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DEVELOPMENT OF TECHNOLOGY AND DETERMINATION OF THE CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN THE MEDICAL STICK WITH EXTRACTS OF MEDICINAL VEGETABLE RAW MATERIALS

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Abstract

One of the main directions of development of the modern pharmaceutical industry is the creation of medicinal preparations out of vegetable raw material. Medical sticks are a dosage form in demand in dermatology due to the ease of use, compactness, hygiene and economy, efficiency and ensuring the optimal duration of pharmacological effect on the skin. This dosage form is widely used both as an anesthetic, deriving, haemostatic (for cuts and skin injuries), antiseptic, wound healing, antiviral agent. The objective of the work is to study the rheological properties of a medical stick based on medicinal vegetable extracts to determine the temperature parameters of the technological process and to develop the methods for analyzing biologically active compounds in its composition.

The object of the study was the experimental samples of a medical stick on a hydrophobic basis with 60 % oil extract of a mixture of medicinal vegetable raw materials and mango extract.

We have suggested the composition of a medical stick, which contains cocoa butter, beeswax, paraffin, anhydrous lanolin, carnauba and candelilla waxes as the thickeners. The components of the base were selected in such a way so that to ensure consistent, technological and consumer properties of the stick, namely hardness, spreadability, stickiness, adhesion, gloss of the film, etc. An oil extract of the mixture of medicinal vegetable raw materials containing sage grass, eucalyptus leaves, marigold flowers, chamomile flowers in a ratio (2:1:1:1) was used as a component of the base and at the same time as a biologically active substance.

Experimental samples of the sticks were obtained by pouring. The production of the finished product consisted of the following technological operations: weighing, melting of hydrophobic components of the base taking into account their melting temperature, the introduction of oil extract and mangiferin, homogenization, cooling, pouring into molds.

Rheological studies were performed in order to determine the temperature regime of the technological process of the medicine production. In order to standardize the medicinal preparation, we determined the content of pigment compounds (the general number of carotenoids calculated as violoxanthin and the general number of chlorophyll calculated as chlorophyll *a*), as well as the content of xanthones calculated as mangiferin. Quantitative determination of the general number of xanthones calculated as mangiferin in the stick sample was performed by spectrophotometric method. The temperature parameters of the medical stick fabrication were determined by means of rheological studies.

Keywords: *medical stick, vegetable extracts, temperature parameters, rheological properties*

Introduction

One of the main directions of development of the modern pharmaceutical industry is the creation of medicinal preparations out of vegetable raw material. This tendency is confirmed by the fact that about a half of all the preparations introduced into medical practice over the last 25 years contain biologically active compounds of natural origin.

Medical sticks are a dosage form in demand in dermatology due to the ease of use, compactness, hygiene and economy, efficiency and ensuring the optimal duration of pharmacological effect on the skin. This dosage form is widely used both as an anesthetic, deriving, haemostatic (for cuts and skin injuries), antiseptic, wound healing, antiviral agent, etc. [2, 7].

The objective of the work is to study the rheological properties of a medical stick based on medicinal vegetable extracts to determine the temperature parameters of the technological process and to develop the methods for analyzing biologically active compounds in its composition.

Methods

The object of the study was the experimental samples of a medical stick on a hydrophobic basis with 60 % oil extract of a mixture of medicinal vegetable raw materials and mango extract.

Experimental samples of the sticks were obtained by pouring. The production of the finished product consisted of the following technological operations: weighing, melting of hydrophobic components of the base taking into account their melting temperature, the introduction of oil extract and mangiferin, homogenization, cooling, pouring into molds.

