D. V. Babaskin et al /J. Pharm. Sci. & Res. Vol. 10(4), 2018, 697-700

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Modifying Factors of Transdermal Delivery of Biologically Active Substances of Peloid: Experimental Research

D. V. Babaskin, T. M. Litvinova, L. I. Babaskina, O. V. Savinova, E. A. Winter
Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University)
119991, Russian Federation, Moscow, Trubetskaya St., 8-2

Abstract

For improving transdermal delivery of biologically active substances (peloids) and efficiency of peloid therapy, and for controlling this process, it is highly important to study the modifying factors related to delivery of active ingredients of peloids. The research was aimed at studying transdermal delivery of biologically active substances (flavonoids) of peat peloid compositions containing the phytocomplex, in model experiments *in vitro*.

Material and methods. Peat peloid compositions with the phytocomplex. The quantitative content of flavonoids in peloid compositions was determined with the use of spectrophotometry, and calculated in terms of quercetine, the prevailing flavonoid of the phytocomplex. The kinetics of flavonoids' transdermal delivery from peloid compositions were studied in Franz diffusion cells.

Results and discussion. The main parameters of the process have been determined, and the flavonoids' delivery speed dependence on the initial concentration in the peloid composition has been found. The effect of dimethyl sulfoxide on flavonoids' delivery in peat peloid compositions has been studied. The almost 1.5-2 times increase in the velocity of releasing biologically active substances from the compositions containing dimethyl sulfoxide within 20-40 minutes of the experiment has been shown. The technological methods of layer-by-layer application of peloid composition and peat peloid have been proposed with the purpose of increasing the efficiency of the complex method of peloid and phytotherapy and rational use of the phytocomplex. **Conclusion.** The kinetics of biologically active substances' (flavonoids) transdermal delivery from peat peloid compositions containing the phytocomplex have been studied in model experiments *in vitro*. The obtained results provide the basis for further pharmacological studies of peloid compositions containing phytocomplex.

Keywords: mud or peloid therapy, transdermal delivery, biologically active substances of peloid, flavonoids.

INTRODUCTION

The therapeutic action of peloids largely depends on the presence of large amounts of biologically active substances, including flavonoids [1-4]. Flavonoids are known to have anti-inflammatory, wound-healing, antimicrobial, immunomodulatory, antioxidant, diuretic and other effects on the human body [5-11].

To increase the pharmacological activity of peloids, extracts of medicinal plants containing flavonoids may be introduced into them [12]. When developing new peloid compositions, the most acute is the issue of ensuring control over delivery of biologically active substances into the organism with the possibility of influencing and controlling this process.

This paper shows the possibility of using biologically active substances of transdermal delivery on the example of peloid compositions containing the phytocomplex in adjusting the process of osteoarthritis patients' rehabilitation. The previous studies have shown the need of improving the technology of the combined use of the phytocomplex and the peloid [12]. Studying transdermal delivery of active substances of peloid compositions into the organism is also important for the possible industrial production of a new preparation based on the phytocomplex, and suitable for peloid therapy. This opens wider perspectives for using the complex method of peloid and phytotherapy.

The phytocomplex is a dry extract from the herb and roots of marsh cinquefoil, creeping alfalfa, and stems and cones of common hop (Technical Specifications 9375-021-00003938-11 "Dry extract of cinquefoil, alfalfa and hop (phytocomplex)") [13]. The main active ingredients of the phytocomplex are flavonoids, polysaccharides, coumestans, tannins, phenol carbonic acids, essential oils, macro- and micronutrients, vitamins, which ensure anti-inflammatory, analgesic and other effects, which allows using it in medicine for inflammatory-degenerative diseases of the musculoskeletal system, including osteoarthritis.

In the literature, there are currently non-systematic works devoted to the issues of controlling the process of transdermal delivery of biologically active substances from peloid compositions containing the herbal formulation. This shows the relevance of these studies.

The work was aimed at studying transdermal delivery of biologically active substances (flavonoids) of peat peloid compositions containing the phytocomplex, in model experiments *in vitro*.

