the cells.¹⁰ The experiments involving animals were carried out as per the ethical guidelines and housed at appropriate conditions 12 hour light and 12 hour dark side (CPSCA No 226).

In vitro permeation studies^{12,13}

The skin permeation rates of lisinopril from various ratios of solvents were determined to evaluate the effect of the formulation factors. The *in vitro* permeation studies were performed using horizontal glass diffusion cells. The dorsal side of the excised skin was used as the model skin barrier. The skin was mounted between the donor and receptor half-cells of each skin permeation system with the *stratum*

corneum facing the donor half-cell. A thin film of petroleum jelly was spread on the lapped glass surface to the cell to provide a watertight seal. Lisinopril was added to various pure solvents and mixed solvents (10 mg/ml). The resulting drug solution or suspension was added to the donor cell. The receptor phase contained 5.0 ml of double distilled water in order to maintain sink conditions. The cells were clamped and immersed in a water bath 37 ± 0.5 °C placed on the magnetic stirrer. The maximum capacity of each of the donor and receiver compartments was 5.0 ml and the surface area of skin exposed to the solution was 5.0 cm². The medium of the diffusion cell was stirred at the rate of 200 rpm using small glass magnetic bead. At regular intervals i.e., 1, 2, 3, 4, 5----10 h, 0.5 ml samples were withdrawn from the receiver compartment and replaced with an equivalent quantity of distilled water to maintain a constant volume. The samples were assayed for lisinopril as previously described.

Permeation data analysis and statistics

The drug concentration was corrected for sampling effects according to the equation described by Hayton and Chien:¹⁴

$$C'_n = C_n (V_t / V_t - V_s) (C'_{n-1} / C_{n-1})$$

Where, C'_n is the corrected concentration of n^{th} sample, C_n is the measured concentration of drug in the n^{th} sample, C_{n-1} the corrected concentration of the drug in the $(n-1)^{\text{th}}$ sample, V_t the total volume of the donor solution, V_s is the volume of the sample withdrawn. The corrected drug concentration was divided by the area of the skin exposed to the donor solution to calculate the cumulative amount of drug permeated per unit area.

The cumulative amount of drug permeated per unit area was plotted against time and the linear portion slope of the plot was estimated as steady-state flux ($\mu\text{g}/\text{cm}^2/\text{h}$).¹⁵

The permeability coefficient (K_p in cm/h)¹⁶⁻¹⁸ was calculated by

$$K_p = J_{ss} / C_v$$

Where, J_{ss} is the steady state flux and C_v is the concentration of lisinopril in the donor compartment. A value of $P < 0.05$ was considered statistically significant.

Statistical analysis

Statistical comparisons were made using the student's t-test, a value of $p < 0.05$ was considered to be significant.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S [1/n_1 + 1/n_2]^{1/2}}$$

$$n_1 + n_2 - 2 = \text{d.f} \quad \frac{S^2 = (n_1 - 1) S.D_1^2 + (n_2 - 1) S.D_2^2}{\text{d.f}}$$

Where \bar{X}_1 and \bar{X}_2 are the means, n_1 and n_2 are number of samples, $S.D_1$ and $S.D_2$ are standard deviation of two sets of data and d.f is the degree of freedom.

RESULTS AND DISCUSSION

A standard curve was constructed for lisinopril in the range of 10-50 $\mu\text{g}/\text{ml}$ (Fig. 1). A good linear relationship was observed between the log molar concentration and log absorbance with a high correlation coefficient ($R^2=0.9994$).

