

The analysis of the permeability process of calcium antagonists in developing transdermal forms with a cardiovascular effect

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Abstract

Aim. The aim of the work was to evaluate the possibility of using calcium antagonists, namely, nifedipine and amlodipine besylate, while conducting transdermal delivery, that included the analysis of *in vitro* permeability process as a primary preformulation stage of pharmaceutical development of a transdermal dosage form, determination of qualitative and quantitative characteristics of a permeability process and the expediency analysis of development of a therapeutic transdermal system (TTS) with a cardiovascular effect.

Materials and methods. The active pharmaceutical ingredients (API) of nifedipine and amlodipine besylate. The study has been carried out *in vitro* by a dialysis method using a modified diffusion device of the Valia-Chien design.

Results. Character analysis, description of the mathematical model and definition of the kinetic parameters in the process of permeability of the studied medicinal products (MP) of nifedipine and amlodipine besylate allowed to evaluate their potential for creating TTS as being positive and appropriate. The implemented methodological approaches allow to substantiate the further algorithm for the development of cardiovascular TTS with the mentioned API.

Keywords

Nifedipine, amlodipine besylate, *in vitro* permeability, a transdermal therapeutic system

Introduction

Cardiovascular diseases, in particular, ischemic heart disease and arterial hypertension are the main causes of disability and mortality among the population. Nowadays, a numerical increase in the incidence of this type of pathology may be present. The pharmacotherapy of the mentioned pathological states usually requires a long time, an individual approach and a complex adjustment, taking into account all the parts of the pathological process.

The first line of the medicinal products in treating hypertensive disease includes calcium antagonists, which show antianginal and antihypertensive properties. Among the groups of the calcium channel blockers the medicinal products of 1,4-dihydropyridine type- nifedipine and amlodipine are widely spread.

Nifedipine is a short-acting calcium antagonist, widely known in the world of medical practice. Its main drawback is a short-duration half-life period, which takes only 2–4 hours. For these purposes, nifedipine requires

an increase in the number of administration during the day and it is accompanied by the occurrence of side effects. After oral administration, it undergoes an intensive metabolism, which leads to a decrease in bioavailability (40–60%) (Oparin et al. 1998).

The next calcium antagonist generations of the dihydropyridine group differ from nifedipine by a longer effect. A vivid representative of the third generation is amlodipine, characterized by a sufficiently high bioavailability (64–80%) and slight fluctuations in the maximum and minimum concentration in the blood within 24 hours (Preobrazhensky et al. 2008; Preobrazhensky et al. 2011; Prikhodko et al. 2011; Bagry 2012).

The design of modern innovative dosage forms (DF) for the medicinal products of this group are based on seeking an alternative way of the delivery of active ingredients (Pastore et al. 2015). Some of the perspective DF are TTS which, thanks to the continuous controlled profile of the MP introduction through the skin, provide concentration stability and a long therapeutic level of the substance in the bloodstream, in a way that facilitates prolonging the therapeutic effect. TTS, as compared to the peroral DF, have the potential to prevent hepatic metabolism in the first pass, eliminate the risks of gastrointestinal adverse reactions development, in this way increasing their safety profile (Flowers 2008; Bala et al. 2014; Kadam et al. 2014). During the use of transdermal patches, a decrease in the dosage frequency is achieved and a high systemic bioavailability of MP is ensured. TTS are quite easy to use and allow to maintain compliance by patients. In case of the occurrence of an adverse reaction, it is possible to discontinue the use of the patch immediately, the risk of overdose is minimized.

Only the oral formulations of nifedipine and amlodipine besylate are currently presented in the pharmaceutical market and none of them are presented in transdermal forms. Nevertheless, a number of scientists have been carrying out research and development of the transdermal forms of these medicinal products for a long time (McDaid and Deasy 1996; Ahmed et al. 2010; John et al. 2013; Yasam et al. 2016). Besides, the range of TTS in the Ukrainian pharmaceutical market is limited and includes only anti-inflammatory and antipyretic medicinal products. That is why the product-line expansion of TTS and the agents that can be used for a mixed pharmacotherapy of cardiovascular diseases is a task of vital importance.