MATERIAL AND METHODS

The studied peloid compositions were based on peat peloid from the "Communa" deposit in the Tula region, Russia. Some of its properties were as follows: humidity - 71.0±0.4%; pH value - 7.2; ash content - 18.8±0.3% (in terms of dry substance); shear resistance – 2,430±10 dyne/cm^2; slush mineralization- 0.70±0.02 g/dm^3; decomposition degree - 48.2±0.5%; and organic carbon content - 42.0±0.2%.

In this study, 5% (PC1), 10% (PC2), and 15% (PC3) peloid compositions with the phytocomplex were used.

The quantitative content of flavonoids in peloid compositions was determined with the use of spectrophotometry [12], and calculated in terms of quercetine, the prevailing flavonoid of the phytocomplex. The absorption spectra of peloid compositions, peat peloid and quercetine (Q 0125, Sigma) were previously studied. It was found that peat peloid did not displace the maximum optical density of quercetine, intensity of which was used for photometry. In addition, quercetine had the absorption spectrum similar to that of flavonoid absorption in peloid compositions. The study was performed on spectrophotometer TitrtekMCC 1340 (Finland) at the wavelength of 370 nm.

The content of flavonoids in PC1 was 0.38±0.02%; in PC2 – 0.74±0.03%; in PC3 – 1.15±0.03%.

The kinetics of flavonoids' transdermal delivery from peloid compositions were studied in Franz diffusion cells (No. 4G-01-00-09-05, SESGmbH-Analysesysteme, Germany) in the V6-SFCS system through Carbosil-P (TU 66-2-512-92) membranes at the temperature of 42.0°C. Model media were the 70% ethanol solution (as quercetine is very poorly soluble in water) and the 0.9% sodium chloride solution. Samples were taken at regular

intervals, with complete replacement of the model media (this system may be considered as flow through in the first approximation), and when 4 ml samples were taken, they were returned back, and the source medium was topped to the required volume if necessary (a closed system).

The results were statistically processed using application SPSS. Statistics. v17. Multilingual-EQUiNOX (SPSSInc).

RESULTS AND DISCUSSION

During the study of the kinetics of flavonoids' delivery from peloid compositions with various concentrations of the phytocomplex into the 70% ethanol in a closed system, it was shown that within 12 h of the experiment, 26% of flavonoids had diffused into the model environment from peloid composition of PC1, 17% − from peloid composition of PC2, and approximately 13% − from peloid composition of PC3 (Figure 1). The equilibrium state was reached after 11-12 hours. The rate of flavonoids' release from peloid compositions with various contents of the phytocomplex at the beginning of the experiment was directly proportional to the initial concentrations of biologically active substances in the compositions, and decreased sharply by the time of reaching the equilibrium state.

Periodic replacement of the model environment (a flow-through system) allowed more complete understanding of kinetics of flavonoid delivery from peloid compositions containing the phytocomplex. The maximum flavonoid delivery rate from the compositions was achieved after 30 minutes of the experiment (Table 1).

