THE STUDY OF PHYSICO-CHEMICAL AND TECHNOLOGICAL PROPERTIES OF ACTIVE SUBSTANCES AND THEIR MIXTURE FOR THE DEVELOPMENT OF A PLANT MEDICINE WITH CARDIOTONIC AND IMMUNOMODULATING ACTION

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ABSTRACT

INTRODUCTION: To develop phytomedicine with a cardiotonic and immunomodulating effect in the form of hard gelatin capsules, to predict the rational composition and optimal technology for obtaining a combined medicine the determination of physico-chemical and pharmaco-technological properties of the lyophilized sunflower protein substance, the dry extract of hawthorn flowers and fruits and the mixture of these biologically active substances in the ratio 1.4: 1 have been used.

MATERIALS AND METHODS: Physical and technological methods on State Pharmacopoeia of Ukraine 2.0 have been used.

RESULTS: The results of the studies of microscopy, bulk volume, settled volume, settling qualities, bulk density, tapped density, flowability, angle of repose and moisture absorption of the lyophilized sunflower protein substance, the dry hawthorn extract and their mixture were analyzed.

The extract does not have flowability because of the fineness of the powder and rough surface of particles. It was necessary to grind additionally the protein substance to achieve the homogeneity of the mixture of the two active substances. The re-milled protein substance showed good flowability. But the flow rate of the mixture was lower. The mixture of powders was characterized by the low bulk density and tapped density, a significant settling quality of the mixture.

CONCLUSION: Analyses of the data obtained pointed to the need to additionally grind the lyophilized sunflower protein substance and to introduce glidants into the mass for the incapsulation, or the obtaining granules. Moisture regulators must be added to the composition for the incapsulation because of the significant moisture absorption of the powder mixture.

Keywords: hawthorn flowers and fruits dry extract, lyophilized sunflower protein substance, physico-chemical properties, technological properties

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INTRODUCTION

One of the primary medical, biological, social and demographic world problems are cardiovascular diseases (CVD) (angina, heart failure, coronary artery disease, atherosclerosis, etc.). CVD cause more than half of all deaths across the European region (1,2). CVD adversely affect the productivity, quality and life expectancy. In recent years, modern clinical

cal medicine notes also a weakened immune system among the many risk factors for the occurrence and the development of CVD, such as heredity, obesity, bad habits, hypodynamia, stress and age (3).

Marketing research showed that drugs with simultaneously cardiogenic and immunomodulating actions were presented in insufficient quantities on the pharmaceutical market of Ukraine. Therefore, in view of the progressive problem of the CVD prevalence in Ukraine, the development of a medicine with cardiogenic and immunomodulatory effects is relevant for practical medicine and pharmacy (4,5).

Hard gelatin capsules were suggested as the optimal medicine dosage form, due to the relative simplicity of the technological process to other solid dosage forms and convenience of taking the drug.

The following active ingredients were used to create the medicine: we selected the dry extract of hawthorn flowers and fruits as a cardiogenic drug and the lyophilized sunflower protein substance as an immunomodulator. The producer of the dry extract is the research-and-production company "Vilarus" Ltd. in Ladyzhyn (Ukraine). The lyophilized sunflower protein substance was obtained under the direction of Professor A.I. Bozhkov at the Institute of biology at V.N. Karazin Kharkiv National University. The immunomodulating properties of the sunflower protein substance have been experimentally proven (6).

The flavonoids (rutin, quercetin, vitexin, hyperoside) are not less than 1.5% in the flowers and fruits of hawthorn. Also, flowers and fruits contain organic acids (citric, oleanolic, ursolic, cratagic, caffeic, chlorogenic acids), carotenoids, tannins, pectin, and vitamins. The active ingredients of hawthorn improve the coronary and the cerebral blood circulation, reduce the excitability of the central nervous system and heart muscle, have the effect of enhancing the contraction of the heart muscle. Hawthorn promotes normalization of heart rate (7-9).

There are 14% of dry matter, 67.2% of oligosaccharides, 23.3% of amino acids and oligopeptides, 11.5% of lipids, less than 1% of vitamins and minerals, less than 1% of organic acids in the lyophilized sunflower protein substance. The free amino acids in the protein substance are threonine (3%), valine (23%), cysteine (16%), methionine (11%), leucine

(10%), arginine (4%), isoleucine (4%), and lysine (1%). The composition of the lyophilized sunflower protein substance includes vitamins: B1 (0.38 mg/l); B2 (3.24 mg/l) and PP (8.3 mg/l) and a small amount of microelements: iron, calcium, phosphorus.

