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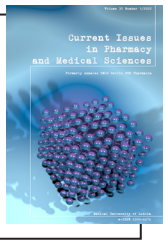

Biopharmaceutical aspects of the development of transdermal forms of Lisinopril dihydrate

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ABSTRACT

Prevention and treatment of arterial hypertension is of great importance. Improvement of existing medicines through the use of alternative routes of administration can enhance the pharmacotherapeutic characteristics of active pharmaceutical ingredients (APIs). Transdermal therapeutic systems (TTS) allow for delivery of a drug through intact skin into the systemic circulation, while minimizing side effects. The development of transdermal formulations of antihypertensive drugs, Lisinopril dihydrate in particular, has practical and scientific significance. Optimization of the algorithm for the development of transdermal drugs involves *in vitro* preformulation studies of the membrane permeability of APIs and the identification of factors that affect this process.

The tests were performed by dialysis through a semipermeable hydrophilic membrane using a Valia-Chien diffusion device. The effect of the initial concentration of Lisinopril dihydrate on the flux rate I_s was investigated. Different donor concentrations of Lisinopril dihydrate were tested at three levels, 10, 20 and 30 mg/ml, accordingly. It was noted that the permeation process of Lisinopril dihydrate under model conditions corresponds to zero-order kinetics and is characterized by a uniform speed. The correlation coefficient (R^2) for all the kinetic equations was 0.999. A linear dependence of the flux velocity of Lisinopril dihydrate on the initial concentration was indicated. The concentration of Lisinopril dihydrate in the donor solution of 30 mg/ml was found to be optimal for further stages of pharmaceutical development of the transdermal formulation. The studies of Lisinopril dihydrate permeability allow to give a positive assessment of the acceptability of this API for use in the transdermal formulation and the development of TTS.

INTRODUCTION

According to the World Health Organization (WHO), hypertension is one of the leading causes of premature death. Indeed, statistics state that, today, 1.28 billion adults aged 30 to 79 years worldwide are currently living with hypertension. However, 46% of all adults with hypertension are unaware of their disease. Moreover, less than half of all adults (42%) with hypertension are diagnosed and treated, and only 21% (one in five) of those with hypertension have it under control [1].

One of the global targets for non-communicable diseases is to reduce the prevalence of hypertension by 33% between 2010 and 2030. The main objective of antihypertensive therapy is to achieve and stabilize a target blood pressure level of 140/90 mmHg, as recommended by WHO (2021) [2]. However, the problem of uncontrolled hypertension in clinical practice has not lost its significance. If patients do not reach the target blood pressure level, clinicians should adjust their medication or seek to optimize adherence to the treatment algorithm [3]. Lack of patient compliance, especially when lifelong therapy is required, can compromise adequate blood pressure control.

The first-line drugs in the treatment of hypertension are angiotensin-converting enzyme (ACE) inhibitors [4,5]. Lisinopril is a long-acting ACE inhibitor widely known in world medical practice, that is actively used for the treatment of hypertension and heart failure, and for prevention of myocardial infarction, as well as for diabetic nephropathy [6]. Lisinopril is not a prodrug. It is not metabolized in the liver and is slowly and partially absorbed orally, with the time to reach maximum concentration varying from 6 to 8 h.

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However, lisinopril is characterised by low oral bioavailability, ranging between 10-30% after a single dose [7,8].

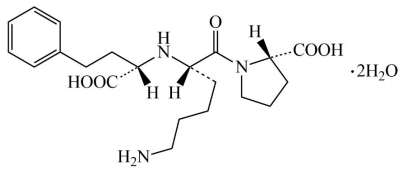
The development of modern innovative transdermal dosage forms has been of undeniable practical and scientific importance [9]. Transdermal therapeutic systems (TTS) are products for the alternative controlled administration of drugs through the skin. Over the course of many years of research, it has been demonstrated that these systems have important advantages over oral and injectable routes of drug delivery. TTSs are able to deliver drugs at a defined, consistent rate, hence maintaining a constant delivery of APIs, which reduces the risk of side effects. The transdermal route allows safe and reproducible delivery of medicines with optimal patient compliance. The ability to use transdermal patches independently also makes them the most convenient personalised approach to providing therapeutic care [10].