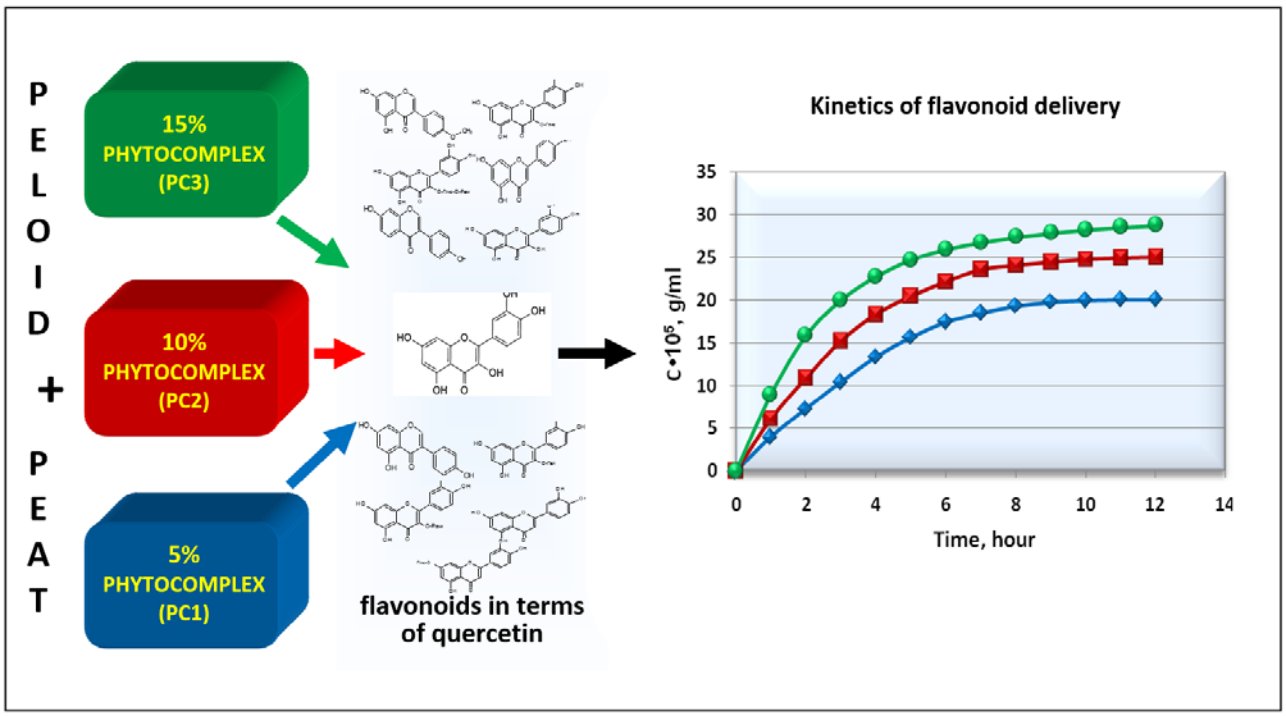

Figure 1. The process of flavonoids' delivery from peloid compositions with various concentrations of the phytocomplex in the 70% ethanol solution through Carbosil-P membranes after reaching the equilibrium state in a closed system at 42°C (C is flavonoid concentration in the model environment)

Table 1. The kinetics of flavonoids' delivery from peloid compositions with various concentrations of the phytocomplex into 70% ethanol solution through Carbosil-P membranes in a flow-through system at 42°C

Time	Velocity (V) and the proportion (P) of flavonoids' delivery					
	PC1		PC2		PC3	
	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %
10 min	3.12±0.02	0.68	4.81±0.02	0.54	7.10±0.03	0.51
20 min	5.87±0.02	1.97	9.25±0.03	1.58	13.30±0.03	1.48
30 min	6.41±0.03	3.38	9.97±0.02	2.71	13.68±0.04	2.47
40 min	5.16±0.02	4.51	6.02±0.02	3.39	8.66±0.04	3.10
1 h	4.23±0.02	6.37	5.98±0.02	4.73	6.53±0.03	4.04
2 h	3.71±0.01	11.25	4.62±0.02	7.85	6.05±0.02	6.67
4 h	3.27±0.02	19.86	4.25±0.02	13.60	5.72±0.02	11.65
8h	2.30±0.01	31.96	3.35±0.01	22.65	4.62±0.02	19.68
12h	1.52±0.01	39.96	2.66±0.01	29.84	3.71±0.02	26.13
24 h	0.87±0.01	53.62	1.68±0.01	43.44	2.09±0.01	37.05
48 h	0.62±0.01	73.11	1.26±0.01	63.89	1.96±0.01	57.54
96 h	0.24±0.01	88.24	0.60±0.01	83.41	1.02±0.01	78.93
144 h	0.13±0.01	96.73	0.25±0.01	91.53	0.38±0.01	86.85

Table 2. Kinetics of flavonoids' delivery from DMSO containing peloid compositions into 70% ethanol solution through Carbosil-P membranes in a flow-through system at 42°C

