MODELING OF THE COMPOSITION OF A SEMISOLID PROPOLIS PREPARATION, AND EVALUATION OF ITS QUALITY

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Abstract

The aim of the study was to select the optimal composition of semisolid using natural materials, and to evaluate the quality of such preparation. Investigation of rheological properties, evaluation of sensory characteristics, detection and identification of phenolic acids using high-performance liquid chromatography (HPLC) were performed. The results of the rheological investigations and the study of sensory properties were evaluated, and the optimal composition of the ointment was selected: olive oil, beeswax, cocoa butter, cholesterol, soft propolis extract, and substances that improved the sensory characteristics. The base of the ointment ensures the stability of the semisolid when the active component is soft propolis extract. The results of the quantitative study by high-performance liquid chromatography identified phenolic acids and determined their amounts and showed that the predominant phenolic acids in the propolis ointment were ferulic acid and coumaric acid. The modeled hydrophobic semisolid is a stable homogenous system with positive sensory characteristics.

Rezumat

Studiul şi-a propus cercetarea unor formulări farmaceutice semisolide cu componente de origine naturală: ulei de masline, ceară de albine, unt de cacao, colesterol, extract de propolis. Au fost efectuate prin tehnici moderne, proprietăţile fizico-chimice ale acestui sistem. De asemenea, prin cromatografie de lichide de înaltă performanţă (HPLC) au fost identificate principalele principii active prezente în sistemul original, natural dezvoltat.

Keywords: propolis; ointment; sensory characteristics; phenolic acids; rheology

Introduction

The popularity and increasing application of transdermal therapeutic semisolid preparations in medical practice is conditioned by objective advantages of such drug forms, i.e. safety, relatively simple use, and acceptability to the patients. The development of the technologies of semisolid drug forms has also been stimulated by studies and practical use of new excipients. The increasing popularity of cosmetic products from natural raw material poses a difficult task to researchers and manufacturers

– to produce stable products from natural raw materials, which would meet contemporary requirements [1,2,3]. Thus, the production of a semisolid transdermal preparation containing not only natural active ingredients, but also a natural base would be relevant. When modeling the base of the ointment, yellow beeswax and olive oil were selected as major ingredients [1,2]. The selection of the appropriate base is one of the main tasks in the production of a semisolid transdermal drug form. It is complicated to model semisolid natural preparations that would meet the quality criteria for modern cosmetic preparations, and to create a preparation whose sensory criteria would equal those of synthetic creams or ointments. Lithuanian scientists have introduced some novelties in the field of pharmaceutical and cosmetic preparation technologies [4]. Rheological studies may indicate the shelf-life of the product, and spreadability determines the local or systemic effect of semisolid preparations, because better spreadability ensures wider field of contact, thus affecting penetration and absorption [5,6]. Suitable selection of the ingredients of the base ensures easy flow of the preparation from the containers, its stability during storage, and good distribution on the skin [7]. Spreadability depends on the proportions of the ingredients in the ointment, as well as on their physical-chemical and organoleptic characteristics [8]. Rheological studies may help predict the state of the ointment throughout the storage period. Improper selection of the ingredients results in the sedimentation of the solid particles, poor skin coverage, and escape of the ointment from the tube due to gravity [7]. For this reason it is relevant to investigate the effect of the ingredients (in varying amounts) on base stability, sensory characteristics, rheologic properties, and homogeneity. It is also expedient to include propolis into the composition of a semisolid preparation – ointment. Propolis is a crude medicinal substance that enriches the preparation with antimicrobial [9,10,11,12], anti-inflammatory, antifungal [13], and antioxidant properties [14].

The aim of this study was to select optimal composition of the semisolid preparation using exclusively natural substances, and to evaluate the quality of the preparation with respect to the expectations of a modern consumer.

Materials and Methods

Materials: soft propolis extract (close corporation Valentis, Lithuania), yellow beeswax (close corporation “Bitutė”, Lithuania), carnauba wax (Carl Roth GmbH, Germany), olive oil (Carl Roth GmbH, Germany), cocoa butter (Henry Lamotte GmbH, Germany), cholesterol (Sigma Aldrich Chemie GmbH).