Thus, the controlled release transdermal dosage form of Lisinopril dihydrate is promising and competitive for increasing the bioavailability and efficacy of the drug and reducing the number of doses in long-term use, which can significantly cut-back the duration and cost of treatment. In scientific publications, considerable attention has been paid to the development of transdermal delivery of lisinopril [11-13]. However, TTS with lisinopril are currently not available on the global commercial pharmaceutical market. In this regard, we consider it relevant to study the possibility of using Lisinopril dihydrate for transdermal delivery and note the prospects for the development of drugs of this API in the form of TTS.

Proper development of new medicinal products is only possible with well-founded research design. It should be noted that the effectiveness of the transdermal route is limited by the stratum corneum factor, which is a barrier to exogenous substances, including APIs. The primary goal of TTS development is to select a drug substance and assess the acceptability of its administration in a given dosage form. The successful delivery of a drug through the skin depends on the physical and chemical properties of the API. The development of a specific TTS should thus be preceded by *in vitro* membrane permeation studies of the drug, the main advantage of which is the ability to control the experimental conditions and, therefore, the ability to control permeation changes due to the influence of various factors.

MATERIALS AND METHODS

The object of the study was Lisinopril dihydrate produced by Zhejiang Huahai Pharmaceutical Co, China. Lisinopril dihydrate (Figure 1) is a white or almost white crystalline powder, soluble in water, poorly soluble in methanol, and practically insoluble in anhydrous ethanol and heptane [14]. Its molecular formula is $C_{21}H_{31}N_3O_5$, $2H_2O$. M_r 441.5.



(2*S*)-1-[(2*S*)-6-Amino-2-[[(1*S*)-1-carboxy-3-phenylpropyl]-amino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate

Figure 1. Chemical structure of lisinopril dihydrate [14]

log$P_{o/w}$ 1.22. The physicochemical parameters of lisinopril hydrochloride (water solubility, molecular weight, distribution coefficient) make lisinopril dihydrate a good candidate for inclusion in a transdermal delivery system.

The *in vitro* permeability of the selected API through a semipermeable hydrophilic membrane was studied via dialysis using a Valia-Chien diffusion device (Station Horizontal Cell, Crown Glass, Germany). The experiment was performed at a temperature of 37±0.5°C. Model solutions with different contents of Lisinopril dihydrate (mg/ml) – 10, 20 and 30 – were employed as donor solutions. Phosphate buffered solution (pH 7.4) was implemented as a diffusion medium. The experimental solutions in the device chambers were mixed using magnetic stirrers (SES H3 Stirrer with Cell Clamps, SES GmbH – Analysesysteme, Germany). Every hour, a sample of the acceptor solution was replaced with a new one, taking this factor into account in further calculations. The content of Lisinopril dihydrate in the dialysate sample was determined spectrophotometrically (Specord 200, Analytik Jena GmbH+Co. KG, Germany).

RESULTS AND DISCUSSION

Quantitative parameters of the diffusion process of membrane permeability of the tested substance were determined by Fick's law. The permeation of Lisinopril dihydrate through a semipermeable hydrophilic membrane was evaluated by analyzing the certain parameters of the API content in the sample dialysate X_i, specific flux Q(t), steady-state flux I_s, permeability coefficient K_p and diffusion lag time Θ (Table 1).