Time	Velocity (V) and the proportion (P) of flavonoids' delivery					
	PC2 + 12% DMSO		PC2 + 15% DMSO		PC2 + 12% DMSO*	
	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %	$V \cdot 10^5$, g/ml·h	P, %
10 min	5.47±0.02	0.62	13.34±0.03	1.50	5.43±0.03	1.09
20 min	13.72±0.03	2.16	18.04±0.03	3.53	13.67±0.03	3.83
30 min	10.86±0.03	3.38	16.76±0.04	5.42	10.62±0.03	5.95
40 min	9.85±0.03	4.49	9.83±0.03	6.53	9.75±0.03	7.91
1 h	8.40±0.03	6.39	9.37±0.03	8.64	7.93±0.03	11.09
2 h	5.97±0.03	10.42	6.64±0.03	13.12	5.26±0.03	17.41
4 h	4.66±0.02	16.72	5.87±0.02	21.06	1.91±0.02	22.00
8 h	3.51±0.02	26.20	3.95±0.02	31.73	1.52±0.02	29.33
12 h	2.90±0.02	34.05	3.31±0.01	40.68	1.19±0.01	35.05
24 h	2.47±0.02	54.04	2.27±0.01	59.11	1.04±0.01	50.08
48 h	1.42±0.01	77.00	1.35±0.01	80.95	0.99±0.01	78.54
96 h	0.47±0.01	92.18	0.39±0.01	93.44	0.19±0.01	89.61

Note. * – layer-by-layer application of peloid composition and peloid in the rate of 1:3.

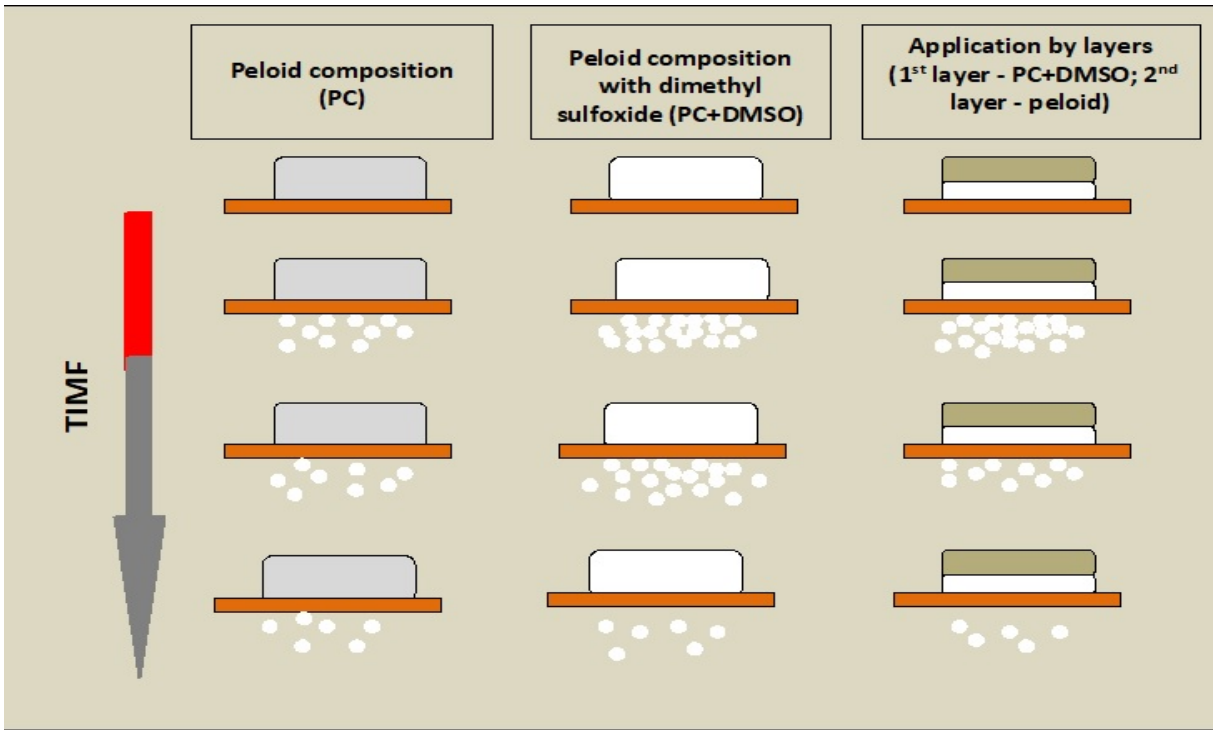

Figure 2. Factors influencing the process of transdermal flavonoids' delivery from peloid compositions (the time of the peloid therapy procedure marked in red)