Investigation of rheological properties, and determination of the viscosity index and the flow consistency coefficient

The viscosity of semisolid preparations (Pa·s) was determined using the SV-10 viscosimeter (A&D Company, Limited, Japan). The studied substance was placed into a special container for measurement. Subsequently the container was fixed on the working surface of the device, and sensors were submerged into the studied substance. The flow curves of the bases of semisolid preparations were obtained using the Carri-med CSL100 rheometer (TA Instruments, Germany), by applying the cone-and-plate geometry (cone diameter – 40 mm, angle – 2°, and sample thickness – 150 μm), at the temperature of 20°C. The shear rate was increased during 2 minutes from 0 to 500 s^{-1}, the sample was then left at rest for 10 minutes, and then the shear rate was reduced during 2 minutes from 500 s^{-1} to 0 s^{-1}. The flow curves were then compared to Oswald de Waele model equation: $\tau=k\gamma^{n}$, where τ – shear stress, Pa; γ – shear rate, s^{-1}; K – flow consistency coefficient, which is an indicator of viscosity; and n – flow behavior index, which is the indicator of deviation from characteristics typical of Newtonian fluids.

Evaluation of sensory characteristics

A trained groups of evaluators analyzed pre-selected products (samples) and selected the concepts (compiled the vocabulary) for describing the sensory characteristics of the samples. Subsequently, the scales for the evaluation of the intensity of these characteristics were selected and discussed, and the intensity of each characteristic of all products was then marked on separate scales. Using these data, mathematical statistics techniques were applied compile a profile of sensory characteristics for each product, indicating the intensity of each characteristic. This profile could then be used to compare products according to their separate properties and their intensity, to determine the relationship between the sensory quality of products and their individual characteristics. A group of seven evaluators participated in the study. The evaluators were selected and trained according to the LST ISO 8586-1 standard. The evaluation was of a closed type, conducted according to the requirements of the LST ISO 8589 standard in booths constructed in the Sensory Analysis Laboratory of the Food Institute of Kaunas University of Technology.

Detection and identification of phenolic acids using high-performance liquid chromatography

Preparation of the solution. 0.5 g of propolis ointment was mixed with 50 ml of 96% ethanol. The solution was dispersed in an ultrasound bath (Sanorex Digitec, Germany) for 10 minutes, and then placed into a freezer (Samsung, Korea) where it was stored for 21 hours at the temperature of -12°C. A separated ethanolic phase was used for the study.

The substance – methanol – for high-performance liquid chromatography (HPLC) was obtained from Carl Roth GmbH (Karlsruhe, Germany). Purified water for sample preparation was filtered through the 0.22 μm Millipore HPLC grade filter (Bedford, USA). Standard phenolic acids were obtained from ChromaDex (Santa Ana, USA). The examination was performed using Waters 2690 chromatography system model (Waters, Milford, USA) with UV/PDA detection with Waters 2487 UV/Vis and Waters 996 PDA detectors. Isolation was performed using Hichrom column Hypersil H5ODS-150A 150×4.6 mm (Hichrom Ltd., Berkshire, UK) and H5ODS-10C guard-precolumn. Eluent A was methanol, and eluent B - 0.5% volume acetic acid in water. Elution profiles: 0 min –10% of eluent A in eluent B, 28 min - 60% of eluent A in eluent B, and 30 min - 10% of eluent A in eluent B. The change was linear. Flow velocity was 1 ml/min, column temperature was equal to ambient temperature, and the injection volume was 10μl. UV analysis was performed at 290 nm wavelength. The eluted components were identified on the basis of the retention time and comparing to the retention times of standard samples. The identity of the components was also confirmed with a PDA detector, comparing with the standard UV spectrum at 190-400 nm wavelength.

