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Research Article

Fulvic acid transdermal patch: Its properties, optimization and release

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Abstract

There is a high demand for the design of fulvic acids (FA) transdermal patches being natural biologically active substances with a wide action. The difficulties in FA transdermal patches creating are due to the peculiarities of FA obtaining with high purity and solubility, the existence of many several polymorphic forms, as well as the problems of their insertion into dosage forms. In this work, we modified the FA isolation as water-soluble polymorph. This operation allowed us to reduce the impurities in the product, to optimize the formulation of the emulsion-based transdermal patches, to provide a quantitative assessment of FA release. Data of IR, ^{13}C NMR, AAS, ICP-AES-spectra, direct and reverse titration of carboxyl groups and phenolic hydroxyls, potentiometry, molecular weight determination by cryoscopy (M.m=740 g/mol), fluorescent analysis, solubility (3.3 mL of water per gram) confirmed the receipt of the desired polymorph. Optimization of the transdermal patches formulations by Response Surface Methodology and release evaluation and kinetics mathematical modeling using a Franz cell showed the preferences of Pluronic Kolliphor p237 as a transcutant. The obtained water-soluble FA can be used as a potential component of transdermal patches with controlled release parameters.

Keywords: fulvic acid, transdermal patches, pluronics, release

INTRODUCTION

Currently, there is an increased interest in natural substances from renewable raw materials, which are products of soil processing of coal, peat and other objects by microorganisms. These natural substances include humic acids (HA), fulvic acids (FA) and others. This demand is due to their diverse spectrum of biological activity, including antioxidant [1-6], anti-inflammatory [3, 7-17], antitumor [18-21] and antiviral properties [22-24].

The use of FA dosage forms into widespread practice is complicated, first of all, by the instability of the substance during storage and strong polarity, which complicates its transdermal delivery. Moreover, FA tends to aggregate with the formation of complex supramolecular structures, reducing its solubility, stability and activity. The ability to form stable complexes with various metal ions [25-30] not only creates new biological activity, but also complicates the FA release from various dosage forms.

Early, we demonstrated the high effectiveness of the treatment of rheumatoid arthritis by the FA transdermal patches where FA was isolated from the peat [31]. However, it was estimated that obtained FA has formed several polymorphs that had the same composition but differ greatly in solubility [32]. One of the reasons for the several polymorphic forms formation may be metal ions (iron, magnesium, calcium, zinc, etc.), as well as unfavorable drying conditions, which promoted the formation of complex supramolecular structures.

In this work, we optimized the formulation of the transdermal patches with the stable water-soluble FA that is minimally prone to the formation of supramolecular complexes. To achieve this aim, we continued our research to find conditions for FA obtaining with the necessary properties. Optimization of the transdermal patch formulation was carried out using mathematical modeling of the patch formulation and FA release kinetics using a diffusion vertical Franz cell.

MATERIALS AND METHODS

Peat and its processed products must comply with the state requirements (state standard – GOST 4.105-2014) in terms of nitrate nitrogen, ammonia nitrogen, phosphorus, chlorine, heavy metals. Tests for heavy metals during peat harvesting were carried out according to the state requirements (GOST R 53218-2008). The peat sample received from the supplier was sifted and crushed during sample preparation according to the state standard (GOST R 54332-2011).

We obtained fulvic acid (FA) from the peat in the Nizhny Novgorod region (Russia) by acid-base hydrolysis and purification according to the Lamar method [32]. Sodium hydroxide (99.0% purity), sulfuric acid (99.9% purity), Superlite DAX-8 (Sigma-Aldrich, USA), Cationite KU 2-8 H^+ (NevaReactiv, Russia) were used. Drying of the FA solution was

carried out by freeze drying (from –80 to –40°C for 8 hours – LGJ-10, Vikumer, Beijing, China) [32]

IR spectra were obtained using a infrared spectrophotometer with a Fourier converter "IRAffinity-1S" (Shimadzu, Japan) in the region of 4000-500 cm^{-1} in the mixtures with KBr.

Registration of solid-state ^{13}C NMR spectra was performed using a JNM-ECX400 spectrometer (JEOL, Japan - 9.39 T, 100.5 MHz) in the solid phase at room temperature.

The determination of the heavy metals content in the FA was carried out: 1) using a atomic absorption spectrophotometer AA-7000 (Shimadzu, Japan) with hollow cathode lamps and standard samples. Samples were treated with a mixture of concentrated acids H_2SO_4:$HClO_4$ (6:1) to release metals (wet mineralization); 2) by atomic emission spectroscopy with inductively coupled plasma (ICP AES) using a Prodigy High Dispersion ICP spectrometer (Teledyne Leeman Labs, USA).

Fluorescence spectra were recorded with a CM 2203 spectrofluorimeter (Solar, Belarus). One-dimensional excitation spectra were recorded in the wavelength range of 300–500 nm at a fixed emission wavelength λ_{Em}=520 nm. One-dimensional emission spectra were recorded in the wavelength range of 400–550 nm at a fixed excitation wavelength λ_{Ex}=360 nm.

Direct and reverse titrations were carried out in accordance with the methods [33].

We used Response Surface Methodology (RSM) computer modeling methodology for the design and optimization the transdermal patches formulations (Minitab Statical Software). The emulsion base was taken as the model, in which we included FA and pluronics (Kolliphor p237, Kolliphor p338 - BASF Pharmaceuticals, Germany) as transcutants [31]. The independent variables were the concentrations of pluronics and plasticizer (glycerin, 99.9% purity), which varied at two levels (low and high). The dependent variable was the concentration of released FA. Trial versions of transdermal patches were prepared in accordance with the design proposed by the program. ANOVA results showed that these models were significant (Table 1).

