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INTRODUCTION

The relevance of the topic. Biologically active substances (BAS) occupy an important place in pharmacy due to their complex pharmacological action and the absence of harmful impurities [1]. In particular, flavonoids are one of the important classes of BAS, which are widely used due to their pronounced antioxidant effect [2, 3, 4]. They also have anti-inflammatory, antiviral, antitumor and immunomodulatory effects [5, 6].

Increasing the yield of flavonoids during extraction from medicinal plant raw materials is an urgent task for the pharmaceutical, cosmetic and food industries, as this improves the efficiency and quality of the final products containing this extracted product [7]. Various methods and strategies are used to achieve this goal [8, 9, 10, 11, 12].

Physicochemical conditions such as temperature, extraction time, raw material to extractant ratio, pH influence the process of flavonoid extraction from plant materials [13, 14, 15, 16]. Studying these parameters helps to select the optimal conditions for maximum yield of flavonoids. Such methods of process intensification (MW) as and ultrasound (US) radiation, temperature are of great importance [8, 9, 11, 12]. The nature of the extractant and the use of additives such as surfactants play a special role [17].

The quality and quantity of extracted flavonoids from plant raw materials is largely determined by the colloidal properties and processes occurring at the interface of phases: the surface properties of the "particles of plant raw materials - extractant solution", the degree of fragmentation of raw materials, the nature of wetting and swelling of dried plant raw materials, the rate of diffusion of flavonoids from crushed particles into solution, the rate and degree of desorption of extracted substances. The phenomena occurring at this interface depend on the size of the surface, on the ability to change the charge of the surface depending on the nature and composition of the solvent, pH, surfactant additives, electrolytes, temperature, mechanical action and other factors. Physicochemical conditions such as temperature, extraction time, ratio of raw materials to extractant, and pH affect the process of extracting flavonoids from plant raw materials. Studying these parameters helps to choose the optimal conditions for maximum flavonoid yield [8, 18]. Such methods of process intensification as MW and US radiation and temperature are important. The nature of the solvent and the use of additives such as surfactants play a special role.

An in-depth study of the extraction process expands the understanding of the underlying mechanisms. The interfacial layer that forms between the vegetable raw material and the solvent is a key element of the extraction process. It contains the processes of dispersion, adsorption, desorption and diffusion of flavonoids. Studying the mechanisms of these processes makes it possible to improve the extraction efficiency and control the quality of the resulting flavonoids [8, 18].

Research in this area may lead to the development of new and more effective methods of flavonoid extraction, which, in turn, will improve the quality and safety of resulting products in the pharmaceutical, cosmetic and food industries.

The degree of development of the research topic. There are many studies in the field of flavonoid extraction covering various aspects of this process. Some of them focus on optimizing extraction conditions to increase yield and product quality, while others seek to understand the mechanisms of interaction between solvents and flavonoids in order to improve process efficiency [8, 9, 10, 11, 12].

At the same time, the colloidal patterns of flavonoid extraction from plant raw materials have not been studied in practice. For example, the specific effects of individual surfactants and mixtures of surfactants on this process have not been studied. There are no studies examining the relationship of the surface charge of plant material particles with the processes of their swelling and desorption. Despite significant efforts in this area, there are still many unresolved issues. For example, there is potential for further investigation of the effects of various factors such as solvent type, temperature, pressure, and extraction duration on the yield and quality of flavonoids. It is also important to deepen the understanding of the influence of various physicochemical methods of exposure to ultrasound or microwave treatment on the processes of crushing, wettability, swelling of plant particles, on the degree of desorption of flavonoids and on the final characteristics of the product. In addition, some aspects of the interaction between flavonoids and plant material components, such as other phytochemical compounds and cellular structures, remain poorly understood. Understanding these interactions can help optimize the extraction process and increase its efficiency.

The extraction of BAS from plant raw materials depends on various factors, and each type of raw material requires an individual approach. Plants contain a complex of compounds that can be isolated using a variety of extraction methods. Substances extracted together with flavonoids can also affect yield of the target components [19].

It should be note that, as a rule, researchers consider the extraction process from a biochemical, pharmacological, pharmaceutical and phytotechnological point of view [1, 8, 11, 15, 18], without paying sufficient attention to the colloidal aspects occurring in the interfacial layer - "the surface of the particles of plant raw materials is an extractant solution." Extraction from a colloidal point of view can be considered as the desorption of substances contained in the solid phase into a solution. It is determined by the surface properties of the "particles of vegetable raw materials - extractant solution". The phenomena occurring at this interface depend on the size of the surface, on the ability to change the charge of the surface depending on the nature of the extractant, pH, surfactant additives, electrolytes,

temperature, mechanical action and other factors. The study of these processes is undoubtedly of interest both from a scientific and practical point of view.

Thus, despite the significant amount of existing research, the field of flavonoid extraction remains subject to active research, offering many opportunities for further discoveries and innovations.

The purpose and objectives of the study. The purpose of this work is to study the effect of colloidal properties of plant material dispersions on the process of flavonoid extraction.

To achieve this goal, it was necessary to solve the following tasks:

1. determine the composition of the Sophora Japonica fruits (SJF);
2. to study the effect of grinding, wetting and swelling of dried vegetable raw materials on the extraction of biologically active substances;
3. to evaluate the dependence of the surface charge of plant particles on the composition of the extractant and its effect on the extraction process;
4. to determine the value of the electrokinetic charge of the surface of crushed particles of vegetable raw materials on the process of extracting flavonoids from the SJF.
5. to study the effect of the nature and pH of the solvent, surfactant additives, and electrolytes on the rate and completeness of flavonoid extraction from SJF;
6. to study the influence of physical factors (temperature, MW and US, freezing) on the physicochemical processes occurring in the interfacial layer;
7. using the method of multiple extraction, determine the total content of flavonoids in the SJF;
8. to study the processes of desorption of flavonoids from plant raw materials;
9. determine the antioxidant activity of the resulting extract.

Scientific novelty of the research. The scientific novelty of the dissertation consists in the development of colloidal factors that determine the process of flavonoid extraction from plant raw materials.

For the first time, it was determined that in the area of the isoelectric point of the surface of the SJF particles, there is no minimum swelling, unlike solutions of high-molecular compounds (HMC). This is due to the fact that the SJF particles have a cellular structure and do not fold into a globule, as in HMC solutions.

It has been determined that surfactants have a significant effect on the extraction of flavonoids from plant raw materials. This effect depends on the type of surfactants used and their concentration and is due to an improvement in the wettability of the surface of the particles of crushed SJF, a decrease in interfacial tension and an increase in the solubilization of flavonoids. The use of a mixture of surfactants in the process of flavonoid extraction from SJF demonstrates a synergistic effect.

The value of swelling of SJF on the process of flavonoid extraction was estimated. The factors influencing the process of swelling of SJF have been established.

It was proven that the pH of the medium significantly affects the completeness of extraction. With an increase in pH, the content of flavonoids in the extraction increases due to an improvement in the solubility of flavonoids in the extractant, facilitating their diffusion and the destruction of the skeleton of the dried plant cell.

Established for the first time that the effect of ethyl alcohol content in the solvent on the extraction process depends on the chosen method. Extraction methods may have different requirements for the extractant and have different effects on the yield of the target components and the extraction quality.

The processes of the influence of MW and US radiation on the extraction of flavonoids from SJF have been studied and it has been determined that such an effect is insignificant without additional exposure to temperature after processing. However, if heated extractions are performed after preliminary irradiation of plant raw materials, the extraction of flavonoids increases significantly.

The theoretical and practical significance of the work. The results of studying the colloidal properties of plant particle dispersions, such as swelling, electrokinetic parameters, $\mathrm{pH}_{zero\ charge\ point}$ and $\mathrm{pH}_{isoelectric\ point}$ and the influence of various factors on them (nature of the liquid phase, temperature, pH, electrolytes, surfactants), analysis of the processes of desorption of BAS from plant raw materials contribute to the development of colloidal chemistry of natural plant dispersed systems.

The practical significance of the dissertation work is to optimize methods for extracting flavonoids, which, in turn, will improve the quality of the final products. In particular, surfactant mixtures have been developed, the use of which allows doubling the yield of flavonoids. The extract obtained using extractants with surfactant additives can be used in the cosmetic or pharmaceutical field to create creams, ointments, micellar solutions with antioxidant and capillary-strengthening effects. Flavonoids obtained from the fruits of Sophora japonica can also be used to obtain medicinal shampoos, as they help in the fight against dandruff. Optimal conditions for processing the fruits of Sophora japonica with US and MW radiation have been developed; as well as a temperature regime to increase the degree of flavonoid extraction, which will improve the technological processes associated with extraction from plant materials.

Methodology and research methods. The work used static and dynamic methods of flavonoid extraction from Sophora Japanese fruits (maceration and percolation) with varying process temperatures, as well as using US and MW radiation.

Spectrophotometric methods were used to quantify flavonoids.

A conductometric method, potentiometric titration, and electrophoresis were used to analyze the extraction.

Provisions to be defended

1. The main colloidal parameters of the dispersion of crushed particles of the SJF affecting the yield of flavonoids during extraction are: particle size, swelling degree, electrokinetic potential, $pH_{zero\ charge\ point}$ and $pH_{isoelectric\ point}$, dispersion viscosity.

2. A process has been developed for obtaining extracts with a high content of biologically active substances, namely, containing the maximum possible amount of flavonoids in a given type of plant material. The following has been determined:

2.1 the influence of the nature of surfactants and their mixtures on the mechanism of flavonoid extraction from plant materials;

2.2. the effectiveness of choosing a solvent as an extractant;

2.3. the effect of ultrasound radiation on the extraction of flavonoids during heating;

2.4. The effect of microwave radiation on the process of extracting flavonoids from crushed SJF.

The features of the desorption process and its influence on the efficiency of flavonoid extraction from plant raw materials are established.

The mechanism of intensive destruction of plant cells, facilitating the extraction of flavonoids, is described.

Main scientific results. The dissertation defines the influence of colloidal factors and physicochemical conditions on the extraction of target components - flavonoids from SJF.

1. The optimal SJF particle sizes for the extraction of flavonoids are determined - 0.1 - 0.2 cm. Particle sizes less than 0.1 cm complicate the extraction of target components due to the formation of mucus and filtration difficulties; particle sizes greater than 0.2 cm are less effective due to a decrease in the contact surface of the extractant with the particles [20, 21] (the personal contribution of the author is 90%, the articles are written by Vasil'yeva P.A. under the supervision of Dmitrieva I.B.).

2. The significance of swelling of plant materials on the process of flavonoid extraction is estimated. Factors influencing the swelling process of SJF particles have been determined [22] (the author's personal contribution is 70%):

• it has been shown for the first time that in the region of the isoelectric point of the surface of Japanese pagoda tree particles, a minimum of swelling is not observed, unlike in HMC solutions. This is due to the fact that the particles have a cellular structure and do not fold into a globule, as in HMC solutions;

• the highest degree of swelling occurs for particles with a size of 0.1 - 0.2 cm, since with a decrease in the particle size, the viscosity of the extractant increases and the diffusion of the solvent in the cell decreases;

• the highest degree of swelling is noted in a solution of a non-ionic surfactant (Laureth-2). Ionic surfactants are characterized by stronger hydration, which does not contribute to swelling and can lead to the reverse process - dehydration of particles;

• an increase in temperature significantly increases the degree of swelling of the SJF due to an increase in the rate of solvent diffusion and more active destruction of plant cells;

• ultrasound radiation contributes to an increase in the degree of swelling of the SJF due to greater destruction of the plant cell membrane [20, 21, 23] (the author's personal contribution to the indicated articles is 90%, the articles were written by Vasil'yeva P.A. under the supervision of Dmitrieva I.B.).

3. The effect of various surfactants and their mixtures on the extraction of flavonoids from SJF was assessed [20, 21, 22] (the author's personal contribution is 90%, 90% and 70%, respectively). The highest yield of flavonoids was noted in the presence of a non-ionic surfactant. Cationic and anionic surfactants do not affect the degree of extraction of flavonoids from SJF. Nonionic and zwitterionic surfactants and their mixtures have higher surface activity and significantly reduce the interfacial tension at the "cell - surfactant solution" boundary, promoting intensive destruction of plant cells, and thereby facilitating the extraction of flavonoids.

4. It was determined that in the alkaline pH region, the value of the electrokinetic potential of Sophora particles increases in absolute value due to an increase in the sorption of OH^- ions both on the surface of the particles and on the active centers of flavonoid molecules, which enhances their transition to the diffusion layer of the DEL and desorption from Sophora particles [20, 21] (the author's personal contribution to the noted articles is 90%, the articles were written by Vasil'yeva P.A. under the supervision of Dmitrieva I.B.). Comparison of the yield of flavonoids in the $pH_{isoelectric\ point}$ and in the alkaline region at high values of |ζ|-potential shows a twofold increase in the yield of flavonoids.

5. It was determined that the effect of ultrasound and microwave radiation during extraction with heating significantly increases the degree of flavonoid extraction due to the destruction of the plant cell, improved desorption and dissolution of biologically active substances in the extractant [20, 21, 22] (the personal contribution is 90%, 90% and 70%, respectively).

6. The results of studying the desorption of flavonoids from plant materials made it possible to establish that the kinetics of desorption corresponds to first-order reactions. Calculation of the degree of flavonoid extraction based on the results of desorption showed that their yield is higher in aqueous-alcoholic solutions than in water, which is associated with an increase in the solubility of flavonoids in aqueous-alcoholic solutions and is consistent with the results of the extraction study.

The degree of reliability and approbation of the results. The reliability of the results of the dissertation work is confirmed by their high reproducibility, the use of standardized methods and a generally recognized modern complex of physical and chemical methods of analysis. The main results of the dissertation work were reported at the following conferences:

The main results of the dissertation work were reported and discussed at the International Scientific Conference of students, postgraduates and Young Scientists "Lomonosov-2019", "Lomonosov-2020", "Lomonosov-2023", Moscow, 2019, 2020, 2023; the All-Russian conference "Surface phenomena in dispersed systems", dedicated to 125-anniversary of the birth of the outstanding Soviet scientist, Academician of the USSR Academy of Sciences Peter Alexandrovich Rebinder, Moscow, 2023; All-Russian scientific and practical conferences with international participation "Modern achievements of chemical and biological sciences in preventive and clinical medicine", St. Petersburg - 2020, 2021, 2023, 2024; All-Russian scientific conferences of students and postgraduates with international participation "Young pharmacy – the potential of the future", St. Petersburg - 2021, 2022, 2024; X and XII Interuniversity Conference-competition (with international participation) of scientific works of students named after Corresponding Member of the USSR Academy of Sciences Alexander Alexandrovich Yakovkin, St. Petersburg – 2021, 2022.

Based on the materials of this dissertation, 18 publications have been published, including 5 articles in scientific journals included in the list of peer-reviewed scientific publications (3 of which correspond to the scientific specialty 1.4.10 Colloid Chemistry (Chemical Sciences)), recommended by the Higher Attestation Commission of the Russian Federation, 3 abstracts of reports at scientific conferences.

CHAPTER 1. LITERATURE REVIEW

1.1 Colloidal factors influencing the process of extraction of BAS from plant materials

Extraction of biologically active substances from plant raw materials is the process of isolating various compounds with physiological activity and useful properties from plants (solid body) using an extractant (extracting liquid).

Extraction consists of the following main stages: wetting of plant raw materials and swelling, formation of internal primary juice and mass exchange [24]. Each stage depends on certain factors that can either increase or decrease the speed and completeness of extraction.

1.1.1 Grinding of plant material

Grinding of plant materials helps to increase the yield of target components, since the degree of grinding affects the contact surface of the phases (the larger it is, the faster the diffusion occurs) [19, 25].

However, the use of very fine plant powders for extraction may not be recommended for the following reasons [24, 25, 26]:

- ***losses:*** Very fine particles can easily be sprayed and washed out together with the solvent during the extraction process, which will lead to the loss of valuable plant components.
- ***reduction of diffusion of target components:*** Very fine powder forms a pasty or gelatinous mass with the extractant, the extraction of which is difficult, since it has a high resistance to the passage of the extractor.
- ***difficulties in filtration:*** *Fine particles can clog the pores of the filter or cake and prevent the passage of the extractant between them. They also contain many destroyed cells, from which a large amount of ballast substances, insoluble particles and colloids pass into the extract. The result is a cloudy, difficult to clean liquid.*

Usually, the following grinding size is recommended for various plant materials (coarser grinding is not advisable, since the process of extraction of medicinal substances slows down in the layer, and during prolonged infusion, a lot of ballast substances pass into the extraction): leaves, flowers and herbs - up to particles of 3-5 mm in size, stems, roots and bark - up to particles of 1-3 mm in size, fruits and seeds - up to particles of 0.3-0.5 mm in size (since the membrane of their cells is covered with hydrophobic substances) [10, 18, 27].

The nature of grinding of plant materials has a great influence on the extraction process and the quality of the extract. For example, the Kharkov Research Chemical and Pharmaceutical Institute conducted a study of the influence of various methods of grinding plant materials on the efficiency of extraction. It was found that grinding of plant materials is advisable to carry out on roller crushers, since in this case dynamic equilibrium in the solid-liquid system occurs faster.

The cells of dry plant material are filled with air. Air and porous partitions of organelles resist the passage of the solvent. During rolling, the raw material is crushed, as a result of which air cavities rupture and microcracks form. This leads to more effective destruction of plant tissues, accelerates the process of penetration of the extractant and dissolution of the extracted substances. To intensify the extraction process for seeds and fruits, a combined grinding method is recommended: first in a hammer or impact-centrifugal mill, and then in a roller crusher to a particle size of 0.1 - 0.2 mm.

1.1.2 The process of wetting and swelling of dried plant material as the first stage of extraction

An important stage of the extraction process is the swelling of dried plant material. Plant material undergoes significant changes during drying: dry residue is obtained from the cell juice, the inner part of the cell is filled with air, the cell wall and membranes of the cell organelles acquire the properties of porous partitions after drying [28].

The cell framework has diphilic properties, but the hydrophilicity of the cellulose is expressed much more strongly than its hydrophobicity. In this regard, the use of hydrophilic extractants is more effective at this stage [29]. This process is accompanied by an increase in the mass and volume of plant materials [30].

The cell membrane of plant materials has a complex composition: it is based on water-soluble cellulose, pectins - soluble and insoluble in water, lignin. The cell membrane is inclusions of cutin, suberin, which are not wetted with water. The dried plant cell has its own characteristics: during the drying process, the cells of plant materials lose moisture, the protoplasm wrinkles, and the contents of the cells turn into a dry residue. The inner part of such a cell consists of air, and the dried material is a spongy structure. Unlike a living cell, where the extractant penetrates into the cell through osmosis through semi-permeable protoplasm, the extractant penetrates into the dried plant material through a porous partition [26].

When processing crushed plant material, the extractant penetrates through the pores into the cell due to wetting and capillary forces. Surface tension and viscosity of the solvent are of great importance in the extraction process. When absorbed, the liquid should spread over the cell surface, which leads to a significant increase in the contact surface and accelerates the process of dissolution of the extracted substances. The greater the surface tension of the extractant, the more difficult it is for the plant material to be saturated with liquid [18].