Rheological studies were performed in order to determine the temperature regime of the technological process of the medicine production. The study was performed using a rheometer Rheolab QC, Anton Paar, Austria using a system of coaxial cylinders C-CC27 / SS. The device meets the requirements of ISO 3219. Measurements of the rheological curve were performed in three stages:

a) linear increase in shear rate from 0,1 s^{-1} to 150 s^{-1} with 45 measuring points and 1 s of point measuring time;

b) constant shear at a shear rate of 150 s^{-1}, one measuring point lasting 1 s;

c) linear decrease of shear rate from 150 s^{-1} to 0,1 s^{-1} with 45 measuring points and 1 s of point measuring time.

Rheological measurements were performed at different temperatures, namely in the order of its reduction from 70 °C at a pitch of 5 °C till complete structuring of the disperse system.

In order to standardize the medicinal preparation, we determined the content of pigment compounds (the general number of carotenoids calculated as violoxanthin and the general number of chlorophyll calculated as chlorophyll *a*), as well as the content of xanthones calculated as mangiferin.

The content of the general number of pigment substances in the medical stick was determined by spectrophotometric method according to the following procedure. A batch of the stick of around 0.500 g is placed in a volumetric flask with a capacity of 10.0 ml, 5 ml of 96% ethanol is added, dissolved by heating in a water bath and stirring. Cooled. Made up to the mark with 96% alcohol and filtered.

The absorbance of the resulting solution is measured on a SPECORD 200 spectrophotometer in a 10 mm cuvette at a wavelength of 443 nm (general number of carotenoids calculated as violoxanthin) and 666 nm (chlorophylls calculated as chlorophyll *a*). Compensation solution – 96 % ethanol.

The content of the general number of carotenoids in milligrams per 100.0 g of product is calculated using a formula:

$$X = \frac{A \cdot 10 \cdot 1000}{m \cdot A_{1cm}^{1\%}}, \text{ where}$$

A – is an absorbance of MHRM (medicinal vegetable raw materials) oil extract in 96 % ethanol;

m – is a batch of the oil extract, g;

$A_{1cm}^{1\%}$ – is a specific absorption of violoxanthin at 443 ± 3nm, which is equal to 2500.

The content of the general number of chlorophylls in milligrams per 100.0 g of product, is calculated using a formula:

$$X = \frac{A \cdot 10 \cdot 1000}{m \cdot A_{1cm}^{1\%}}, \text{ where}$$

A – is an absorbance of MHRM oil extract in 96 % ethanol;

m – is a batch of the oil extract;

$A_{1cm}^{1\%}$ – is a specific absorption of chlorophyll *a* at 665 ± 3nm, which is equal to 944,5.

Quantitative determination of the general

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number of xanthones calculated as mangiferin in the stick sample was performed by spectrophotometric method according to the following procedure. A batch of the sample of around 1,000 g is placed in a 100 ml conical flask, 20 ml of 70% ethyl alcohol is added, and all is refluxed for 30 min, cooled, and then the supernatant fluid is filtered into a volumetric flask with a capacity of 50.0 ml. 15 ml of 70% ethyl alcohol is added to a conical flask, again refluxed for 30 min, cooled, and then the supernatant fluid is filtered into the same volumetric flask. The extraction is repeated again with 10 ml of 70% ethyl alcohol. The content of the volumetric flask is made up to the mark with 70% ethyl alcohol.

0.5 ml of the obtained solution is placed in a volumetric flask with a capacity of 25.0 ml, 10 ml of 70% ethyl alcohol is added, 0.5 ml of diluted acetic acid is added, and then all is made up to the mark with 70 % alcohol.

Compensation solution. Ethyl alcohol 70%.

The absorbance of the test solution is measured with a spectrophotometer at a wavelength of 367 nm in a cuvette with a layer thickness of 10 mm against the compensation solution.

The content of xanthones calculated as mangiferin (X,%) is calculated using a formula:

$$X = \frac{A \cdot 50 \cdot 25}{A_{1см}^{1\%} \cdot 0{,}5 \cdot m}, \text{ where}$$

A – is an absorbance of the test solution;

$A_{1см}^{1\%}$ – is a relative rate of mangiferin absorption, which is equal to 325,1;

m – is the weight of the sample batch, g.