In practical terms, the efficiency of the peloid compositions largely depends on the completeness and the velocity of biologically active substances' delivery during the first 30-40 minutes. To increase the velocity of releasing flavonoids within the first hour, dimethyl sulfoxide (DMSO) was introduced into peloid composition PC2 (TS 6-09-3818-89) in the concentrations of 10, 12 and 15%. DMSO was also chosen due to its anti-inflammatory, analgesic and antimicrobial effects. It was previously found that DMSO did not displace the maximum optical density of the quercetine, and did not affect the nature of the spectrum. Significant influence of DMSO on flavonoids' delivery from peloid compositions has been established. Thus, from composition PC2 containing no DMSO, almost complete extraction of flavonoids was observed after 144 h of the experiment (Table 1), and from compositions containing 12-15% of DMSO – after 96 h (Table 2). Significant influence of DMSO was manifested during the first hours of the experiment. For example, after 20 min of the experiment, velocity of flavonoids' delivery from peloid compositions containing 12-15% of DMSO increased almost 1.5-2 times, and reached the maximum value.

The obtained results of flavonoids' extraction from PC2-based peloid compositions containing 12% and 15% of DMSO into 0.9% sodium chloride solution were also the evidence of improving the velocity of flavonoid delivery.

Analysis of the physicochemical properties of peloid compositions containing DMSO showed that the optimal criteria were characteristic of composition PC2 that contained 10-12% of DMSO.

Although the velocity of flavonoids' delivery from peloid compositions increased with the introduction of DMSO, the percentage of biologically active substances released during the first hour of the experiment remained relatively low. In order to increase the degree of flavonoids' extraction from the compositions, and to ensure more rational use of the phytocomplex, the possibility of layer-by-layer application of peloid and peloid composition was studied: first, the membrane was covered with the peloid composition, then the main peat peloid was applied (Figure 2). The following composition and peloid shares were used: 1:2, 1:3, 1:4, 1:7, 1:14 (accordingly, composition layer thickness was approximately equal to 5, 4, 3, 2 and 1 mm). Experiments with peloid composition PC2 containing 12% of DMSO were made using Carbosil-P membranes in 70% ethanol solution in a flow-through system at 42^{o}C.

It has been found that velocity of flavonoids' delivery is preserved with the layer-by-layer application of the composition and the peloid in the ratio of 1:2 within 40 min, in the ratio of 1:3 – within 30 min, and in the ratio of 1:4 - within 20 min. Layer-by-layer application of the composition and peat peloid in the ratio of 1:7 and 1:14 decreased the flavonoids' release rate within the first minutes of the experiment, compared to the monolithic composition. The share of flavonoids' extraction in case of layer-by-layer application of the composition and peat peloid in the ratio of 1:2, 1:3 and 1:4 increased by almost 40-70% within the first hour, compared to the monolithic use of peloid composition.

After introducing DMSO into the composition, velocity of flavonoids' delivery was preserved in case of layer-by-layer application of the composition and peloid in the ratio of 1:2 within 30 min, in the ratio of 1:3 - within 20 min (Table 2). The share of flavonoids' extraction in case of layer-by-layer application of the composition and peat peloid in the ratio of 1:2 and 1:3 increased almost 1.5 to 1.7 times within the first hour, compared to the monolithic use of the peloid composition.

CONCLUSION

1. The kinetics of biologically active substances' (flavonoids) transdermal delivery from peat peloid compositions containing 5 to 15% of the phytocomplex have been studied in model experiments *in vitro*. The main parameters of the process have been determined, and the flavonoids' delivery speed dependence on the initial concentration in the peloid composition has been found.
2. The effect of DMSO on flavonoids' delivery in peat peloid compositions has been studied. The considerably increased velocity of releasing biologically active substances from the compositions was observed within the first hour of the experiment.
3. The technological methods of layer-by-layer application of peloid composition and peat peloid have been proposed with the purpose of increasing the efficiency of the complex method of peloid and phytotherapy and the rational use of the phytocomplex.