Table 1. Statistical parameters obtained from ANOVA

Responses	R-sq	Adj R-sq	F-Values	P-Values
Plasticizer				
Q_{FA}, mg·cm^{-2}	98,92%	96,81%	216,89	0,000
Kolliphor p237				
Q_{FA}, mg·cm^{-2}	99,44%	98,42%	886,87	0,000
Kolliphor p338				
Q_{FA}, mg·cm^{-2}	98,78%	97,33%	197,25	0,001

The FA permeability and release study was carried out using a Franz diffusion cell with a volume of 4.35 mL under conditions close to physiological, at a temperature of 37 ° C. Phosphate buffer with pH 7.4 was used as the release medium. A cellulose acetate membrane (d–0.45 μm) with an area of 1.3 cm^2 (OE 67, 0.45 μm, 25 mm, Cytiva Whatman, UK) was used. Fluorescence spectroscopy was used to assess the FA release and penetration from the transdermal patches.

All the experiments were carried out three times (n=3), and the data are expressed as mean ± S.D.

RESULTS AND DISCUSSION

To design the FA transdermal patches with the desired FA polymorph, at the first stage, researches for the modification of FA obtaining methods is needed. It let us to isolate only one polymorph with the necessary properties. At the second stage, the justification and optimization of the transdermal patches formulation was carried out using mathematical modeling methods. The final determination of the desired transdermal patch formulation was made based on FA release studies using a Franz diffusion cell and evaluation of FA release kinetic modeling.

Modification of the FA obtaining method from the peat in the form of the water-soluble polymorph

The general scheme for FA obtaining is: 1) primary processing of peat until homogeneity and the absence of microelements are achieved, in accordance with the state standards (GOST R 54332-2011); 2) subsequent extraction of water-soluble sodium salts of HA and FA after alkaline hydrolysis and removal of other organic residues; 3) separation of HA and FA salts during treatment with sulfuric acid; 4) sorption purification of acidic solutions of FA; 5) drying. We shown that this scheme produced several polymorphs [32].

Suggested procedures: the long-term treatment with concentrated sulfuric acid for 1 hour at the temperature of 60-80°C, repeated purification on DAX-8 resin [34] and subsequent protonation, final freeze-drying from –80 to –40°C for 8 hours, led to the stable samples of FA polymorph with high solubility and storage stability. The simplified scheme for FA optimal isolation from peat is shown in figure 1.

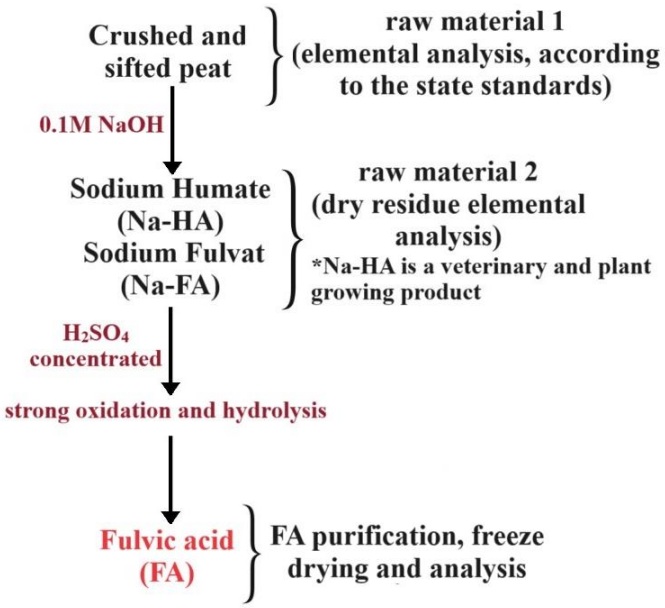

Figure 1: Optimal scheme for FA obtaining from the peat

The nitrogen assay by the Kjeldahl method in the analyzed FA sample did not exceed 0.17% in all cases. It suggests that nitrogen-containing compounds are present only as impurities (Table 2).

Table 2: Elemental composition of the peat and products obtained from it

N	Samples	Elemental composition		Product Procedures
		Metals (content in ppm)	Nitrogen, sulfur, phosphorus (content in %)	
1	Peat (crushed and sifted)	Ca – 10, Fe – 15, Si – 20, Mg – 10. No heavy metals (Pb, As, Hg, Cd)	0,20	The peat samples were taken mechanically. Processing of the samples included sequential grinding and sieving procedures. (State standard – GOST R 54332-2011).
2	Na-HA + Na-FA	Ca – 2, Fe – 10, Si – 10, Mg – 3	[N] – 0,17	Sample 1 treated with 0.1 M NaOH, ultrasound and heating (80°C)
3	FA	Ca – 2, Fe – 10, Si – 10, Mg – 3	[N] – 0,11	Sample 2 treated with H_2SO_4 concentrated. Purification by the Lamar method.

The high carboxyl and phenolic groups concentration in the FA samples were found. The IR spectra contained characteristic of C=O stretching vibrations of associated carboxyl groups, phenolic hydroxyls and carbonyl groups, as well as the solid-state ^{13}C NMR spectra contained signals the mentioned groups (Table 3). In this case, the [COOH]:[Ph-OH] ratio was 4:2, and the C:O ratio according to EDX was not less than 60:40.