Results

We have suggested the composition of a medical stick, which contains cocoa butter, beeswax, paraffin, anhydrous lanolin, carnauba and candelilla waxes as the thickeners. The components of the base were selected in such a way so that to ensure consistent, technological and consumer properties of the stick, namely hardness, spreadability, stickiness, adhesion, gloss of the film, etc. [1, 6, 8, 9].

An oil extract of the mixture of medicinal vegetable raw materials containing sage grass, eucalyptus leaves, marigold flowers, chamomile flowers in a ratio (2:1:1:1) was used as a component of the base and at the same time as a biologically active substance.

In addition to the oil extract, which should provide anti-inflammatory, reparative and antioxidant effect, we introduced 5% mango extract to the stick composition (manufacturer: Xi'an Xin Sheng Bio-Chem Co., Ltd, China). Mango extract, containing more than 95% xanthones calculated as mangiferin, is used in a number of medicinal preparations that show antiviral activity against DNA viruses and moderate anti-inflammatory effect. Mangiferin (xanthone glycoside) has a moderate bacteriostatic effect on gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*), fungi (*Microsporum canis*) and pathogenic protozoa (*Entamoeba histolytica, Thrichomonas vaginalis*) [10-12, 14].

According to a number of authors, the mechanism of action of mangiferin is based on the inhibition of bacterial nuclease, without pronounced inhibition of viral neuraminidase. This causes the violation of virus penetration into the cell and its reproduction. On the other hand, mangiferin has an immunostimulatory effect on cellular and humoral immunity and the ability to induce the production of γ-interferon in blood cells. Mangiferin is also a low-toxic compound showing no mutagenic or local irritant properties [3, 15].

There are medicines with mangiferin known - Alpizarin (Pharmcenter "Vilar", Russia, 2% and 5% ointment), Mangogerpin (Bivi Pharma, Vietnam - 5% and 10% cream, capsules of 0.1 g) – which are classified as antiviral agents.

To substantiate the temperature regime of the medical stick manufacturing technology and storage conditions (use), it is necessary to investigate the effect of temperature on the rheological parameters (plasticity, elasticity, structural viscosity, thixotropy, type of flow) of the medicinal preparation. The process of stick preparation consists in step-by-step loading of the base components into a reactor and their fusion at the temperature corresponding to the melting temperature of the components. The introduction of active substances occurs at a temperature of 60 °C, the mass gets fully homogenized and gradually cools down while stirred. A critical parameter of the manufacturing technology is the temperature of pouring the mass into the mold, at which the mass is fluid so that to ensure accurate dosing and to prevent setting of the mass in the equipment transport systems. To determine the dependence of the fluidity of the mass on the temperature, rheological studies were performed in the order of decreasing the temperature from 70°C, which corresponds to the technological process of making sticks. The results of the research are shown in fig. 1-3 and table 1, 2.

As a result of rheological studies of the mass it was found that the composition is in a liquid state and has a Newtonian type of flow at a temperature of 70 °C, 65 °C, 60 °C and 55 °C. When the temperature drops below 55 °C, the system gets gradually structured and acquires thixotropic properties. Within the temperature range from 50 °C to 45 °C the mass is characterized by a plastic type of flow with weak thixotropic properties, as evidenced by the areas of hysteresis, and has a low lower limit of the flow.

The results show that the process of fusion of the base components can be carried out at a temperature of 70-60 °C, the process of introducing the active components into the base of the sticks as well as the homogenization can be carried out at a temperature of 60-55 °C, because at this temperature the system has a low viscosity, which will ensure uniform distribution of substances and energy savings. The process of pumping the finished composition of the sticks from the reactor to a filling machine must be carried out within the temperature range of 60-55 °C. The mass begins to structure at temperatures below 55 °C, and at 45 °C the mass does not show self-flowing.

