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Transdermal Delivery of Biologically Active Substances During Electrophoresis of the Phytocomplex in Model Experiments

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Abstract

Scientific justification for choosing a therapeutically significant dosage of the phytocomplex in the working solution for electrophoresis, increasing transdermal delivery of biologically active substances during electrophoresis of the phytocomplex, and control of this process require knowledge of the kinetics of the phytocomplex active ingredients' delivery during electrophoresis. This work is aimed at studying the process of transdermal delivery of biologically active substances (flavonoids) during electrophoresis of the phytocomplex in rehabilitation of patients with osteoarthritis in model experiments *in vitro*. Working solutions with various concentrations of the phytocomplex for electrophoresis were used. The quantitative content of flavonoids in the working solutions was determined spectrophotometrically and calculated in terms of rutin, which was the predominant flavonoid in a phytocomplex. The kinetics of flavonoids' delivery from the working solutions was studied in modified (for 3 chambers with electrodes) Franz diffusion cells through Carbosil-P membranes. The main parameters of the processes have been determined; dependence of the rate of flavonoids' delivery on the initial concentration in the working solution and the type of electric current has been found. The influence of dimethyl sulfoxide on flavonoids' delivery from the working solutions of the phytocomplex during electrophoresis has been studied. Almost 1.5-2 times increase in the release rate of biologically active substances from the working solutions containing dimethyl sulfoxide within 20-30 minutes of the experiment has been shown. The kinetics of transdermal delivery of biologically active substances (flavonoids) during electrophoresis of the phytocomplex in model experiments *in vitro* has been studied. The obtained results provide the basis for further study of the nature and the mechanism of the biologically active substances' action in the phytocomplex proposed for electrophoresis during rehabilitation of patients with osteoarthritis.

***Keywords**: biologically active substances, flavonoids, iontophoresis or electrophoresis, transdermal delivery.*

INTRODUCTION

Medicinal electrophoresis is a complex pharmaco-physiotherapeutic treatment method, in which the medicinal preparation and constant or pulsed electric current act on the organism of the patient [1]. These factors can interfere, therefore, the response of the organism is not simply the sum of effects from the preparation and electrical current; it is more specific and complex, involving the nervous and the endocrine systems [2, 3]. Medicines that are resistant to the action of electric current and at the same time preserve their pharmacological activity, by entering the body of the patient from the medicinal form in therapeutically significant quantities, may be used for electrophoresis [4-8].

In recent years, preparations of plant origin have been increasingly used for electrophoresis [9, 10]. The proposed phytocomplex for electrophoresis is a dry extract from the green and roots of marsh cinquefoil, alfalfa stems or cones, and common hops (Specification 9375-021-00003938-11 "Extract of cinquefoil, alfalfa, and dry hops (phytocomplex)") [11]. It contains a set of biologically active substances that have analgesic, anti-inflammatory, antioxidant and other effects, allowing it to be used in medicine for inflammatory-degenerative diseases of the musculoskeletal system, including osteoarthritis.

The main biologically active substances in the phytocomplex are flavonoids [12]. During electrophoresis, their penetration through the skin obeys the Fick's law, according to which the flux of the particles *(I)* diffusing through the plane perpendicular to the direction of diffusion is directly proportional to concentration gradient *(dc/DX)*:

$$I = -D(dc/dx) \qquad (1)$$

where *D* is the diffusion coefficient.

The second Fick's law is usually used for analyzing the majority of diffusion experiments:

$$dc/dt = D(d^2c/dx^2) \qquad (2)$$

From equation (2) it follows that the change in the concentration over time *(dc/dt)* at distance *x* from the initial plane is proportional to the rate of changing the concentration gradient towards *x* at moment *t*. For the practical use, equation (2) should be integrated under appropriate boundary conditions [13, 14].

Currently, there are no systematic works in the literature about the penetration of flavonoids from the medications through the skin during electrophoresis. Hence the relevance of these studies is obvious.

The present work is aimed at studying the process of transdermal delivery of biologically active substances (flavonoids) during electrophoresis of the phytocomplex in rehabilitation of patients with osteoarthritis in model experiments *in vitro*.

MATERIALS AND METHODS

5% (1), 10% (2) and 15% (3) working solutions of the phytocomplex were used in the work. The quantitative content of flavonoids in the working solutions was determined spectrophotometrically and calculated in terms of rutin, which was the predominant flavonoid in the phytocomplex. For obtaining more reproducible results, the reaction of forming a complex of rutin with aluminum chloride was performed in the presence of acetic acid. At the same time, the optical density of the standard sample of rutin (R-5143, Sigma) prepared similar to the tested solution was determined. The study was performed on spectrophotometer Titrtek MCC 1340 (Finland) at the wavelength of 415 nm.

