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Research Article

THE INFLUENCE OF THE EXCIPIENTS ON THE ANTI-INFLAMMATORY EMULGEL BIOPHARMACEUTICAL QUALITY PARAMETERS

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ABSTRACT

Objective: *Boswellia serrata* dry extract (BSDE) has clinically proved its anti-inflammatory, anti-gout, and analgesic effect. In the course of this study, compositions and technologies of the emulgels, containing dry Boswellia extract, were developed.

Materials and Methods: Rheological characteristics of gels were determined with rotational viscosity method using coaxial rotation viscometer Lamy Rheology RM 200. The stickiness was determined using a lever mechanism which separates two preprocessed glass plates of the same area from each other. Spreadability was determined with a weighted amount of gel placed on a glass plate (10 cm × 10 cm), covered with a cover glass of the same size and a 100 g measuring plane placed on top of it.

Results: The composition containing 20% of turpentine oil was shown to have the highest yield strength, which can positively affect the aggregative stability of the dosage form. The spreadability of all samples lies within the average value of the spreadability of the commercial gels. The stickiness of all samples was determined to be in the range from 3.6 to 4.9 N. The composition containing turpentine oil showed maximum stickiness after application and its minimum decrease by 5 min.

Conclusion: The advantages of the composition including turpentine oil as a solvent for BSDE were experimentally established.

Keywords: Emulgel, Dosage forms development, *Boswellia serrata*, Spreadability, Stickiness.

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INTRODUCTION

Basic medical way of treating inflammatory diseases nowadays is using nonsteroidal anti-inflammatory drugs. The related diseases occurring during the treatment stimulate the search of an alternative safer medicinal substance [1]. Among the substances of vegetable origin, the Boswellia extract was traditionally used as an anti-inflammatory agent [2]. Main pharmaceutical components of Boswellia are pentacyclic triterpene acids. The clinical trials have proven the anti-inflammatory, anti-gout, and analgesic effects of Boswellia extract [3-5]. At present, Boswellia extract is used in Russia, the Middle East, Europe, and the USA in the compositions of oral medications and dietary supplements for diseases of the bone tissue [5, 6]. According to the U. S. FDA, the range of drugs for external use based on the Boswellia extract is small, so its expansion through the development of new delivery systems, such as emulgel, is an actual task. The novelty of the study is that for the 1st time, the development of a pharmaceutical composition was carried out on the basis of a combination of a *Boswellia serrata* dry extract (BSDE) with eucalyptus essential oil and turpentine oil, which could potentially increase the absorption of boswellic acids (BAs) and enhance the pharmacological effect [7, 8].

MATERIALS AND METHODS

Materials

BSDE, Carbopol ETD 2020 (*USP 39, EPh 9.0*), Lanette® SX (USP 39, EPh 9.0), triethanolamine (TC 2423-168-00203335-2007), Kollicream IPM (USP 39, EPh 9.0), and methylparaben and propylparaben (USP 39, EPh 9.0) were used.

Solvents

ethanol 96% (State Pharmacopeia XIV, Russia), turpentine oil (PA 000644-190713), and Corymbia citriodora essential oil (State standard ISO 3044-2017, Russia) were used.

Obtaining gel samples

A weighted amount of methylparaben and propylparaben taken at 3:1 ratio was dissolved in purified water mixed by magnetic mixer. Carbopol ETD 2020 was added to the solution, the solution was left for 30 min to swell, after that 0.5 ml of triethanolamine were added and thoroughly mixed. To the following systems consequently were added: BSDE solution in the specified solvent (Table 1), previously melted on the water bath at 70 ± 2°C Lanette SX emulsifier, emollient Kollicream IPM. This system was mixed using turbine mixer for 10 min.