An important stage of pharmaceutical development is the standardization of the medicinal preparation. We have developed the methods for quantitative determination of the group of biologically active compounds contained in the extracts and responsible for the pharmacological effect of the developed dosage form.

To standardize a medical stick by the general number of pigment compounds, an alcohol extraction from samples was prepared, and the absorption spectrum of the absorption in the visible part of the spectrum was investigated. It was found that the alcohol extraction from the medicinal preparation is characterized by the presence of absorption maxima at wavelengths of 443 nm and 666 nm, which are inherent to the absorption maxima of the alcohol solution of the oil extract of medicinal vegetable raw materials (Fig. 4)

The results of quantitative determination of the general number of carotenoids calculated as violoxanthin in the stick as well as metrological characteristics of the results obtained are shown in table 3.

The general number of carotenoids calculated as violoxanthin in the medicinal preparation must be no less than 2.0 mg [5].

The results of quantitative determination of the general number of chlorophylls calculated as chlorophyll *a* in the stick as well as the metrological characteristics of the results obtained are shown in table 4.

The general number of chlorophylls calculated as chlorophyll *a* in the medical stick must be no less than 2.20 mg [13].

The literature [4] describes a spectrophotometric method for determining the quantitative content of xanthones calculated as mangiferin using the calculated specific absorption index of mangiferin, which is 325.1. To use the method for quantifying the general number of xanthones in the stick, an alcohol extract from the stick was prepared according to the method offered and the UV-absorption spectrum of the resulting solution was recorded within the range from 300 to 400 nm. It was found that the UV-spectrum of alcohol extraction from the stick is characterized by the presence of two absorption maxima at wavelengths of 320 nm and 367 nm, which correspond to the absorption maxima of the standard sample of mangiferin by the position (Fig. 5).

The results of quantitative determination of the general number of xanthones calculated as mangiferin in the stick sample by spectrophotometric method are given in table. 5.

To determine the reproducibility of the method, the general number of xanthones calculated as mangiferin in six different batches of the stick was determined.

The general number of xanthones calculated as mangiferin should be no less than 4.90 g in the stick.

Discussion

The technology of the medical sticks with medicinal vegetable extracts was studied.

The temperature parameters of the medical stick fabrication were determined by means of rheological studies.

The methods of quantitative determination of biologically active compounds of the extracts from medicinal plants containing in the developed medical stick were suggested.

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Table 1 Value of shear stress of the stick mass depending on the temperature

Shear rate, s^{-1}	Value of shear stress in the forward and backward flow rheograms, Pa													
	70 °C		65 °C		60 °C		55 °C		50 °C		45 °C		40 °C	
3,51	0,71	0,43	1,26	0,15	1,19	0,46	1,22	1,91	21,9	1,45	16,0	7,09	1410	31,4
10,3	0,92	0,88	1,89	0,96	1,46	0,95	2,45	2,69	22,4	2,93	19,9	10,3	597	42,2
20,5	1,46	1,07	1,65	1,88	2,08	1,75	4,2	3,39	18,3	5,42	22,6	13,4	472	56,7
30,8	1,83	1,41	1,77	1,64	3,14	2,38	4,83	4,22	16,4	6,38	25,4	16,5	357	68,4
41,0	1,96	1,82	2,24	2,74	2,68	2,33	6,63	6,15	15,7	9,08	29,8	21,4	297	80,1
51,2	2,55	2,37	2,5	2,16	4,17	2,68	7,15	6,73	17,5	9,53	31,1	23,7	276	90,7
85,3	3,25	3,24	3,57	3,79	4,58	4,16	11,2	10,6	19,9	15,1	41,9	34,7	259	130
123	4,39	4,17	4,7	4,48	6,71	5,91	14,9	14,3	24,0	21,2	51,5	45,6	240	160
150	4,99	5,04	5,78	5,66	6,64	6,3	17,6	17,4	27,0	26,3	58,5	57,7	213	203

Table 2 Value of structural viscosity of the stick mass depending on the temperature