Among the various factors affecting passive permeability, the concentration of the active substance plays the most important role. To evaluate this effect for the tested substance, the experiment was planned at three different concentrations of the drug. The results obtained (Table 1,

Table 1. Quantitative parameters of lisinopril dihydrate permeability through a semipermeable membrane *in vitro*

The concentration of API in the donor chamber C_s, mg/mL	Sampling time *t*, hr	Quantity of API in a dialysis sample $X_i \cdot 10^{-3}$, g	The concentration of API in the dialysate sample C_i, mg/mL	Specific flux of API Q(t) mg/cm²
10	1	8.0194	0.2970	1.9323
	2	8.2771	0.3066	3.9270
	3	8.4272	0.3121	5.9575
	4	7.8872	0.2921	7.8579
	5	7.9831	0.2957	9.7817
20	1	17.3685	0.6433	4.1853
	2	18.9323	0.7012	8.7473
	3	19.2355	0.7124	13.3822
	4	18.0926	0.6701	17.7419
	5	17.6453	0.6535	21.9936
30	1	23.1800	0.8585	5.5854
	2	23.5511	0.8723	11.2606
	3	25.7876	0.9551	17.4745
	4	26.7616	0.9912	23.9233
	5	25.4011	0.9408	30.0442

Figure 2) for the content of X_i and the concentration of C_i of the API in the sample show that the amount of passage of Lisinopril through the hydrophilic membrane during the experiment is proportional to the initial concentration of the latter in the donor solution. This was predictable, since an increasing of increase in the API concentration in the donor chamber increased the concentration gradient, which is the driving force of mass transport. The specific flux Q(t), which shows the total amount of a substance that passed through a unit area of the membrane during the experiment, varies in the same ratio.

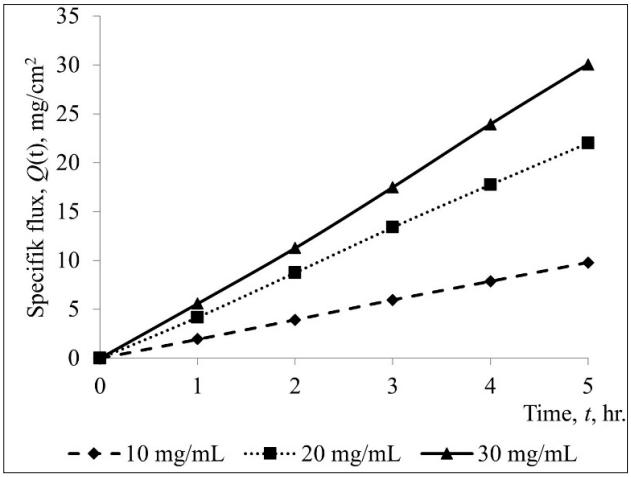


Figure 2. Dependence of the *in vitro* permeability of lisinopril dihydrate from the initial concentration

Based on the analysis of the obtained values of X_i and C_i it can be stated that the passage of Lisinopril dihydrate through the hydrophilic membrane is uniform. This was confirmed by the study of the convergence of the obtained experimental values of the kinetic parameters. The statistical equivalence of the obtained data was evaluated by way of the study of samples of experimental values arranged in ascending order. Accordingly, variations in the variants of the obtained samples can be considered insignificant if the values of their extreme variants do not exceed the boundary values of the confidence interval calculated by the maximum permissible value the half-width of the confidence interval $max\Delta_x$. Building upon the requirements of the Pharmacopoeia regarding the relative uncertainty of the quantitative analysis of the given drug (Δ_{As}= 0.32) and based on the relative tolerance of its quantitative content in TTSB = 25% (Ph. Eur. Chap. 2.9.6), the value of $max\Delta_x$ was set at 8% (Equation 1):

$$max\Delta_x = 0.32 \times B = 0.32 \times 25 = 8\% \qquad (1).$$

The results of the study of the convergence of the experimental values of the kinetic parameters of permeability of Lisinopril dihydrate depending on the initial concentration of the latter are given in Table 2. The results in Table 2 show that the values of the variant of all samples do not exceed the boundary values of the confidence interval X_{low} and X_{high}. Therefore, all the experimental values of the parameters under study are within the confidence interval and vary insignificantly. It can be thus stated that *in vitro* permeation of Lisinopril dihydrate through a hydrophilic membrane within the studied concentrations is carried out at a constant rate, which corresponds to zero order.