Rheological characteristics of gels were determined with rotational viscosity method using coaxial rotation viscometer Lamy Rheology RM 200 (France) and "cylinder in cylinder" type measuring geometry, shear rate from 0 to 350 s^{-1} at 20°C temperature. To measure the thixotropic behavior of the samples, a rotation test was performed – determination of viscosity and shear stress at successive shear rate changes: "Small shear-large shear-small shear" (from 0 to 350 s^{-1} and from 350 to 0 s^{-1}). Yield strength and plastic viscosity were calculated by the Casson fluid model.

$$\sigma^{1/2} = \sigma_{\gamma}^{1/2} + (\eta_{\rho}\gamma)^{1/2}$$

where,
σ – shear stress;
σ_{γ} – yield strength;
η_{ρ} – plastic viscosity;
γ – shear rate.

To estimate, the consumer characteristics of the gels stickiness and spreadability were determined.

To determine spreadability, a weighted amount of gel was placed on a glass plate (10 cm × 10 cm), covered by a cover glass of the same size. On top, in the center of the measuring plane, a 100 g weight was placed for 60 s. Due to viscoelastic deformation, happening due to cover glass weight and a 100 g weight, higher than the yield strength the sample spread on the glass plate forming a stain, its diameter was used to determine the spreadability [9, 10].

The stickiness was determined using a lever mechanism which separates two preprocessed glass plates of the same area from each other. A dialysis membrane or Dial D14b with pores 12–14 kDa (Orange Scientific, Belgium) was placed on the lower plate. A 1.0 g weighted amount of gel sample was placed on the plate with a putty knife and spread over the area. A weight was placed on the second lever arm. The stickiness was calculated as a multiplication of the mass of the weigh, at which the glass plates separated, on the acceleration of gravity (g= 9.81 m/s^2).

The aggregative stability of gels was estimated using the "accelerated aging" method according to the SF XIV. The samples were stored in the 50 ml glass wide mouth bottles capped with a polymer stopper and a screw cap at 40 ± 2°C temperature in the climate chamber Binder KBF 115 (Germany), relative air humidity 60%. The aggregative stability was measured after 3, 6, 9, 14, 21, and 30 days of experiment by the rate of lamination of the samples after centrifuging. 5 ml of the samples were placed in the centrifuge tubes and were centrifuged on the Biosan LMC-3000 (Germany) centrifuge for 5 min at 3000 rounds per minute speed.

The weight loss on drying was determined using gravimetric method after incubating the samples in cuvettes with a constant area of 28 cm^2 at 20 ± 2°C temperature and 45% relative air humidity in the climate chamber Binder KBF 115 (Germany) [11].

RESULTS

The main pharmaceutically active substances in Boswellia are BAs that are insoluble [12]. BA low solubility combined with specifics of the through skin absorption would be an obstruct for getting into the focus of inflammation. Therefore, before being added to the dosage form, Boswellia extract was dissolved in the lipophilic solvent chosen accordingly to its possible ability to increase the absorption of BA. According to Chen *et al*. and Lin *et al*. [7,13], such compounds include lipophilic fluids containing terpenes: eucalyptus oil and turpentine oil (compositions 2 and 3). These substances also have anti-inflammatory, bactericidal action and are used for treating musculoskeletal system diseases. For comparison, in the compositions 1 and 4, ethanol was used as a solvent.

Flow curves and viscosity curves made basing on the results of viscosity researches are shown in Fig. 1.

The viscosity of sample 1 after testing in the "small shear-large shear-small shear" ranges (from 0 to 350 s^{-1} and from 350 to 0 s^{-1}) has decreased from 6.69 Pa*s to 0.65 Pa*s which is almost 10 times lower which indicates the destruction of the structure.

Samples 2, 3, and 4 have thixotropic properties – their viscosity after applying shear stress and its following removing is restored to initial values within 300 s.

Fig. 2 shows the flow curves of the samples. A pseudoplastic flow type with expressed yield strength is characteristic for compounds 2, 3, and 4. Equivalent values of shear stress at the same shear rates on the ascending and descending loops of the cycle indicate the absence of violations in the structure of viscous-plastic bodies. The sample 1 shows reverse trend with increasing shear rates, shear stress drops sharply, the curve loses its classic loop shape and deviates to the Y-axis. At a shear rate of about 200 s^{-1}, sample 1 is irreversibly destroyed. Therefore, the determination of plastic viscosity for sample 1 was not performed.