It was oligosaccharides that played a major role in the shaping and strengthening of human immunity from all these components, and it was threonine, from these amino acids, that stimulated the immune system (6).

One dose of the studied active substances for capsules was chosen according to the literature database (4,6,10). The ratio of lyophilized protein substance and dry extract was 1.4: 1, respectively.

The purpose of this scientific work was the study of physical, chemical and technological properties of the dry extract of hawthorn flowers and fruits and the lyophilized sunflower protein substance obtained by the technology we have modified on the stage of grinding.

MATERIALS AND METHODS

In carrying out the work, the physical and technological methods of the State Pharmacopoeia of Ukraine 2.0 (11) are used to obtain the reproducible and reliable data.

Determining the shape and particle size of the extract was performed using fluorescent microscope type "Liumam R1", which allows the observation and photographing of the image of the object in the transmitted light with lighting from above through an Opak illuminator and a lens, and also from below through the microscope condenser. Samples of the extract were prepared in silicone oil (the polydimethylsiloxane PMS-100). Photomicrographs of a mixture of the dry extract of hawthorn and the lyophilized sunflower protein substance after re-grinding were obtained also with this microscope in the transmitted light without silicone oil.

The research of microscopy of the protein substance before the grinding and the mixture of the protein substance with the extract was carried out using MBS-9 microscope in the reflected light. Digital photographs were taken using ocular camera DCM 300. The particle size was determined by the ScopePhoto 3.1.

Determination of fractional composition of the active pharmaceutical ingredients was carried out using the sieving method with a set of sieves with normal accuracy and a cell size 1; 0.7; 0.5; 0.355; 0.25; 0.18 and 0.09 mm.

The bulk volume, settling qualities, bulk density and tapped density were determined using a 545P-AK-3 device for powder vibratory compacting (Ukraine).

The determination of the moisture content of the active substances was carried out by Auto Express Moisture Tester Sartorius MA-150.

RESULTS AND DISCUSSION

The dry extract of the flowers and fruits of hawthorn is a fine, odorless, light yellow powder. Dominating particles have isodimetric nearly spherical shape with an average size of 35 μm , a rough surface

and the ability to aggregate in the extract according to the microscopic analysis (Fig. 1a). The particles have a porous structure. It may be predicted a lack of flowability of powder from these results.

The lyophilized sunflower protein substance that had the appearance of solid plates was ground by a laboratory grinder and sieved through a sieve with a mesh diameter of 1 mm. The resulting micrographs of the ground sunflower protein substance (Fig. 1b) shows that the powder has isodimetric shaped dense particles with a size of 80-700 μm , with a homogeneous structure and a rough surface.

Active ingredients were mixed in a laboratory mixer (a mixture micrograph shown in Fig. 1c). In the study of technological properties of the mixture on different devices it turned out that the mixture is subjected to separation. Therefore, to prevent the possibility of this process during the encapsulation,

Fig. 1. Photomicrographs of active substances powders: a) the dry extract of hawthorn fruits and flowers; b) the lyophilized sunflower protein substance before the secondary grinding; c) a mixture of the dry extract of hawthorn and the lyophilized sunflower protein substance before the secondary grinding; d) a mixture of the dry extract of hawthorn and the lyophilized sunflower protein substance after re-grinding

the protein substance powder is further crushed and sieved through a sieve with holes with a diameter of 0.355 mm. After re-grinding and mixing the protein substance with the dry extract a mixture separation was not observed (Fig. 1d).

Fig. 2 presents the results of the fractional analysis of the dry extract of hawthorn and the lyophilized sunflower protein substance before re-grinding

Fig. 2. Fractional composition of the dry extract of hawthorn and the lyophilized sunflower protein substance before re-grinding

ing. According to fractional analysis, agglomerates of particles with the predominant size of 0.09-0.18 mm are formed in the dry extract. The destruction of agglomerates was seen during the mixing of the two active ingredients. The protein substance before re-grinding is a powder with the predominant fractions of particles which have a size of <0.09-0.18 mm. The existence of 15.11% fractions with particle sizes of more than 0.355 mm lead to the separation of the two substances from the mixture. The separation as indicated above was leveled by additional grinding and sieving of the protein substance powder.