Table 2. Convergence study results of the experimental values of the kinetic parameters of the permeability of lisinopril dihydrate

	Initial concentration in donor solution, C_s, mg/mL		
	10	20	30
Investigation of values convergence of drug quantity in the dialysate sample, $X_i \cdot 10^{-3}$, g			
Variants of samples, x_i	7.8872	17.3685	23.1800
	7.9831	17.6453	23.5511
	8.0194	18.0926	25.4011
	8.2771	18.9323	25.7876
	8.4272	19.2355	26.7616
$\bar{X}$	8.1192	18.2549	24.9363
X_{low}	7.4697	16.7945	22.9414
X_{high}	8.7687	19.7153	26.9312
Investigation of values convergence of drug concentration in the dialysate sample, C_i, mg/mL			
Variants of samples, x_i	0.2921	0.6433	0.8585
	0.2957	0.6535	0.8723
	0.2970	0.6701	0.9408
	0.3066	0.7012	0.9551
	0.3121	0. 7124	0.9912
$\bar{X}$	0.3007	0.6761	0.9236
X_{low}	0.2766	0.6220	0.8497
X_{high}	0.3248	0.7302	0.9975
Investigation of values convergence of specific flux change of the drug, ΔQ(t), mg/cm²			
Variants of samples, x_i	1.9004	4.1853	5.5854
	1.9238	4.2517	5.6752
	1.9323	4.3597	6.1209
	1.9947	4.5620	6.2139
	2.0305	4.6349	6.4488
$\bar{X}$	1.9563	4.3987	6.0088
X_{low}	1.7998	4.0468	5.5281
X_{high}	2.1128	4.7506	6.4895

A graphical interpretation of the *in vitro* permeability of Lisinopril dihydrate for each concentration is presented in Figures 3-5. In all experiments, the obtained kinetic equations have the form of a general linear regression $Y = A + Bx$.

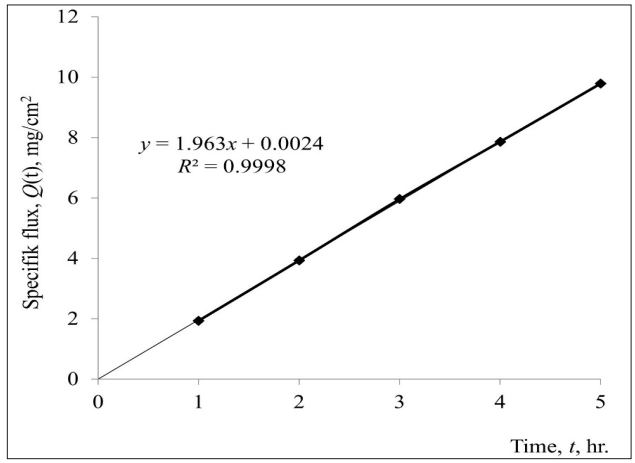


Figure 3. Kinetics of the *in vitro* membrane permeability process of lisinopril dihydrate, concentration 10 mg/mL (pH 7.4)

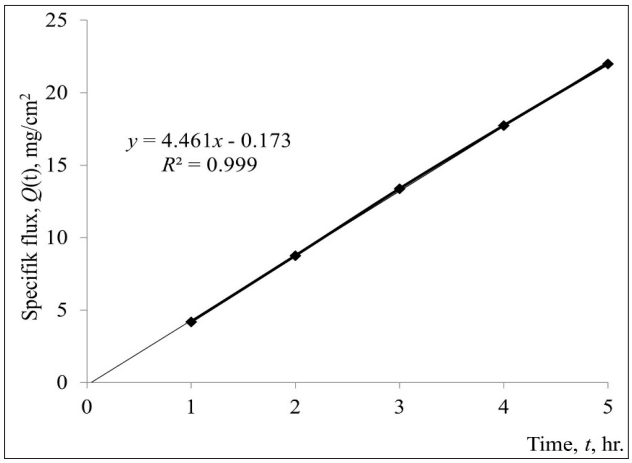


Figure 4. Kinetics of the *in vitro* membrane permeability process of lisinopril dihydrate, concentration 20 mg/mL (pH 7.4)