Table 1: *Boswellia serrata* dry extract gel samples composition

Number of ingredients, %	Composition				
	1	2	3	4	5
BSDE	5.0	5.0	5.0	5.0	5.0
Ethanol 96%	30	-	-	28	-
Turpentine oil	-	20.0	-	-	20.0
Eucalyptus oil	-	-	20.0	2	-
Carbopol ETD 2020	0.75	0.75	0.75	0.75	0.75
Triethanolamine	0.5	0.5	0.5	0.5	0.5
Lanette SX	3.0	3.0	3.0	3.0	3.0
Kollicream IPM	0.5	0.5	0.5	0.5	2.0
Methylparaben: propylparaben	0.04	0.04	0.04	0.04	0.04
Purified water	To 100 ml				

BSDE: *Boswellia serrata* dry extract

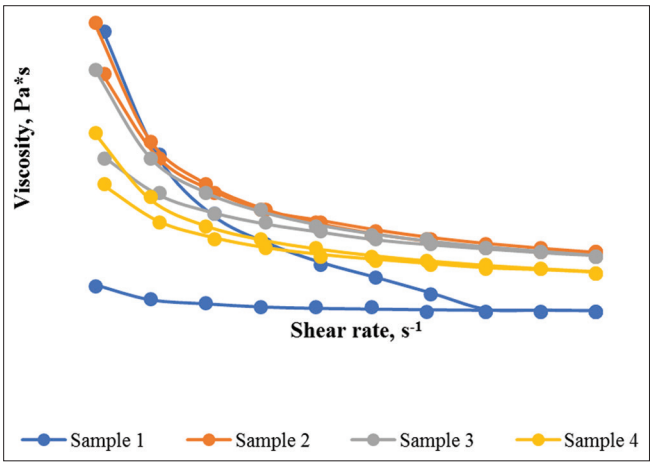

Fig. 1: BSDE samples viscosity curves

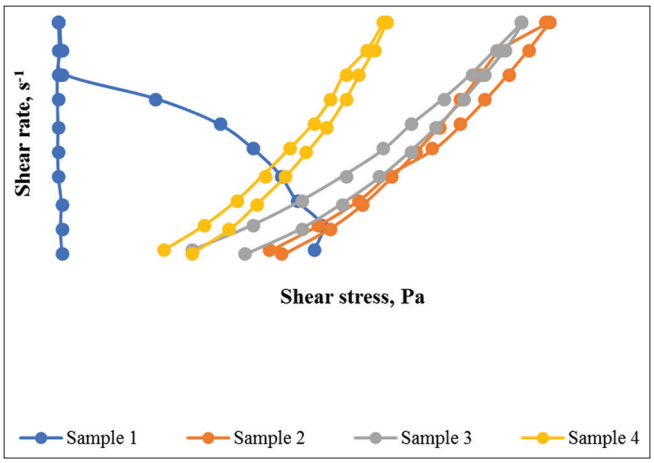

Fig. 2: BSDE samples flow curves

The interpretation of the rheological characteristics was performed according to the Casson fluid model, used to assess the rheological properties of materials with yield strength that does not flow at low shear rates or has such high viscosity values that, for technological applications, they can be neglected [14]. The main rheological characteristics of samples 2–4 are given in Table 2.

Sample 2 has the highest yield strength. High-yield strength characterizes the high strength of the gel structure, which can positively affect the aggregative stability of the dosage form.

Spreadability, stickiness, and weight loss on drying (drying ability) were determined to evaluate consumer characteristics.

The spreadability determining is not a pharmacopoeial test and has no standard values. Therefore, to determine the optimal spreadability range, a comparative study of the following commercial drugs in the form of gels for external use was conducted: Troxevasin® 2% (Balkanpharma-Troyan, Bulgaria); Artrosilene® 5% (Dompe, Italy); Nise® 1% (Dr. Reddy's Laboratories Ltd., India); Deep Relief® (Mentholatum Company Limited, Great Britain); Bystrumgel®, 2.5% (Acrichin, Russia); Diclofenac®, 5% (Synthesis, Russia); Dolobene® (Merckle, Germany); and Chondroxide®, 5% (NIZHFARM, Russia). The results are given in Fig. 3.