The absorption spectra of the phytocomplex and rutin had been previously studied. It has been found that the phytocomplex does not shift the maximum of rutin's optical density, the intensity of which will be used for photometering. In addition, rutin had the differential absorption spectrum similar to the differential absorption spectrum of flavonoids in the phytocomplex.

The kinetics of flavonoids transdermal delivery from the working solutions of the phytocomplex during electrophoresis was studied in modified (for 3 chambers with electrodes) Franz diffusion cells (SES GmbH-Analysesysteme, Germany) through Carbosil-P membranes (Specification 66-2-512-92) at the temperature of 42.0°C. The working solution of the phytocomplex was placed into the central chamber, the side chambers were filled with the model medium, 0.9% solution of sodium chloride (natural saline solution). Electrophoresis was performed using various types of electric current used in rehabilitation of patients

with osteoarthritis: diadynamic currents (DDC) - 100 Hz full-wave continuous current for 1 minute, short-period modulated current with the modulation frequency of 1.5 seconds for 3 minutes with constant component of the apparatus; sinusoidal modulated currents (SMC) in the DC mode in case of I and IV kind of operation for 5 minutes each, with 100 Hz modulation frequency, the modulation depth of 75%, the duration of the half-periods for 2 and 3 seconds, current of 5 mA using COMBI 500 (Gymna Uniphy, Belgium-Germany). Samples were taken at certain intervals, with complete replacement of the model medium (in the first approximation, this system might be considered flow-through), and with sampling 4 ml from each camera with subsequent returning (a closed system).

Resistance of standard samples of the following main flavonoids in the phytocomplex to the action of DDC and SMC was identified: rutin (R 5143, Sigma), apigenin (42251 Fluka), apigenin-7-glucoside (44692, Fluka), biochanin A (14,563, Aldrich), hyperoside (00180585, Fluka), daidzein (D 7802, Sigma), isoquercitrin (00140585, Fluka), quercetin (Q 0125, Sigma), quercitrin (00740580, Fluka), and working solutions of the phytocomplex. Absorption spectra of the biologically active substances and working solutions were recorded before and after the action of electric current. The quantitative content of flavonoids was determined by spectrophotometry at the wavelengths of 360 nm (rutin), 339 nm (apigenin), 333 nm (apigenin-7-glucoside), 326 nm (biochanin A), 365 nm (hyperoside), 302 nm (daidzein), 362 nm (isoquercitrin), 370 nm (quercetin), 363 nm (quercitrin) and 415 nm (flavonoids in the working solutions of the phytocomplex reduced to rutin). The last determination was performed after the reaction of formation of the complex of rutin with aluminum chloride in the presence of acetic acid. It has been shown that the maximum electrophoretic mobility of the tested flavonoids in the phytocomplex was achieved by using both working cathode and anode with DDC and SMC.

The results were statistically processed in application SPSS.Statistics.v17.Multilingual-EQUiNOX (SPSS Inc).

RESULTS AND DISCUSSION

In studying the kinetics of flavonoids' delivery from the working solutions with various concentrations of the phytocomplex during electrophoresis of DDC and SMC in a closed system, it has been found that within the 6 hours of the experiment, approximately 50% of flavonoids diffused into the model medium from working solution 1, about 30% - from solution 2, and about 25% - from solution 3. The balance was established after 4 to 6 hours. The rate of flavonoids' delivery from the working solutions with various content of the phytocomplex at the beginning of the experiment was directly proportional to the initial concentrations of biologically active substances in the working solutions and dramatically decreased by the time equilibrium was established (Figure 1).

Periodic replacement of the model medium (a flow-through system) allowed obtaining a more complete picture of the kinetics of flavonoids' delivery from the working solutions of the phytocomplex during electrophoresis (Table 1). The maximum rate of flavonoids penetration through the membrane from solution 1 during electrophoresis with DDC and SMC was achieved after 30 minutes of the experiment, and from solutions 2 and 3 - after 20 minutes of the experiment. The "time lag" was 1-2 minutes. During the first 20-30 minutes of the experiment (the approximate duration of the electrophoresis procedure) 9-17% flavonoids diffused into the model medium from working solution 1, 8-14% - from solution 2, and 6-13% − from solution 3. In using DDC electrophoresis, during the first two hours of the experiment, the rate and extent of flavonoids' delivery from the working solutions of the phytocomplex was higher than in using SMC electrophoresis. Complete extraction of flavonoids from the working solutions by DDC electrophoresis required less time than extraction by SMC electrophoresis (Table 1).