Since the cell remains a concentrated solution as a result of drying – “primary juice”, due to the difference in osmotic pressures, soluble substances leave the cell, and the solvent penetrates into it; “colliding” of osmosis and dialysis processes leads to swelling of the plant material.

Filling the cell capillaries with an extractant can take a long time, since air prevents the movement of liquid. This process can be accelerated by vacuuming. If the extractant wets the raw material well, the

process of filling the cell is accelerated, thus the spreading coefficient depends on the wetting angle (wettability of the raw material) and the surface tension of the extractant.

Surface tension is calculated using the following formula (1):

$$d_{13} = d_{23} + d_{12}\cos q \quad (1),$$

where d_{13}, d_{23}, d_{12} – surface tension at the phase boundary; phase numbers 1 - extractant, 2 - air (gas), 3 – solid, q - contact angle.

If $d_{13} > d_{23} + d_{12}$ the liquid phase spreads over the surface of a solid, the driving force of the wetting process is spreading, and the spreading coefficient S is determined by the formula (2):

$$S = d_{13} - d_{23} - d_{12} \quad (2)$$

Thus, the better the raw material contacts the extractant, the easier and more BAS will pass from the cell sap into the extractant. When in contact with the extractant, soluble substances dissolve, high-molecular compounds (HMC) and colloidal substances swell, then the infinitely swelling substances pass into the sol, and some gels are peptized. The degree of swelling of the raw material depends on the chemical nature of the liquid. The strongest swelling is caused by water, the least by non-polar solvents. The swelling of the raw material during extraction with alcohol depends on the water content in it. Being a hydrophilic substance, alcohol competes with the cellular colloid for water [18].

The dry residue in the cell is a complex of substances that can be both soluble and insoluble. Soluble substances can be adsorbed to insoluble substances [26; 31; 32]. Then the extractant diffuses into the cell under the action of capillary forces.

The swelling process of plant materials depends on many factors, including:

- the type of medicinal raw material (leaves, flowers, roots, fruits, etc.), as well as its chemical composition [33, 34, 35];
- particle size [19, 36];
- charge of particles of medicinal plant raw materials [37],
- nature of the extractant;
- pH value, ionic strength and osmotic activity of the extractant [38, 39];
- presence of surfactants in the solution [40],
- contact time of particles with the extractant [41],
- temperature at which the swelling process is carried out [9, 27].

The composition of plant material significantly affects the process of its swelling. The determining factor is the ability of high-molecular compounds, which make up the plant cell, to interact with the extractant [19].

When swelling in water - the presence of polar groups in the HMV macromolecule. The swelling degree can be assessed for substances with limited swelling. The presence of hemicellulose and lignin

in the cellulose suggests that the swelling of the plant cell can be limited, since these substances have a branched and cross-linked structure, and the free space of this structure is filled with an extractant upon contact [19].

Proteins and polysaccharides have the ability to swell in water, and the water-polymer interaction is of an adsorption nature. Moreover, when heated, some proteins, such as albumins, can dissolve [19].

There are two types of swelling: limited and unlimited. With unlimited swelling, the increase in mass and volume of the polymer occurs throughout the entire time and ends with the dissolution of the polymer. With limited swelling, the increase in volume and mass occurs over a certain period of time and then remains unchanged.

The quantitative characteristic of swelling is the degree of swelling, which shows the relative increase in mass and volume of the HMV during the hydration process [19]. It depends on the substances that make up the framework of the plant cell (chain rigidity), as well as the affinity of the cell to the solvent. It is calculated using formulas 3 or 4, depending on the research methods:

$$\alpha_t = \frac{m_t - m_0}{m_0} \quad (3)$$

$$\alpha_t = \frac{V_t - V_0}{V_0} \quad (4)$$

where m_t and V_t mass and volume of raw materials respectively through swelling time; t, m_0 and V_0 mass and volume at the initial moment of time.

The maximum (limit) degree of swelling is the degree of swelling at the time when the swelling process ceases, i.e. the volume and mass of the polymer cease to increase. Since the degree of swelling with unlimited swelling increases with increasing time and is ultimately accompanied by dissolution of the polymer, the maximum degree of swelling can be determined for infinitely swelling substances (which also include plant materials). The swelling rate indicator in this case is the swelling constant, which is calculated using the formula [30] (5):

$$K_t = ln \frac{\alpha_{max}}{\alpha_{max} - \alpha_t} \quad (5)$$

where K_t – swelling rate constant, min^{-1}; α_{max}– maximum swelling degree; t – time, min.

In limited swelling, the maximum degree of swelling reaches a limiting value, after which it remains constant throughout the entire time. This occurs due to strong intermolecular interactions in the polymer; the contribution of free energy due to the enthalpy of displacement becomes equal to the contribution of free energy due to the elastic retraction forces in the network; there is no driving force for swelling and the extractant cannot separate the macromolecules, swelling equilibrium is achieved [42].

There are two types of sorption centers with different binding energies. Primary sorption centers are hydrophilic centers of the cell membrane HMB (cellulose, hemicellulose and lignin). Secondary sorption centers are sorption centers of the first and subsequent water layers [30].

Swelling of plant materials consists of two stages [19]. In some studies, the swelling process of plant materials is divided into three stages (the first is fast, the second is slow, and the third is stationary) [43]. But with any division, the division mechanism is similar.

At the first stage, a weak exothermic reaction occurs, during which free water is bound. This process occurs most quickly. Water absorption occurs during interaction with primary sorption centers, and water molecules are directly bound to the -OH groups of the HMWS of the plant cell membrane. [26]. And at the second stage, osmotic activity is important, during which free water is absorbed, filling the voids in the cell framework [19].

Changing the ***pH value*** of the environment can also affect the maximum degree of swelling, but this effect is individual and depends on the chemical composition of the raw material. Crushed medicinal plant raw materials have a certain value of the isoelectric point. Proteins included in the composition of the raw material at the isoelectric point are able to fold into globules and, thereby, their degree of swelling decreases [19]. However, all components influence the isoelectric point of medicinal plant materials and the dependence of swelling may be individual.

In the study “Carbohydrate Gel Beads as Model Probes for Quantifying Non-ionic and Ionic Contributions behind the Swelling of Delignified Plant Fibers” by Rose-Marie Pernilla Karlsson et al., it was determined that with increasing charge density on gel beads, analogous to plant cell walls, there is a marked increase in water uptake with increasing pH of the solution. In this work, it was determined that the maximum uptake is possible at a pH value of 10. This phenomenon occurs due to the dissociation of carboxyl groups at pH values above the pK_a of the charged groups. In turn, this dissociation increases the osmotic pressure inside the beads due to the imbalance of ion concentrations inside and outside the gel beads as a result of the immobility of the carboxyl groups [38].

An important characteristic of the raw material swelling process is the thermal effect of wetting (Q), which occurs when dried plant material is added to water [36]. Using this value, it is possible to calculate the specific surface area (S) of one gram of the wetted plant [36]. For this, the following is taken into account: wetting occurs uniformly, water molecules combine with the surface of the solid and a non-molecular adsorption layer is formed. The specific surface area of one gram of the wetted plant is determined by formula 6 [40]:

$$S = \frac{Q}{q} \tag{6}$$

where q - the total energy of the water surface between the liquid-vapor phases, which is equal to $116 \cdot 4{,}188 \cdot 10^7$ J/cm^2.

1.1.3 Electrokinetic properties of plant material dispersion

Zeta potential can affect the electrostatic interactions between plant material particles and the solvent. This in turn can affect the ability of the solvent to extract certain components from the plant material.

The point of zero charge and the isoelectric point are important in the extraction process and affect the behavior of the particle surface in a dispersed system.

The point of zero charge is the pH of the solution at which the surface of the particle has no charge. At this point, the positive and negative ion charges on the surface of the particle cancel each other out, resulting in zero charge. This is important because at a pH below the point of zero charge, the surface of the particle will be positively charged, and at a pH above it will be negatively charged.

The isoelectric point is the pH value at which the particle surface charge is zero. At this point, the transition between positive and negative charge dominance on the particle surface occurs. Beyond the isoelectric point, the particle surface will be positively charged at low pH values and negatively charged at high pH values.

During the extraction process, the isoelectric point and the point of zero charge can affect the interaction of plant material particles with the solvent and other components of the mixture:

- At a pH close to the isoelectric point, the interaction between plant material particles and the extractant may be the smallest, which may complicate the extraction process.

- Around the point of zero charge, the particle surface may be less charged and more susceptible to aggregation, which may also affect the extraction efficiency.

Therefore, taking these parameters into account, as well as controlling the pH of the solution, can be important when developing extraction strategies to maximize the efficiency of extracting BAS from plant material.

The surface charge of the particle and the pH of the solution affect the ***electrostatic interactions*** between the particles and the solvent. This can lead to different adsorption and diffusion forces that affect the efficiency of the extraction process.

The surface charge of the particle can also affect the ***solubility of the BAS*** in the extractant. For example, certain types of solvents or extraction conditions may be preferable for charged particles.

Changes in the pH of the solution can also affect ***interactions with other components***, such as salts, other BAS, or additives that may be present in the plant material or solvent.

1.1.4 Selecting an extractant

Extraction can be carried out using various solvents, such as ethanol, methanol, water, propylene glycol, etc. The choice of the optimal extractant in the technology of phytochemical preparations is of great importance [13, 14, 15].

The extractant must meet the following requirements:

• have the ability to extract certain classes of compounds and minimize the extraction of ballast substances;

• facilitate the desorption of flavonoids and their effective dissolution. Different solvents have different affinities for flavonoids, so their choice can affect the extraction efficiency. Polarity, dielectric conductivity, viscosity and other properties of extractants are of great importance;

• wet the material well. This property ensures effective contact between the extractant and the plant material, which in turn contributes to more effective extraction of active substances;

• be inert to medicinal substances and should not change their pharmacotherapeutic properties. This is important to ensure the safety of active ingredients and the effectiveness of the final medicinal product;

• be safe for humans during subsequent use of the extract;

• be available.

Some authors note that solutions of dimethyl sulfoxide (DMSO), polyethylene oxide 400 (PEO 400), propylene glycol (PG) also have high extraction capacity in relation to flavonoids [17, 44].

However, the processes of solvation of plant material and desorption of BAS in the case of DMSO differ from alcohol solvents due to its aprotic nature. As a result, the overall extraction capacity of DMSO does not exceed that of aqueous solutions of PG and PEO 400. PEO 400 has a high viscosity, which can negatively affect the extraction process [17].

Water and ethyl alcohol are most often used as extractants for the extraction of flavonoids, since many of them are soluble in water, alcohol, or a mixture of both [13]. n particular, flavonoid aglycones are more soluble in alcohol, and glycosides in water. Ethanol is most widely used for the extraction of flavonoids due to its higher yields [28, 45].

Advantages of purified water as an extractor:

• penetrates cell walls well,

• dissolves many medicinal substances (including alkaloid salts, glycosides, tannins better than other extractors),

• availability,

• cheap,

• compliance with all safety requirements,

• pharmacological indifference.

Disadvantages of purified water as an extractor:

• does not dissolve non-polar medicinal substances (e.g. oils, resins, coumarins),

• has high surface tension,

• no antiseptic properties (therefore, aqueous extracts are not stable during storage),

• causes hydrolytic cleavage of many substances (especially at high temperatures),

• has high boiling point (100 °C) and heat of vaporization (539 kcal/kg, or 2258 kJ/kg).

Advantages of ethyl alcohol as an extractant:

• has a bacteriostatic effect (microorganisms do not develop in extracts containing at least 20% ethyl alcohol),

• inactivates enzymes (therefore preventing the course of hydrolytic processes in plant tissues),

• due to the volatility of ethyl alcohol, alcohol solutions easily thicken to the state of thick and powdery substances,

• has lower values of the heat of vaporization (216.4 kcal / kg, or 906.7 kJ / kg) and boiling point than water,

• availability and relative cheapness.

Disadvantages of ethyl alcohol as an extractant:

• fire and explosion hazard,

• pharmacologically not indifferent (has local and general effects on the human body).

Many studies show that it is more effective to extract flavonoids not with pure alcohols, but with solutions of alcohols with water. Water molecules in the extractant better wet the surface of plant materials, which increases extraction [45].

The principle of "like dissolves like" is the basis for choosing a solvent for extraction. Polar extractants, such as water, methanol or ethanol, which have high dielectric constant and are able to dissolve polar compounds well, are most often used to extract polar substances. Non-polar substances, such as essential oils, coumarins and alkaloid bases, are better extracted using non-polar extractants, such as dichloromethane or hexane [10]. The polarity of an extractant can be assessed by its dielectric constant: the higher it is, the more polar the extractant. However, other factors should also be taken into account, such as the solubility of the substance in the extractant, its selectivity and stability.

The viscosity of the extractant also has a great influence on the process of BAS extraction [46]. This is due to the fact that the diffusion coefficient is inversely proportional to the viscosity of the extractant. Solutions with lower viscosity have a greater diffusion capacity, while viscous extractants are characterized by high hydraulic resistance. It is important to note that temperature also has a significant effect on the viscosity of the extractant. Usually, an increase in temperature leads to a decrease in viscosity, which improves diffusion and accelerates the extraction process.

High viscosity of the extractant and its surface tension can hinder the penetration of liquid into the narrow capillaries of cell membranes, which can prevent the complete extraction of target compounds.

pH is important in flavonoid extraction [42]. Typically, the optimal pH depends on the specific type of flavonoid and the solvent used. For example, for acid-sensitive flavonoids such as anthocyanins, the optimal pH may be below 7, while for other flavonoids such as quercetin, the optimal pH may be closer to neutral (around 7) or even slightly alkaline. It is known that at pH values above 8, flavonols are gradually oxidized by atmospheric oxygen. [16].

Changes in pH can affect the stability and solubility of flavonoids, as well as their ability to form complexes with other molecules in solution. Therefore, when extracting flavonoids, it is important to control and maintain an optimal pH value to achieve maximum extraction efficiency [16].

The properties of the solvents used as extractants are presented in Table 1.

Table 1 - Properties of solvents used as extractants

solvent	dielectric constant	boiling point, °C	density at 20 °C, g/cm^3	surface tension at 20 °C, N/m*10^3	viscosity at 20 °C, mPa*s
purified water	78,2	100,0	1,00	72,75	1,00
methyl alcohol	37,9	64,6	0,793	22,99	0,60
ethyl alcohol	25,2	78,39	0,789	22,03	1,20
Acetone	20,7	56,24	0,790	23,70	0,32
propyl alcohol	19,7	97,2	0,804	22,90	2,23
dichloroethane	10,3	83,5	1,26	32,2	0,89
methylene chloride	9,1	40,00	1,33	27,50	0,45
ethyl acetate	6,0	77,15	0,90	23,75	0,49
chloroform	4,7	61,26	1,49	27,14	0,57
ethyl ether	4,2	34,5	0,71	16,49	0,23
benzene	2,3	78,50	0,88	28,87	0,65
Carbon tetrachloride	2,2	76,80	1,595	25,68	0,97
Hexane	1,9	68,74	0,659	1,41	0,31

1.1.5 Selecting an extraction method

Most biological properties of flavonoids are largely determined by the extraction mode.

All existing extraction methods can be classified by the nature of the process into static and dynamic. In static methods, the raw material is periodically saturated with an extractant and left to infuse

for a certain period of time. Dynamic methods involve movement, constant replacement of the extractant or raw material and extractant [24].

According to the periodicity of the process, periodic methods are distinguished, when the supply of raw materials (extractant and/or plant material) to the extraction apparatus is carried out periodically, and continuous methods (with continuous supply of raw materials) [24].

According to the number of extraction cycles (repetitions), single-stage and multi-stage methods are distinguished [24].

The methods commonly used for extracting flavonoids are maceration and percolation. And methods for intensifying the process are used such as heating, using ultrasound or microwave radiation, and others.

Maceration and percolation are traditional extraction methods that have been used for many years to extract BAS from plant materials, such as flavonoids.

Maceration is a process of isolating active components where the raw material is in contact with a solvent for a certain period of time. This process can take a long time, and usually requires a large amount of solvent for effective extraction [11].

Percolation involves the process of passing a solvent through a bed of raw materials, allowing the extracted components to move from one point to another and become enriched with active substances. This method can also be time-consuming and solvent-consuming.

There are multi-step methods for extracting flavonoids, such as remaceration and repercolation (repeated percolation).

Although these methods are easy to implement and have been widely used in the past, they have disadvantages such as long extraction times, high solvent consumption, and sometimes low extraction yields [28].

Modern extraction methods such as supercritical extraction, ultrasonic extraction and microwave extraction, pressurized liquid extraction, pressurized hot water extraction can be more efficient in terms of time, resources and extraction yield. Recently, microwave flavonoid extraction method, or ultra-high frequency extraction (MW) and ultrasonic extraction technology have been widely used [12]. Supercritical fluid extraction and extraction at elevated temperature are also used.

MW is an electromagnetic spectrum of radiation in the range from 300 MHz (radio emission) to 300 GHz (infrared radiation). This heating method uses microwave energy and is based on the direct effect of microwaves on dipole polarization and ionic conductivity molecules (Fig. 1). Unlike conventional heat transfer, MW heating transfers energy directly into the volume of the mixture being extracted. Increasing the temperature of the extractant increases its dissolving capacity and reduces viscosity, which in turn improves the mass transfer process. However, the effect of microwave extraction

is not limited to increasing the temperature of the extractant. MW radiation interacts with the dipoles of polar and polarizable substances in the solvent and raw materials. This process leads to their heating and destruction of hydrogen bonds, which ultimately enhances the migration of dissolved ions and promotes the penetration of the solvent into the raw materials [11].

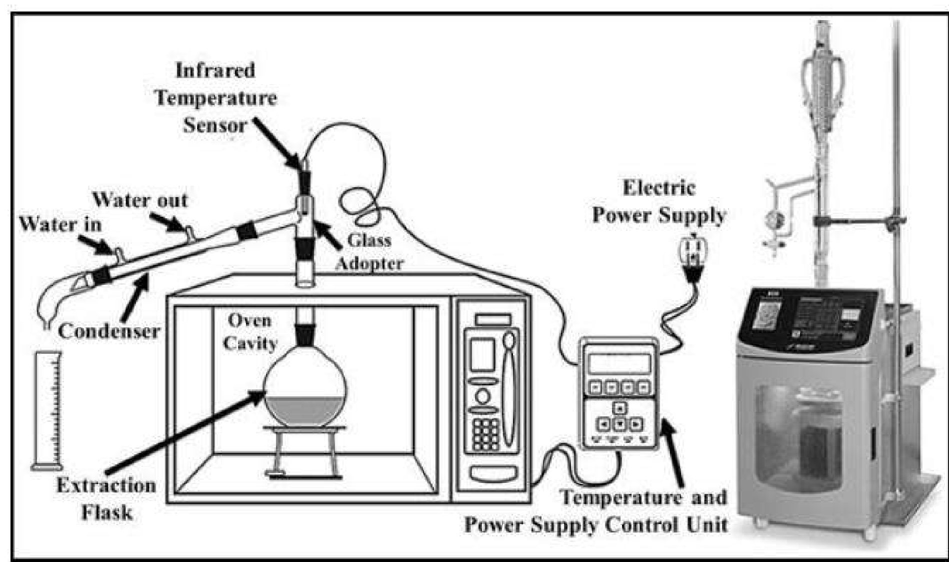

Fig. 1 - Schematic diagram of ultra-high frequency extraction

The peculiarity of MW extraction is that it affects both the raw material and the extractant [47].