The modern approach to the problems of transdermal DF design involves a detailed and comprehensive study of the various biopharmaceutical aspects of their development. The experimental research should be directed to the determination of the target quality profile of a medicinal product. For optimal therapeutic effect with transdermal administration of the product it is necessary to take into account physico-chemical properties of the active substance, the influence of the excipients, which are part of the combinations, and the skin condition. With the aim of defining a more rational approach for developing TTS a pharmaceutical consideration of a transdermal product should be preceded by preformulation studies of the me-

dicinal products permeability *in vitro* through the membrane. The main advantage of such research is a possibility of a control over the experiment conditions and, therefore, the possibility to control changes in permeability due to the influence of different factors (Yasam et al. 2016; Shyteyeva et al. 2017; Shyteyeva et al. 2018).

In this context, the aim of our work is to study *in vitro* the process of calcium antagonist permeability, nifedipine and amlodipine besylate, and determining their application perceptiveness in the creation of transdermal patches with cardiovascular effect.

Materials and methods

The API of nifedipine (Suchem Laboratories company, India) and amlodipine besylate (Hetero Drugs Limited company, India) have been chosen as the objects of the study.

Nifedipine (Fig. 1) is a yellow crystal powder, which is practically insoluble in water, poorly soluble in ethanol. $M_m = 346,34$ Da. $\log P$ (octanol-water) = 2,20. Hydro solubility at 25 °C = 56,3 mg/l (Ukrainian compendium).

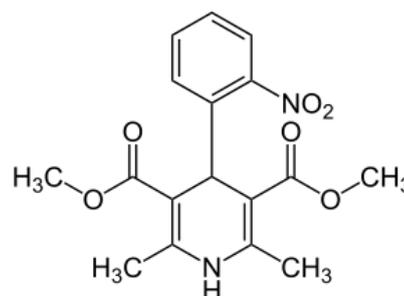


Figure 1. Nifedipine 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester.

Amlodipine (in the besylate form) (Fig. 2), is a yellow crystal powder, which is slightly soluble in water, sparingly soluble in ethanol. $M_m = 408,89$ Da. $\log P$ (octanol-water) = 3. Hydro solubility – 75,3 mg/l at 25 °C (Ukrainian compendium).

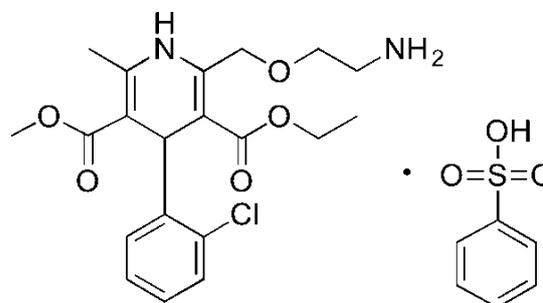


Figure 2. Amlodipine 3,5-Pyridinedicarboxylic acid, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-, 3-ethyl 5-methyl ester.

The study of the permeability of the chosen API through a semipermeable membrane was carried out *in vitro* by a dialysis method using a modified diffusion de-

vice of the Valia-Chien design, which was earlier described in paper (Chien 1987). The initial saturated solutions of the test substances in a phosphate buffer solution were used as donor solutions. A phosphate buffer solution (pH 7.4) served as an acceptor solution. The experiment was conducted under the temperature of $37 + 0,5$ °C (the temperature of human subcutaneous layers). At certain intervals, 1 hour, that were corresponding to 1, 2, 3, 4, and 5 hours from the beginning of the experiment, the entire solution was removed from the acceptor compartment, replacing the sample acceptor solution with a new one, which was taken into account in the calculations. For every test sample the absorption spectra were recorded on the spectrophotometer Specord 200. The optical density of the obtained solutions was determined by the maximum absorption at the appropriate wavelength for each product.

The qualitative characteristics of the permeability process were determined with Fick's law, which describes diffusion processes, including the active substance transfer through the skin or membrane.

Results and discussion

The assessment of the permeability process of the studied substances through a semipermeable membrane was conducted according to the determined values of the flux I_s , permeability coefficient K_p and the diffusion delay time Θ . The experiment results have been presented in Table 1.

Table 1. The qualitative criteria of permeability of API with cardiovascular effect through a semipermeable membrane in a dialysis sample.

