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IN VITRO STUDY OF THE PERMEABILITY OF ENALAPRIL MALEATE THROUGH A SEMIPERMEABLE MEMBRANE IN THE PROCESS OF PHARMACEUTICAL DEVELOPMENT OF A TRANSDERMAL THERAPEUTIC SYSTEM

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Abstract

At the stage of preformulation studies of the pharmaceutical development of the transdermal therapeutic system (TTS) with antihypertensive action, the in vitro permeability process of enalapril maleate was studied. Testing was performed by dialysis through a semipermeable membrane using side-by-side diffusion chambers. The effect of the initial concentration of enalapril maleate on the steady-state flux rate I_s was investigated. Four donor concentrations 10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL of enalapril maleate were tested. Conducted analysis of the nature, description of the mathematical model and determination of the main kinetic parameters of the permeability process of the studied enalapril maleate allow assessing its potential for creating TTS and substantiate a further algorithm for the development of TTS antihypertensive action according to API data. Review articles, research papers, case reports and letters to the editor may be submitted for publication.

Keywords: *in vitro permeability, enalapril maleate, transdermal therapeutic system*

Introduction

The creation of innovative drugs in the form of transdermal therapeutic systems (TTS) is one of the most promising scientific directions of modern pharmaceutical technology. Pharmaceutical development of such drugs involves a thorough study of all biopharmaceutical aspects. The initial stage of development of TTS is the choice of drug substance, assessment of the acceptability of its introduction in this dosage form. In order to determine a more rational approach to the creation of TTSs, the pharmaceutical development of a transdermal drug should be preceded by in vitro preformulation studies of the permeability of the active pharmaceutical ingredient (API) across the membrane. The main advantage of these studies is the establishment at the initial stage of the factors influencing this process, the ability to control the conditions of the experiment, and, therefore, the ability to control changes in permeability based on certain kinetic parameters [1-3].

Hypertension, a hypertensive disease, is one of the most common causes of disability and mortality. Today there is a numerical increase in the incidence of this type of pathology. Pharmacotherapy of these pathological conditions is usually long-term and requires an individual approach and comprehensive correction, taking all parts of the pathological process into account [4].

The search for APIs promising for use in transdermal dosage forms continues intensively. Among the group of antihypertensive drugs, one of the main places is occupied by angiotensin-converting enzyme (ACE) inhibitors. Enalapril maleate has been widely used in clinical practice for decades. Among all ACE inhibitors, it has the widest list of indications for use, including hypertension, chronic heart failure (CHF), coronary heart disease. Enalapril is the gold standard among ACE inhibitors for its ability to control blood pressure. Enalapril maleate has a dose-dependent hypotensive effect, which is observed within 24-36 hours after a single oral administration. The maximum reduction in blood pressure is achieved after 6-8 hours [5, 6].

In the last scientific publications, much attention is paid to the development of transdermal delivery of the enalapril maleate. The use of TTS provides stability of concentration and long-term therapeutic level of the substance in the bloodstream, which contributes to the therapeutic effect prolongation. TTS, in comparison with oral dosage forms, eliminates the risk of gastrointestinal side effects, which increases their safety profile. When using transdermal patches, a reduction in dosing frequency is achieved and high systemic bioavailability of a drug is ensured. TTSs are quite easy to use and can significantly increase compliance with patients [7-9].

To achieve the optimal therapeutic effect with transdermal administration of the drug, it is necessary to take into account both physico-chemical properties of the active substance and external factors, in particular the effect of concentration, composition of the diffusion medium and others. In order to determine a more rational approach to the creation of TTS, in vitro preformulation studies of API permeability through membranes should precede the pharmaceutical development of a transdermal preparation. The main advantage of these studies is the ability to control the conditions of the experiment and, consequently, the ability to control changes in permeability due to the influence of various factors.

In this regard, the aim of our work was to conduct preformulation studies of the pharmaceutical development of transdermal dosage form of TTS antihypertensive action with enalapril maleate. During the study, the nature and kinetic parameters of the in vitro process of permeability of enalapril maleate through a semipermeable membrane, as well as the influence of the initial concentration of the selected API on this process were determined.

Methods

Studies of the permeability of enalapril maleate through a semipermeable membrane were performed in vitro by dialysis using a modified diffusion device of Valia-Chien design [10]. Different concentrations of enalapril maleate were used as donor solutions: 10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL. Phosphate buffer solution (pH 7.4) was used as the diffusion medium. The experiment was performed at a temperature of (37+0.5)°C. At defined intervals, with an interval of 1 h, which

corresponded to 1, 2, 3, 4, and 5 h from the beginning of the experiment, all the solution from the acceptor chamber was removed, replacing the sample of acceptor solution with a new one, which was taken into account. The content of enalapril maleate in the dialysate sample was determined spectrophotometrically.