Table 3: Fulvic acid functional groups analysis

Functional group	Analysis methods			
	Direct potentiometric titration, mmol-eq/g	Reverse titration, mmol-eq/g	FTIR, cm^{-1}	Solid state ^{13}C NMR, ppm
[COOH]	7.1±0.1	7.5±0.3	1608	165-190
[Ph-OH]	4.8±0.1	5.1±0.2	1224	120-140
C=O	-	-	1716	197-198
C-OH in alcohols	-	-	1070	60-80
C:O ratio by EDX	At least 60:40			

According with the obtained data, we assumed that the FA sample from the studied peat have the following structures (Figure 2), which is consistent with the literature data [35].

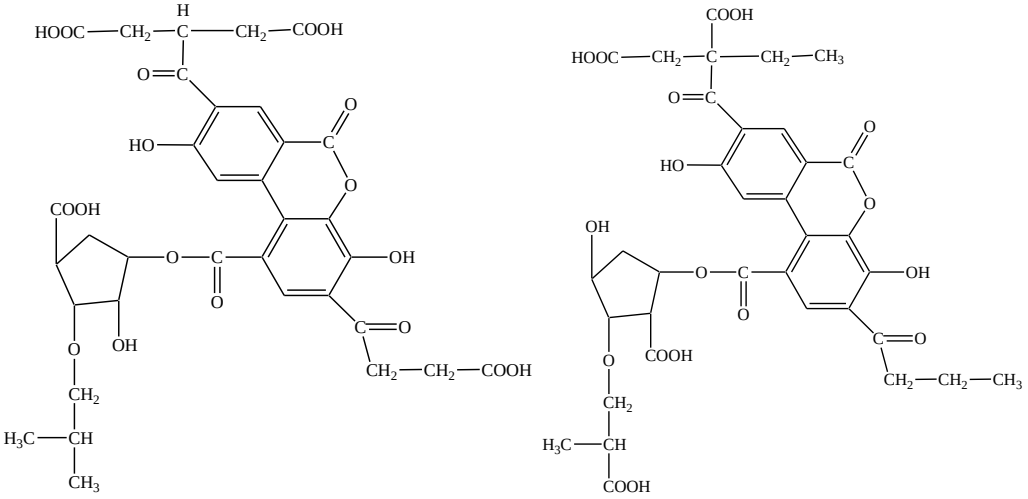

Figure 2: Some possible FA structures [35]. $C_{34}H_{30}O_{18}$; C:O=56.2%:39.6%; M.m.=726 g/mol (M.m. by cryoscopy 740 g/mol) [32]

The resulting freshly prepared samples had good solubility (3.3 mL of water per gram of substance according to the European Pharmacopoeia 11.0) and a high zeta potential (–27.9 ± 0.21 mV). During dry storage in a desiccator, the samples practically did not change their properties, whereas when stored in an aqueous environment, the samples changed their properties. The FA assay during storage of samples in a desiccator, performed by fluorescence spectroscopy in accordance with the literature data [36-38], confirmed their stability (Figure 3).

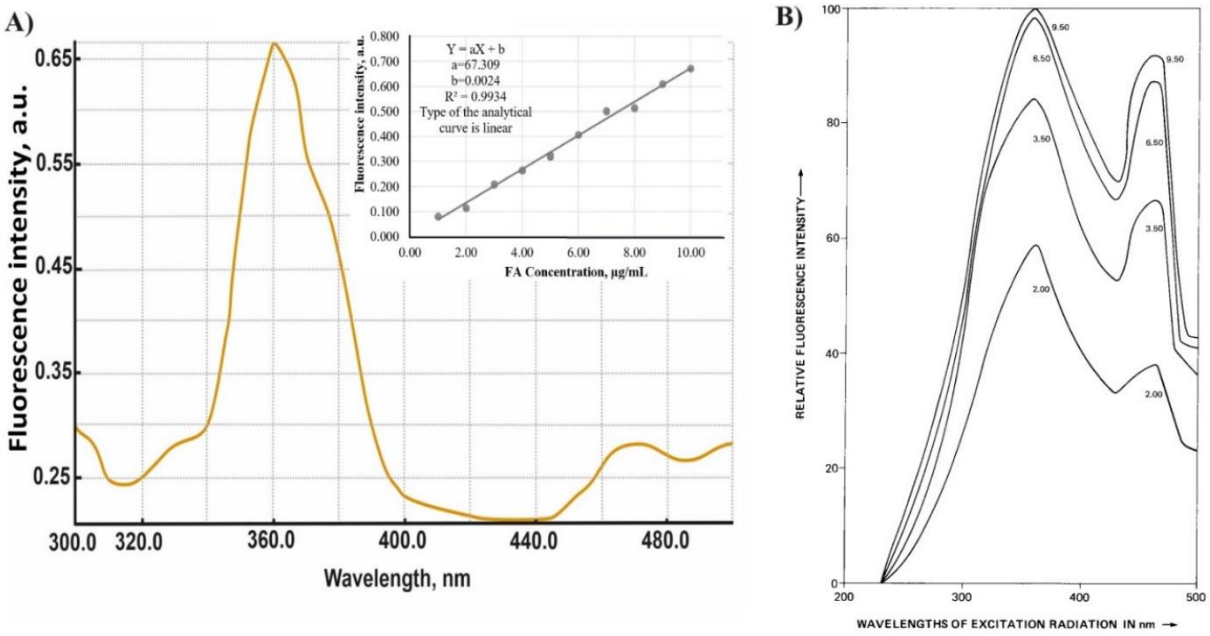

Figure 3: A – excitation spectrum of FA aqueous solution (10 mg·L^{-1}) [31]; B- FA excitation spectra, according to the literature data [37]

Thus, the optimized method for the FA obtaining that we proposed allows us to significantly reduce the amount of trace elements that interfere with the FA release from dosage forms, as well as obtain the stable water-soluble FA sample.