The extraction of flavonoids can be influenced by a large number of parameters, among which the most important are: time, temperature, plant material to solvent ratio, solvent concentration, solvent polarity, irradiation, frequency intensity and MW power [1].

Thus, in the electromagnetic field of high-frequency waves, highly polar dielectric molecules tend to line up in space in accordance with the direction of the changing field. This leads to an increase in the release of extractable substances due to a decrease in the degree of their hydration. As a result, the size of the solvating substance molecules decreases, which enhances the overall mass transfer in the cell-extractant system. In addition, with dielectric heating, the viscosity of the intracellular juice decreases due to the appearance of internal heat sources with an uneven temperature associated with different dielectric properties of cells and tissues, which significantly increases the free diffusion coefficient. This promotes faster coagulation of protein compounds and large polymers of plant origin, which reduces their effect on the diffusion of the bulk of the intracellular contents. A positive effect of MW extraction is also a reduction in the microbial contamination of the processed raw materials by up to 10 times [24].

MW extraction allows to significantly reduce the extraction time of a wide range of compounds, and also reduces the volumes of solvents used, it has also been proven that the yields during the extraction of BAS from many types of raw materials exceed traditional methods, such as maceration,

extraction in a soxhlet apparatus, etc. The main disadvantage of this method is the significant energy costs [1, 48].

However, in some cases, the increase in the yield of BAS does not occur when treated with MW radiation. These changes may occur due to insufficient destruction of the cell membrane or destruction of target components [8].

Ultrasonic extraction is a method used to break down plant material and extract BAS. The method is based on the phenomenon of acoustic cavitation, which consists of the formation of bubbles and subsequent rupture, causing the release of BAS, and this rupture depends on the extraction conditions (Fig. 2) [11].

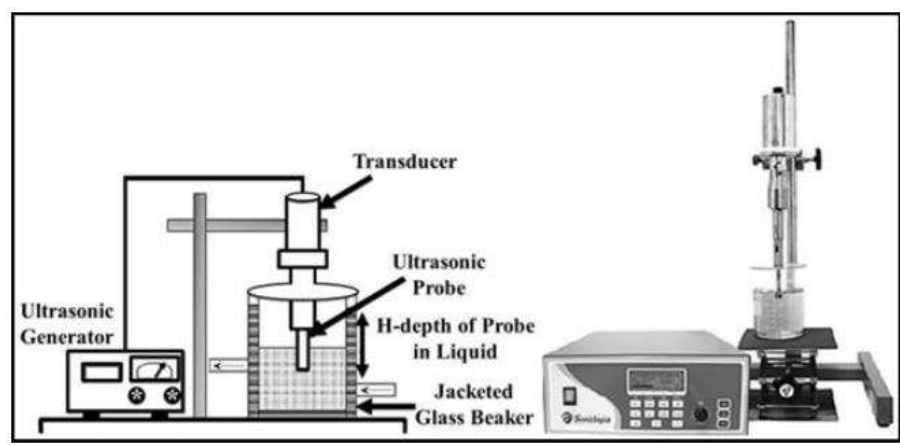

Fig. 2 - Ultrasonic extraction scheme

The cavitation effect created by this technique not only allows the cell walls of plant material to be broken down, but also helps to reduce the particle size, which favors solvent-substrate interactions. There are many variables that can influence flavonoid extraction processes and therefore the number of experiments; to optimize a specific process, experimental matrices are usually used to perform the optimization process to determine the conditions that will help to recover the maximum flavonoid content.

The advantages of this technique include high yields as well as reduced extraction times [8, 48, 49, 50]. Since this method avoids prolonged heating and high temperatures, it can be recommended for the extraction of heat-labile substances [10].

The most rational for the extraction of most types of medicinal plant raw materials are ultrasound intensities in the range of 1.5 - 2.3 W/cm^2 [24].

Some authors also note that in this extraction mode, an increase in temperature during US extraction has a positive effect on the process [24].

In some cases, an increase in the yield of BAS does not occur with this type of extraction. This occurs because the diffusion of the extracted substances into the extractant and the destruction of the components that are already in it occur simultaneously [8].

Supercritical fluid extraction. Any substance at a temperature and pressure above its thermodynamic critical point is a supercritical fluid. Under these conditions, the properties of liquids give rise to high permittivity and low viscosity solvents used to improve the substance transfer process [10].

The most commonly used solvent in this extraction method is carbon dioxide (CO_2). It has numerous advantages such as being flammable, non-toxic, cheap, and very easy to remove due to its volatility. A schematic representation of this technique is shown in Fig. 3.

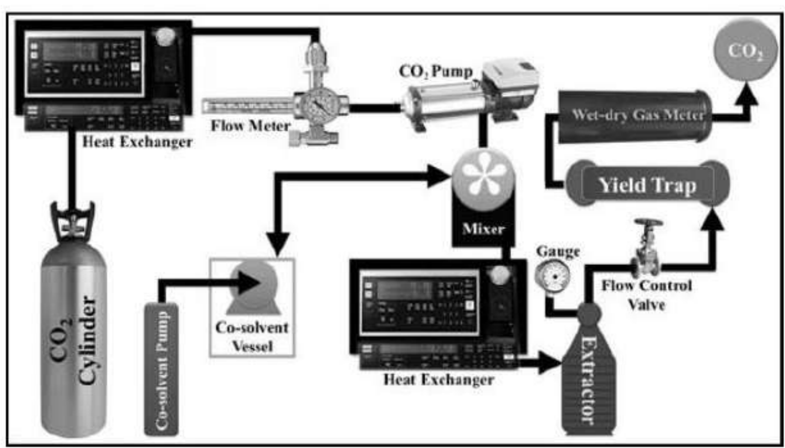

Fig. 3 - Schematic diagram of supercritical fluid extraction

Some advantages of this extraction technique are low temperatures that maintain the integrity of the products, high volatility of the solvents that keep waste at a low level, extraction without phase changes, easy separation of volatile and non-volatile compounds. However, this technique has some limitations: difficult equilibrium between the solute and solvent that may require the use of other separation processes, high pressure that prevents continuous addition of solids to the extract, high operating costs, maintenance costs, etc.

Temperature is one of the most important factors in this process. In this technique, the use of low temperatures is aimed at keeping the product as stable as possible [1].

Extraction at elevated temperature. An increase in temperature can have a significant effect on the extraction process by enhancing diffusion and desorption of active substances from plant material. As the temperature increases, the molecules move faster, the viscosity of the extractant decreases, which facilitates faster diffusion of substances through the cell walls and improves the contact of particles with the extractant [8, 9]. An increase in temperature by 50 °C leads to a 3-fold increase in the diffusion coefficient.

However, an increase in temperature is not always advisable, as it can lead to the destruction of heat-labile medicinal substances, deterioration of dissolution or evaporation of some substances (e.g. essential oils), transition to the extraction of a larger amount of ballast substances (e.g. starch, pectin, inulin), as well as colloids and insoluble substances, due to the rupture of cell walls. This can lead to the

release of a wider range of components into the solution, including ballast substances, which in turn can reduce the quality of the extraction and increase the amount of undesirable compounds, it also complicates further purification of the extract, increases its viscosity and worsens the filtration process.

1.1.6 The influence of surfactants on the extraction of flavonoids from plant materials

To increase the extraction rate and to obtain a more complete extraction, surfactants are used. These substances significantly accelerate the extraction process of many substances from plant materials [17].

Surfactants are organic compounds that have both polar and non-polar groups in their molecule. Surfactant molecules are amphiphilic or diphilic, which means that they have both hydrophilic and hydrophobic parts. Polar groups in a surfactant molecule can include groups such as hydroxyl (–OH), carboxyl (–COOH), amine ($–NH_2$), and others. Non-polar hydrocarbon chains, such as alkyl or aryl groups, provide hydrophobic properties. This diphilic structure determines the surface activity of surfactants, that is, their ability to be attracted to interphase boundaries and adsorbed on them [51].

Surfactants can significantly speed up the extraction process by reducing surface tension, which facilitates the penetration of the solvent into the material. This promotes better wetting of the material surface and increases the available surface for contact between the solvent and the extracted substance.

Different types of surfactants can change the surface tension and viscosity of the solvent, improve washing, increase mass transfer and selectivity of extraction. Some surfactants can also modify interfacial surfaces and improve the phase separation process [52, 53].

Some authors note the ability of flavonoids to be incorporated into surfactant micelles, which also increases their extraction from plant materials [17].

In addition, surfactants can also help break down emulsions and dispersions that can form during the extraction process, which can significantly increase the efficiency of the process.

The polarity of surfactants determines their ability to interact with various components of plant materials.

The molecular structure of surfactants can determine their ability to form micelles or microemulsions, which can also affect their ability to extract flavonoids.

There are various classifications of surfactants, but the main one is related to their ability to dissociate in aqueous solutions: ionogenic, zwitterionic and nonionogenic [51].

Ionic surfactants are further divided into anionic (AS) and cationic (CS).

AS dissociate to form surface-active anions. Most traditional AS, such as salts of higher aliphatic carboxylic acids, alkyl sulfates, alkyl sulfonates, alkyl benzene sulfonates and others, include hydrophobic aliphatic radicals or alkyl aromatic fragments in their structure. Hydrophilic properties are

usually provided by carboxyl, sulfate, sulfonate and phosphate groups. The cation can be a metal ion (e.g. sodium, potassium, calcium), ammonium ion or organic ion (e.g. DEA, TEA).

Some anionic surfactants can cause precipitation of alkaloids and therefore require special care when used in the extraction process.

CS dissociate to form surface-active cations. These are mainly nitrogen- and phosphorus-containing compounds. In addition to these, cationic surfactants also include sulfonium and sulfoxonium compounds, phosphonium compounds and iodonium compounds.

CS can effectively interact with anionic groups of flavonoids, such as carboxyl and phenolic groups, due to their ion-exchange properties. These properties can also contribute to a more efficient extraction of alkaloids from plant material.

Zwitterions, also known as amphiphilic surfactants, are amphoteric surfactants. This means that they can exhibit both cationic and anionic properties depending on environmental conditions such as pH. The appropriate pH of the environment allows them to exist in various ionic forms, which makes them useful in a variety of industrial and scientific applications, including water treatment, detergents, and extraction processes.

The polar group in amphoteric surfactants is electrically neutral, but the opposite charges are separated in space by structural units of the molecule. Betaines and sulfobetaines belong to this type of surfactants.

Non-ionic surfactants do not dissociate into ions in water. These include polyoxyethylene ethers of aliphatic alcohols and acids, alkyl phenols, amines, and other compounds. Non-ionic surfactants can also play an important role in the extraction process, especially in the extraction of glycosides. Their use can help to increase the yield of target compounds from plant material.

An important characteristic of all surfactants is the critical micelle concentration (CMC). CMC is the minimum concentration of a surfactant at which a large number of micelles are formed in the solution, which are in thermodynamic equilibrium with the molecules or ions. Approaching CMC, a sharp change in the physicochemical properties of the surfactant-water system is observed, which is often manifested in the appearance of characteristic kinks in the concentration dependences of various physicochemical parameters. The CMC value depends on the structure of the hydrocarbon radical in the surfactant molecule and the nature of the polar group, as well as on such external factors as the nature of the solvent, the presence of electrolytes and organic compounds, temperature and pressure [51].

Using a ***mixture of surfactants*** instead of individual components may be more effective in extracting flavonoids for several reasons [51]:

Synergistic effect: Different types of surfactants can interact with the flavonoids in the mixture, resulting in a synergistic enhancement of their extraction properties. This may improve the extraction efficiency and increase the yield of flavonoids.

Polarity: Flavonoids can have different polarities, making their extraction more efficient when using a mixture of different types of surfactants, which helps to extract both more polar and less polar compounds.

Improved wettability: A mixture of different surfactants can provide more efficient wettability of flavonoids, which facilitates their more complete isolation from the plant material.

Adsorption properties: Different surfactants can act on the surface of the solid phase (e.g., the cell walls of plant cells), improving the accessibility of flavonoids to the extractant and facilitating their more efficient extraction.

Due to different properties, each type of surfactant can have a different effect on the process of extracting flavonoids from plant materials.

For example, cationic surfactants can be effective in extracting anionic flavonoids, while nonionic surfactants can provide better wettability of plant material. Therefore, the choice of the optimal surfactant mixture depends on the specific extraction conditions and the objectives of the study.

There is a theory that surfactants contribute to better swelling of plant materials not only by reducing the surface tension of the extractant, but also by strengthening the structure of water [40]. In the article "The Effect of Surfactants on the Heat of Wetting and Swelling of Small-Flowered Oregano (Origanum tittanthum)", the authors note that "In the volume of water there are associates (s-structure) formed due to hydrogen bonds between water molecules, as well as mutually unrelated freely moving (translational) molecules (h-structure). At a constant temperature, the ratio of these structures is a constant number. The molecules (or ions) of the surfactant, being located in the "voids" between the s-structures (interstitial dissolution), strengthen the bonds between the water molecules; the probability of the transition of the h-structure to the s-structure increases. The number of translationally moving water molecules decreases. As a result, the value of the thermal effect of wetting the plant dispersion decreases" [40].

An increase in the amount of surfactant in the solution enables mutual binding of molecules of the dissolved substance. At a constant temperature, the ratio of s- and h-structures to some extent becomes unchanged. As a result, the thermal effect of wetting of plant raw materials in the solution remains almost unchanged. An increase in the concentration of the solution leads to bonds between the molecules of the dissolved substance, and the transition of the molecular solution to a micellar one. Between the molecules that make up the micelle, in a sense, there are "voids" (for example, the Hartley micelle), in which the probability of the location (immobilization) of translationally moving water

molecules increases. As a result, the amount of the thermal effect of wetting decreases sharply. An even greater increase in the concentration of surfactant leads to an increase in the number of molecules joining the micelle. A micelle related to the McBean micelle appears, and the density of molecules in the micelle increases relatively, immobilized water molecules are displaced and the number of h-structures in the volume of water increases. This circumstance leads to an increase in the thermal effect of wetting. However, the thermal effect of a micellar solution is significantly lower than the thermal effect of a molecular solution.

Thus, the choice of the optimal type of surfactant depends on the specific extraction goals and the characteristics of the plant material, as well as on the requirements for the quality and quantity of the compounds being extracted.

However, in some cases, the plant material contains natural surfactants that can be quite effective in breaking emulsions and improving the extraction process without the need to add additional surfactants.

In such situations, adding additional surfactants may be unnecessary and not lead to an acceleration of the extraction process, since natural surfactants already perform the necessary functions.

1.2 Processes occurring during the extraction of BAS from plant materials

During the extraction process, both soluble and insoluble substances are washed out of plant materials, such as starch, mucus, proteins and pectin substances.

The process of diffusion occurs through the macropores of the cells, when the molecules of the extractant and dissolved substances move from an area of higher concentration to an area of lower concentration.

The processes of osmosis and dialysis occur through the micropores of the cell membrane. Osmosis is the movement of water from an area with a lower concentration of dissolved substances to an area with a higher concentration. Dialysis is the process of separating molecules by size and charge, when smaller molecules with a lower charge pass through the membrane, and larger molecules with a higher charge are retained.

Some substances inside the cells are bound to each other or to the plant membrane. Thus, during the extraction process, the process of desorption of a number of substances occurs [24].

1.2.1 Mass exchange processes during the extraction of BAS from plant materials

The main physicochemical processes of extraction are: diffusion, which occurs until a dynamic equilibrium of the concentrations of dissolved substances in and outside the cell is reached, as well as adsorption (the process of sorption of dissolved substance molecules on the surface of the solid phase) and desorption (transition of substances from the surface of the solid phase to the extractant) [18].

Diffusion is the process of movement of dissolved substance molecules through the cell membrane. It occurs as a result of the difference in the concentrations of dissolved substances inside and outside the cell. This process is especially important when extracting active components from plant materials, since it determines the speed and efficiency of extraction. This process is called mass exchange, or mass transfer, in an isolated closed system consisting of two or more phases. It occurs spontaneously and occurs until a dynamic phase equilibrium is established, in which the same number of molecules pass from the first phase to the second per unit of time as pass from the second to the first [18].

The transfer of mass from a raw material with a cellular structure occurs through three successive and interrelated stages, which are determined by diffusion processes [24]:

1. "Internal diffusion", which covers all the phenomena of substance transfer within the particles of the raw material.

2. "Free diffusion", where the transfer of the substance occurs within the diffusion boundary layer of the solution of extractive substances that has come to the surface of the particles of plant material.

3. Transfer of the substance by a moving extractant, known as convective diffusion.

At the first stage, the transfer of molecules of dissolved substances occurs first in the extractant present in the intercellular space, then in the extractant filling the micro- and macrocracks, and finally to the surface of the pieces of material. Over a certain period of time, the concentration of the solution of extractive substances is equalized at all points inside the particle of the raw material and on its surface. [24].

Then, the substances diffuse from the surface of the raw material particle through the diffusion boundary layer of dissolved extractive substances into the extractant surrounding the raw material. This boundary layer creates resistance to further movement of the extractable substances into the extractant, and its thickness depends on the hydrodynamic processes occurring at the surface. Consequently, the process is activated by stirring - the higher the stirring speed, the thinner the boundary layer [24].

The intensity factor in mass transfer processes such as diffusion and extraction is usually associated with the difference in concentrations of dissolved substances in different phases. This is a consequence of the second law of thermodynamics, which states that spontaneous processes tend to establish equilibrium by minimizing the potential difference or intensity factors.

According to the nature of diffusion, ***three main stages of extraction*** are distinguished.

1. Diffusion of extractive substances from the interior of cells to their surface.

2. Diffusion of substances through a laminar sublayer surrounding the particle and arising due to friction forces (viscosity forces) of the extractant when flowing through the raw material layer.

3. Convective transfer of extractive substances from the outer surface of the laminar sublayer into the general flow of the solvent. Convective (forced) diffusion is more effective, the more intense the hydrodynamic regime (mixing and circulation). The thickness of the laminar sublayer also depends on the hydrodynamic regime.

The process of mass transfer in a steady state is described by equation (7):

$$dG = K*F*(C_1 - C_2)*dt \quad (7)$$

where K— mass transfer coefficient, m/s; F — surface of plant material through which mass exchange occurs, m^2; t — extraction time, c; C_1 — concentration of substance in solid phase, kg/m^3; C_2 — concentration of substances in liquid phase, kg/m^3.