Shear rate, s^{-1}	Value of structural viscosity in the forward and backward flow rheograms, Pa·s													
	70 °C		65 °C		60 °C		55 °C		50 °C		45 °C		40 °C	
3,51	0,20	0,12	0,36	0,04	0,34	0,13	0,35	0,55	6,19	0,41	4,54	2,02	43200	8,95
10,3	0,09	0,08	0,18	0,09	0,14	0,09	0,24	0,26	2,16	0,28	1,93	0,99	57,2	4,09
20,5	0,07	0,05	0,08	0,09	0,10	0,08	0,21	0,17	0,89	0,26	1,1	0,65	22,9	2,76
30,8	0,06	0,04	0,06	0,07	0,10	0,07	0,16	0,14	0,53	0,22	0,83	0,54	16,6	2,22
41,0	0,05	0,04	0,05	0,06	0,07	0,06	0,16	0,15	0,38	0,22	0,72	0,52	7,24	1,96
51,2	0,05	0,04	0,05	0,04	0,07	0,05	0,14	0,13	0,34	0,18	0,61	0,46	5,39	7,77
85,3	0,04	0,03	0,04	0,04	0,06	0,05	0,13	0,12	0,23	0,17	0,49	0,41	3,04	1,52
123	0,03	0,03	0,04	0,04	0,05	0,05	0,13	0,12	0,19	0,17	0,42	0,37	1,86	1,31
150	0,03	0,03	0,04	0,04	0,05	0,04	0,12	0,16	0,18	0,17	0,39	0,39	1,42	1,35

Table 3 Results of quantitative determination of the general number of carotenoids calculated as violoxanthin in the medical stick, and metrological characteristics

Batch, g	Absorbance	General number of carotenoids, mg	Metrological characteristics
0,5377	0,2779	2,07	$\bar{x}$ = 2,09
0,5145	0,2622	2,04	S^2 = 0,0033
0,5019	0,2547	2,03	S = 0,0575
0,5076	0,2769	2,18	$S_{\bar{x}}$ = 0,0235
0,5048	0,2683	2,13	Δx = 0,1478
0,4964	0,2569	2,07	$\Delta\bar{x}$ = 0,0603 $\bar{\varepsilon}$ = 2,89% ε = 7,08%

Table 4 Results of quantitative determination of the general number of chlorophylls calculated as chlorophyll *a* in the medical stick, and metrological characteristics

Batch, g	Absorbance	General number of chlorophylls, mg	Metrological characteristics
0,5377	0,1201	2,36	$\bar{x}$ = 2,48 S^2 = 0,0064 S = 0,0802 $S_{\bar{x}}$ = 0,0327 Δx = 0,2061 $\Delta\bar{x}$ = 0,0841 $\bar{\varepsilon}$ = 3,38% ε = 8,30%
0,5145	0,1238	2,55	
0,5019	0,1214	2,56	
0,5076	0,1163	2,43	
0,5048	0,1177	2,46	
0,4964	0,1191	2,54	

Table 5 Results of determining the general number of xanthones calculated as mangiferin

Stick batch, g	Absorbance of Solution "Б"	General number of xanthones calculated as mangiferin in the stick sample, g	Metrological characteristics
1,0738	0,6940	4,95	$\bar{x}$ =5,13 S^2 = 0,0234 S = 0,1532 $S_{\bar{x}}$ = 0,0625 Δx = 0,3938 $\Delta\bar{x}$ = 0,1608 $\bar{\varepsilon}$ = 3,14% ε = 7,68%
1,1008	0,7042	4,92	
0,9983	0,6873	5,29	
0,9997	0,6785	5,22	
1,0049	0,6807	5,21	
1,0032	0,6729	5,16	

Figure 1 Dependence of shear stress (τ, Pa) on shear rate (γ, 1 / s) and dependence of structural viscosity (η, mPa · s) on shear rate of the medical sticks at 70 °C – 40 °C temperature