Table 1. The kinetics of flavonoids' delivery from the working solutions of the phytocomplex into the physiological solution through membranes Carbosil-P in a flow-through system at 42°C by DDC and SMC electrophoresis

Time	Rate (*V*) and share (*P*) of flavonoids' delivery					
	5% working solution of the phytocomplex		10% working solution of the phytocomplex		15% working solution of the phytocomplex	
	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %
DDC electrophoresis						
10 min	53.78±0.02	4.98	88.13+0.03	4.08	118.91+0.03	3.67
20 min	65.34+0.03	11.03	108.86+0.04	9.12	151.31+0.03	8.34
30 min	67.07+0.04	17.24	108.21+0.04	14.13	148.07+0.04	12.91
40 min	66.74+0.04	23.42	107.57+0.04	19.11	142.88+0.04	17.32
1 h	65.23+0.03	35.50	105.19+0.04	28.85	139.64+0.04	25.94
2 h	39.28+0.03	57.31	91.69+0.04	54.32	113.62+0.04	46.98
4 h	16.57+0.03	75.72	33.23+0.03	72.78	55.38+0.03	67.49
6 h	7.10+0.02	83.61	14.49+0.03	80.83	26.70+0.03	77.38
8 h	0.23+0.01	83.86	1.84+0.02	81.85	11.64+0.03	81.69
SMC electrophoresis						
10 min	43.52+0.02	4.03	74.74+0.02	3.46	80.35+0.03	2.48
20 min	54.76+0.01	9.10	90.29+0.03	7.64	110.48+0.04	5.89
30 min	57.02+0.03	14.38	90.07+0.04	11.81	106.27+0.04	9.17
40 min	54.32+0.03	19.41	89.87+0.04	15.97	100.76+0.04	12.28
1 h	53.35+0.03	29.29	88.78+0.03	24.19	98.17+0.04	18.34
2 h	36.59+0.02	49.62	73.76+0.03	44.68	89.26+0.04	34.87
4 h	18.16+0.02	69.80	37.96+0.03	65.77	63.58+0.03	58.42
6 h	8.98+0.02	79.78	18.95+0.03	76.30	32.78+0.03	70.56
8 h	4.09+0.01	84.32	9.25+0.02	81.44	15.36+0.02	76.25
10 h	0.21+0.01	84.55	4.09+0.02	83.71	11.53+0.02	80.52

able 2. The kinetics of flavonoids' delivery from the working solution of the phytocomplex containing 10% of DMSO into the physiological solution through membranes Carbosil-P in a flow-through system at 42°C by DDC and SMC electrophoresis

Time	Rate (*V*) and share (*P*) of flavonoids' delivery					
	5% of DMSO in the working solution		10% of DMSO in the working solution		15% of DMSO in the working solution	
	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %	$V \cdot 10^5$, g/ml·h	*P*, %
DDC electrophoresis						
10 min	128.30±0.04	5.94	177.34±0.05	8.21	221.40±0.06	10.25
20 min	147.10±0.05	12.75	201.96±0.06	17.56	233.50±0.08	21.06
30 min	142.99±0.05	19.37	152.28±0.05	24.61	191.59±0.07	29.93
40 min	141.91±0.04	25.94	148.82±0.05	31.50	162.86±0.06	37.47
1 h	110.48±0.04	36.17	120.85±0.04	42.69	123.98±0.06	48.95
2 h	82.62±0.03	59.12	79.31±0.04	64.72	76.75±0.04	70.27
3 h	45.86±0.03	71.86	38.84±0.03	75.51	33.37±0.04	79.54
4 h	23.29±0.03	78.33	19.22±0.02	80.85	13.90±0.03	83.40
5 h	13.36±0.02	82.04	7.96±0.02	83.06	0.25±0.01	83.47
6 h	2.20±0.01	82.65	0.22±0.01	83.12	-	-
SMC electrophoresis						
10 min	107.14±0.04	4.96	135.00±0.04	6.25	219.46±0.05	10.16
20 min	131.11±0.05	11.03	156.60±0.05	13.50	233.50±0.06	20.97
30 min	110.38±0.04	16.14	144.29±0.05	20.18	191.16±0.05	29.82
40 min	92.45±0.04	20.42	104.54±0.04	25.02	141.70±0.05	36.38
1 h	90.18±0.04	28.77	99.58±0.04	34.24	134.78±0.04	48.86
2 h	77.18±0.03	50.21	72.76±0.03	54.45	76.75±0.03	70.18
3 h	52.88±0.03	64.90	52.27±0.03	68.97	33.48±0.03	79.48
4 h	36.65±0.02	75.08	32.22±0.03	77.92	13.93±0.02	83.35
5 h	22.25±0.02	81.26	17.03±0.02	82.65	0.22±0.01	83.41
6 h	2.41±0.01	81.93	2.12±0.01	83.24	-	-