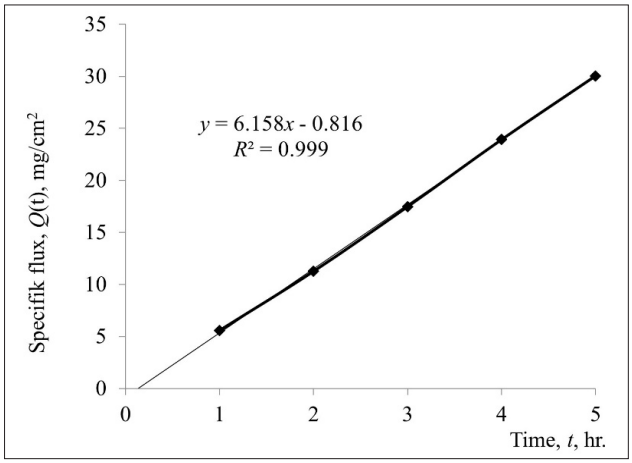


Figure 5. Kinetics of the *in vitro* membrane permeability process of lisinopril dihydrate, concentration 30 mg/mL (pH 7.4)

The dependence of the passage of Lisinopril dihydrate through the hydrophilic membrane on the time for selected concentrations is linear, which is confirmed by the parameters of linear regression. For all the kinetic equations used, the correlation coefficient is R^2=0.999. The *in vitro* kinetic parameters of the permeation process of Lisinopril dihydrate, calculated from the results of statistical analysis, are given in Table 3.

Table 3. Kinetic parameters of the *in vitro* lisinopril dihydrate permeability

The initial concentration of API in the donor solution, C_s, mg/mL	Steady-state flux of drug, I_s, mg·cm^{-2}·hr^{-1}	Diffusion lag time, Θ, min	Permeability coefficient, K_p, cm/hr	Linear correlation coefficient, R^2
10	1.963	−0.06	0.20	0.999
20	4.461	2.34	0.23	0.999
30	6.158	7.80	0.21	0.999

The obtained values of Lisinopril dihydrate flux velocity for all concentrations indicate the high potential of this substance to overcome membrane barriers and allow predicting good permeability through human skin. However, to determine the optimal concentration for further stages of TTS development, it is necessary to take into account all factors of the permeability process in total. Thus, it was noted that the diffusion lag time Θ, which determines the duration of the non-stationary period of the process, for a lisinopril hydrate concentration in the donor solution of 10 mg/ml has a negative value of this indicator. This characterizes the permeability with a delay in the onset of stationary flux from the beginning of the process and is explained by the insufficiency of the selected initial concentration of the substance to saturate the membrane. When the concentration of Lisinopril dihydrate in the donor solution is changed from 20 to 30 mg/ml, the diffusion lag time increases more than three times, but does not exceed 8 min. The maximum flux rate of Lisinopril dihydrate I_s = 6.158 mg·cm^{-2}·h^{-1} was also obtained for a solution with a concentration of 30 mg/ml. The permeability coefficient K_p characterizes the properties of the membrane and varies between 0.20 and 0.23 cm/h. Taking into account all the results of the experiments, we consider that the concentration of Lisinopril dihydrate of 30 mg/ml is sufficient to create a drug substance depot during pharmaceutical development of TTS, which provides the required level of flux for a short non-stationary period.