The obtained results provide the basis for further pharmacological studies of peloid compositions containing phytocomplex in osteoarthritis therapy.

REFERENCES

[1] Antonelli M. and Donelli D. Effects of balneotherapy and spa therapy on levels of cortisol as a stress biomarker: a systematic review. Int J Biometeorol. 2018 Feb 18, doi: 10. 1007/s00484-018-1504-8.

[2] Badalov N. G. and Krikorova SA. Peloid therapy: the theoretical and practical aspects, problems and prospects of its development. Vopr Kurortol Fizioter Lech Fiz Kult., 2012 May-Jun; (3): 50-4. (InRuss).

[3] Morer C, Roques CF, Françon A, Forestier R. and Maraver F. The role of mineral elements and other chemical compounds used in balneology: data from double-blind randomized clinical trials. Int J Biometeorol. 2017 Dec; 61 (12): 2159-2173.

[4] Suárez M, González P, Domínguez R, Bravo A, Melián C, Pérez M, Herrera I, Blanco D, Hernández R. and Fagundo JR. Identification of organic compounds in San Diego de los Baños Peloid (Pinar del Río, Cuba). J Altern Complement Med., 2011 Feb; 17 (2): 155-65.

[5] Batista CM, Alves AVF, Queiroz LA, Lima BS, Filho RNP, Araújo AAS, de Albuquerque Júnior RLC, Cardoso JC. The photoprotective and anti-inflammatory activity of red propolis extract in rats. J Photochem Photobiol B., 2018 Jan 31; 180: 198-207.

[6] Boutaghane N, Alabdul Magid A, Abedini A, Cafolla A, Djeghim H, Gangloff SC, Voutquenne-Nazabadioko L. and Kabouche Z. Chemical constituents of Genista numidica Spach aerial parts and their antimicrobial, antioxidant and antityrosinase activities. Nat Prod Res., 2018; 15: 1-7.

[7] Dawé A, Mbiantcha M, Yakai F, Jabeen A, Ali MS, Lateef M. and Ngadjui BT. Flavonoids and triterpenes from Combretum fragrans with anti-inflammatory, antioxidant and antidiabetic potential. Z Naturforsch C. 2018 Feb 16; pii: /j/znc. ahead-of-print/znc-2017-0166/znc-2017-0166. xml.

[8] Ghasemzadeh A. , Karbalaii MT. , Jaafar HZE. And Rahmat A. Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. Chem Cent J., 2018 Feb 17; 12 (1): 17.

[9] Ribeiro D, Proenca C, Rocha S, Lima JLFC, Carvalho F, Fernandes E. and Freitas M. Immunomodulatory effects of flavonoids in the prophylaxis and treatment of inflammatory bowel diseases: a comprehensive review. Curr Med Chem., 2018 Feb 14. doi: 10. 2174/0929867325666180214121734.

[10] Shi D, Xu M, Ren M, Pan E, Luo C, Zhang W. and Tang Q. Immunomodulatory Effect of Flavonoids of Blueberry (Vaccinium corymbosum L.) Leaves via the NF-κB Signal Pathway in LPS-Stimulated RAW 264. 7 Cells. J Immunol Res., 2017; 2017: 5476903.

[11] Zeng X, Xi Y. and Jiang W. Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: a review. Crit Rev Food Sci Nutr., 2018 Feb; 12: 1-44.

[12] Babaskin DV. The release of biologically active compounds from peat peloids. Vopr Kurortol Fizioter Lech Fiz Kult. 2011 Sep-Oct; (5): 52-4. .

[13] Babaskin DV, Babaskina LI. and Babaskin VS. Sposob polucheniya fitopreparata dlya lecheniya osteoartroza i sposob reabilitatsii bol'nykh osteoartrozom. Patent RUS No. 2453325, 2012; 17 (I): 94. (InRuss). http://www. freepatent. ru/patents/2453325 Accessed January 10, 2017.