The average value of the spreadability of the commercial gels was 4.575 cm (minimal value 2.8 cm, maximum – 5.6 cm) (Fig. 3). All obtained

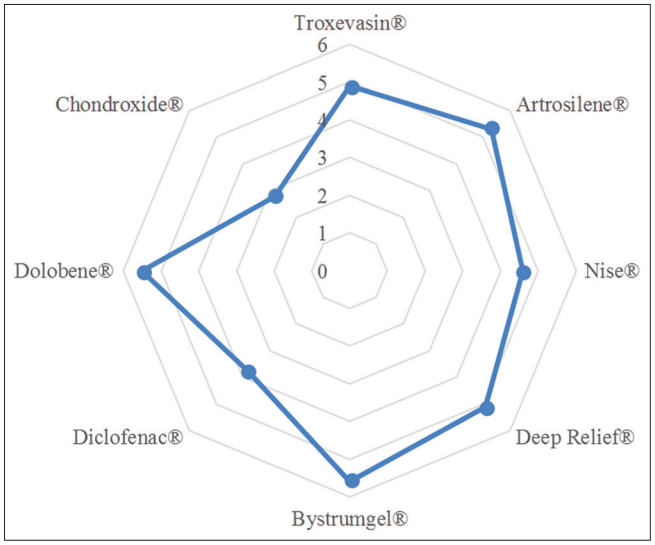

Fig. 3: Profilogram of the spreadability of the commercial drugs in the form of gels for external use

BSDE gel samples have comparable values of the spreadability – from 4.1 to 4.5 cm (Table 2).

The stickiness of drugs in the form of gels is primarily due to the presence of solutions and polymeric dispersions in their composition. The stickiness of the gel containing BS extract, obtained from *Boswellia serrata* resin, is also due to the sticky properties of the extract. Therefore, the determination of the stickiness of the experimental gel samples was of interest from the viewpoint of comfort of use. For the majority of topical dosage forms, the stickiness value decreases as the sample dries. Therefore, the indicator was measured immediately after the application of the gel, as well as after 3 and 5 min. The results are given in Table 2 and Fig. 4.

Table 2 shows the values of stickiness, determined immediately after applying the samples to the measuring surface. The stickiness of all samples is ranged from 3.6 to 4.9 N. Sample 4 had the least stickiness, in its compound a mixture of eucalyptus oil and 96% ethanol (1:14) was used to dissolve BSDE, sample 2 had the greatest stickiness, its solvent was turpentine. The dynamics of stickiness while drying is shown in Fig. 4. Already after 5 min after application, all samples stickiness had reduced: 16% for sample 3, containing volatile essential oil from *Corymbia citriodora*; 8%, 6%, and 10% for samples 1, 2, and 4, respectively.

It is remarkable that sample 2, which has the highest yield strength value, showed maximum stickiness after application and its minimum decrease by 5 min. For the correction of the indicator, the concentration of the emollient Kollicream IPM in the composition of the sample was increased from 0.5% to 2.0%, which led to a decrease of stickiness after applying the gel to 3.62 N (composition 5).

The final stage of the evaluation of the technological parameters of the samples of the gels was the determination of the aggregative stability by the method of "accelerated aging," the storage conditions, and the justification of the packaging.