In the study of the pharmaco-technological properties, it was found that the flow rate of the dry hawthorn extract is characterized as infinite time (no flowability) (Table 1). Data from studies of the re-milled lyophilized protein substance showed good flowability. But the flow rate for the mixture of the

Table 1. The physicochemical and pharmacotechnological properties of the dry hawthorn extract and the re-milled lyophilized protein and their mixture

Indicator	Ground lyophilized sunflower protein	Dry hawthorn extract	Mixture of sunflower protein and hawthorn extract (1.4 : 1)
Bulk volume, (V_0), ml	161±2.5 ²	223±2 ³	182.5±0.5 ⁴
Settled volume, (V_{10}), ml	152±2 ²	193±1 ³	162.5±0.5 ⁴
Settled volume, (V_{500}), ml	121±1 ²	152±2 ³	120.5±0.5 ⁴
Settled volume, (V_{1250}), ml	117±0.5 ²	141±1 ³	117.2±0.3 ⁴
Settled volume, (V_{2500}), ml	117±0.3 ²	132±1 ³	117.2±0.3 ⁴
Settling qualities, (V_{10} - V_{500}), ml	31±1	41±1	42±0.5
Bulk density, (m/V_0), g/ml	0.62±0.01	0.18±0.01	0.27±0.01
Tapped density, ($m/V_{1250;2500}$), g/ml	0.85±0.01	0.30±0.02	0.43±0.01
Flowability, g/sec	7.47±0.36	A powder does not fall	1.87±0.04
Flowability, sec / 100 g	13.38±0.65	An infinite time	53.31±1.01
Angle of repose, degrees	25±1	-	40±1
Carr index, %	27±1	41±0.2	36±0.3
Hausner ratio	1.38±0.02	1.67±0.02	1.56±0.01
Moisture content, %	5.12±0.11	2.64±0.60	5.08±0.44
Moisture absorption in 100% rel. hum., 25°C, 24 h, %	10.21±1.01	19.34±2.41	13.86±2.25

Note:

1. $n = 5$; $P = 0.95$.

2. For 100 mg

3. For 40 mg

4. For 50 mg

lyophilized protein substance – the dry extract (ratio 1.4:1, respectively) was several times smaller because of the envelopment of protein substance particles with small particles of the extract. The good flowability of the lyophilized protein substance and insufficient flowability for the mixture were confirmed in the measurement of the angle of repose as an indirect characteristic of the flowability. Although the Carr index and the Hausner ratio indicate that the protein substance flowability is unsatisfactory, the powder must be shaken and stirred when dosed on the automatic equipment. These indicators show very poor flowability for the extract, and bad flowability for the mixture because of the large physical interaction of particles between them.

The ground sunflower protein substance without addition of the extract can be associated with middle powders by the tapped density ($0.6 \div 1.1 \text{ g/ml}$) (12). The dry extract and the mixture of medicinal substances were characterized by a slight bulk density and tapped density ($<0.6 \text{ g/ml}$), which provides the basis for the classification of these powders as light powders. There were also high settling qualities of the mixture, which is a negative phenomenon to achieve homogeneity of the dosage during the work of capsule filling machines.

The results of the studies on the absorption of moisture by active substances and their mixture at 100% relative humidity in a desiccator at 25°C for 24 h showed that the protein substance has the ability to absorb moisture, the substance became darker in color, but no significant changes in the consistency occurred.

In contrast, the dry hawthorn extract has turned into a dense mass of dark brown color during the absorption of moisture, that is, it is very hygroscopic substance. The mixture of substances became a thick, ductile, sticky mass of dark brown color.

As can be seen from the results obtained, a mixture of powders of the lyophilized sunflower protein substance and the hawthorn extract is characterized by the poor flowability, low bulk density and tapped density, a significant settling quality, requiring the introduction of auxiliary antifriction glidants into the composition of the mass for the encapsulating, or the obtaining a granulate to improve these indicators. The composition of the mass also requires the

addition of moisture regulators because of the moisture absorption, which can be observed when filling capsules and storage of a finished product.

CONCLUSION

The studied physical, chemical and technological properties of the dry hawthorn extract and the lyophilized sunflower protein substance and their mixture allowed the prediction of the technological methods in the manufacture of capsules on the basis of these active substances.

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