Optimization of FA transdermal patches formulations

FA is a substance with high polarity and hydrophilicity. This is due to its molecular structure, which is dominated by polar carboxyl and phenolic functional groups capable to form hydrogen bonds. These FA properties make it a suitable component for use in emulsion-based formulations. However, for effective FA transdermal delivery, the introduction of transcutants is necessary. Previously, we showed the high FA efficiency in the transdermal patches *in vivo* [31].

In this work, we studied the effect of the transcutants Kolliphopr p237 and Kolliphor p338 on the FA release from the emulsion transdermal patches. It is very useful to examine the influence of these factors on release using 3D surface plots for all variables. Figure 4 shows surface and contour plots describing the effect of transcutants and plasticizer on FA release. Contour plots complement the surface plots, allowing you to highlight lines of equal levels of FA release and identify areas where the greatest or least release was observed, depending on the combination of transcutant and plasticizer.

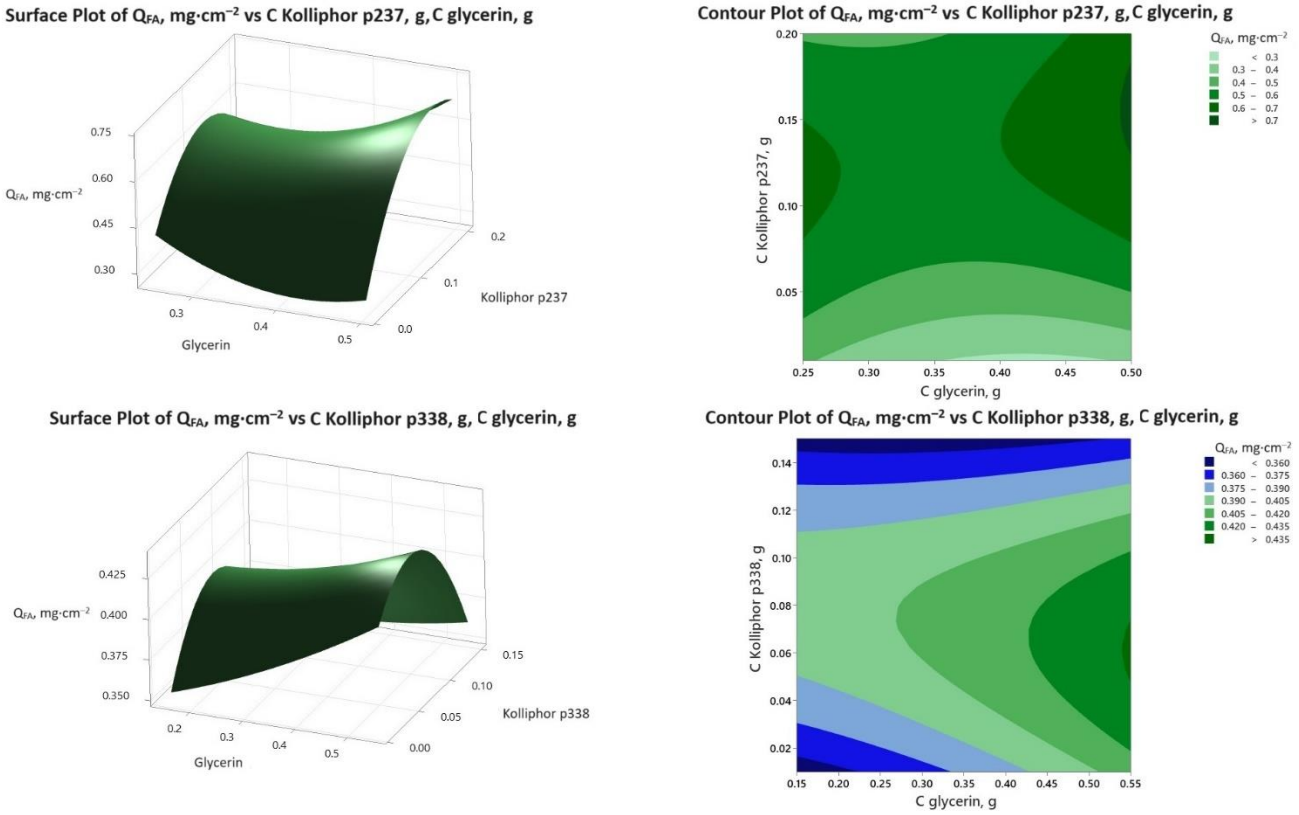

Figure 4: Surface plots and Countor plots, describing the impact of Kolliphor p237 and Kolliphor p338, on drug release at 500 min

This approach to data analysis allows us to study the effect of the transcutants on the FA release at various concentrations. It helped in the optimization transdermal patch formulations.

Thus, the optimal concentrations of the pluronics and plasticizer in the emulsion system were selected (xanthan, glycerin, PVP K17, PEO 400, PEO 1500, Tween 80, emulsion wax, water): 1 – Kolliphor p237 0.1 g (1.16%), glycerin 0.45 g (5.11%); 2 – Kolliphor p338 0.085 g (1.05%), glycerin 0.45 g (5.11%).

Evaluation of FA release from the transdermal patches

From the obtained graphs, as well as the permeability test, it is shown that the best transcutant for the FA in the emulsion transdermal patch is pluronic Kolliphor p237 (Figure 5).