The mass transfer coefficient is calculated using formula (8):

$$K = \frac{1}{\frac{R}{D_{in}} + \frac{d}{D_l} + \frac{1}{\beta}} \quad (8)$$

where D_{in}, — coefficient of internal diffusion of the substance (due to the presence of a number of porous partitions inside the cell, it is approximately 10 times less than the coefficient of molecular diffusion in the liquid); D_l, — coefficient of molecular diffusion of the substance in the liquid laminar sublayer; R — average radius of the particle size of the plant material (or 1/2 the particle size if it is not round), m; d — thickness of the average diffusion layer around the particle (laminar sublayer), m; β — coefficient of mass transfer (convective diffusion).

The main factor determining the rate of mass transfer is the internal resistance of the solid phase. A decrease in particle size leads to a decrease in resistance.

According to the Fick-Shchukarev law, the amount of dissolved substance G (kg) that has diffused through a certain layer of solvent (extractant) is directly proportional to the difference in concentrations of this layer (C_1 — C_2) (kg/m), time t (h), surface area of the layer E (m^2) and inversely proportional to the thickness of the diffusion layer d (m) (equation 9).

$$G = -\frac{D*F*t(C1-C2)}{d} \quad (9)$$

where D — diffusion coefficient equal to the amount of substance capable of diffusing per unit time through a unit surface with a concentration difference of one, m^2/h.

The diffusion coefficient depends on the type of diffusing substance (K_0), is directly proportional to the temperature (T) and inversely proportional to the viscosity of the extractor (μ) (equation 10).

$$D = \frac{K_0*T}{h} \quad (10)$$

where K_0— constant, independent of temperature and inversely proportional to the particle radius (g) of the dissolved substance (equation 11).

$$K_0 = \frac{R}{6*N_0*p*r} \quad (11)$$

where R — gas constant (8,314 462 618 153 24 J/(mol·K)); N_0 - Avogadro's number (6,023 x 102); г — radius of diffusing particles, m; T — absolute temperature, degrees; μ — viscosity, kg*s/m^2.

Thus, the diffusion coefficient increases with increasing temperature and decreases with increasing viscosity of the medium and the particle size of the extracted substance. The smaller the particle radius, the faster the diffusion [18].

At the third stage, convective diffusion occurs, which differs from molecular diffusion in that the substance is transferred not by individual molecules, but by volumes of its solution. This process occurs due to the movement of the extractant relative to the raw material [24].

The more intense the mixing, the higher the convective diffusion coefficient (β). It becomes maximum during turbulent motion. Therefore, when calculating the mass transfer coefficient, the third component can be neglected. In this case, the first component of the denominator, i.e. the value of the internal diffusion coefficient (D_{in}), becomes decisive for the extraction process, since free diffusion of substances in the laminar sublayer (the second component) has an insignificant effect due to its small thickness (d). For the analytical calculation of the mass transfer coefficient (β), an expression obtained from the mass transfer equation (equation 12, 13) is often used:

$$\beta=\frac{m}{F*\Delta C*\tau} \quad (12)$$

$$\Delta C=\frac{\Delta C_н-\Delta C_k}{2,3*lg\frac{\Delta C_i}{\Delta C_f}} \quad (13)$$

where m — amount of extracted substance; F — surface area of solid phase extraction; ΔC — mean logarithmic difference in concentrations; ΔC_i и ΔC_f — initial and final differences in concentrations, respectively.

The Fick-Shchukarev equation can also be expressed in differential form (Fick's first law) (equation 14):

$$dG = -D*F*\frac{\partial c}{\partial x}*d\tau \quad (14)$$

where $\frac{\partial c}{\partial x}$ — concentration gradient, showing the change in concentration over an infinitely small period of time (dr) per unit length of the normal (∂x). As the process proceeds, the concentration gradient decreases [18**Ошибка! Источник ссылки не найден.**].

Fick's second law describes the dynamic process that occurs when the concentration gradient changes (equation 15):

$$\frac{dc}{d\tau} = D*\frac{\partial^2 c}{\partial x^2} \quad (15)$$

The equation shows the change in the concentration of a substance at a certain point depending on time. In spatial form, the equation has the following form (16):

$$\frac{dc}{d\tau} = D_{м} * (\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}) \quad (16)$$

where $D_{м}$, — coefficient of mass conductivity.

Thus, diffusion continues until a dynamic equilibrium of concentrations in the solid-liquid system is reached. Therefore, during the extraction process, it is necessary to maintain the maximum difference in concentrations, which in practice is achieved by mixing, circulating the extractant, or replacing the extract with a pure extractant (this can be done periodically and continuously).

It is worth noting that the amount of substance that has diffused through the raw material layer is directly proportional to the duration of the process. During extraction, not only BAS but also ballast substances pass into the extract. Therefore, the end of the extraction process should be judged not by the sum of extractives, but by the number of target components. Which (for example, alkaloids, glycosides) have a lower molecular weight (300-500) and diffuse faster than HMV, in this case, increasing the duration of extraction is inappropriate (the qualitative composition of the extract deteriorates due to the high content of ballast substances). Thus, it is necessary to strive to increase the completeness of extraction with the help of factors leading to the intensification of the extraction process.

1.2.2 Kinetics of the process of extraction of BAS from plant raw materials

The kinetics of the extraction process can be described by various mathematical models, depending on the extraction conditions and the properties of the plant material. This allows us to obtain the kinetic characteristics of the extraction process, which makes it possible to study the process more deeply and develop mathematical models describing it. Several widely used approaches are used to analyze the kinetics of such processes, such as first- or second-order equations, the Peleg model, the Minchev and Minkov model, and others. They are used to describe the dynamics of component extraction from various types of plant materials [8].

A first-order equation is an equation that describes the kinetics of a process that depends linearly on the concentration of the component to be extracted (17):

$$\frac{dt}{dc} = -k_1 c \quad (17),$$

where c - concentration of the extracted component, t - time, and k_1 - first-order rate constant.

A second-order equation is an equation that describes the kinetics of a process that depends quadratically on the concentration of the extracted component (18):

$$\frac{dt}{dc} = -k_2 c^2 \quad (18),$$

where k_2 - second-order rate constant.

The Peleg model is an empirical model widely used to approximate extraction curves (19):

$$\frac{X}{X_\infty}=kt^n \quad (19),$$

where X - amount of the extracted component at time t, X_∞ - total amount of the extracted component, k и n - model parameters.

The Minchev and Minkov model is another empirical model used to describe the extraction kinetics (20):

$$X=kt^m(1-e^{-kt}) \quad (20),$$

where k and m - model parameters.

These models are used to approximate experimental data on the kinetics of the extraction process and help to better understand and optimize the process.

To obtain reliable kinetic characteristics of the process of extraction of BAS from plant material, it is necessary to take into account the influence of all extraction conditions.

The multifactorial nature of the process of extraction of biologically active substances (BAS) from plant raw materials causes certain difficulties when obtaining kinetic characteristics. The obtained kinetic data are usually applicable only to a specific extraction method, which is determined by the type of raw material, process conditions and composition of the components. Therefore, a comparison of kinetic parameters for the extraction of components from plant raw materials using different extraction methods is often not carried out and requires additional research [8].

The extraction process begins with the surface of the plant material particles. Gradually, the extractant penetrates the internal pores of the particle as they pass into the volume of the solution. The surface on which the interaction between the extractant and the extracted component occurs gradually shifts inside the particle. In this process, the resistance to mass transfer in the region between the said surface and the outer surface of the particle increases over time. If $\beta_в(t)$ is used to denote the effective mass transfer coefficient through this region, then the flow of the extracted component $j(t)$, related to the unit surface of the particle, can be expressed using the mass transfer equation (21) [54]:

$$j(t) = \beta_в(t)[C_n - C_m(t)] \quad (21),$$

where C_n – concentration of the extracted component on the surface where the reactants interact, and it corresponds to the concentration of the saturated solution; C_m – concentration of the extracted component on the surface of the plant material particle.

The same specific mass flow $j(t)$ can be described through the mass transfer equation applied to the external diffusion boundary layer of the particle (22) [50]:

$$j(t) = \beta_n(t)[C_m(t) - C(t)] \quad (22),$$

where β_n - coefficient of mass transfer from the particle surface to the volume of the extractant, which depends only on the hydrodynamic conditions in the apparatus; C(t) – concentration of the extracted component in the volume of the solution.

From equations 1.2.2.5 and equations 1.2.2.6, the following can be obtained (23):

$$j(t) = \frac{C_n - C(t)}{\frac{1}{\beta_B(t)} + \frac{1}{\beta_n}} \quad (23),$$

This means that the extraction rate decreases over time due to a decrease in the driving force of mass transfer and an increase in diffusion resistance inside the particle. It is the process of mass transfer inside the particle that ultimately determines the duration of the extraction process at a given extraction level [54].

There is also an equation for the specific flow through the rate of change of concentration in the volume of the suspension C(t) (24) [50]:

$$j(t) = \frac{V}{S}\frac{C(t)}{dt} \quad (24),$$

where S – total surface area of all particles in suspension; V – its volume of extractant.

From equations 1.2.2.6 and 1.2.2.7 the following follows (25) [54]:

$$\beta_B(t) = [\frac{C_n - C(t)}{\frac{VC(t)}{S\ dt}} - \frac{1}{\beta_n}]^{-1} \quad (25)$$

1.2.3 The phenomenon of hydrodynamics in a layer of plant material

In the extraction process, a special place is occupied by the ***phenomenon of hydrodynamics in the layer of plant material***. When the extractant moves through a porous heterogeneous layer of plant material inside channels of complex shape, the speed of the liquid is non-uniform due to interaction with solid particles. In such conditions, it is convenient to use a fictitious (average) speed, which is defined as the ratio of the volumetric flow rate of the liquid to the cross-sectional area of the flow. Fictitious speed allows you to simplify calculations by representing a complex system of liquid movement through a porous heterogeneous layer as an equivalent flow with a uniform speed. This facilitates the application of the laws of hydrodynamics to determine the pressure difference required to move a certain amount of liquid at the desired speed through the material layer.

The efficiency of the diffusion process in extraction depends on the following factors: the specific load of the extractor, the total length (height) of the layer of plant material in the extractor, its porosity, the speed and principle of extractant supply, as well as the uniformity of the extractant movement.

The specific load of the extractor is the amount of the extracted substance per unit volume of the extractor. The higher the specific loading, the more substance can be extracted in a given period of time, provided that other process parameters remain constant.

Overall length (height) of the bed. The length of the material bed in the extractor (specific loading) determines the path that the extractant must travel in order to. Increasing the bed length can improve the efficiency of the process, allowing more complete use of the available surface area of the material.

Porosity is the proportion of the material volume occupied by cavities or pores that can be filled with liquid. Porosity depends on the specific loading of the raw material in the extractor, the size and shape of the particles of the ground plant material. With an increase in the specific loading of the extractor, the overall porosity of the bed can decrease due to denser packing of the material. This can lead to a decrease in the volume of pores available for liquid and, consequently, an increase in the resistance to the passage of liquid through the material bed. When the material bed is pressed, the pores can be compressed or even closed, which leads to a decrease in the available space for liquid penetration. This creates a "dead pore space" - areas where liquid cannot penetrate due to closed or too narrow pores. Reducing the available surface for contact between the material and the extractant reduces the efficiency of mass exchange, since the exchange of substances occurs on the surface of the material.

The presence of air prevents the efficient and rapid penetration of the extractant into the raw material. To remove it, methods of vacuuming the mixture of raw material and extractant or replacing the extractant with a gas that is easily soluble in it, at the stage of wetting the raw material, with subsequent replacement of the gas with the required extractant are often used [24].

Extraction agent feed rate. Increasing the extraction agent feed rate can increase the rate of diffusion of BAS from the material into the extraction agent and accelerate the achievement of equilibrium between the concentrations in the material and the extraction agent. This can lead to a higher extraction rate and an increased yield of target compounds. However, it is important to consider that increasing the extraction agent feed rate can also cause undesirable effects, such as turbulence or uneven distribution of the extraction agent inside the extractor. This can lead to non-uniformity of the extraction process and a decrease in its efficiency.

Extraction agent feed principle. When the extraction agent is fed from above, the material layer in the extractor is unevenly compressed. The difference between the pressure of the liquid surrounding the particle and the pressure of the overlying particles plays a key role in this process. The greatest resistance to liquid passage occurs in the lower part of the extractor, where the liquid pressure is highest and the compressive forces are greatest. The solution to this problem is to install intermediate gratings or feed the extraction agent from above.

Uniformity of extraction agent movement. It is important that the extractant is uniformly distributed over the extractor cross-section to ensure uniform contact with the material and extraction of the substance without the formation of channels or layers. To increase the volumetric load of the extractors, crushed raw materials tend to be loaded with a higher density. When plant materials are crushed, particles of different sizes form a mixture.

As a result of the formation of a layer in the extractor, elastic particles increase the contact surface and bends, and small particles can close the channels between large ones, increasing the tortuosity of the

liquid path. When the raw material swells, the elastic properties of the layer decrease, which leads to its deformation under the influence of the extractant pressure. These processes contribute to the formation of tortuosity and unevenness of the channels, increasing the hydraulic resistance of the layer.

Changes in the direction and speed of liquid movement in a layer consisting of tortuous channels of variable cross-section often lead to a loss of pressure. This loss is due to both the viscous forces caused by the friction of the liquid against the material particles and the inertial forces associated with the tortuous movement along the channels. The influence of each of these components on the total pressure loss depends on the structure of the layer and can be described by equation 26, where the first term of the equation expresses the pressure loss due to friction forces, and the second - due to inertial forces.

$$\frac{\Delta P}{L} = aw + \delta \rho w^2 \quad (26),$$

where $\frac{\Delta P}{L}$ — pressure loss per unit layer height, N/m^2*s; w — fictitious (average) speed of a medium moving through a layer, m/s; p — density of the medium, kg /m^3; a and d — coefficients that are a function of variables characterizing the properties of the environment and the properties of the layer.

D.O. Kollerov proposed an equation that includes a larger number of hydrodynamic parameters characterizing the layer (equation 27):

$$\frac{\Delta P}{Lw} = K_0 (\frac{L_g}{L})^2 * S_0^2 \frac{(1-\varepsilon)^2}{\varepsilon^3} * \mu + K_x \left(\frac{L_g}{L}\right)^3 * S_0 \frac{1-\varepsilon}{\varepsilon^3} * \rho * w \quad (27)$$

where S_0 — specific surface area of the material, m^2/m^3; Ɛ — porosity (porosity) of the raw material layer, m^3/m^2; $\frac{Lg}{L}$ — relative length of pore channels (tortuosity), m/m; L— layer height, m; Lg — actual length of pore channel, m; μ— dynamic viscosity coefficient, N*s/ m^2; K_0 — coefficient equal to 2.5 (form factor); K_x — coefficient equal to 0.227 for plant materials.

It should be noted that increasing the rotor speed significantly enhances the extraction process. The effect of the rotor speed is especially noticeable at the initial stage of the process, when the surface layers of plant material particles are extracted [54].

The elasticity of the raw material depends on its type. And the layer of plant material reaches its greatest resistance after complete swelling.

1.3 Flavonoids as a class of compounds

1.3.1 Structure and classification of flavonoids

Flavonoids got their name from the Latin word flavus - yellow, since the first substances isolated from plants belonging to this group had a yellow color [55].

You think that flavonoids were discovered by Nobel laureate Szent-Györgyi, who in 1930 isolated the substance citrine from lemon peel, which regulates capillary permeability from lemon peel.

Flavonoids were first classified as vitamins P (due to their ability to increase capillary permeability) and vitamin C (because some flavonoids had properties similar to vitamin C). However, some sources indicate that the study of flavonoids began in the first half of the 19th century, when in 1814 Chevrolet isolated a crystalline substance from oak bark, called quercetin [56].

Flavonoids are a large group of phenolic compounds, the structure of which is based on a skeleton of 15 carbons (C_6 -C_3 -C_6), grouped into two benzene rings (A and B), linked together by a three-carbon chain (propane bridge). By means of the propane bridge, a heterocycle is formed in most flavonoids, which is a derivative of pyran or γ-pyrone [57]. A significant number of flavonoids can be considered as derivatives of 2-phenylchroman (flavane, Fig. 3) or 2-phenylchromone (flavone, Fig. 4) [58, 59].

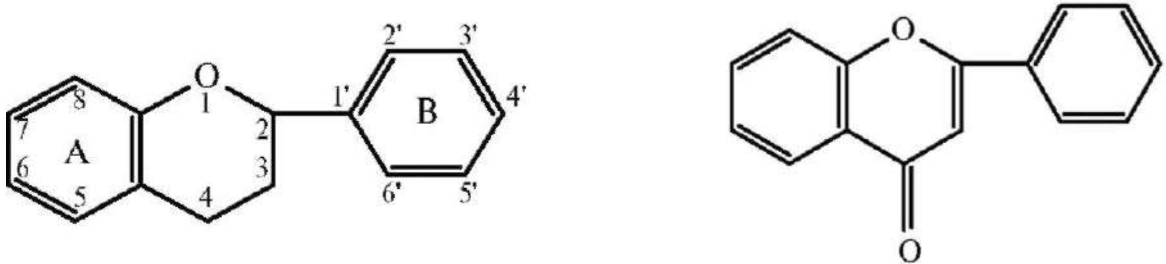

Fig. 3 - Flavan

Fig. 4 - Flavone

Flavonoid glycosides are compounds in which one or more monosaccharide residues are attached to an aglycone, which is the basic structural unit of flavonoids. These sugars are usually attached to hydroxyl groups on the C_3 or C_7 carbon atoms of the aglycones [60].

Flavonoids are water-soluble pigments present in the plant kingdom as secondary plant metabolites that can be found specifically in the cytosol and stored in the vacuole of plant cells [1].

The classification is based on [58]:

1) the degree of oxidation and hydroxylation of the propane fragment;

2) the position of the side phenyl radical;

3) the size of the heterocycle.

Differences in oxidation state and degree of unsaturation of the heterocyclic ring (or lack thereof in the case of chalcones) result in six distinct classes: isoflavones, flavanones, flavanones, flavan-3-ols, flavonols and anthocyanidins, each with specific characteristics [58].

Depending on the position of the B ring, the following subgroups of flavonoids are distinguished:

I. Flavonoids proper (euflavonoids) with a side phenyl radical at C_2.

II. Isoflavonoids with a phenyl radical at C_3.

III. Neoflavonoids with a phenyl radical at C_4.

IV. Biflavonoids.

Flavonoids obtained from plant materials are called bioflavonoids. They are of great interest, since they have a variety of pharmacological effects, they are also metabolically close to the human body, which ultimately determines the prospects and demand for substances of natural origin [28]. They represent a group of plant components called phenolic compounds [13].

1.3.2 Distribution of flavonoids

More than 7,000 flavonoids have been identified and are considered to be substances that have a therapeutic effect on human health [1]. In addition to monomeric flavonoids, there are dimeric forms of flavonoids. In this case, flavonoids are linked to each other by carbon bonds or condensed with other phenolic compounds: phenolic and hydroxycinnamic acids, lignans, isoprenoids and alkaloids [58].

Flavonoids are found in varying amounts in almost all plants, and are also found in algae, fungi, microorganisms and insects. In plants, flavonoids are found in leaves, flowers and fruits, and less often in stems and underground organs [2].