CONCLUSIONS

As a result of the study, the qualitative and quantitative characteristics of the *in vitro* permeation process of Lisinopril dihydrate were determined. Based on the analysis of the obtained experimental values of API content in the dialysate sample X_i and the gradient of specific flux per unit time ΔQ(t), it was noted that the permeation process of Lisinopril dihydrate under model conditions corresponds to zero order kinetics and is characterized by a uniform speed. The high value of the correlation coefficient (R^2=0.999) for the obtained kinetic equations confirms the linear dependence of the passage through membrane on time.

The studies demonstrated the ability of the molecules of the selected substance to overcome membrane barriers and allow to give a positive assessment of the acceptability of this API for the development of TTS. Moreover, the proportional dependence of the flow rate of Lisinopril dihydrate on the initial concentration was shown. In addition, the optimal concentration of Lisinopril dihydrate was found to be 30 mg/ml for further experimental studies in the pharmaceutical development of the transdermal formulation.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

The authors declare that ethical approval was not required.

INFORMED CONSENT

The authors declare that informed consent was not required.

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REFERENCES

1. *International Society of Hypertension. World Hypertension Day*; 2023. [https://ish-world.com/world-hypertension-day-2023/]
2. *Guideline for the pharmacological treatment of hypertension in adults*. Geneva: World Health Organisation; 2021. [https://apps.who.int/iris/bitstream/handle/10665/344424/9789240033986-eng.pdf]
3. Skolnik N. *The new blood pressure targetin primary care*. Medscape. 2023. [https://www.medscape.com/viewarticle/987184?form=fpf]
4. Ahmad H, Khan H, Haque S, Ahmad S, Srivastava N, Khan A. Angiotensin – converting enzyme and hypertension: A systemic analysis of various ACE inhibitors, their side effects, and bioactive peptides as a putative therapy for hypertension. *JRAAS*. 2023;2023: 7890188.
5. Bakris GL. *Drugs for Hypertension*. University of Chicago School of Medicine; 2022. [https://www.msdmanuals.com/professional/cardiovascular-disorders/hypertension/drugs-for-hypertension]
6. Zheng W, Tian E, Liu Z, Zhou C, Yang P, Tian K, et al. Small molecule angiotensin converting enzyme inhibitors: A medicinal chemistry perspective. *Front Pharmacol*. 2022;13:968104.
7. *Lizynopryl* (*Lisinopril*). *Kompendium*. *Likarski preparaty*. [https://compendium.com.ua/uk/akt/76/3045/lisinoprilum/]
8. Olvera LE, Parmar M, Pendela VS, Jamie MT. Lisinopril. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2023.
9. Dumitriu BO, Paduraru AM, Stefan CS, Dinu M, Cocos DI, Nwabudike LC, et al. Strategies for improving transdermal administration: New approaches to controlled drug release. *Pharmaceutics*. 2023;15:1183.
10. Ramadon D, McCrudden MTC, Courtenay AJ, Donnelly RF. Enhancement strategies for transdermal drug delivery systems: current trend sand applications. *Drug Deliv Transl Res*. 2022;12(4):758-91.
11. Jitendra B, Pathaka AK, Subhash P. Development and optimisation of transdermal system of lisinopril dehydrate: Employing permeation enhancers. *Iran J Pharm Sci*. 2010;6(4).
12. Rajendra M, Srinivas A. Formulation and characterisation transdermal patches for enhancing the bio-availability of lisinopril dihydrate. *Int J Med Nanotechnol*. 2015;2(6):306-10.
13. Aparanjitha R, Sunitha RM, Sarangapani M. Formulation And In vitro evaluation of transferosomal patches for enhanced drug delivery of lisinopril dihydrate. *J Scient Res*. 2020;64(3):105-9.
14. *Lisinopril dihydrate*. EuropeanPharmacopoeia. Strasbourg: EDQM; 2016: 2010-2.