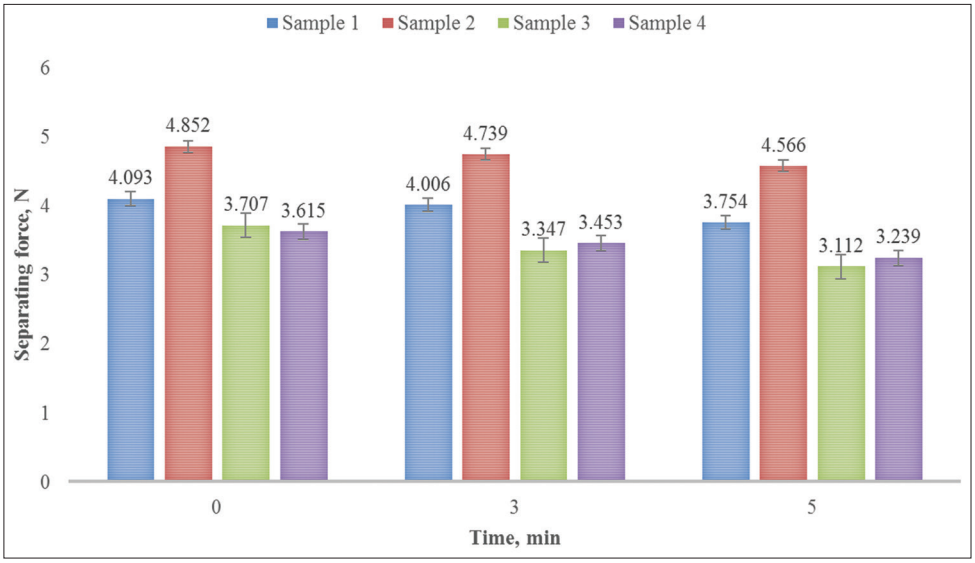

Fig. 4: Changes in the stickiness of BSDE gel samples

Table 2: *Boswellia serrata* dry extract gel samples rheological characteristics

Sample number	Plastic viscosity, Pas	Yield strength, Pa	Spreadability, cm	Stickiness, n
1	-	5.45±0.21	4.5±0.05	4.093±0.18
2	0.369±0.021	116.7±1.52	4.1±0.05	4.852±0.22
3	0.466±0.023	89.4±1.35	4.4±0.05	3.707±0.15
4	0.303±0.015	71.8±1.37	4.4±0.05	3.615±0.13

Values are given as mean±SD. SD: Standard deviation

Table 3: The percentage of delamination of *Boswellia serrata* dry extract gels stored by the method of "accelerated aging"

Sample number	BSDE gels delamination (%), storage time (days)					
	3	6	9	14	21	30
1	0.00	12.00±0.60	29.00±1.50	-	-	-
3	30.00±1.50	-	-	-	-	-
4	0.00	14.50±0.73	35.00±1.75	-	-	-
5	0.00	0.00	0.00	0.00	0.00	0.05±0.03

Values are given as mean±SD. -: No further research has been carried out, BSDE: *Boswellia serrata* dry extract, SD: Standard deviation