Flavonoids in plants are found both in the form of aglycones and in the form of glycosides. Flavonoid glycosides contain glucose, galactose, xylose, arabinose, and rhamnose as sugars. Flavonoid glycosides usually contain no more than three monosaccharide residues [60].

In plants, most flavonoids are present in the form of glycosides, which are better soluble in cell sap. The main group of bound flavonoids are O-glycosides; C-glycosides (glycoflavonoids) are less common. The most common sugar residues are: from hexoses - glucose, galactose; from pentoses - xylose, arabinose; from methylated pentoses - rhamnose; from uronic acids - glucuronic acid [1].

Flavonoids are known for their antioxidant properties and the ability to protect the body from damage caused by oxidants. Oxidants such as UV rays, pollution, and chemicals in food can cause damage to cells and tissues in the body by increasing the levels of free radicals. Free radicals are unstable molecules that can cause damage to cells, DNA, and other biological molecules. Flavonoids can help prevent or reduce such damage by acting as antioxidants that neutralize free radicals and protect cells from oxidative stress [2, 3].

Flavonoids contain variable amounts of phenolic hydroxyl groups in their chemical structure and excellent chelating properties for iron and other transition metals, which give them high antioxidant capacity; therefore, they play a significant role in protecting against oxidative damage and exert therapeutic effects. They have a wide range of effects, including coronary heart disease or atherosclerosis cancer [5, 61].

1.3.3 Pharmacological properties of flavonoids

As is known, the direction of the pharmacological effects of bioflavonoids is determined by chemical differences in their structure. Thus, diosmin, obtained from plants of the genus Rutaceae, is known to primarily have proven capillary-protective, venotonic, lymph-stimulating and anti-edematous

effects. Linarin is a flavonoid glycoside with analgesic and anti-inflammatory activity. Isorchoifolin is a flavone that reduces vascular permeability, improves microcirculation and exhibits anti-inflammatory and weak antiradical activity. Hesperidin, in turn, is classified as a citrus flavonoid that strengthens the capillary network and has proven antioxidant, anti-inflammatory and high endothelioprotective properties. The anti-inflammatory activity of this substance is due to the inhibition of the oxygenase pathway, arachidonic acid metabolism, and prostaglandin E2 synthesis. Literature data indicate that hesperidin reduces platelet aggregation and is able to prevent microvascular disorders by inhibiting the enzyme hyaluronidase, which regulates the permeability of capillary walls [6].

Flavonoids are known for their antioxidant properties and the ability to protect the body from damage caused by oxidants. Oxidants such as ultraviolet rays, pollution, and chemical compounds in food can cause damage to cells and tissues in the body by increasing the level of free radicals. Free radicals are unstable molecules that can cause damage to cells, DNA, and other biological molecules. Flavonoids can help prevent or reduce such damage by acting as antioxidants that neutralize free radicals and protect cells from oxidative stress [4].

The severity of the choleretic effect increases in the series: flavones-chalcones-flavanones. Flavonols mainly affect the liver's detoxifying function, the mechanism of action is associated with changes in oxidation-reduction processes in the mitochondria of liver cells.

Flavonoids have a pronounced antispasmodic effect (aglycones are stronger than glycosides) [18]. Their antispasmodic effect on the coronary vessels and vessels of internal organs is slightly inferior in strength to coumarins.

Most flavonoids have a moderate diuretic effect, the mechanism of which is primarily due to the dilating effect on renal vessels. The antiulcer effect is most pronounced in glycosides of flavonols and chalcones. This activity is associated with the inclusion of these compounds in specific biochemical reactions occurring in the stomach wall [62].

Isoflavonoids exhibit estrogenic activity [18].

Thus, flavonoid compounds have a strong antioxidant effect with complex pharmacological activity, which includes capillary-strengthening, antiallergic, antispasmodic, anabolic, antithrombotic, venotonic, hypocholesterolemic, antiatherosclerotic, cardiostimulating, antitumor, antiviral, hepatoprotective and endothelioprotective effects [1, 43, 58, 63]. Anti-inflammatory (including a decrease in neurophosphorus in neurodegenerative diseases such as Parkinson's, Alzheimer's, multiple sclerosis, amyotrophic lateral sclerosis and others [64]), antiviral and antiallergic effects of flavonoids, as well as their protective role against cardiovascular diseases [6, 65, 66, 67], cancer [68] and various pathologies have been noted. However, these effects are manifested in high doses of flavonoids [1].

he State Register of Medicines Approved for Use in the Russian Federation includes only the antioxidant diquertin, which is dihydroquercetin (taxifolin), a flavonoid obtained from the wood of Siberian larch. However, it is believed that medicinal plants containing flavonoids rutin, dihydroquercetin, hyperoside, quercetin, bisapigenin, are a promising source of antioxidants and drugs. The most probable manifestation of antioxidant activity is possible in the case of flavonol glycosides - rutin and hyperoside, the probability of manifestation of antioxidant activity of quercetin and bisapigenin is quite high [1, 6].

There is data in the literature that the antioxidant effect of flavonoids is realized by a combined mechanism and the most effective combinations or mixtures of flavonoids (primarily natural ones), which is due to the synergism of their action. Thus, the weaker antioxidant hesperidin promotes the restoration of more active diosmin molecules spent on neutralizing radicals, acting as a synergist for the latter in the reactions of chain termination of the free radical process of lipid peroxidation. In addition, isoflavonoid aglycones, unlike glycosides, are able to be absorbed from the stomach, so it can be assumed that due to the presence of diosmin in the composition, when taking a combination of the above flavonoids, the effect will occur faster [63].

ost flavonoids have high P-vitamin activity, that is, the ability to reduce the fragility and permeability of capillary walls. These properties are most pronounced in flavans and flavanones, especially rutin [69]. For biological activity to occur, two hydroxyl groups must be present in positions 5 and 7 of the benzopyrone nucleus and a substituent in positions 3' and 4' of the phenyl radical [18]. The absence of hydroxy groups in positions 3 and 3' in flavonoid genins significantly affects capillary-strengthening activity. An increase in action is observed when moving from aglycones to monosides, and a decrease in the series of biosides and triosides. Some authors note that only three drugs available on the pharmaceutical market are recommended for the treatment of chronic venous edema: MPFF, rutin and hydroxyethylrutosides, butcher's broom extract [70].

This proves the relevance of using medicinal raw materials containing rutin and other flavonoids for the creation of medicinal and cosmetic products [7, 71, 72]. Products based on herbal medicinal raw materials can be considered safe, which increases their demand in the market [73].

1.3.4 Physicochemical properties of flavonoids

Most flavonoids are solid crystalline substances with a specific melting point and are odorless [58].

Flavones, flavonols, chalcones, aurones are yellow, catechins, leucoanthocyanidins, flavanones, isoflavones are colorless. Anthocyanins have the brightest color and impart red or blue coloration to plant tissues depending on the pH of the environment (in an acidic environment they are red (cation salts), in an alkaline environment they are blue (anion salts) [58].

The physicochemical properties of flavonoids are greatly influenced by the form in which they are presented in the extract (Fig. 5).

Glycosidic form Aglycone form (quercetin)

Fig. 5 - Forms of routine

Flavonoids have different solubility depending on the form they are in (Table 2).

Table 2 - Solubility of flavonoids

Solvents	**Flavonoid glycosides***	**Flavonoid aglycones**
water	soluble	insoluble
water-alcohol mixtures	soluble	-
alcohols	soluble when heated	well soluble in lower alcohols (methyl and ethyl)
ethyl acetate	soluble when heated	-
organic solvents	insoluble	highly soluble
alkali solutions	-	highly soluble

* - containing more than three sugar residues

Lipophilicity of flavonoids is determined by its distribution between phases of different polarity, which occurs due to the presence of various functional groups.

Glycosides and aglycones of flavonoids are soluble in alkalis with the formation of phenolates. Glycosides are hydrolyzed by acids and enzymes [18**Ошибка! Источник ссылки не найден.**].

Hydrolysis of flavonoids occurs in different ways. For example, O-glycosides are easily hydrolyzed to aglycone and carbohydrate residue under the action of dilute mineral acids and enzymes. C-glycosides are difficult to break down even under the action of concentrated acids (HCl or CH_3COOH) or their mixtures under prolonged heating [58].

Flavonoids are relatively resistant to oxidation, with the exception of catechins and leucoanthocyanidins. The latter are oxidized in the presence of oxygen, under the action of light and alkalis, turning into colored compounds - condensation products, up to high-molecular polymer forms [58].

The following chemical ***properties of substances*** of this class were also noted [58]:

- OH group in position 7 is capable of diazotization;
- the ability to form complexes with metal salts of varying degrees of stability due to carbonyl and phenolic oxy groups;
- the ability to be reduced by atomic hydrogen in an acidic medium in the presence of magnesium or zinc;
- interaction with alkalis with the formation of yellow phenolates, which change to orange or brown when heated.

The antioxidant properties of flavonoids can be associated with [67]:

- the enthalpy of dissociation of the O–H bond, where a relatively low value facilitates the reaction of H evolution between the antioxidant and the radical;
- the ability to donate electrons, such as the ionization potential (relative adiabatic ionization potential);
- factors that stabilize the corresponding radical after the release of hydrogen;
- electrochemical properties, such as oxidation-reduction potentials;
- solubility.

The physicochemical properties of the main representatives of the flavonoid class are presented in Table A.1 Appendix A.

1.3.5 Methods for determination of flavonoids

To determine flavonoids, qualitative reactions based on the formation of colored complex compounds are used. The main specific reaction for flavonoids is the cyanidin test (Shinoda test). When magnesium powder and conc. HCl are added to extracts containing flavonoids, a red color appears (formation of pyrilium salts during the reduction of the flavonoid fragment with hydrogen) (Fig. 6) [57, 72].

Fig. 6 - Reaction of flavonoids with magnesium (Shinoda test)

A similar reaction occurs with a mixture of zinc dust in the presence of hydrochloric acid – the formation of cyanidin chloride from dihydroquercetin (Fig. 7) [73]:

Fig. 7 - Reaction of flavonoids with zinc dust

Other reactions to flavonoids - with ammonia solution, sodium hydroxide and iron (III) chloride - are not very specific. With ammonia solution, a brown-yellow coloration was observed, characteristic of flavones. With iron chloride - a green coloration due to the formation of complex compounds of flavonoids at phenolic hydroxyls.

In the methods of qualitative and quantitative determination of flavonoids, the complexation reaction with aluminum chloride is most often used. This reaction is recommended by the State Pharmacopoeia for the determination of flavonoids [57, 73].

During this reaction, complexes of flavonoids with aluminum (III) ions are formed (Fig. 8) [73]. n this reaction, it is necessary to maintain a constant pH value, since this is critical; for this, a fixed value of acetic acid is added to the solution [16].

Fig. 8 - Reaction of flavonoids with aluminum (III) chloride

An important characteristic of flavonoid glycosides is ***optical activity*** [58, 72].

When studying flavonoids by ***UV-spectrophotometry***, two main absorption peaks were found: in the wavelength range of 240-400 nm, one of which is associated with the absorption of the B-ring system, the other is associated with the absorption including the benzoyl system of the A-ring. It was also noted that the presence of functional groups attached to the flavonoid framework can cause a shift in absorption, for example, quercetin from 367 nm to 371 nm [67].

Some flavonoids have a second absorption band with a maximum in the range of 300-550 nm. This band is clearly visible in flavonols and flavones, i.e. classes of flavonoids in which there is a conjugation between the B and C rings (a double bond in the C_2-C_3 position) [57].

Spectrophotometric methods for the determination of phenolic compounds in medicinal plant materials are based on several processes, such as derivatization with 2,4-dinitrophenylhydrazine, reduction of the phosphomolybdenum-tungsten complex, and complexation with aluminum ions with or without preliminary nitrosation. However, these methods do not provide the ability to clearly distinguish between different subgroups of polyphenols depending on their structure.

As a result of complexation with an aluminum ion after nitrosation, rutin and luteolin among flavones and flavonols showed the greatest shift of maxima to 531 and 517 nm, respectively. At the same time, quercetin, as a flavonol, showed a significantly shorter-wave maximum of 477 nm, and dihydroquercetin, as a flavanonol, demonstrated an absorption band maximum in the long-wave region (507 nm). Flavonols and other flavones with hydroxyl groups in positions 3 and/or 5 give absorption in the region of 410-430 nm. In the presence of $NaNO_3$ in an alkaline medium, absorption in the region of 510 nm is observed during the interaction of catechins and some other polyphenols with $AlCl_3$ [74].

Therefore, although complexation with an aluminum ion does not provide unambiguous identification of specific subgroups of flavonoids and hydroxycinnamic acids, this method allows reducing the influence of accompanying substances on the optical density of the solutions being studied [74].

The Folin–Ciocalteu method is widely used to determine the total content of phenolic compounds in extracts from medicinal plant material [74, 75]. It is based on the reduction of a phosphomolybdotungsten complex, which results in the appearance of an absorption band at 765 nm. Recalculation of the results to the mass of the gallic acid equivalent in a given mass of the sample allows for the quantitative determination of polyphenols in the extracts. The spectrophotometric technique using the Folin–Ciocalteu reagent for the determination of the total content of polyphenols in complex materials has a number of advantages, including specificity, accuracy, linearity, and repeatability [74].

Since flavonoids belong to the group of polyphenols that are widely distributed in plants, their presence in plant materials affects the analytical results. If flavonoids predominate among other phenolic compounds, the results obtained using the aluminum chloride complexation and Folin–Ciocalteu methods will be similar. For example, the analysis of Sophora, which contains mainly rutin, calendula (with rutin and isoquercetin) and yarrow (with luteolin and apigenin) showed similar results. However, in some cases, such as licorice root, hawthorn fruits and calendula flowers, the content of polyphenols determined by the complexation method with aluminum chloride was lower than when using the Folin-Ciocalteu reagent. This is explained by the fact that these samples contain compounds that either do not

react with aluminum chloride or absorb its complexes in the ultraviolet region of the spectrum: for example, catechins, phenolic acids and ascorbic acid in hawthorn fruits, liquiritin and gallotannin in licorice root, gallic acid in calendula [75].

Flavonoids are also determined by ***thin-layer chromatography***. Flavonoids on chromatograms usually have a weak or absent color, which makes it difficult to detect them in the visible spectrum. To increase the efficiency and specificity of the analysis of chromatographic methods, reagents are used that are capable of forming colored compounds and fluorescing when examined in UV light. To visualize spots on a chromatographic plate, an alcohol solution of aluminum chloride, zirconium chloride with citric acid, a solution of potassium hydroxide, a solution of antimony trichloride in tetrachloromethane, a 1% solution of vanillin in concentrated hydrochloric acid, a solution of ferric alum, ammonia vapors and others are used.

The TLC method is recommended by the State Pharmacopoeia of the Russian Federation, as well as other Pharmacopoeias, for example, the EAEU, for determining flavonoids.

Studies describe relationships between the chemical structure of flavonoids and their behavior during chromatography [76, 77]:

1.The Rf value decreases with an increase in the number of hydroxyl groups in the molecule.

2. Methylation of hydroxyl groups causes an increase in the Rf value of aglycones.

3. Glycosidation causes a decrease in the Rf value. The formation of bioside leads to a smaller decrease in the Rf value than the formation of diglycoside.

4. Acetylation can contribute to both an increase and a decrease in Rf.

5. Ortho- and vicional positions of substituents lead to an exception to these rules in the direction of increasing Rf.

Flavones, flavonol-3-glycosides, flavanones, chalcones are detected on chromatograms as brown spots, flavonols and their 7-glycosides - as yellow or yellow-green spots [58].

1.4 Antioxidant properties of extracts

Flavonoids are known for their antioxidant properties (AO). They are able to protect the body's cells from the effects of free radicals, thereby helping to reduce oxidative stress and prevent cell damage.

AO of substances in plant extracts is characterized by the rate constant of the reaction of peroxyl radicals with an inhibitor [78].

Free radicals and oxidative stress are the main factors that underlie the physicochemical mechanisms of cell damage. AO of flavonoids make them important in protecting cells from oxidative stress, and also make them potentially useful in the prevention of various diseases, such as cancer, cardiovascular diseases and some neurodegenerative diseases [44].

Radicals such as hydroxyl radical, superoxide radical and peroxide radicals can be formed in the body as a result of oxidative processes and contribute to cell and tissue damage. Flavonoids have the ability to neutralize these radicals by donating hydrogen or electrons, which makes them effective antioxidants. After interaction with radicals, the resulting flavonoid radicals can undergo disproportionation reactions, resulting in the formation of more stable products [79].

There are optical, electrochemical, and chromatographic methods for determining AO [80]. Electrochemical methods are the simplest and most informative, and the most promising of these is the potentiometric method. It is based on measuring potentials after a chemical reaction between the antioxidant and the reagent. The change in potential occurs as a result of a reaction that can be described by equation 26 [79].

$$\text{n}Me_{Ox}\text{L} + \text{AO} = \text{n}Me_{Red}\text{L} + \text{AO}_{Ox} \tag{26}$$

where Me_{Ox}L – oxidized form of metal;

Me_{Red}L – restored form of metal;

AO_{Ox} – antioxidant oxidation product.

AO is a characteristic of the system as a whole, which is influenced by the complex composition of the extract [79].

1.5 Conclusions based on the results of the literature review

Extraction of flavonoids is an effective way to obtain environmentally friendly BAS from plants that meet the concept of "green chemistry".

Flavonoids are a large group of phenolic compounds that have a variety of pharmacological properties. The main action of flavonoids is antioxidant with complex pharmacological activity, which includes capillary strengthening, antiallergic, antispasmodic, anabolic, antithrombotic, venotonic, hypocholesterolemic, antiatherosclerotic, cardiostimulating, antitumor, antiviral, hepatoprotective and endothelioprotective action. In this regard, flavonoids obtained from medicinal plant materials are used in various industries such as: food, pharmaceutical, cosmetic.

The presence of flavonoids in nature is very diverse - they are contained in almost all plants, are also found in algae, fungi, microorganisms and insects. In plants, flavonoids are found in leaves, flowers and fruits, less often in stems and underground organs.

Flavonoids are derivatives of 2-phenylchromane or 2-phenylchromone. In turn, flavonoids in plants are found in the form of glycosides or aglycones. Glycosides are compounds in which one or more monosaccharide residues are attached to an aglycone, which is the main structural unit of flavonoids.

The form in which flavonoids are found affects their physicochemical properties. Also, different types have their own physicochemical characteristics. When extracting flavonoids from plant materials,

it is important to understand which of them predominates in the composition. This substance is used to quantitatively determine the amount of flavonoids in the raw material.

Extraction consists of the following main stages: wetting of plant materials and swelling, formation of internal primary juice and mass transfer. During the extraction process, the following processes occur: adsorption, desorption, diffusion, and in special cases, dialysis and osmosis. Mass exchange processes can be described by the basic equations of mass transfer and mass transfer. A special place is occupied by the phenomenon of hydrodynamics in the layer of plant material, since when the extractant moves through a porous heterogeneous layer of plant raw materials inside channels of complex shape, the speed of the liquid is non-uniform due to interaction with solid particles. In this case, it is worth assessing the specific pressure of the extractant and the principle of its supply.