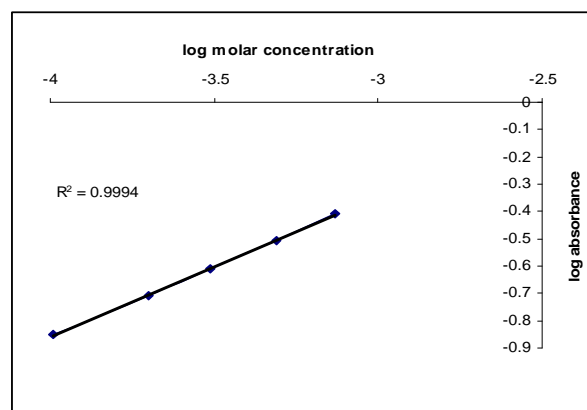


Fig. 1 Calibration plot for lisinopril

To quantify solubility of lisinopril in pure and ternary solvent systems (**S1**, **S2** and **S3**), the solvent mixture design was employed. The solubility of lisinopril in pure solvents and mixed solvent system are shown in table 1. The solubility of lisinopril in water, propylene glycol and ethanol was 98.47 mg/ml, 14.2 mg/ml and 1.01 mg/ml respectively, indicating a higher solubility in water than

that in propylene glycol and ethanol. The solubility of the drug in ternary systems **S1**, **S2** and **S3** was 60.56, 50.32 and 33.22 mg/ml, respectively. The solubility of lisinopril in solvent system was higher than that of pure solvents of propylene glycol and ethanol but was lower than that of water, which may be due to decrease in dielectric constant of mixed system. The highest solubility of drug in a ternary system was observed in **S1** system, which was approximately 1.2 and 1.8 times more than that in **S2** and **S3**, respectively. Thus, solubility of lisinopril was reduced drastically in ternary solvent by increasing fraction of alcohol and decreasing fraction of propylene glycol, therefore, proper combination of different composition of the ternary solvent could solubilize drug up to 60.56 mg/ml. However, the solution of drug in these solvent

become turbid on dilution with alcohol by more than 50% and it is suspected that drug will precipitate in gel formulation. Therefore, different combination of solvents of **S1**, **S2** and **S3** were taken for investigation.

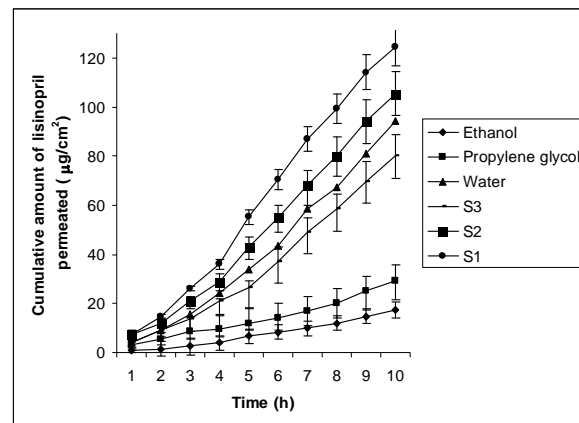


Fig. 2: Amount of lisinopril permeated from various pure and mixed solvent systems across rat abdominal skin

Table 1: Solubility of lisinopril in various pure and mixed solvents at 25 °C

S. No	Solvent	Solubility of lisinopril (mg/ml) at 25°C
1.	Water	98.47
2.	Propylene glycol	14.2
3.	Ethanol	1.01
4.	S1 (propylene glycol: ethanol: water::50:30:20 v/v)	60.56
5.	S2 (propylene glycol: ethanol: water::40:40:20 v/v)	50.32
6.	S3 (propylene glycol: ethanol: water::30:50:20 v/v)	33.22

The skin permeation profile of lisinopril from water, propylene glycol, ethanol and ternary solvent systems containing various ratios of propylene glycol, ethanol and water i.e., **S1**, **S2** and **S3** are shown in figure 2. The flux and permeability coefficients of lisinopril from various pure and mixed solvent systems are given in table 2.

Although the solubility of lisinopril in water was higher than other pure solvents and ternary systems, **S1** and **S2**, the skin permeation rate from water was lower than these systems. The steady state flux of lisinopril in water, propylene glycol and ethanol was 10.18 ± 1.03 , 3.21 ± 0.91 and $2.01 \pm 0.23 \mu\text{g}/\text{cm}^2/\text{h}$ respectively

Table 2: Steady state flux and permeability coefficient of lisinopril from pure and mixed solvent system