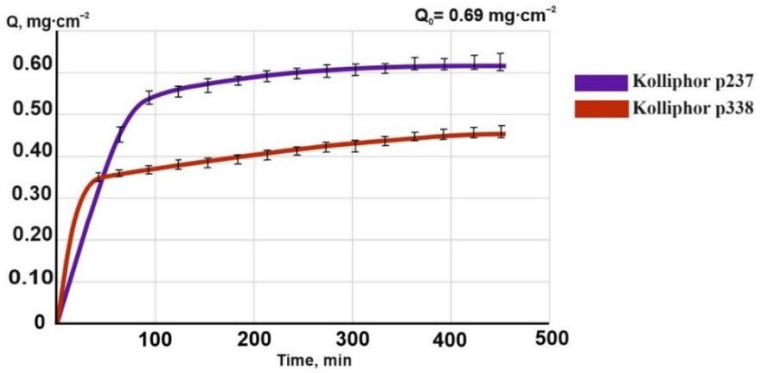

Figure 5. Dependence of FA release on time through cellulose acetate membrane. FA concentration was established by fluorescence excitation spectra.

The initial surface concentration of the FA in the patches on the cellulose acetate membrane with an area of 1.3 cm² was 0.69 mg/cm². The amount of FA released from the initial patch content of 16 mg was:

• (0.619±0.002) mg/cm² (Kolliphop p237),

• (0.455±0.002) mg/cm² (Kolliphop p338).

The assessment of the FA release kinetics from transdermal patches was carried out using mathematical modeling, the models of which are presented in Table 4.

Table 4: Kinetic models of FA release from the patches used

Release models	The equation	$y = f(x)$
Korsmeyer-Peppas, [39-41]	$\frac{Q_\tau}{Q_e} = k_{KP}\tau^n$ $ln\frac{Q_\tau}{Q_e} = nln\tau + lnk_{KP}$ where $ln\frac{Q_\tau}{Q_e}$ – the drug proportion released by τ; n – release rate	$x = ln\tau$ $y = ln\frac{Q_\tau}{Q_e}$
Higuchi, [40-43]	$Q_\tau = k_H\tau^{1/2} = [D\varepsilon/t(2A - \varepsilon C_s)C_s]\tau^{1/2}$, where D – diffusion coefficient; C_s – solubility of the drug in the acceptor liquid; ε – porosity; A – the drug concentration; t – tortuosity	$x = \tau^{1/2}$ $y = Q_\tau$
Pseudo-second order reaction, [44, 45]	$\frac{d[Q]}{d\tau} = k_2[Q]^2$ $\frac{\tau}{Q_\tau} = \frac{1}{k_{2'}Q_e^2} + \frac{\tau}{Q_e}$ where Q_e – maximum amount of the drug in the acceptor chamber	$x = \tau$ $y = \tau/Q_\tau$

Figure 6 shows graphical interpretations of the release process using the Higuchi model, the Korsmeyer-Pepppas model, and the pseudo-second order release model.

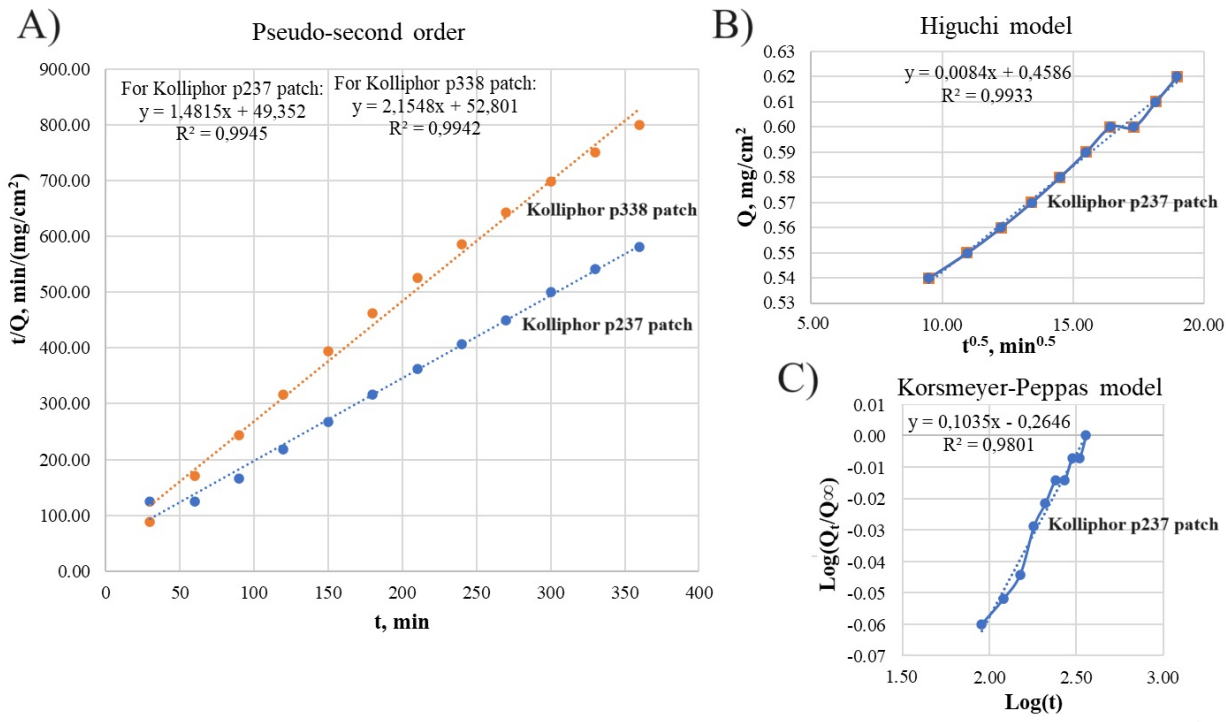

Figure 6. Kinetic model of FA release from the transdermal patches according to the dissolution profile