Obtaining kinetic characteristics of the extraction process is of great importance in understanding the extraction process. To analyze the kinetics of such processes, first- or second-order equations, the Peleg model, the Minchev and Minkov model, and others are used.

To date, there are a large number of studies aimed at extracting flavonoids from plant materials. The variety of extraction methods is explained by the multifactorial nature of the process. There is no single best way to extract all flavonoids from the entire variety of plant materials. In each case, the selection of a method is individual.

This process is affected by the type of raw material, its composition, grinding. Since ballast substances can pass into the extractant during the extraction process, which also affect the extraction process.

In the process of selecting an extractant, the affinity of the extractant to the components being extracted, its viscosity, pH value, and surface tension are of great importance. It should also wet the material well and be accessible.

The influence of surfactants can have a significant effect on the extraction of BAS from plant materials. This occurs due to a decrease in surface tension, which facilitates the penetration of the solvent into the material, as well as wetting the plant shell. Some authors note the ability of flavonoids to be embedded in surfactant micelles, which also increases their extraction from plant materials. The nature of the surfactant has a significant impact on the extraction process.

The surface charge of the particle and the pH of the solution can affect the electrostatic interaction between the particles and the solvent. This can lead to different adsorption and diffusion forces that affect the efficiency of the extraction process. The surface charge of the particles can also affect the solubility of the BAS in the extractant.

There are classical methods for extracting flavonoids, such as maceration and percolation. There are also methods for intensifying the process: the use of MW and US radiation, temperature, etc. In each

case of extraction, the effect of these factors is different: sometimes their use helps to increase the yield and reduce the extraction time, and sometimes it causes the destruction of substances. Since flavonoids have pronounced antioxidant properties, their evaluation in the extract is of practical interest. Free radicals and oxidative stress are the main factors that underlie the physicochemical mechanisms of cell damage. The antioxidant properties of flavonoids can have a positive effect on human health, helping to combat various diseases and stimulating the overall health of the body.

CHAPTER 2. RESEARCH METHODS AND MATERIALS

2.1 Characteristics of the materials used

The fruits of Sophora japonica TU 10.89-066-14721358-2017 were used as the object of the study. Sophora japonica belongs to the legume family, grows in eastern countries, but is cultivated in Russia. The medicinal plant material (fruits) are indehiscent, flattened-cylindrical, greenish-brown, multi-seeded beans up to 10 cm long, 0.5-1 cm wide, with a clearly visible yellowish seam (Fig. 9).

Fig. 9 - Fruits of Sophora japonica

This raw material is of interest because it contains a variety of biologically active substances (BAS), the main amount of which are flavonoids. Rutin is the predominant one among them. Also, medicinal plant raw materials correspond to the concept of "green chemistry", have fewer side effects than synthetic substances. This allows the use of an extract from the fruits of Sophora japonica, which has a wide range of action [66, 68].

Rutin, or rutoside, is a glycoside that combines the flavonol quercetin and the disaccharide rutinose (α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranose) Fig. (10) CAS номер: 153-18-4.

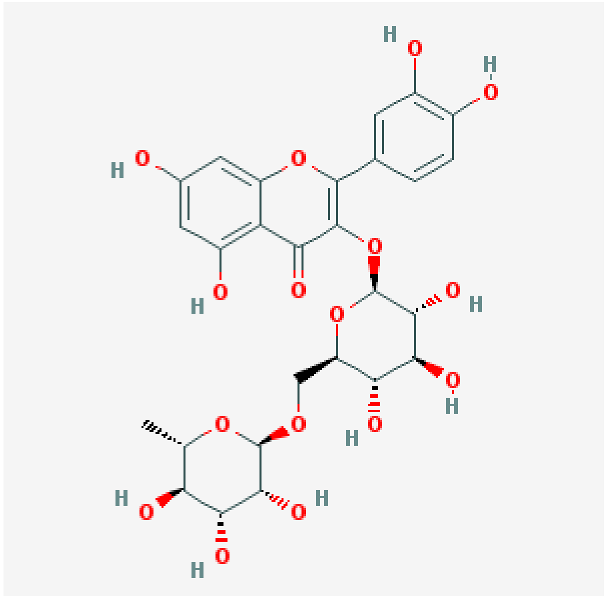

Fig. 10 - Routine formula

Distilled water with a specific conductivity value of $æ = 2 \cdot 10^{-6} Ом^{-} \cdot см^{-1}$, rectified ethyl alcohol 95% manufactured according to GOST 5962-201 were used as an extractant.

The reagents presented in Table 3 were used in the work.

Table 3 - Characteristics of reagents used

The reaction	Used reagent	Purity	Manufacturer
	Determination of flavonoids		
Candinavia alloy	magnesium hydrochloric acid conc., butyl alcohol	 ch. p. ch. p.	Merck KGaA, Darmstadt, Germany JSC "ECOS-1", Russia JSC "ECOS-1", Russia
Reaction Wilson	boric acid, oxalic (atenciosaacid)	ch. p. ch. p.	JSC "ECOS-1", Russia JSC "ECOS-1", Russia
With aluminium chloride	aluminium chloride (III), acetic acid (ice)	ch. p. ch. p.	PKF "Intermetkhim", LLC, Russia JSC "Inreactive", Russia
With ferric chloride	ferric chloride (II)	ch. p.	OOO "Recreativ", Russia
With ammonia	Ammonia	pure for analysis	OOO ACTIVCONTROL, Russia
quantification	Standard routine	standart	Rutin, party 332, Sichuan Guangsong Pharmaceutical Co., Ltd., China is

Continuation Table 3 - Characteristics of reagents used

The reaction	Used reagent	Purity	Manufacturer
the Definition of simple phenols			
With ferric sulfate	ferrous sulfate (II), butyl alcohol	ch. p. pure for analysis	OOO "Expression", Russia PKF "Intermetkhim", LLC, Russia
With geltonai-tion with alum	sulfate iron(III)-ammonium	pure for analysis	OOO "Greativ", Russia
Determination of polysaccharides			
With ethyl alcohol	ethyl alcohol	96%	Marsprojekt , OOO, Russia
With sodium chloride	sodium chloride	ch. p.	AO ". Lenreactive JSC", Russia
With hydrochloric acid	hydrochloric acid conc.	ch. p.	ECOS-1 JSC, Russia
Determination of saponins			
With lead acetate lead	acetate lead	ch. p.	of GRANCHEM LLC, Russia
Foaming/ creating a pH extractant	hydrochloric acid 0.1 N, sodium hydroxide	Fix ch. p.	. OOO "Neva Reagent", JSC "LenReaktiv", Russia
Foaming/ creating a pH extractant	hydrochloric acid 0.1 N, sodium hydroxide	Fix. ch. p.	OOO "Neva Reagent", JSC "LenReaktiv", Russia
Determination of alkaloids			
Reaction with tannin	Tannin	tech.	LLCLivagos, Russia
Determination of tannins			
With gelatin	gelatin,	tech.	GRANCHEM LLC, Russia
With potassium bichromate	potassium bichromate	techn.	CHEMTT LLCKHIMTT LLC, Russia
With iron-ammonium alum	iron(III) - ammonium sulfate	pure for analysis	Yugreactive LLCYugreactive LLC, Russia
With	sodium nitrate sodium nitrate, hydrochloric acid conc.	ch. p. ch. p.	Lenreactive JSCЛенРеактивLenreactive JSC, Russia ECOS-1 JSC, Russia

Continuation Table 3 - Characteristics of reagents used

The reaction	**Used reagent**	**Purity**	**Manufacturer**
Other			
Process study Salting	out Sodium chloride	ch. p.	AO "of Lenreactive JSC, Russia
	Sodium iodite	ch. p.	Merck KGaA, Darmstadt, Germany
	Sodium rhodanide	ch. p.	of GRANCHEM LLC, Russia
Determination of the effect of surfactants	lauret-2	-	Ataman Chemistry, Turkey
	lauril sarkosinate	-	Ataman Chemistry, Turkey
	miramistin	-	Ataman Chemistry, Turkey
	Cocamidopropylene glycol-coldimonium Chloride Phosphate	-	Ataman Chemistry, Turkey

To prepare working solutions and samples, measuring vessels and equipment presented in Table 4 were used.

Table 4 - Measuring glassware and equipment used for preparing working solutions and samples

Measuring utensils/Equipment	Accuracy class	Error limit/measurement accuracy
Measuring utensils		
10 ml cylinder	1	± 0,10 ml
25 ml cylinder	1	± 0,25 ml
25 ml flask	1	-
5 ml pipette	1	-
10 ml burette	1	± 0,05 ml
5 ml microburette	2	± 0,02 ml
Equipment		
Analytical balance Shinko HTR-220 CE	1	± 0,00005 g
Analytical balance CAS MWP-3000H	2	± 0,05
pH meter 150-M	-	± 0,02 pH
Electric stove Kitfort KT-175	-	-
Digital thermometer Hanna Instruments HI98509 Checktemp 1	-	± 0,2°C

2.2 Methods

2.2.1 Preparing Sophora Japanica fruits

Dried Japanese pagoda tree fruits were ground in a Kinematica POLYMIX ® PX-MFC 90 D dry mill and then sifted using a set of fractional sieves according to GOST 6613-866613-86 [81]. The following sizes of Japanese pagoda tree fruit particles were used in the work: less than 0,0315 cm; 0,0315 – 0,05 cm; 0,05 – 0,1 cm; 0,1 – 0,2 cm; 0,2 – 0,5 cm; 0,5 – 1,0 cm. The particle size was determined by the objectives of the study.

2.2.2 Getting the extract

The extract was obtained by one of the methods corresponding to the objectives of the study.

10 ml of extractant were added to the sample of raw material. The required amount of extractant was calculated as follows. The ratio of raw material to extractant was taken as 1:4, respectively, since this value is noted as the most effective for extraction from fruits [82]. The amount of required extractant was calculated using the formula (27):

$$V_{extractant}=V_{extract}+(m_{raw\ materials}*K) \quad (27),$$

where $V_{extract}$ – volume of extraction required;

$m_{raw\ materials}$ – weight raw materials;

K – the water absorption coefficient was taken to be equal to 2.

The following extractants were used in the work (depending on the research task): distilled water; distilled water acidified with 0.1 mol/l hydrochloric acid to a certain pH value (2.80 ± 0.05; 3.90 ± 0.05; 5.29 ± 0.05); distilled water alkalized with 0.01 mol/l sodium hydroxide to pH (8.00 ± 0.05; 8.23 ± 0.05; 9.00 ± 0.05); ethyl alcohol solution of various concentrations; surfactant solutions (laureth-2, sodium lauryl sarcosinate and cocamidopropylene glycol dimonium chloride phosphate).

2.2.2.1 Obtaining an extract by maceration

The maceration method is static and consists of infusing the raw material in an extractant [83, 84, 85].

10 ml of the extractant were added to a 5 g sample of raw material. It was left sealed for an hour. Then 20 ml of the extractant were added and it was left to infuse for 30 minutes.

The resulting extract was filtered using de-ashed “blue ribbon” filters.

2.2.2.2 Obtaining the extract by percolation method

The percolation method is dynamic and involves passing an extractant flow through the raw material at a given rate [83, 84].

10 ml of the extractant were added to a 5 g sample of raw material. It was left sealed for an hour. Then the swollen raw material was loaded into the lower percolator (3), at the bottom of which gauze

was installed, 10 ml of the extractant were added. The upper percolator (2) was placed above the percolator, and the remaining amount of the calculated extractant (10 ml) was poured into it.

The extractant feed was set so that the feed rate was equal to the outlet rate. The extract was collected in a glass receiver (4). The setup diagram is shown in Fig. 11.

The resulting extract was filtered using ash-free "blue ribbon" filters.

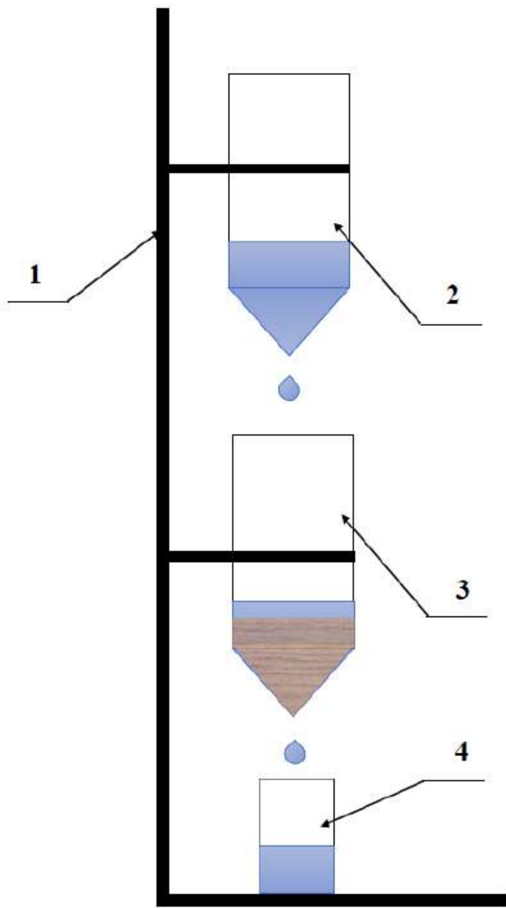

Fig. 11 - Scheme of the percolation extraction setup. 1 – stand, 2 – upper percolator, 3 – lower percolator (with gauze), 4 – receiver

2.2.2.3 Obtaining an extract by maceration with heating

A 5 g sample of raw material was placed in a round-bottomed flask with a ground joint (3) and 10 ml of extractant was added. The mixture was kept at room temperature for an hour. Then 20 ml of extractant were added to the flask, connected to a reflux condenser (2) and heated in a boiling water bath (4) at 100 °C for 15 minutes. After extraction, the flask was removed from the condenser, the extract was cooled, and filtered using ash-free filters “blue ribbon” [66, 87]. The setup diagram is shown in Fig. 12.

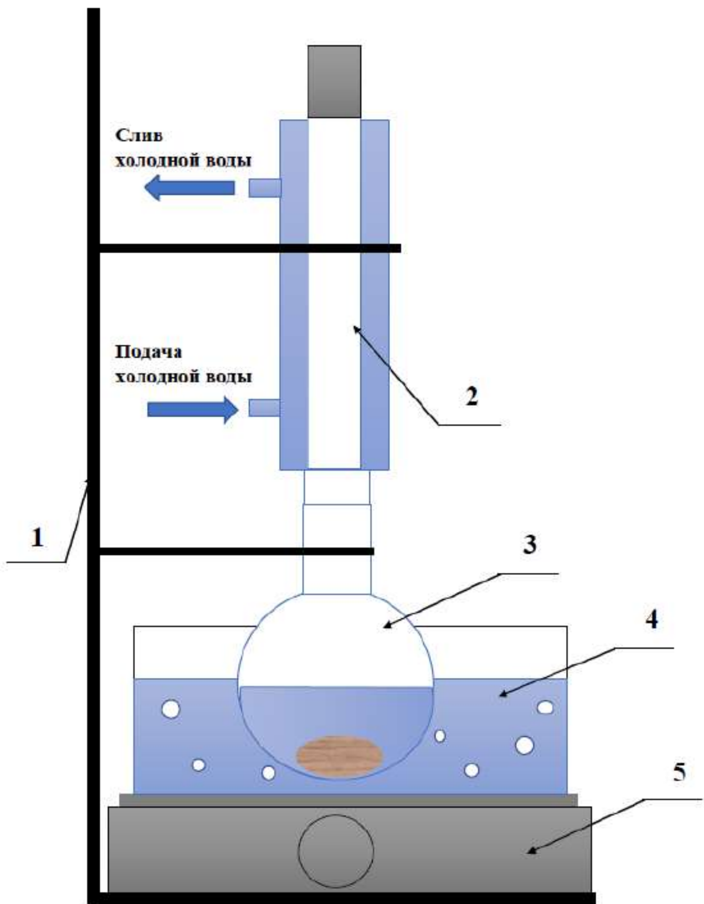

Fig. 12- Schematic diagram of the extraction setup with heating. 1 – stand, 2 – reflux condenser, 3 – flask with raw material and extractant, 4 – water bath, 5 – hotplate

2.2.3 Determination of swelling parameters

The degree of swelling was determined by the change in the volume of the raw material in the cylinder before and after swelling. A 10 ml graduated cylinder was filled with weighed portions of Japanese pagoda tree particles (2.0 ± 0.025 g) and 6 ml of the extractant was added.

The cylinders were placed in a LOIP LT-105a circulating liquid thermostat. The measurement was carried out at a temperature of 20 ± 0.1 ºC and 60 ± 0.1 ºC.

The time of fixation of the change in the volume of fruits in the cylinder was recorded at the initial moment of time, after 10, 20, 30, 40, 60, 90, 120 and 180 minutes [86].

The degree of swelling was calculated using the formula (28):

$$\alpha=\frac{V-V_0}{V_0} \qquad (28)$$

where α – the swelling degree, V - volume occupied by the Sophora Japanese fruit particles after time (t), ml; V_0 - the volume occupied by the Sophora Japanese fruit particles at the beginning of the process, ml.

The work studied the effect of temperature, composition of aqueous solutions (in distilled water, at different pH, electrolyte additives, surfactants, in a 50% aqueous solution of ethyl alcohol) on the swelling of Japanese pagoda tree particles.

The degree of swelling was determined in distilled water with a pH of 5.8 ± 0.05, in solutions of hydrochloric acid with a pH of 3.56 ± 0.05 and sodium hydroxide with a pH of 7.94 ± 0.05.

Sodium chloride solutions of concentrations of 0.001 M, 0.01 M, 0.1 M were chosen as electrolyte additives; solutions of sodium iodide and thiocyanate with a concentration of 0.001 M. The choice of electrolytes was determined by the fact that the anions of these electrolytes can be placed in one lyotropic series, and the sodium ion is more indifferent for assessing the effect on the swelling of plant materials.

Laureth-2, sodium lauryl sarcosinate and cocamidopropylene glycol dimonium chloride phosphate were used as surfactant additives.

The swelling rate constant for limited swelling of high-molecular compounds (HMC) was determined using equation (29) [19].

$$ln = \frac{\alpha_{max}}{\alpha_{max} - \alpha} = K\tau, \quad (29)$$

where K – swelling rate constant, min^{-1}; – maximum (limit) degree of swelling; τ – time, min.

The swelling rate constant (the slope of the linear dependence) was determined from the graph of the lg$(\alpha_{max}/(\alpha_{max} - \alpha))$ dependence on time [30].

2.2.4 Determination of ζ-potential

The ζ-potential was determined using electrophoresis – the moving boundary method [88, 89].

To study the dependence of the electrokinetic potential on the contact time of dispersed particles with the dispersion medium, measurements were taken immediately after obtaining the extract (using the maceration method described in section 2.2.2.1), after 30, 45 and 60 minutes [90]. The extract was introduced through a burette (2-4), then a tap (2) was opened to feed it into a U-shaped tube (1). The time during which the extraction boundary (sol) changed, the direction of displacement and the electric field strength were recorded. The setup diagram is shown in Fig. 13 [91].