API	Number of a chosen sample, n	Sampling time, t, h	API quantity in a dialysis sample, $X_i \cdot 10^{-4}$, g	API concentration in a dialysis sample, $C_i \cdot 10^{-3}$, mg/ml	Specific flux of API, $Q_i \cdot 10^{-2}$, mg/cm ²
Nifedipine	1	1	30,771	113,9666	74,1470
	2	2	28,539	105,7000	142,9157
	3	3	31,751	117,5962	219,4241
	4	4	28,727	106,3962	288,6458
	5	5	27,725	102,6851	355,4529
Amlodipine besylate	1	1	45,090	167,0000	108,6507
	2	2	45,839	169,7740	219,1060
	3	3	46,097	170,7296	330,1831
	4	4	47,584	176,2370	444,8434
	5	5	48,010	177,8148	560,5301

According to the obtained results of the API quantity, X_i and its concentration C_i in a dialysis sample (Table 1), it can be noted that these indices practically do not change within the duration of the experiment, that the passage of all the tested substances through the selected membrane has been carried out uniformly that corresponds to the zero-order kinetics with respect to the concentration gradient of donor and acceptor solutions.

The statistical equivalence of the obtained data was evaluated on the basis of a study of samples with experimental values, organized in ascending order. Changes in the variant X_i of the received samples can be considered insignificant if the values of their extreme variants do not exceed the limit values of the confidence interval, calculated by the maximum permissible half-width of the confi-

dence interval ($\max \Delta_x$). The value $\max \Delta_x$ was defined on the basis of the relative uncertainty of quantitative analysis of the given API (Δ_{As}) (equation 1) based on the relative tolerance of API quantitative content in TTS B = 25 % according to the State Pharmacopoeia of Ukraine (SPhU) requirements (State Pharmacopoeia of Ukraine).

$$\max \Delta_x = 0.32 \cdot B = 0.32 \cdot 25 = 8.0\% \quad (1)$$

The limit values of the confidence interval were determined by equations 2 and 3:

– the upper limit

$$X_{high} = \bar{X} \cdot \left(1 + \frac{\max \Delta_x}{100} \right) \quad (2)$$

– the lower limit

$$X_{low} = \bar{X} \cdot \left(1 - \frac{\max \Delta_x}{100} \right) \quad (3)$$

The convergence estimation results of experimental values of process parameters of the examined API permeability through a membrane have been presented in Table 2.

According to the results, presented in Table 2, it can be seen that the variant values of all the selections X_i do not exceed the limit values of the confidence interval X_{low} and X_{high} . Thus, all the obtained experimental values of the studied parameters are within the limits of the confidence

interval \bar{X} and change insignificantly.

According to the results, presented in Table 3, relative uncertainty for probability 95% does not exceed the maximum allowed uncertainty of the analysis results (< 8,0%).

As a result, one can take the position that nifedipine and amlodipine besylate permeability through a semipermeable membrane from the solution in the model conditions of the experiment occurs at a constant rate.

The graphic interpretation of the *in vitro* permeability process of the tested substances through a semipermeable membrane and the statistical analysis parameters of the obtained results are presented in Figure 3 and in Figure 4.

It has been observed that in all experiments the obtained kinetic equations have the form of a general linear regression ($Y = A + B \times X$). For the obtained kinetic equa-

Table 2. The research results of the convergence estimation of experimental values of process parameters of permeability *in vitro*.

Estimation parameters	Selection of values in the dialysis sample		
	API content ($X_i \cdot 10^{-4}$, g)	API concentration ($C_i \cdot 10^{-3}$, mg/ml)	API flux ($I_s \cdot 10^{-2}$, mg/cm ² h)
Nifedipine			
Selection variants,	27,725	102,6851	66,8072
X_i	28,539	105,7000	68,7687
	28,727	106,3962	69,2217
	30,771	113,9666	74,1470
	31,751	117,5962	76,5084
	29,503	109,2688	71,0906
\bar{X}			
X_{low}	27,142	100,5273	65,4034
X_{high}	31,863	118,0103	76,7778
Amlodipine besylate			
Selection variants,	45,090	167,0000	108,6506
X_i	45,839	169,7740	110,4554
	46,097	170,7296	111,0771
	47,584	176,2370	114,6602
	48,010	177,8148	115,6867
\bar{X}	46,524	172,3111	112,1060
X_{low}	42,802	158,5262	103,1375
X_{high}	50,246	186,0960	121,0745

Table 3. Metrological characteristics of the method for determining the quantitative parameters of API permeability in the dialysis sample.