Results and Discussion

The results obtained (Table 1, Fig. 1) show that the amount of enalapril maleate passing through the semipermeable membrane is proportional to its initial concentration in the donor solution according to Fick's law.

Based on the analysis of the results of the study on the quantity of drug in the dialysate Xi and the value of the specific flux of drug gradient per hour ΔQ(t) it was noted that these values do not change from the second hour from the beginning of the experiment for all studied concentrations of enalapril maleate in the donor solution.

Graphical interpretation of the in vitro permeability of enalapril maleate for each concentration is presented in Fig. 2-5. In all experiments, the obtained kinetic equations have the form of a general linear regression $Y=A+B\times X$.

The linear dependence of enalapril maleate on the membrane over time is confirmed by linear regression parameters. The correlation coefficient for the obtained kinetic equations, within the experiment time, was not less than 0.999.

The coefficient of permeability of the studied API through the membrane, K_p, cm/h, was calculated taking into account the gradient of concentrations of donor and acceptor solutions, based on Fick's law:

$$K_p=I_s \times \Delta C_s \quad [1]$$

The main quantitative characteristics of the dependence of the enalapril maleate permeability on its initial concentration through the semipermeable membrane in vitro, calculated from the results of statistical analysis, are shown in Table 2.

According to the results (Table 2), it was found that with increasing the initial concentration of enalapril maleate from 10 mg/mL to 40 mg/mL, the steady-state flux of drug I_s increases 4.6 times. The obtained values of the steady-state flux of enalapril maleate for all concentrations indicate the high potential of this substance in overcoming membrane barriers, and predict, including good permeability and human skin. But to determine the optimal concentration for the next stages of creating TTS, it is necessary to take into account all the factors of the permeability process together.

The diffusion delay time determines the duration of the non-stationary period of the process. A negative value of this indicator indicates a lack of membrane saturation. During the experiment, it was found that the diffusion delay time for the concentration of enalapril maleate in the donor solution of 10 mg/mL and 20 mg/mL varies slightly from 7.2 to 9 min. At a concentration of enalapril maleate in the donor solution of 30 mg/mL, the diffusion delay time increases more than twice to 18.6 min. At a concentration of enalapril maleate in the donor solution of 40 mg/mL, the duration of the non-stationary period increases significantly, the diffusion delay time is 1 h 23 min. The permeability coefficient K_p characterizes the properties of the membrane and varies between 0.34 and 0.44 cm/h.

Taking into account all the results of the experiments, we consider the best initial concentration of enalapril maleate 30 mg/mL, which provides the required level of the steady-state flux of drug in a short non-stationary period.

Statistical equivalence of the obtained data was assessed based on a study of samples of experimental values (starting from the second hour), arranged in ascending order. Changing the variants of the obtained samples can be considered insignificant if the values of their extreme variants do not exceed the confidence interval limit values calculated from the value of the maximum allowable confidence interval half-width ($max\Delta_X$). The value of $max\Delta_X$ was determined based on the relative uncertainty of the quantitative analysis of this drug (Δ_{As}), based on the relative tolerance of the quantitative content of drug in TTS B = 25% according to the requirements of Ph. Eur. Chapter 2.9.6 [11] (equation [2]):

$$[max\Delta_x] = 0.32 \cdot B = 0.32 \cdot 25 = 8.0\ \% \quad [2]$$

The results of the study of the convergence of experimental values of kinetic parameters of permeability of enalapril maleate depending on its initial concentration are shown in Table 3.

According to the results given in Table 3, it can be seen that the variant values of all samples do not exceed the confidence interval limits X_{low} and X_{high}. Therefore, all the obtained experimental values of the studied parameters are within the confidence interval $\bar{X}$ and change slightly. Based on this, it can be argued that the permeability of enalapril maleate through the membrane in vitro within the studied concentrations from the second hour is carried out at a constant rate corresponding to zero-order kinetics.

The studies carried out to determine the nature and quantitative characteristics of the enalapril maleate permeability showed, first of all, the ability of the selected substances molecules to overcome membrane barriers and make it possible to give a positive assessment of the acceptability of this API for use in transdermal form and the creation of TTS. The development of TTS for enalapril maleate is promising and relevant. Carrying out the next stages of the pharmaceutical development of TTS with enalapril maleate will make it possible to introduce a new transdermal therapeutic system with antihypertensive action into medical practice in the future.