From the data presented in Figure 6, it is clear that the best model (correlation coefficient R^2) described the process of the FA diffusion according to the mechanism of desorption from the polymer matrix is a pseudo-second order reaction. The effective release constants calculated from the equation are respectively equal to:

$$k_{2'} = \frac{1}{b \cdot Q_e^2} = \frac{1}{49{,}352 \cdot 0{,}675^2}$$
$$= 0{,}044\frac{cm^2}{mg \cdot min} \ (for\ the\ Kolliphor\ p237\ patch),$$

$$k_{2'} = \frac{1}{b \cdot Q_e^2} = \frac{1}{52{,}801 \cdot 0{,}464^2}$$
$$= 0{,}088\frac{cm^2}{mg \cdot min} \ (for\ the\ Kolliphor\ p338\ patch),$$

where b is a parameter reflected the diffusion process (the area cut off on the Y axis)

The Higuchi and Korsmeyer-Peppas models can only be applied when more than 50% FA was released (Figure 6).

Thus, the optimization of transdermal patches formulations and kinetic modeling data, showed that the transcutant Kolliphor p237 provided controlled FA release. The FA release

from the patch probably occurs via a diffusion-limited desorption mechanism from the polymer matrix, which follows from the correspondence of the release process to a pseudo-second-order equation.

CONCLUSIONS

In general, we obtained and characterized fulvic acids from the peat in the Nizhny Novgorod region of Russia in the form of the polymorph with good solubility, high aggregative stability, and practically non-forming of supramolecular assemblies during storage. The work modified the technique previously used by us to obtain various polymorphs of fulvic acids [32]. The main stages of the process included hydrolysis with the alkali (0.1 M NaOH) and strong oxidative destruction with sulfuric acid, followed by stepwise sorption purification and freeze-drying. The modified method makes it possible to reduce significantly the amount of trace elements that interfere with fulvic acid released from dosage forms.

In the work, we optimized the formulations of the fulvic acid emulsion-based transdermal patches using Response Surface Methodology to identify the optimal transcutant for fulvic acid. It has been shown that the optimal transcutant for fulvic acid in the emulsion system (xanthan, glycerin, PVP K17, PEO 400, PEO 1500, Tween 80, emulsion wax, water) is the pluronic Kolliphor p237 at a concentration of 1.10-1.16%.

The study of the fulvic acid kinetic release features from the patches showed that the process corresponds to the Higuchi and Korsmeyer-Peppas model only when the fulvic acid release is more than 50%. The most adequate mathematical description the fulvic acid release process from the patches corresponds to a pseudo-second-order reaction according to the diffusion-limited desorption mechanism from the polymer matrix. The effective release constants of fulvic acid by the pseudo-second-order reaction differed by almost twofold ($k_{2'} = 0.044 \frac{cm^2}{mg \cdot min}$ for the Kollipfor p237 patch и $k_{2'} = 0.088 \frac{cm^2}{mg \cdot min}$ for the Kolliphor p338 patch).

Thus, our results allow us to use the method for fulvic acid obtaining with better physicochemical properties. This fulvic acid polymorph suitable for use in emulsion-based transdermal patches with pluronic Kolliphor p237 as a transcutant. To use transdermal patches with fulvic acid in widespread medical practice, it is necessary to optimize the formulations of the patches and mathematical modeling of the fulvic acid release kinetics. These patches can be effective in the treatment of various inflammatory diseases, as we have previously shown [31].