<i>m</i>	<i>v</i>	$\bar{X}_i \cdot 10^{-4}$	$\bar{X} \cdot 10^{-4}$	<i>S</i> ²	\bar{S}	<i>P</i>	<i>t</i> (<i>P</i> , <i>v</i>)	Confidence interval, ϵ , %	$\Delta X \cdot 10^{-4}$
Nifedipine									
5	4	27,725	29,503	2,839	0,7535	0,95	2,78	29,503±2,0946	7,10
		28,539							
		28,727							
		30,771							
		31,751							
Amlodipine besylate									
5	4	45,090	46,524	1,510	0,5495	0,95	2,78	46,524±1,5277	3,28
		45,839							
		46,097							
		47,584							
		48,010							

tions, within the time of the experiment, the correlation coefficient (*R*²) was not less than 0,999.

The main quantitative characteristics of the permeability process of the studied API *in vitro*, calculated on the basis of a statistical analysis, have been presented in Table 4.

Table 4. The kinetic parameters of the permeability process of API with cardiovascular effect *in vitro* through a semipermeable membrane.

API	API steady-state flux, I_s , mg/cm ² h	Time of diffusion delay, Θ , min	Permeability coefficient, K_p , cm/h	Linear correlation coefficient, <i>r</i>
Nifedipine	0,7083	- 3,06	0,075	0,9997
Amlodipine besylate	1,1295	3,28	0,124	0,9999

According to the obtained results (Table 4), the kinetic parameters of the amlodipine besylate permeability process are 1.6 times higher than the same indices for nifedipine. Thus, the value of the steady-state flux rate I_s , obtained for nifedipine, is 0.7083 mg/cm² h, and for

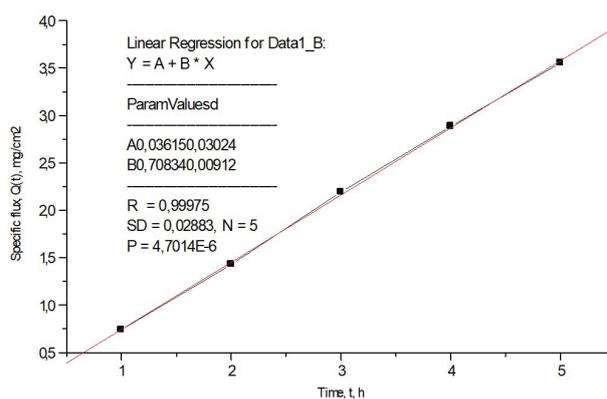


Figure 3. The kinetics of the nifedipine permeability process through a semipermeable membrane *in vitro*.

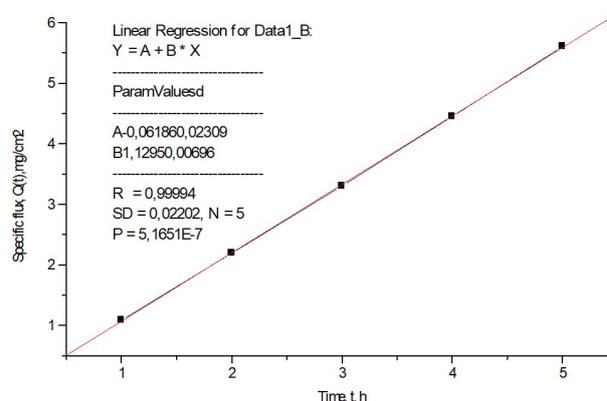


Figure 4. The kinetics of the amlodipine besylate permeability process through a semipermeable membrane *in vitro*.

amlodipine besylate is 1.1295mg/cm² h. In the same ratio for these substances the values of the permeability coefficient K_p were defined, that is 0.075 cm/h for nifedipine and 0.124 cm/h for amlodipine besylate. The diffusion delay time determines the duration of the non-stationary period of the process. In the frame of the experiment, the non-stationary period for the permeability process of both nifedipine and amlodipine besylate is defined within 3 minutes but at that point, the negative significance of this indicator for nifedipine indicates a lack of the membrane saturation.

Conclusions

In the result of the carried out research it was defined that nifedipine and amlodipine besylate permeability process in the simulative conditions is characterized by uniform velocity. Based on the statistical analysis, the linear dependence of this process was confirmed. The obtained quantitative values of the steady-state flux velocity and the coefficient of permeability indicate the potential of the selected substances in overcoming membrane barriers, and allow to predict a positive assessment of the acceptability of the selected API for the use in the design of TTS.

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