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Table 1. Quantitative parameters of enalapril maleate permeability through a semipermeable membrane in vitro

The concentration of API in the donor chamber, C_s, mg/mL	Number of a chosen sample, n	Sampling time, t, h	Quantity of drug in a dialysis sample, $X_i \times 10^{-3}$, g	Specific flux of drug, Q(t), mg/cm^2
10	1	1	12.4910	3.0097
	2	2	11.2952	5.7312
	3	3	11.3724	8.4715
	4	4	11.2642	11.1975
	5	5	10.9343	13.8325
20	1	1	30.4608	7.3401
	2	2	26.8024	13.7986
	3	3	28.4838	20.6625
	4	4	26.0577	26.9414
	5	5	25.5105	33.0883
30	1	1	47.4724	11.4389
	2	2	38.4113	20.6944
	3	3	37.1823	29.6538
	4	4	36.0529	38.3413
	5	5	36.3799	47.1075
40	1	1	121.8189	29.3539
	2	2	52.5146	42.0081
	3	3	51.7826	54.4860
	4	4	51.2530	66.8364
	5	5	50.5682	79.0215

Table 2. Kinetic parameters of the dependence of the enalapril maleate permeability on its initial concentration through the semipermeable membrane in vitro

The initial concentration of API in the donor solution, C_s, mg/mL	Steady-state flux of drug, I_s, mg/cm^{2}hr	Diffusion delay time, Θ, min	Permeability coefficient, K_p, cm/h	Linear correlation coefficient, *r*
10	2.7112	-7.2	0.34	0.9999
20	6.4639	-9.0	0.43	0.9996
30	8.8984	-18.6	0.39	0.9998
40	12.4160	-82.8	0.44	0.9999

Table 3. The results of the study of the convergence of the experimental values of the kinetic parameters of the permeability of enalapril maleate depending on its initial concentration

	Initial concentration in donor solution, C_s, mg/mL			
	10	**20**	**30**	**40**
Investigation of convergence of values of quantity of drug in a dialysate sample, $X_i \cdot 10^{-3}$, g				
Variants of samples, x_i	10.9343	25.5105	36.0529	50.5682
	11.2642	26.0577	36.3799	51.2530
	11.2952	26.8024	37.1823	51.7826
	11.3724	28.4838	38.4113	52.5146
$\bar{X}$	11.2165	26.7136	37.0066	51.5296
X_{low}	10.3192	24.5765	34.0461	47.4072
X_{high}	12.1138	28.8507	39.9671	55.6520
Investigation of the convergence of the values of the concentration of drug in the dialysate sample, C_i, mg/mL				
Variants of samples, x_i	0.4050	0.9448	1.3353	1.8729
	0.4183	0.9651	1.3474	1.8983
	0.4190	0.9927	1.3771	1.9179
	0.4212	1.0550	1.4226	1.9450
$\bar{X}$	0.4159	0.9894	1.3706	1.9085
X_{low}	0.3826	0.9102	1.2609	1.7558
X_{high}	0.4492	1.0686	1.4802	2.0612
Investigation of convergence of values of change of specific flux of drug, ΔQ(t), mg/cm²				
Variants of samples, x_i	2.6349	6.1469	36.0529	12.1851
	2.7215	6.2790	36.3799	12.3504
	2.7260	6.4585	37.1823	12.4779
	2.7403	6.8639	38.4113	12.6542
$\bar{X}$	2.7057	6.4371	37.0066	12.4169
X_{low}	2.4892	5.9221	34.0461	11.4235
X_{high}	2.9222	6.9521	39.9671	13.4103