Results and Discussion

In the modeling of the base of the ointment, beeswax and olive oil were selected as the main components. Chemical compounds in beeswax have emulsifying properties and thus complement the substance with plasticity and compatibility with other natural ingredients [2, 3]. In the production of cosmetic preparations – such as creams, ointments, or lip balm – beeswax is used as a consistence-providing and moisture-preserving ingredient [2, 3, 15]. According to literature, beeswax in semisolid preparations makes 5% to 30% of the base mass [2, 15, 16]. In forming the semisolid matrix of the preparation, olive oil was selected as a hydrophobic fluid to be dispersed in beeswax. Olive oil was selected due to its characteristics – it well mixes with solid substances, animal fat, wax, or paraffins [15]. To select the optimal base of the ointment, beeswax and olive oil ratios were changed in base samples, and evaluated their viscosity (Table I).

Table I
The effect of temperature on the values of the flow consistency coefficient (K) ($Pa \cdot s^n$) and the flow behavior index (n) in ointment bases

Wax/olive oil ratio	Groups	40ºC		50ºC		60ºC	
		K	n	K	n	K	n
1/1	I	311.18	0.02117	65.085	0.0791	4.6416	0.0591
1/2	II	21.606	0.0885	18.542	0.017	0.0846	0.5548
1/3		6.6804	0.0636	3.8741	0.1231	0.1197	0.505
1/5		2.6959	0.1952	1.9961	0.1196	0.0412	0.5804
1/8	III	0.5777	0.4226	0.4476	0.2098	0.0332	0.5866
1/10		0.2197	0.525	0.1597	0.4718	0.0285	0.6078

The flow consistency coefficient was measured by evaluating the effect of the technological parameter (temperature) on the viscosity of the ointment. The selected temperature range was from 40°C to 60°C, and was based on the peculiarities of the production of hydrophobic ointments and the beeswax melting temperature [15,16]. According to the values of consistency coefficient the results were grouped into three groups. As seen from the data presented in Table I, independently of the temperature, the highest K value was observed when beeswax content was 50%. K value in the second group of mixtures ranged between 21 and 0.08. Meanwhile, in the third groups of samples, the flow consistency constants were lower – from 2 to 0.02. Increasing the olive oil concentration in the samples resulted in alterations in the flow behavior index (Table I). The results of the study showed that lower temperature increases the viscosity of the ointment bases. Thus, generalized results of the study suggest the importance of appropriate selection of technological parameters, such as the melting temperature of the base, stirring velocity, the cool-down temperature of the ointment base, duration, and the speed of stirring during the cool-down period. The results of the study showed that in order to produce homogenous hydrophobic ointment base, the cooling phase of the ointment base should be gradual, with insignificant reduction of temperature. For further investigation the ointment base containing less than 10% of beeswax was selected. To increase the plasticity of the ointment base, spermaceti was added into its composition [15].

To select optimal composition of the propolis ointment, ointment samples were produced containing substances enhancing their sensory characteristics where a soft extract of propolis was used as active substance (Table II).

Table II

Selection of the composition of the ointment

Components (%) of the ointment	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Yellow beeswax	10.0	10.0	10.0	-	-	10.0	10.0
Carnauba wax	-	-	-	10.0	10.0	-	-
Honey	1.0	-	-	-	-	-	-
Propolis (soft extract)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Spermaceti	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cholesterol	-	-	-	-	0.3	0.3	0.3
Cocoa butter	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Lemon-balm essential oil	0.06	0.03	-	0.03	0.06	0.06	0.06
Buckthorn oil	-	-	-	-	-	-	0.1
Olive oil	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100

To improve the sensory characteristics of the ointment base, the following substances were included into its composition: buckthorn oil for color, and lemon-balm essential oil for scent. In addition to that, experimental studies have proven that buckthorn oil has antioxidant characteristics, protecting the organism against negative effect of free radicals. Lemon-balm essential oil is used in the treatment of allergy, hay fever, asthma, eczema, and herpes; in cosmetics, it is used for care of lips and greasy porous skin and greasy hair [17,18]. Honey as an antibiotic substance can be applied when treating suppurating wounds and skin burns [19]. Thus, all these substances may not only improve the sensory characteristics of propolis ointment, but also strengthen its therapeutic properties.