REFERENSES

1. Rodríguez NC, Urrutia EC, Gertrudis BH, Chaverri JP, Mejía GB, “Antioxidant activity of fulvic acid: A living matter-derived bioactive compound” Journal of Food, Agriculture & Environment, 2011; 9(3):123-127.
2. Zykova MV, Schepetkin IA, Belousov MV, Krivoshchekov SV, Logvinova LA, Bratishko KA, ... & Quinn MT, “Physicochemical characterization and antioxidant activity of humic acids isolated from peat of various origins” Molecules, 2018; 23(4):753. DOI: https://doi.org/10.3390/molecules23040753
3. Khuda F, Anjum M, Khan S, Khan H, Sahibzada MUK, Khusro A, ... & Emran TB, “Antimicrobial, anti-inflammatory and antioxidant activities of natural organic matter extracted from cretaceous shales in district Nowshera-Pakistan” Arabian Journal of Chemistry, 2022; 15(2):103633. DOI: https://doi.org/10.1016/j.arabjc.2021.103633
4. Shikalgar TS, Naikwade NS, “Evaluation of cardioprotective activity of fulvic acid against isoproterenol induced oxidative damage in rat myocardium” International Research Journal of Pharmacy, 2018; 9(1):71-80. DOI: 10.7897/2230-8407.09111
5. Aeschbacher M, Graf C, Schwarzenbach RP, Sander M, “Antioxidant properties of humic substances” Environmental science & technology, 2012; 46(9):4916-4925. DOI: https://doi.org/10.1021/es300039h
6. Csicsor A, Tombácz E, “Screening of Humic Substances Extracted from Leonardite for Free Radical Scavenging Activity Using DPPH Method” Molecules, 2022; 27(19):6334. DOI: https://doi.org/10.3390/molecules27196334
7. van Rensburg, CE, “The antiinflammatory properties of humic substances: a mini review” Phytotherapy Research, 2015; 29(6):791-795. DOI: https://doi.org/10.1002/ptr.5319
8. Sabi R, Vrey P, van Rensburg CEJ, “Carbohydrate-derived Fulvic acid (CHD-FA) inhibits Carrageenan-induced inflammation and enhances wound healing: efficacy and Toxicity study in rats” Drug Development Research, 2012; 73(1):18-23. DOI: https://doi.org/10.1002/ddr.20445
9. Schepetkin IA, Xie G, Jutila MA, Quinn MT, “Complement-fixing activity of fulvic acid from Shilajit and other natural sources” Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 2009; 23(3):373-384. DOI: https://doi.org/10.1002/ptr.2635
10. Schepetkin I, Khlebnikov A, Kwon BS, (2002). “Medical drugs from humus matter: Focus on mumie”, Drug development research, 2002; 57(3):140-159. DOI: https://doi.org/10.1002/ddr.10058
11. Güngen G, Ardic F, Fındıkoğlu G, Rota S, “The effect of mud pack therapy on serum YKL-40 and hsCRP levels in patients with knee osteoarthritis”, Rheumatology international, 2012; 32:1235-1244. DOI: https://doi.org/10.1007/s00296-010-1727-4
12. Verrillo M, Parisi M, Savy D, Caiazzo G, Di Caprio R, Luciano MA, ... & Piccolo A, “Antiflammatory activity and potential dermatological applications of characterized humic acids from a lignite and a green compost”, Scientific Reports, 2022; 12(1):2152. DOI: https://doi.org/10.1038/s41598-022-06251-2
13. Winkler J, Ghosh S, “Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes”, Journal of diabetes research, 2018. DOI: https://doi.org/10.1155/2018/5391014
14. Junek R, Morrow R, Schoenherr JI, Schubert R, Kallmeyer R, Phull S, Klöcking R, “Bimodal effect of humic acids on the LPS-induced TNF-α release from differentiated U937 cells”, Phytomedicine, 2009; 16(5):470-476. DOI: https://doi.org/10.1016/j.phymed.2008.10.003
15. Chien SJ, Chen TC, Kuo HC, Chen CN, Chang SF, “Fulvic acid attenuates homocysteine-induced cyclooxygenase-2 expression in human monocytes”, BMC Complementary and Alternative Medicine, 2015; 15(1):1-8. DOI: https://doi.org/10.1186/s12906-015-0583-x
16. Motojima H, Yamada P, Han J, Ozaki M, Shigemori H, Isoda H, “Properties of fulvic acid extracted from excess sludge and its inhibiting effect on β-hexosaminidase release”, Bioscience, biotechnology, and biochemistry, 2009; 73(10):2210-2216. DOI: https://doi.org/10.1271/bbb.90316
17. Snyman JR, Dekker J, Malfeld SCK, Van Rensburg CEJ, “Pilot study to evaluate the safety and therapeutic efficacy of topical oxifulvic acid in atopic volunteers”, Drug Development Research, 2002; 57(1):40-43. DOI: https://doi.org/10.1002/ddr.10116
18. Pant K, Gupta A, Gupta P, Ashraf A, Yadav A, Venugopal S, “Anti-proliferative and anticancer properties of fulvic acid on hepatic cancer cells”, Journal of Clinical and Experimental Hepatology, 2015; 5(2). DOI: https://doi.org/10.1016/j.jceh.2015.07.005
19. Pant K, Singh B, Thakur N, “Shilajit: A humic matter panacea for cancer”, International Journal of Toxicological and Pharmacological Research, 2012; 4(2):17-25.
20. Huang WS, Yang JT, Lu CC, Chang SF, Chen CN, Su YP, Lee KC, “Fulvic acid attenuates resistin-induced adhesion of HCT-116 colorectal cancer cells to endothelial cells”, International Journal of Molecular Sciences, 2015; 16(12):29370-29382. DOI: https://doi.org/10.3390/ijms161226174

21. Jayasooriya RGPT, Dilshara MG, Kang CH, Lee S, Choi YH, Jeong YK, Kim GY, "Fulvic acid promotes extracellular anti-cancer mediators from RAW 264.7 cells, causing to cancer cell death in vitro", International Immunopharmacology, 2016; 36:241-248. DOI: https://doi.org/10.1016/j.intimp.2016.04.029

22. Zhernov YV, Konstantinov AI, Zherebker A, Nikolaev E, Orlov A, Savinykh MI, ... & Perminova IV, (2021). "Antiviral activity of natural humic substances and shilajit materials against HIV-1: Relation to structure", Environmental Research, 2021; 193:110312. DOI: https://doi.org/10.1016/j.envres.2020.110312

23. Hafez M, Popov AI, Zelenkov VN, Teplyakova TV, Rashad M, "Humic substances as an environmental-friendly organic wastes potentially help as natural anti-virus to inhibit COVID-19", Sci. Arch, 2020; 1(2):53-60. DOI: http://dx.doi.org/10.47587/SA.2020.1202

24. Socol DC, "Clinical review of humic acid as an antiviral: Leadup to translational applications in clinical humeomics", Frontiers in Pharmacology, 2023; 13:1018904. DOI: https://doi.org/10.3389/fphar.2022.1018904

25. Zhou P, Yan H, Gu B, "Competitive complexation of metal ions with humic substances", Chemosphere, 2005; 58(10):1327-1337. DOI: https://doi.org/10.1016/j.chemosphere.2004.10.017