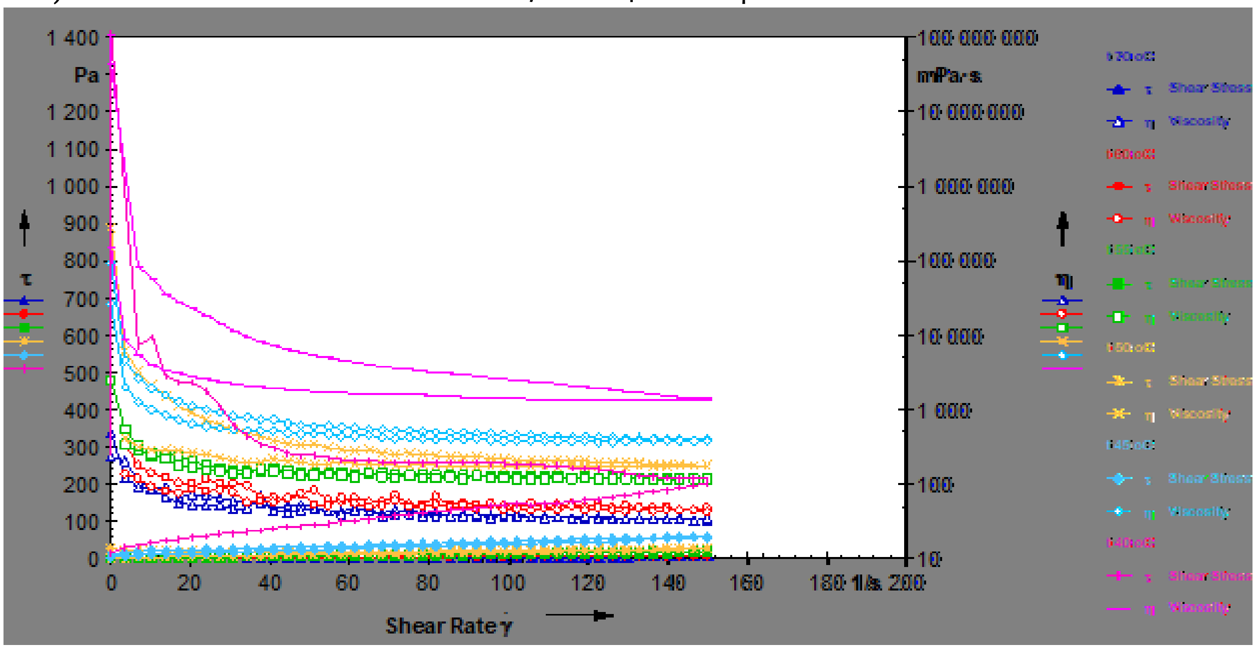

Figure 2 Dependence of shear stress (τ, Pa) on shear rate (γ, 1 / s) and dependence of structural viscosity (η, mPa · s) on shear rate of the medical sticks at 55 °C – 45 °C temperature