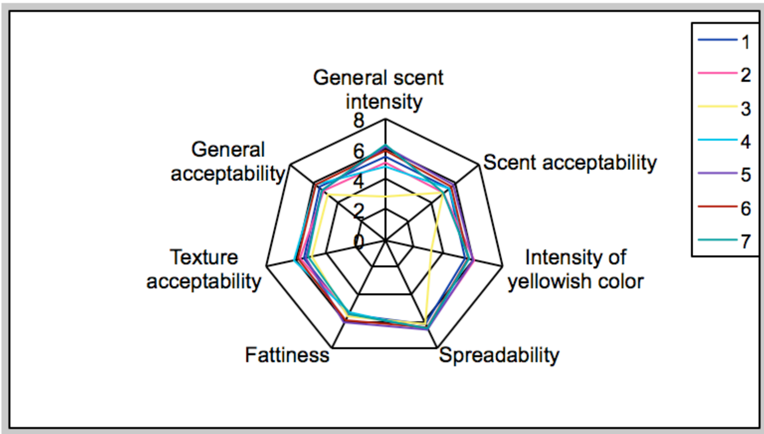

Figure 1

Results of the investigation of sensory characteristics

As seen from the presented data (Figure 1), the scent intensity of sample No. 3 was the weakest, and its color – the palest. Its spreadability – indicating how easily and equally the ointment can be distributed on the surface of the skin – was also lower, although this difference was not significant. However, this could affect the evaluation of the texture and general acceptability of the samples – which was significantly poorer, compared to the evaluation of samples No. 4 and No. 6. These two samples were evaluated as most acceptable. Sample No. 6 differed from sample No. 7 only in its general intensity of scent and yellowish color. No statistically significant difference was found between samples No. 2 and No. 4 concerning either of the studied characteristics. Samples No. 5, 6, and 7 also received equal evaluations.

Stability tests showed that structural changes in ointment of the selected composition No.5 were shrinkage of the ointment matrix with resulting expulsion of the constituents (drops visible on the ointment surface). In addition to that, crystal changes were detected – wax crystals increased in size, resulting in granules that were felt when applying the ointment on the skin and subsequently melted on skin surface. Such changes in the crystal form may affect the therapeutic effect of the ointment. Experimentally compositions of ointments containing cocoa butter as the spreadability-enhancing substance [6] were selected. On the basis of the results of previous examinations of the sensory characteristics, buckthorn oil was added to achieve acceptable color of the preparation.

Using high-performance liquid chromatography, phenolic acid content (Table III) in propolis ointment of the selected compositions No. 5, 6 and 7 was identified and quantified.

Table III

Results of the investigation of active substance content using high-performance liquid chromatography (mean ± SD, n=3)

Active substances μg/ml	**Ointment No. 5**	**Ointment No. 6**	**Ointment No. 7**
Chlorogenic acid	17.760±0.04	10.581±0.17	13.462±0.01
Vanillic acid	1.655±0.12	1.188±0.01	1.180±0.05
Caffeic acid	3.797±0.08	2.382±0.01	2.711±0.11
Vanillin	29.152±0.04	23.181±0.02	24.722±0.05
Coumaric acid	51.243±0.11	42.426±0.04	44.881±0.05
Ferulic acid	35.463±0.07	33.480±0.30	35.045±0.04
Rosemary acid	0.816±0.01	0.511±0.01	0.687±0.21
Cinnamon acid	3.817±0.23	2.316±0.71	2.523±0.01

The results of the study showed that the predominant phenolic acids in the preparation – like in crude propolis [14] – were ferulic acid and coumaric acid.

Conclusions

The modeled hydrophobic semisolid is a stable homogenous system with positive sensory characteristics. The base of the ointment ensures the stability of the semisolid preparations when the active component is soft propolis extract. High-performance liquid chromatography of the propolis ointment identified phenolic acids and determined their amounts, which may also be considered as one of the quality evaluation indices.

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