26. Güngör EBÖ, Bekbölet M, "Zinc release by humic and fulvic acid as influenced by pH, complexation and DOC sorption", Geoderma, 2010; 159(1-2):131-138. DOI: https://doi.org/10.1016/j.geoderma.2010.07.004

27. Boguta P, Sokołowska Z, "Zinc Binding to Fulvic acids: Assessing the Impact of pH, Metal Concentrations and Chemical Properties of Fulvic Acids on the Mechanism and Stability of Formed Soluble Complexes", Molecules, 2020; 25(6):1297. DOI: https://doi.org/10.3390/molecules25061297

28. Zherebtsov SI, Malyshenko NV, Bryukhovetskaya LV, Lyrshchikov SY, Ismagilov ZR, (2015). "Sorption of copper cations from aqueous solutions by brown coals and humic acids", Solid Fuel Chemistry, 2015; 49:294-303. DOI: https://doi.org/10.3103/S0361521915050110

29. Olaniran AO, Balgobind A, Pillay B, "Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies", International journal of molecular sciences, 2013; 14(5):10197-10228. DOI: https://doi.org/10.3390/ijms140510197

30. Adusei-Gyamfi J, Ouddane B, Rietveld L, Cornard JP, Criquet J, (2019). "Natural organic matter-cations complexation and its impact on water treatment: A critical review", Water research, 2019; 160:130-147. DOI: https://doi.org/10.1016/j.watres.2019.05.064

31. Konnova MA, Volkov AA, Solovyeva AG, Peretyagin PV, Melnikova NB, "Anti-Inflammatory Property Establishment of Fulvic Acid Transdermal Patch in Animal Model", Scientia Pharmaceutica, 2023; 91(4):45. DOI: https://doi.org/10.3390/scipharm91040045

32. Konnova MA, Volkov AA, Kostryukov SG, Melnikova NB, "Features of Obtaining and Properties of Fulvic Acid from the Peat of Nizhny Novgorod Region", Saudi J Med Pharm Sci, 2023; 9(9):617-628. DOI: https://doi.org/10.36348/sjmps.2023.v09i09.004

33. Melnikova N, Solovjeva O, Vorobyova O, Solovyeva A., Peretyagin P, Didenko N, Korobko V, (2017). "The Humic Acids of Peat. Physico-Chemical Properties and Biological Activity in Erythrocytes", Int. J. Pharm Sci. Rev. Res, 2017; 45(2):278-856.

34. Lamar RT, Olk DC, Mayhew L, Bloom PR, "A new standardized method for quantification of humic and fulvic acids in humic ores and commercial products", Journal of AOAC International, 2014; 97(3):721-730. DOI: https://doi.org/10.5740/jaoacint.13-393

35. Samios S, Lekkas T, Nikolaou A, Golfinopoulos S, "Structural investigations of aquatic humic substances from different watersheds", Desalination, 2007; 210(1-3):125-137. DOI: https://doi.org/10.1016/j.desal.2006.05.038

36. Miano TM, Martin JP, Sposito G, (1988). "Flourescence spectroscopy of humic substances", Soil Science Society of America Journal, 1988; 52(4):1016-1019. DOI: https://doi.org/10.2136/sssaj1988.03615995005200040021x

37. Ghosh K, Schnitzer M, "Fluorescence excitation spectra of humic substances", Canadian Journal of Soil Science, 1980; 60(2):373-379. DOI: https://doi.org/10.4141/cjss80-040

38. Bertoncini EI, D'Orazio V, Senesi N, Mattiazzo ME, "Fluorescence analysis of humic and fulvic acids from two Brazilian oxisols as affected by biosolid amendment", Analytical and Bioanalytical Chemistry, 2005; 381:1281-1288. DOI: https://doi.org/10.1007/s00216-005-3054-2

39. Singhvi G, Singh M, "In-vitro drug release characterization models", Int J Pharm Stud Res, 2011; 2(1):77-84.

40. Nazir S, Khan MUA, Al-Arjan WS, Abd Razak SI, Javed A, Kadir MRA, "Nanocomposite hydrogels for melanoma skin cancer care and treatment: In-vitro drug delivery, drug release kinetics and anti-cancer activities", Arabian Journal of Chemistry, 2021; 14(5):103120. DOI: https://doi.org/10.1016/j.arabjc.2021.103120

41. Dash S, Murthy PN, Nath L, Chowdhury P, "Kinetic modeling on drug release from controlled drug delivery systems", Acta Pol Pharm, 2010; 67(3):217-223.

42. Higuchi TJJOPS, "Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices", Journal of pharmaceutical sciences, 1963; 52(12):1145-1149. DOI: https://doi.org/10.1002/jps.2600521210

43. Tsai W, Tsai H, Wong Y, Hong J, Chang S, Lee M, "Preparation and characterization of gellan gum/glucosamine/clioquinol film as oral cancer treatment patch", Materials Science and Engineering, 2018; 82:317-322. DOI: https://doi.org/10.1016/j.msec.2017.05.040

44. Khamizov RK, "A Pseudo-Second Order Kinetic Equation for Sorption Processes", Russian Journal of Physical Chemistry A, 2020; 94(1):125-130. DOI: https://doi.org/10.1134/S0036024420010148

45. Plazinski W, Dziuba J, Rudzinski W, "Modeling of sorption kinetics: the pseudo-second order equation and the sorbate intraparticle diffusivity", Adsorption, 2013; 19:1055-1064. DOI: https://doi.org/10.1007/s10450-013-9529-0