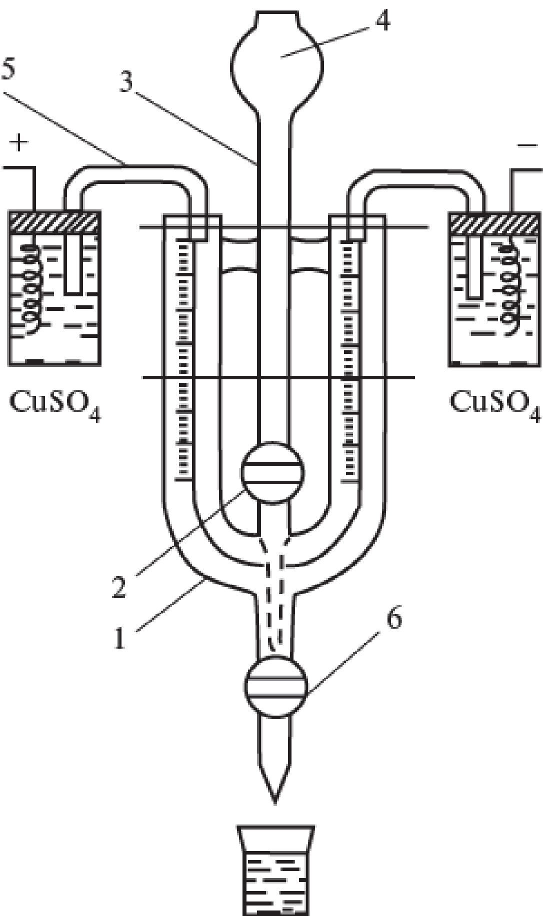

Fig. 13 - Scheme of the electrophoresis setup using the moving boundary method: 1 – U-shaped tube, 2 – tap for introducing sol (extraction), 3-4 – burette, 5 – agar-agar bridges, 6 – tap for draining liquid

Calculations of the ζ-potential were carried out using formula 30.

$$\zeta = \frac{h * \eta * l}{t * \varepsilon * \varepsilon_0 * E} \tag{30}$$

where h – the displacement of the sol boundary, m; η - viscosity of the dispersed medium (0,0011 N·s/m^2); l – distance between the electrodes, m; t – electrophoresis time, c; ε – permittivity of the medium; ε_0 – dielectric constant ($8.85*10^{-12}$ A*c/(B*м)); E – electric field strength, V.

2.2.5 Determination of sorption of H^+ and OH^-

Sorption of H^+ и OH^--ions on particles of an aqueous dispersion of Sophora was determined using the method of continuous potentiometric titration of the initial solution, extract and aqueous dispersion of Japanese Sophora fruits [92, 93, 94]. The extract was obtained by the maceration method described in section 2.2.2.1.

Titration was carried out with a solution of KOH (0.025 mol·l^{-1}) or HCl (0.01 mol·l^{-1}). The titrant was added in portions of 0.1 ml at intervals of 30 seconds and the pH values were recorded.

For dosing the titrant, 5 ml microburettes were used. KOH solutions were prepared with distilled water, previously freed from atmospheric CO_2 by boiling for 30 minutes.

Potentiometric titration is carried out in the cell shown in Fig. 14 - 16.

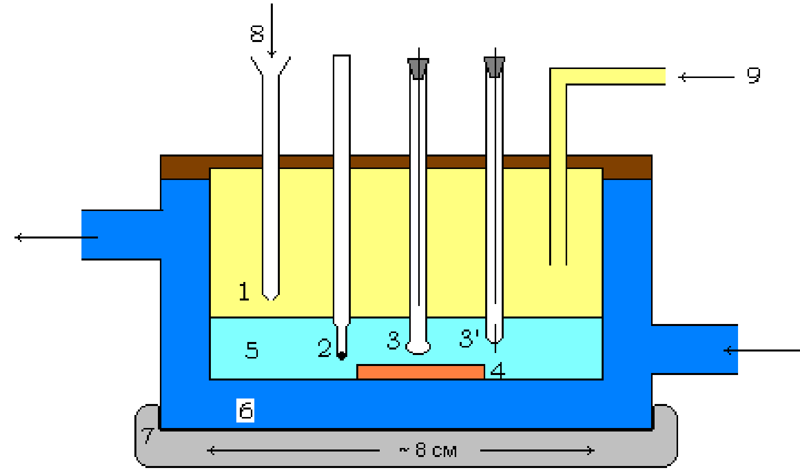

Fig. 14 - Titration cell: 1- main working cuvette: the space occupied by the gas - nitrogen or air without CO2 is highlighted in yellow; the working solution is highlighted in blue; 2 - thermometer; 3 - glass electrode; 3' second electrode (AgCl); 4 - magnetic stirrer; 5 - working solution; 6 - thermostat (filled with water inside;) 7 - stand; 8 – connects to the burette where the titrant is located (HCl or NaOH (see Fig. 15)); 9 – connects to the gas cylinder (see Fig. 16). The lid is indicated in brown (it is made of plexiglass, fits tightly (without special attention to tightness), but since the gas is slowly passed through the entire time of the experiment, 2-3 holes are made in it with a needle so that excess gas can escape from the cuvette.

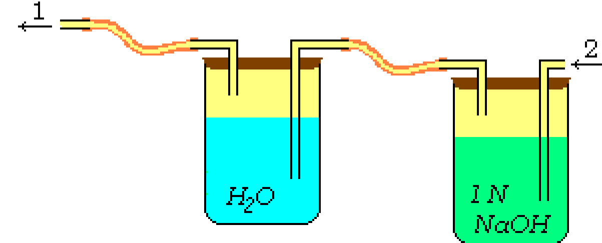

Fig. 15 - Supplement to Figure 14: 1 – connection with the right branch of Figure 14; 2 – to the gas cylinder

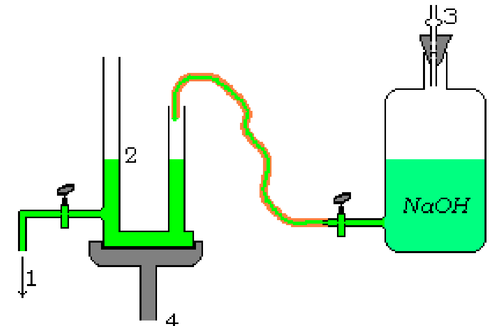

Fig. 16 - Supplement to Figure 14: 1 – connection on the left in Figure 14; 2 – burette with alkali or acid (alkali is written in the figure as an example); 3 – tube with soda lime

2.2.6 Definition of surface tension

Determination of the critical micelle concentration (CMC) of surface-active substances (SAS); the value of the surface tension of water-alcohol solutions, as well as extracts based on them, was determined on a Du Nouy tensiometer using the ring tear-off method at a temperature of 20 °C [93, 95, 96, 97, 98].

Before starting work, using a screw for tightening the thread (4) and an indicator with a vernier (5), the zero division of the reading limb was set and the screw connected to the elastic thread (7) was rotated (until the balance arm with the ring suspended on it was in a horizontal position).

A series of solutions (water-alcohol extractants and extracts based on them) of different concentrations were prepared. The studied solutions were poured into a cuvette (8) on a movable table (9) with a screw (10). The measurements began with the most dilute solutions. The table was raised until the ring touched the surface of the liquid being studied, then the thread (1) was twisted using the screw (4). The position of the pointer on the limb was noted at the moment the ring was torn off the surface of the liquid. The setup diagram is shown in Fig. 17 [99].

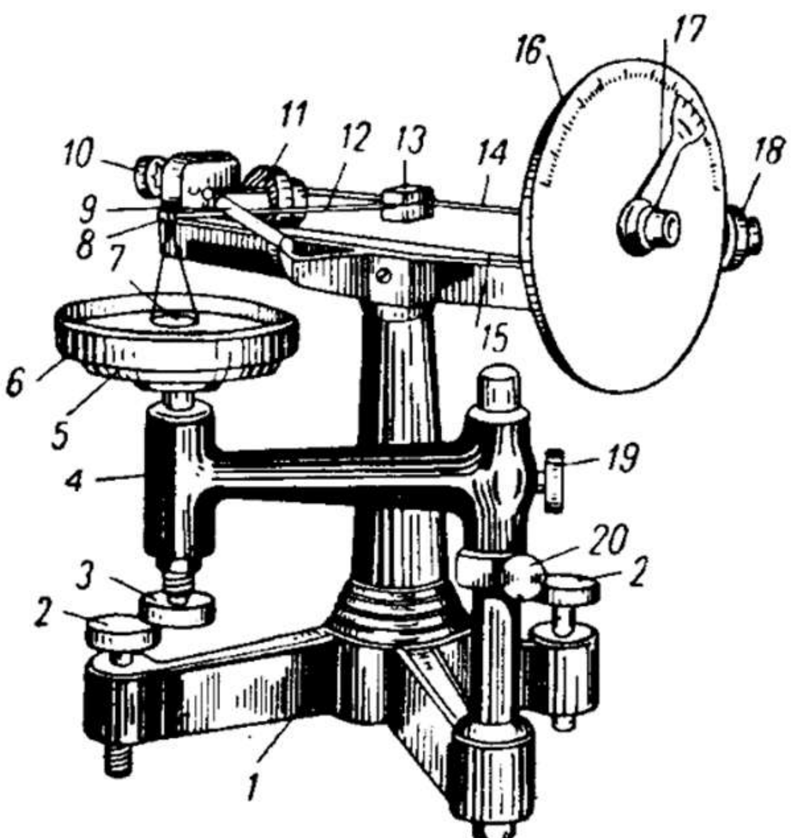

Fig. 17 - Schematic diagram of the Du Nouy tensiometer installation: 1 – stand, 2 – mounting screws (for horizontal installation of the device), 3 – micrometer screw (for vertical installation of the platform), 4 – rotating bracket, 5 – platform, 6 – flat-bottomed cup, 7 – platinum-iridium wire ring, 8 – hook, 9 – movable frame, 10 – screw for tensioning the wire, 11, 12 – lever, 13 – prism, 14 – steel wire, 15 – plate, 16 – disk, 17 – pointer, 18 – screw for turning the pointer, 19, 20 – screws for the bracket

The CMC of the anionic surfactant was also determined by the conductometric method [100, 101, 102]. A series of solutions of the anionic surfactant of different concentrations were prepared. The electrical conductivity was determined using a METLER TOLEDO LE703 conductometer, starting with the most dilute solution. The setup diagram is shown in Fig. 18 [91].

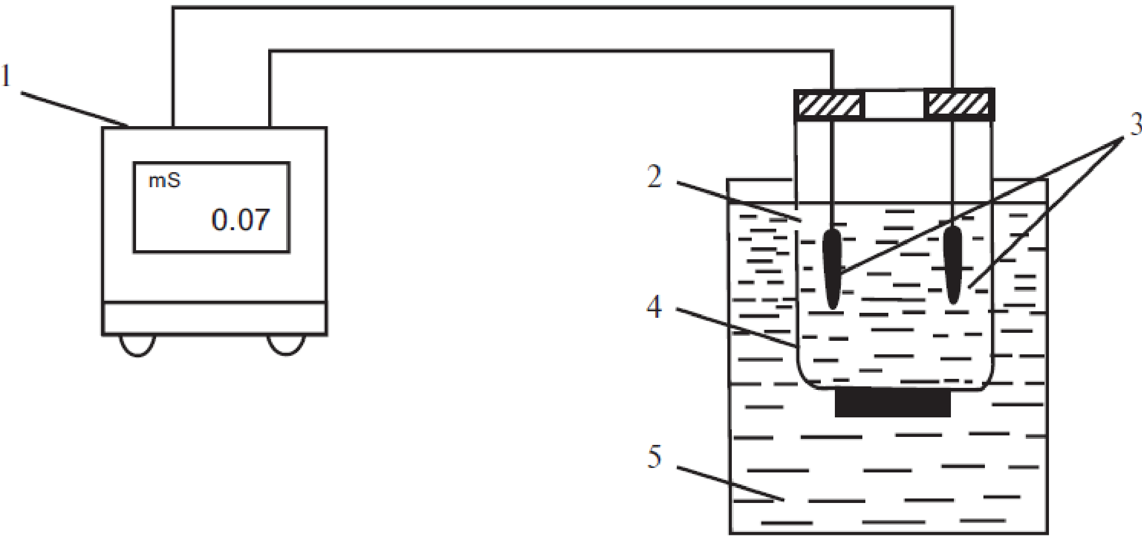

Fig. 18 - Conductometric measurement of specific electrical conductivity: 1 – measuring block; 2 – hole for filling with solution; 3 – measuring plates; 4 – conductometric cell; 5 – test solution

2.2.7 Determination of antioxidant activity

The antioxidant activity (AOA) of the extracts was determined by the EMF method based on the change in the potential of oxidation-reduction electrodes of the $\mathrm{Me}^{ox}/\mathrm{Me}^{red}$ п during their chemical interaction with antioxidants (flavonoids in the extract). The measurement was performed on an Anion 4100 potentiometer.

The essence of the method lies in measuring the electrical potential that occurs during the oxidation of the substances under study on the surface of the working electrode at a certain potential and comparing the obtained signal with the signal of the antioxidant standard measured under the same conditions. This approach allows one to directly determine the antioxidant potential and antioxidant capacity of the substance under study, summarily assessing the number of easily oxidized groups (-OH, -SH, -NH_2).

The oxidation-reduction electrode Fe^{+3}/Fe^{+2} . The setup diagram is shown in Fig. 19.

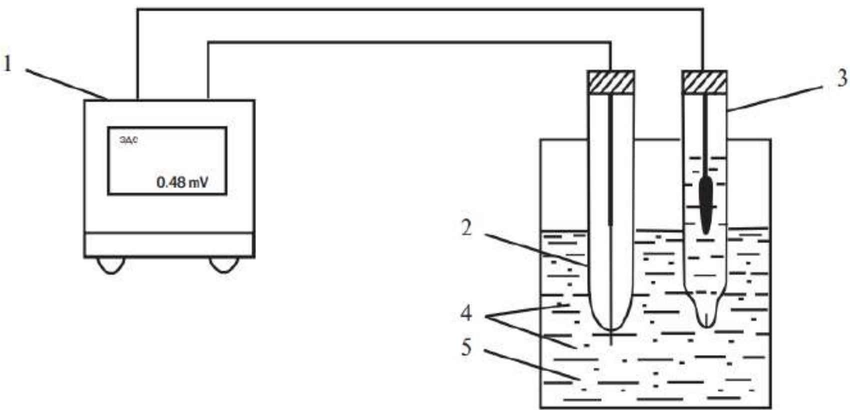

Fig. 19 - Setup for potentiometric determination of AOA: 1 – measuring unit – potentiometer; 2 – oxidation-reduction electrode consisting of an inert electrode – usually a platinum electrode placed in the test solution and redox reaction - Fe^{+3}/Fe^{+2}; 3 – silver chloride electrode (reference electrode); 4 – test solution; 5 – solution Fe^{+3}/Fe^{+2}.

Solutions of 0.02 M iron (III) sulfate and 0.02 M iron (II) sulfate heptahydrate were prepared. The solutions were then combined in a 3:1 ratio to obtain a solution with iron (III) and (II) ions.

A 0.01 M ascorbic acid solution was prepared.

The EMF of the following solutions was measured over time (0, 5, 10, 15, 20, 25, and 30 minutes):

5 ml of extract;

15 ml of a combined solution of 0.01 M iron (III) sulfate and 0.01 M iron (II) sulfate heptahydrate in a 1:3 ratio.

5 ml of 0.01 M ascorbic acid solution;

15 ml of a combined solution of 0.01 M iron (III) sulfate and 0.01 M iron (II) sulfate heptahydrate in a 1:3 ratio.

The solutions were prepared by serial dilution.

2.2.8 Quantitative determination of flavonoids in the extract

It is accepted to determine the sum of flavonoids in extracts, the predominant amount of which is rutin, by carrying out a complexation reaction with aluminum chloride (III) in a weakly acidic medium. In this case, a bathochromic shift of the absorption band of flavonoids from 330-350 to 390-410 nm into the visible region of the spectrum is observed [103]. The mechanism of the complexation reaction of flavonoids with aluminum chloride is shown in Fig. 20.

Fig. 20 - Mechanism of the reaction of complex formation of flavonoids with aluminum chloride

Rutin was used as a standard for determining flavonoids in the extract [104]. In this case, the determination of most bioflavonoids is carried out in the wavelength range of 408 - 420 nm [105].

The spectrum was plotted using the complexation reaction of rutin with a solution of aluminum chloride (III). Reference and stock solutions were prepared using the following procedure [66, 87].

Preparation of comparison solution (solution A):

3 ml of the extract, 0.2 ml of 1% acetic acid were pipetted into a 25 ml flask, then brought to the mark with 95% ethyl alcohol.

Preparation of the initial solution (solution B):

3 ml of the extract,126 0.2 ml of 1% acetic acid, 5 ml of 10% aluminum (III) chloride solution were pipetted into a 25 ml flask, then brought to the mark with 95% ethyl alcohol.

Solutions A and B were kept for 30 minutes. The absorption spectrum of rutin solutions of different concentrations was recorded on an SF 2000 spectrophotometer and the analytical wavelength (wavelength corresponding to the absorption maximum) was determined. Then, solutions of the standard rutin sample of various concentrations were prepared and the optical density was determined using a KFK-3KM photocolorimeter at an analytical wavelength. A calibration graph was constructed based on the data obtained [106].

The reaction of complexation of flavonoids contained in the obtained extracts with a solution of aluminum chloride (III) was carried out in a similar manner. Then the extract was diluted 30 times.

The quantitative content of flavonoids in the extract was determined using a KFK-3KM photocolorimeter at a wavelength of 410 nm, a cuvette thickness of 1 cm or 0.5 cm (depending on the obtained optical density).

The quantitative content of flavonoids in the extracts was calculated using the formula (31):

$$C=\frac{D*C_{st}}{D_{st}}*Kd*V_{extract}/l \tag{31}$$

where D – optical density of the test solution,

D_{st} – optical density of the standard rutin solution of concentration C_{st}, mg/ml

C_{st} – concentration of the standard rutin solution,

Kd – dilution factor,

$V_{extract}$ – volume of the obtained extract, ml

l – cuvette thickness, cm

2.2.9 Determination of the influence of MW- and US- exposure on the quantitative content of flavonoids in the extract

A 5 g sample of raw material was placed in a 100 ml round-bottomed flask, 10 ml of distilled water (extractant) were added, and the mixture was left to infuse for 1 hour.

Then another 20 ml of extractant was added to the flask and the mixture was treated with MW or ultrasound radiation.

MW exposure.

The flask with raw material and extractant was placed in a 500 ml porcelain cup with cold water at 20 0C, this setup was placed in a Mystery MMW-2315G microwave chamber and irradiated for a set time [4]. Every minute, the water in the bath was replaced with cold water to avoid excessive heating of the raw material, thus maintaining the set water temperature in the range of 25 ± 5 °C.

The study was carried out in a microwave chamber at a power of 900 W and a field frequency of 2450 MHz (intensity 10 mV / cm^2).

US- exposure. The flask with the raw material and extractant was placed in a PSB.2835.05 US bath with an operating frequency of 35 kHz, an intensity of 0.5 - 1.0 W/cm^2 and irradiated for a specified time [85, 107].