Figure 1. Dependence of the permeability of maleate enalaprile on concentration

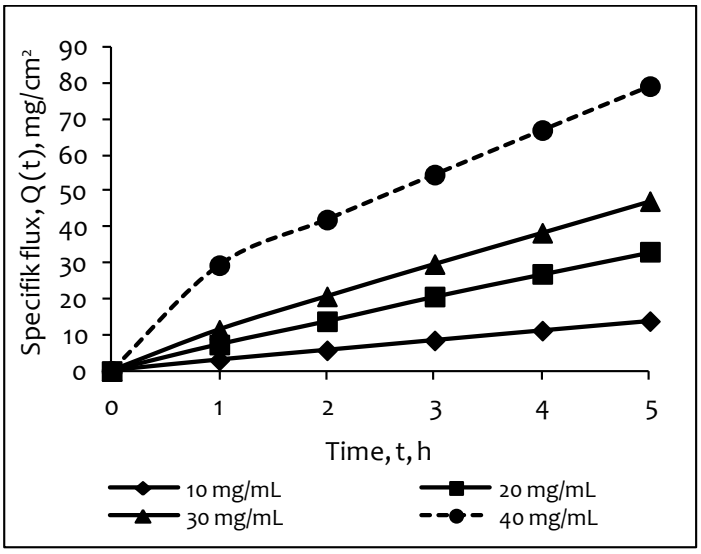

Figure 2. Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 10 mg/ml)

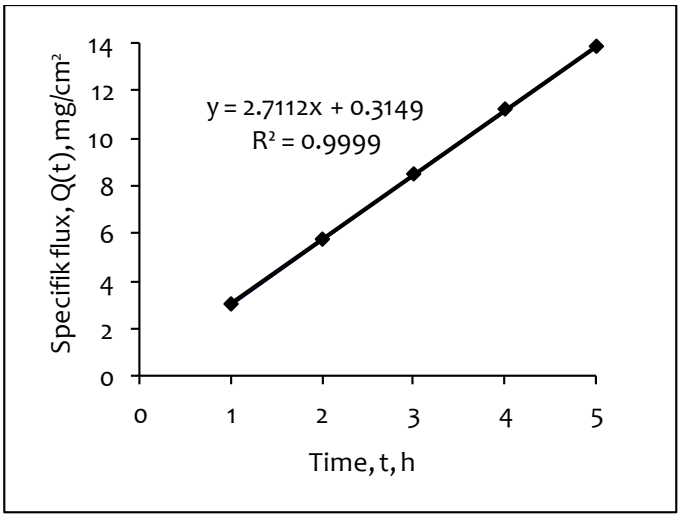

Figure 3. Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 20 mg/ml)

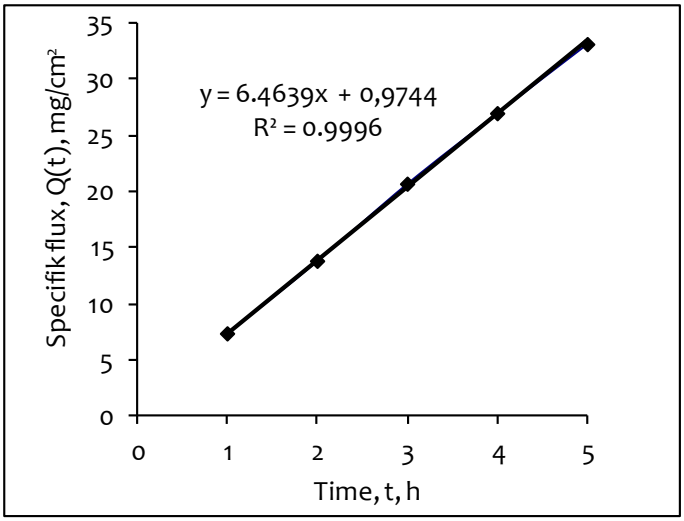

Figure 4. Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 30 mg/ml)

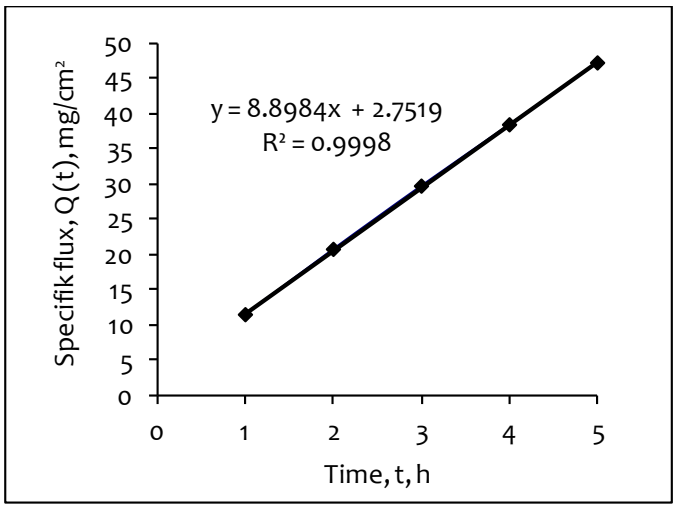

Figure 5. Kinetics of the in vitro membrane permeability process of enalapril maleate (initial concentration 40 mg/ml)