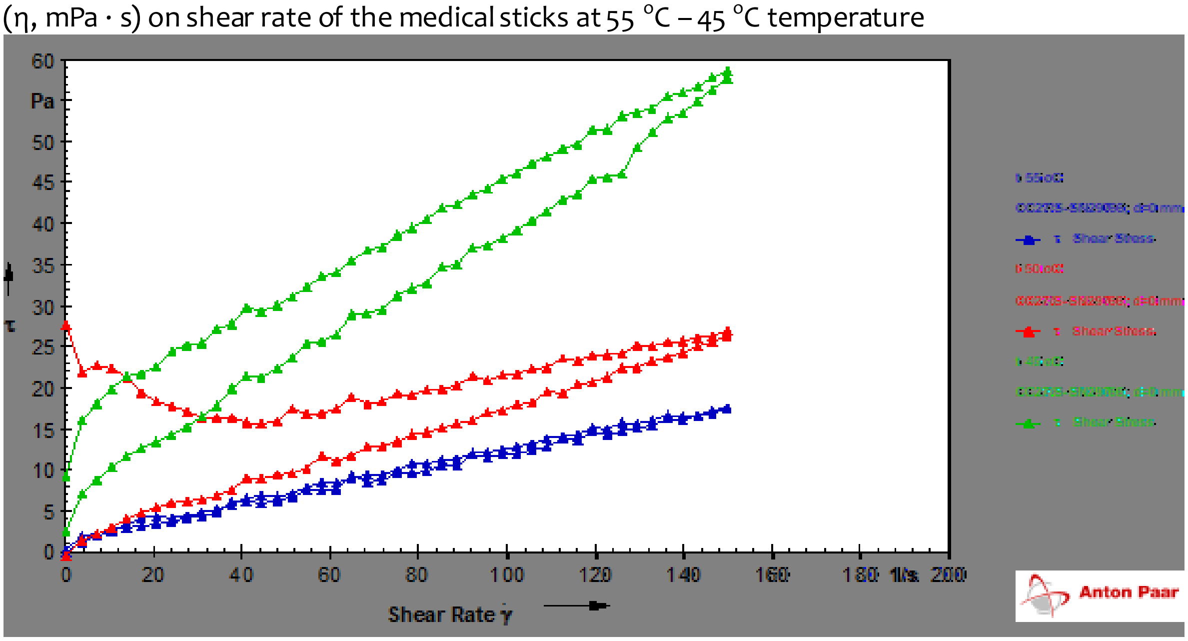

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Figure 3 Dependence of shear stress (τ, Pa) on shear rate (γ, 1 / s) and dependence of structural viscosity (η, mPa · s) on shear rate for the medical sticks at 40 °C temperature