S. No	Solvent	Steady state flux, $\mu\text{g}/\text{cm}^2/\text{h}$	Permeability coefficient, $\text{Kp} (\text{cm}^2/\text{h}) \times 10^{-4}$
1.	Water	10.18 ± 1.03	2.036 ± 0.67
2.	Propylene glycol	3.21 ± 0.91	0.642 ± 0.72
3.	Ethanol	2.01 ± 0.23	0.402 ± 0.01
4.	S1 (propylene glycol: ethanol: water::50:30:20 v/v)	14.32 ± 1.98	2.864 ± 0.63
5.	S2 (propylene glycol: ethanol: water::40:40:20 v/v)	13.67 ± 1.68	2.734 ± 0.91
6.	S3 (propylene glycol: ethanol: water::30:50:20 v/v)	9.32 ± 1.76	1.864 ± 0.92

Despite its higher solubility of lisinopril in propylene glycol than alcohol, the permeability of lisinopril is not significantly different from alcohol ($p < 0.05$, t test) i.e., more or less the same. The reason for this may be due to the similar thermodynamic activity of lisinopril in propylene glycol and alcohol, which is considered to be main driving force.¹⁹ According to literature, propylene glycol also acts as carrier as it partitions into the skin and thereby promotes the movement of the drug into and through the skin.²⁰ In the present investigation, this particular mechanism is not appearing dominating because the partitioning of the drug contained in propylene glycol from the donor compartment to the skin barrier phase was low as evidenced by the low permeability coefficient (Table 2). As far as permeation of lisinopril from ethanol was concerned, it was low though not exactly zero as was expected due to poor solubility of drug. This may be due to extraction of lipids from *stratum corneum*, which is commonly associated with the use of alcohol and thus leads to enhanced percutaneous absorption of drugs.²¹

The skin permeation rate of lisinopril from the **S1** mixture was found to be higher as compared to other solvent systems, **S2** and **S3**. The steady state flux of lisinopril in **S1**, **S2** and **S3** was 14.32 ± 1.98 , 13.67 ± 1.68 and $9.32 \pm 1.76 \mu\text{g}/\text{cm}^2/\text{h}$ respectively. This may be due to the varying influence of solvent systems on the biophysical properties of the *stratum corneum* i.e., change in solvent system leads

to change in thermodynamic activity.^{22,23} Increased thermodynamic activity of lisinopril by both ethanol and propylene glycol, and reduction in the barrier property of *stratum corneum* by ethanol are thought to be the reasons for the higher flux of lisinopril in ternary solvent system, highest being from **S1**. The decreased permeation of lisinopril from **S2** and **S3** may be because of dehydrating effect of alcohol on the skin; previous reports^{22,23} also confirms that the predominant influence of alcohol might be responsible for decreased permeability of drugs. The penetration enhancing activity of ethanol is furnished by 'push effect'²⁴ and 'pull effect'²⁵ However, at the same time concentration dependent effect of ethanol has been hypothesized²⁵ i.e., at low concentration only lipoidal pathway is affected, while at higher concentration polar pathway is also affected. Therefore, lisinopril being hydrophilic drug, its flux with pure ethanol was lower than that of mixed solvent systems.

The increase in the flux of lisinopril with increasing concentration of propylene glycol in ternary solvent system is thought to be because of variations in the dehydrating effect of propylene glycol on *stratum corneum*, which ultimately found as a change in the thermodynamic activity of drug due to change in the cosolvent concentration. In ternary solvent system hygroscopic requirements of propylene glycol are compensated by the water present in the solvent system

and therefore, water is taken by propylene glycol from lipid bilayer and corneocytes to a minimum extent and hence the natural barrier property of skin is not altered. This may be the reason for having appreciable permeability of drug from mixed solvent system. Whereas, in pure propylene glycol, there is no free water in solvent system thus it extracts water from the lipid bilayer and corneocytes and thereby enhancing the barrier property of *stratum corneum*.²⁶ Present investigation suggest that at optimum ratio of solvent system i.e., 50% propylene glycol, 30%

ethanol and 20% water, could perform greater extraction of the *stratum corneum* lipids by mixed action thus leading to greater permeability of lisinopril, as compared to water, pure ethanol, pure propylene glycol and other propylene-ethanol-water solvent systems.

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