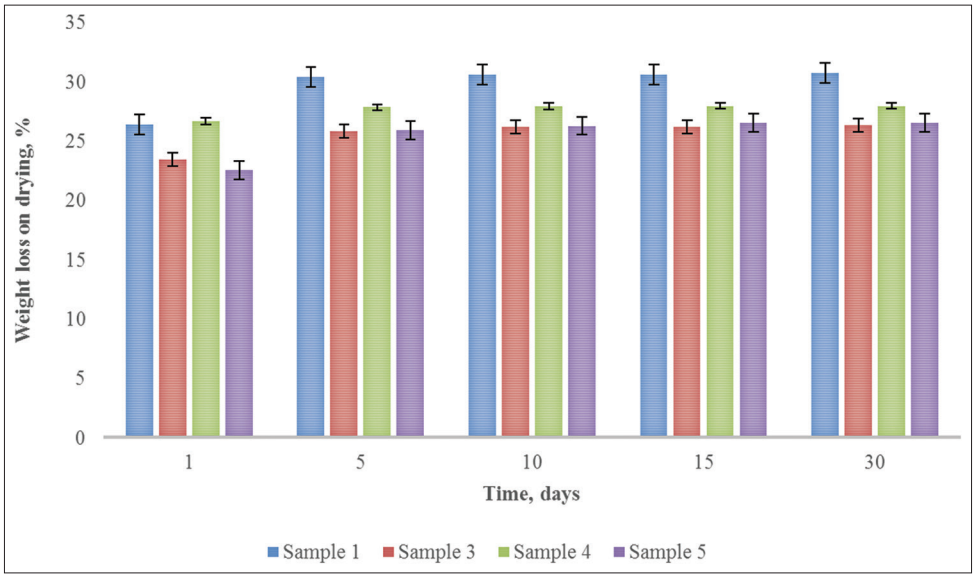

Fig. 5: Weight loss on drying (drying ability) of the BSDE gels samples when stored in cuvettes at a temperature of 20 ± 2°C and relative air humidity of 45% in the climate chamber Binder KBF 115

Table 3 shows the results of determination of the aggregative stability. After storing the samples for 30 days at a temperature of 50 ± 2°C and a relative humidity of 60%, the minimum percentage of delamination was determined for sample 5, which indicates a correlation of the yield strength-the percentage of delamination.

For recommendations on the storage conditions of the obtained dosage form, the weight loss on drying (drying ability) of the samples was determined (Fig. 5).

For the first 24 h of the experiment, the minimum decrease of gel mass during the drying process was 22.53 ± 0.05% for sample 5. For 30 days, this indicator did not change significantly and amounted to 26.51 ± 0.05%. The highest drying ability during the storage is characteristic for composition 1 and by the 30th day of the experiment, it was 30.7 ± 0.05%. The obtained results substantiate the need for a sealed package for a BSDE gel, such as aluminum tubes with bushings of high-pressure polyethylene, and storage conditions at a temperature not exceeding + 25°C and humidity around 45%.

DISCUSSION

The result of this study showed that the most promising solvents for the introduction of the BSDE in the composition of emulgel for external use are terpene oil and a combination of ethyl alcohol and eucalyptus essential oil in a 1:14 ratio. Composition 1 containing 30% of ethyl alcohol 96% had unsatisfactory dynamic viscosity and low-yield strength, affecting the stability during storage. The lack of aggregative stability during storage was confirmed by the method of "accelerated aging." The results of storage were also unsatisfactory for a composition containing an essential oil as a monosolvent – <15 days of storage under natural conditions (temperature 20°C, air humidity 65%).

Compositions 2 and 4, containing turpentine oil and a mixture of ethyl alcohol with eucalyptus essential oil in a 1:14 ratio as a solvent, turned out to be rheologically close. However, at the stage of studying, the spreadability and stickiness, composition 4, containing a multicomponent solvent, turned out to be the best with the highest value of spreading and minimal stickiness among the analyzed compositions. Presumably, this is due to the increased volume of the dispersion medium compared to the other compositions: 30 ml of the combined solvent versus 20 ml in the compositions 1, 2, 3, and 5. However, this composition also had low stability during storage – after the time of the "accelerated aging" experiment, equal to 60 days, the delamination of the composition began, and the residual drops of the dispersion medium released on the surface. Thus, composition 4 may be promising for further research related to the search of optimal concentrations and selection of the type of emulsifier.

Composition 2, containing turpentine oil as a solvent for BSDE, has optimal biopharmaceutical characteristics, excepting the stickiness. The stickiness of this composition was the highest among all analyzed compositions. Apparently, this is due to the own properties of the turpentine oil, which makes up 20% of the total mass of the composition. The pharmaceutical composition was corrected by adding the emollient, which did not affect the rheological parameters of the system, but increased the spreadability (from 4.1 to 4.6 cm) and reduced the stickiness almost 1.5 times.

CONCLUSION

Thus, the advantages of the composition 5, which includes turpentine oil as a solvent for BSDE, were experimentally established. This composition has high gel structure strength, aggregative stability, the lowest drying ability, and optimal consumer properties, which made it possible to determine its perspectives for preclinical trials.

AUTHORS' CONTRIBUTIONS

Demina N. and Krasnyuk I. designed the study. Bakhrushina E. and Korneev M. performed the experiment and analyzed the obtained information.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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