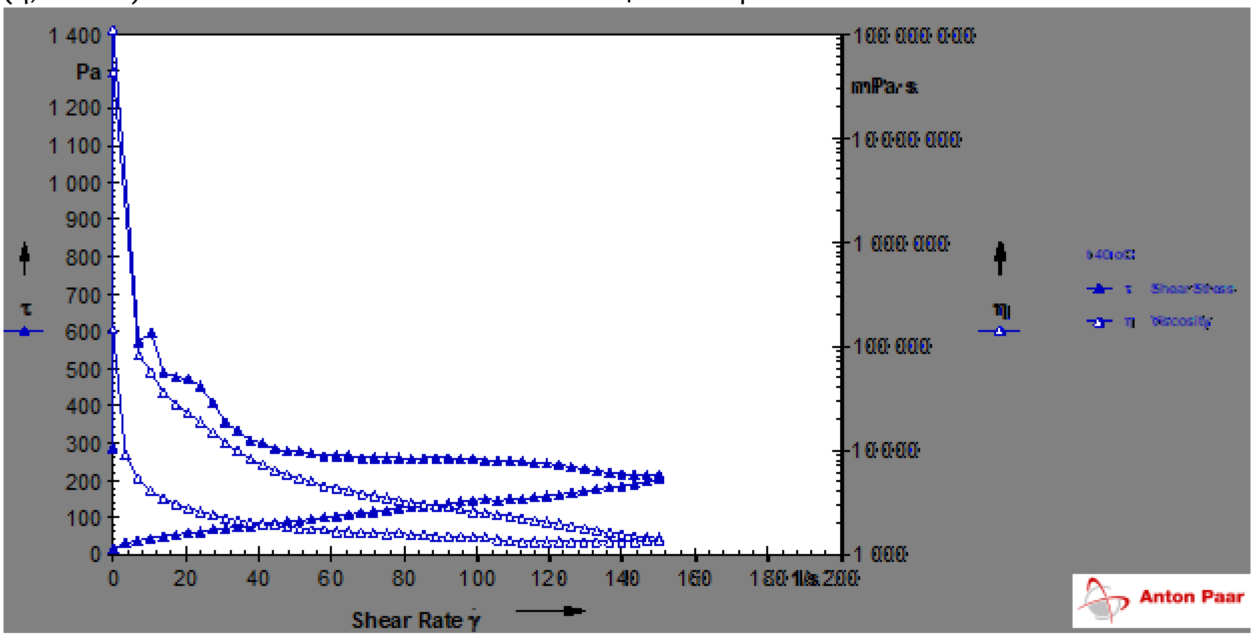

Figure 4. Absorption spectrum of 96% ethanol extraction from the stick

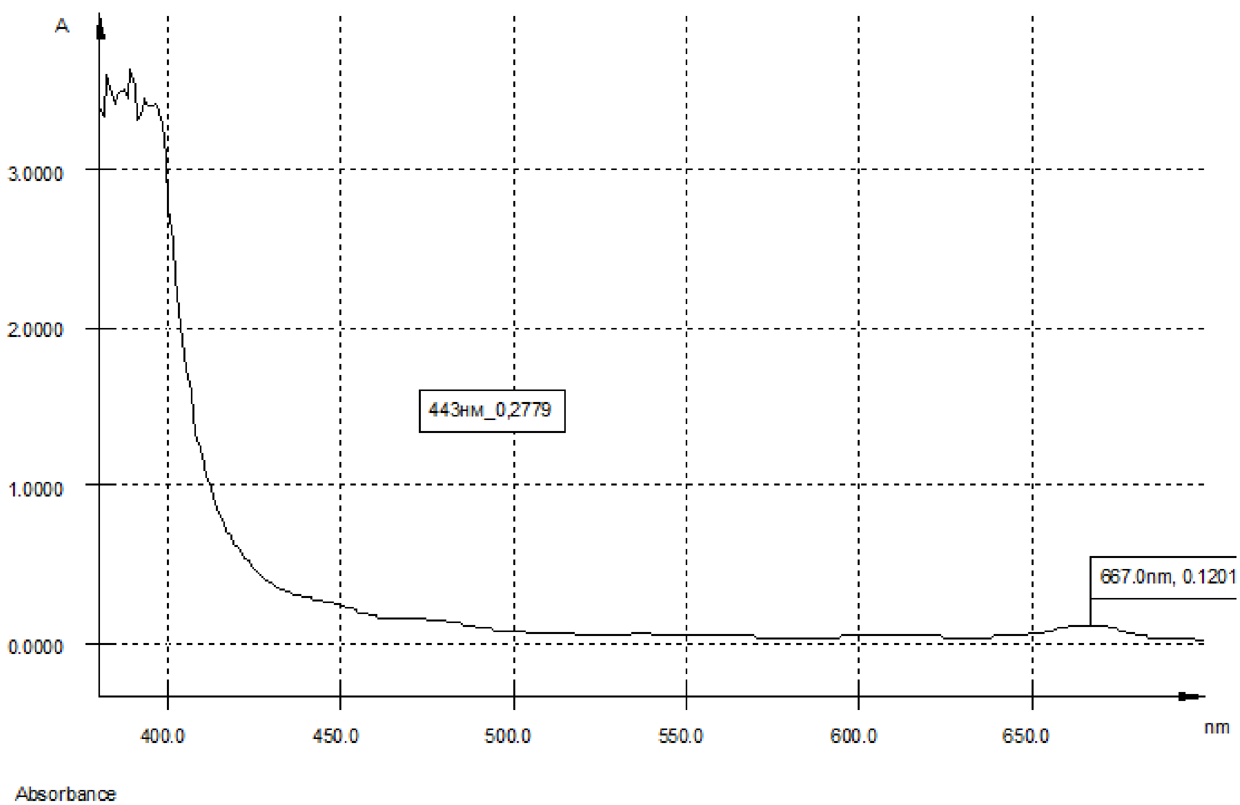

Figure 5. UV-spectrum of alcohol extraction from the medicinal preparation