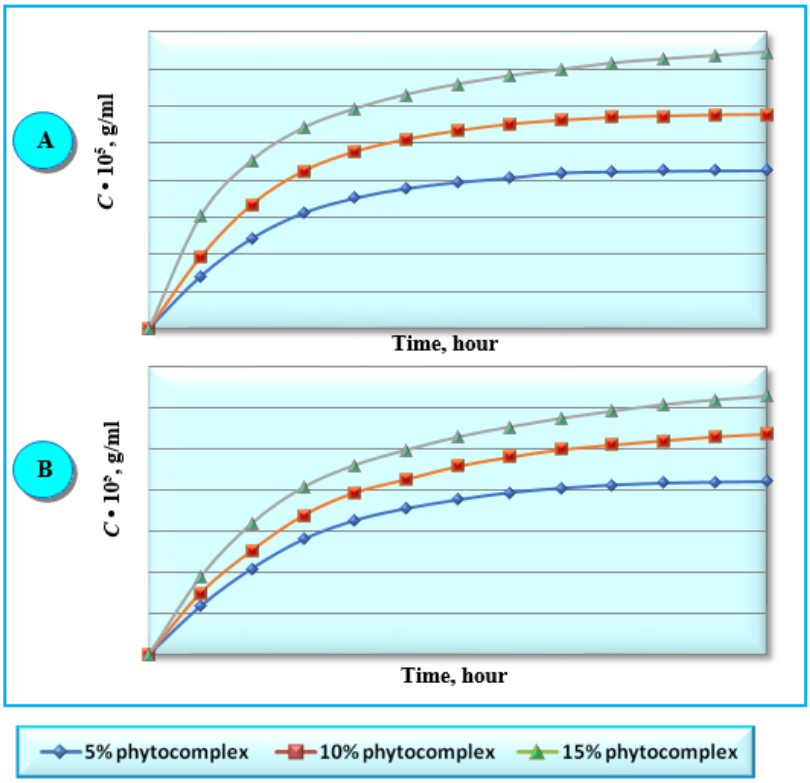

Figure 1. The kinetics of flavonoids' delivery from the working solutions of the phytocomplex into the physiological solution through membranes Carbosil-P during the establishment of equilibrium in a closed system at 42°C by electrophoresis with DDC (A) and SMC (B) (*C* is the concentration of flavonoids in the model medium)

In practice, efficiency of electrophoresis largely depends on the completeness and the rate of biologically active substances' delivery in the first 20-30 minutes. To increase the speed of flavonoids' penetration through the membrane during the first hour, dimethyl sulfoxide (DMSO) was added to the working solutions. The choice of this "carrier" of biologically active substances was also due to its anti-inflammatory, analgesic, and antimicrobial action.

The kinetics of flavonoids' delivery from the working solutions of the phytocomplex containing DMSO was studied in a flow-through system. It had been previously found that DMSO did not shift the maximum optical density of rutin, and did not affect the nature of the spectrum.

Experimental data have shown that DMSO introduction into working solutions of the complex during DDC and SMC electrophoresis significantly affects flavonoids' delivery from solutions: with increasing DMSO concentration in the working solutions of the phytocomplex, the rate of flavonoids' penetration through the membrane increases, and the time of complete migration decreases. Thus, in working solution 2 without DMSO, almost complete flavonoids' extraction with the use of DDC was observed after 8 hours (Table 1); in the solution containing 10% of DMSO – after 6 hours; and in the solution containing 15% of DMSO – afrer 5 hours (Table 2). The influence of DMSO was most significantly manifested in the first few minutes of the experiment. For example, after 20 minutes of the experiment, the rate of flavonoids' delivery from the working solution of the phytocomplex containing 15% of DMSO increased almost 2 times under the action of DDC, compared to the solution without DMSO, and reached the maximum value (Table 2).

CONCLUSION

As a result of studying the kinetics of transdermal flavonoids' delivery from working solutions of the phytocomplex in course of DDC and SMC electrophoresis in model experiments *in vitro*, the main parameters of the processes have been determined; the flavonoids' release rate dependence on their initial concentration in the working solution of the phytocomplex has been established. The influence of electric current and DMSO on the release of biologically active substances from working solutions of the phytocomplex has been studied. It has been shown that the introduction of DMSO into working solutions increases the rate of flavonoids' delivery from the phytocomplex almost 1.5-2 times, especially during the first 20-30 minutes of electrophoresis. The obtained results provide the basis for further study of the nature and the mechanism of the biologically active substances' action in the phytocomplex proposed for electrophoresis during rehabilitation of patients with osteoarthritis.

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