After treatment with MW or US radiation, extraction was carried out using two methods depending on the purpose of the study: maceration without heating or with heating (p. 2.2.2).

2.2.10 Determining the effect of freezing/defrosting on the quantitative content of flavonoids in the extract

A 5 g sample of raw material was placed in a 50 ml beaker, 10 ml of distilled water (extractant) were added, and the mixture was left to stand for 1 hour.

Then another 20 ml of extractant were added to the beaker. The beaker was sealed and placed in an ATLANT XM 4009 freezer (temperature -18 ± 1 °C) for three days.

After which the beaker was defrosted at room temperature and extraction was carried out with heating (section 2.2.2.3).

2.2.11 Obtaining extracts from Japanese pagoda tree fruits under conditions of multiple extraction

At the first stage, extraction was carried out using the percolation method (section 2.2.2.2). Distilled water and 50% ethyl alcohol were used as the extractant.

Then the used meal (raw material remaining after extraction) was left in the lower percolator and a repeated percolation cycle was carried out. The number of cycles was determined by the content of the total amount of flavonoids in the extract after extraction.

2.2.12 Obtaining extracts from Japanese Sophora fruits in order to describe the kinetics of extraction

The extraction kinetics were determined using the maceration method (section 2.3.3.1).

10 ml of the extractant were added to a 5 g sample of raw material. It was left sealed for an hour. Then 20 ml of the extractant were added and left to infuse for 5, 10, 20, 40, 60, 90, 120 and 150 minutes. One extract was used for each point.

The obtained extracts were filtered using ash-free filters "blue ribbon".

2.2.13 Determination of BAS in the extract

Determination of biologically active substances was carried out using the methods described by Aslonova I. Zh. [3]. These methods are also described in other articles, for example, in the work of M. I. Fedorovska [108, 109].

2.2.13.1 Determination of flavonoids

The following reactions were used to determine flavonoids in the extract: "Cyanidin test", reaction with aluminum chloride (Fig. 21), reaction with iron trichloride (Fig. 22), Wilson reaction (Fig. 23), reaction with ammonia (Fig. 24).

Cyanidin test

1 ml
extract

Mg,
HCl conc.

raspberry
coloring

H_2O,
C_4H_9OH

Flavonoid aglycones

Flavonoid glycosides

Reaction with aluminum chloride

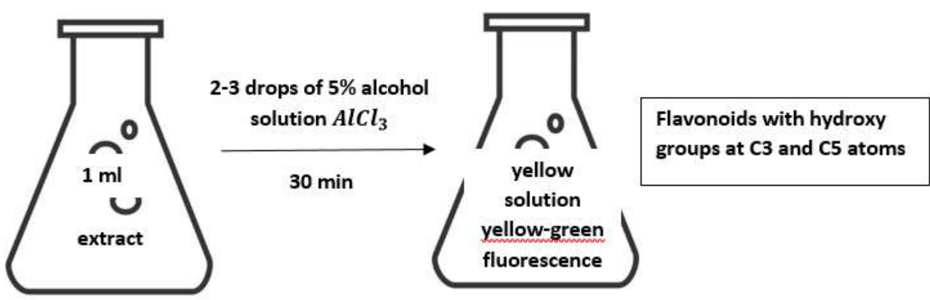

Fig. 21 - Qualitative reaction for determination of flavonoids in the extract

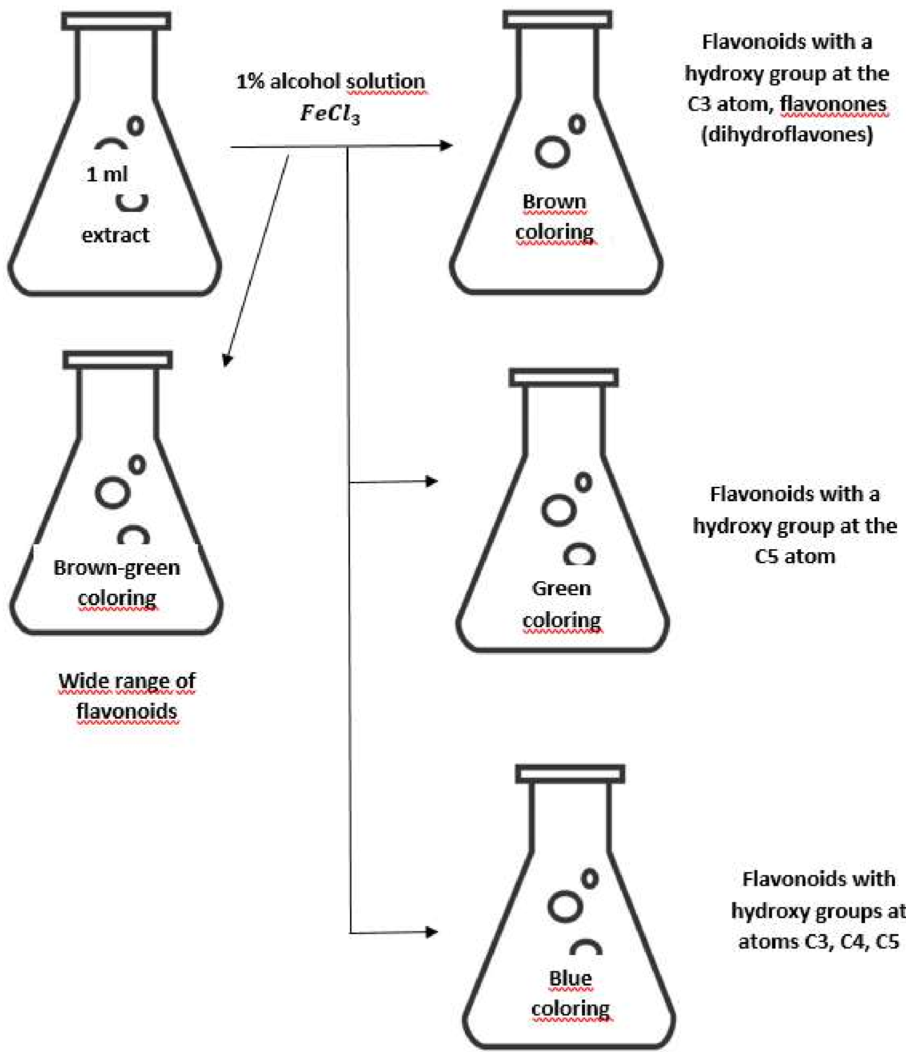

Fig. 22 - Reaction with iron trichloride for determination of flavonoids in the extract

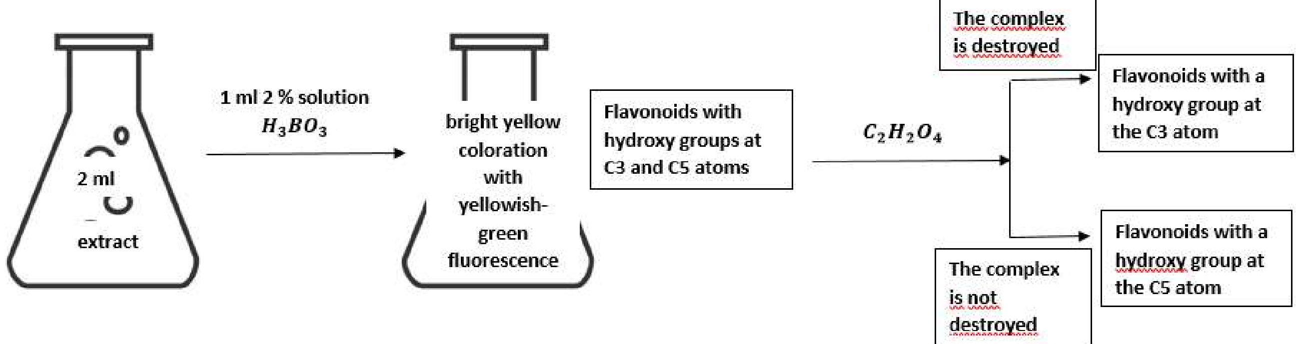

Fig. 23 - Wilson reaction for determination of flavonoids in the extract

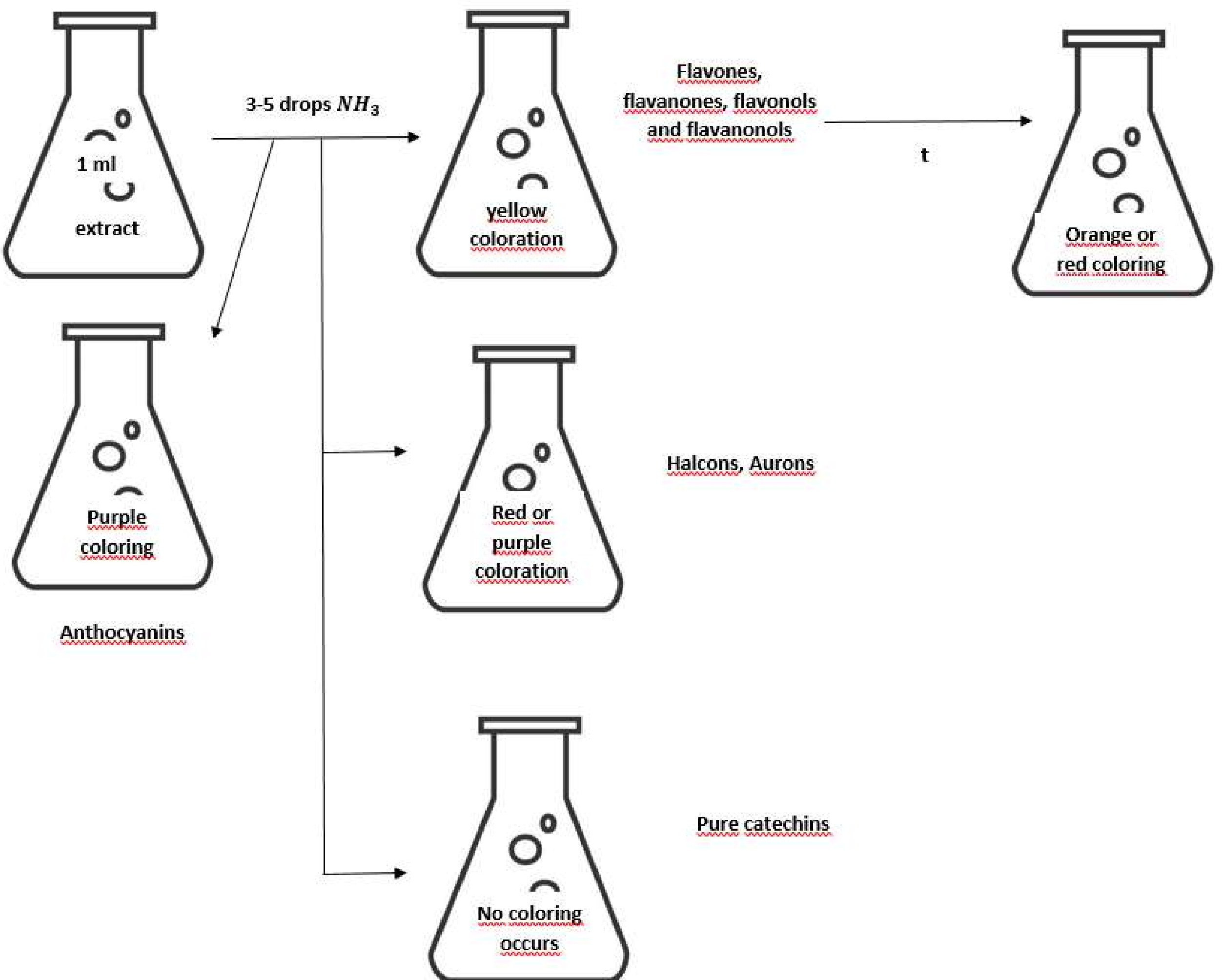

Fig. 24 - Reaction with ammonia to determine flavonoids in the extract

Thin-layer chromatography was also used to determine flavonoids in the extract. A solution of n-butanol, acetic acid and water in a ratio of 4:1:5 (mobile phase) was placed in the chromatographic chamber.

The extract and standard rutin solution were applied with a microsyringe to a Silufor silica gel plate so that the distance between samples was no more than 10 mm. The plate with the applied samples was dried in air. After the mobile phase passed through the plate, it was removed, sprayed with a 5% aluminum chloride solution, dried in air and viewed in a TLC-254\365 UV irradiator at a wavelength of 354 nm.

2.2.13.2 Determination of simple phenols

To determine simple phenols in the extract, reactions with iron (II) sulfate and ferric ammonium alum were used (Fig. 25).

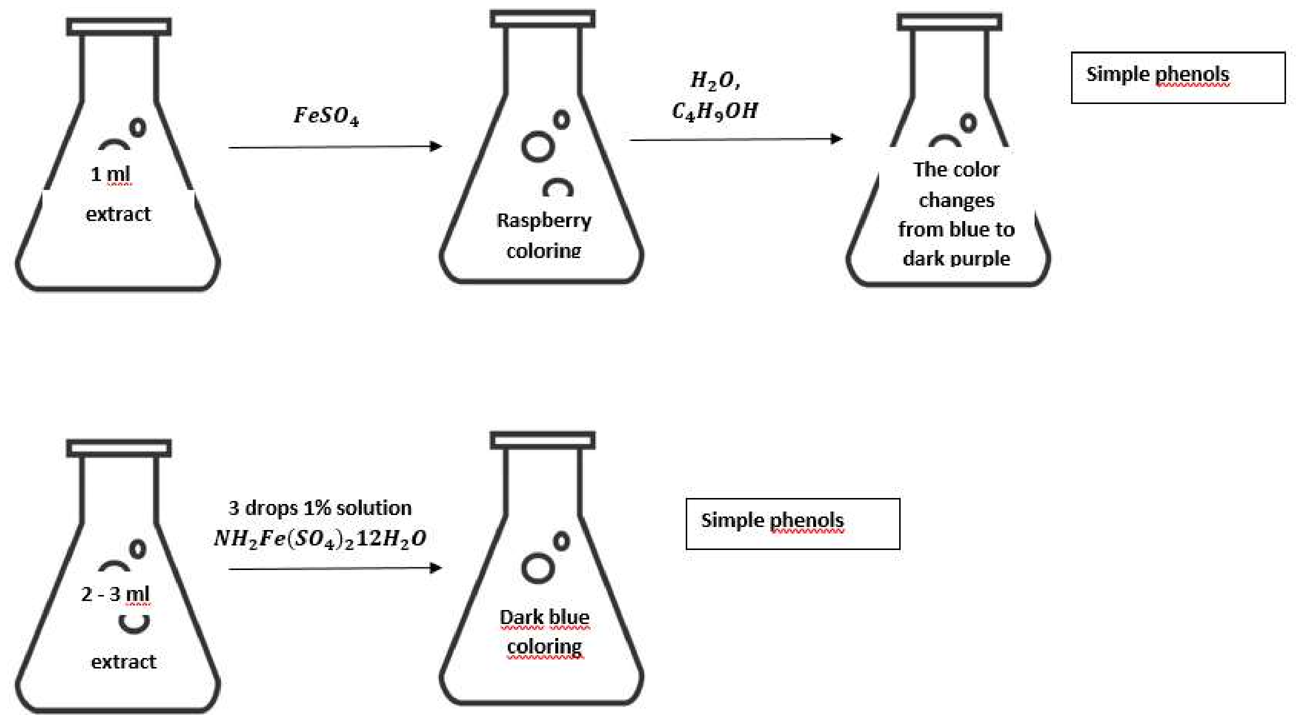

Fig. 25 - Qualitative reactions for the determination of simple phenols in the extract

2.2.13.3 Determination of polysaccharides

To determine polysaccharides in the extract, reactions with ethyl alcohol, sodium hydroxide and concentrated hydrochloric acid were used (Fig. 26).

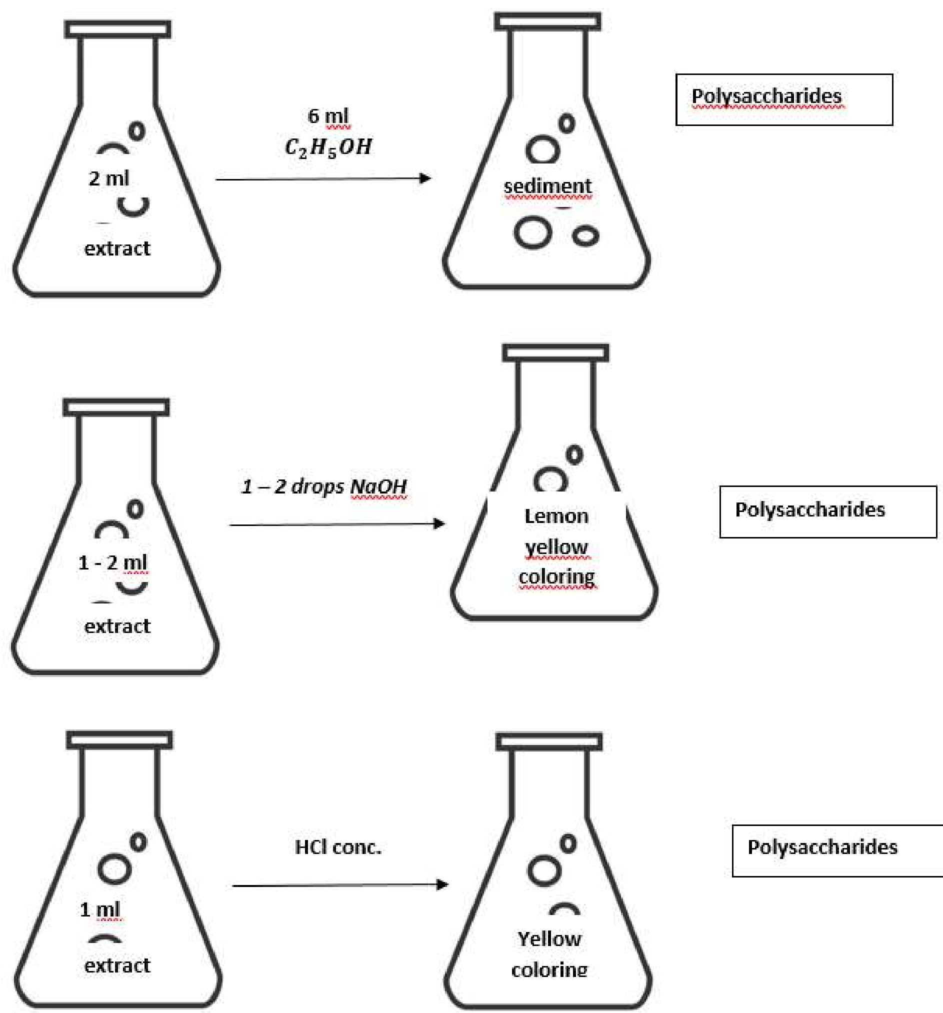

Fig. 26 - Qualitative reactions for the determination of polysaccharides in the extract

2.2.13.4 Determination of saponins

To determine saponins, reactions with 10% lead acetate and foaming (in alkaline and acidic environments) were used (Fig. 27).

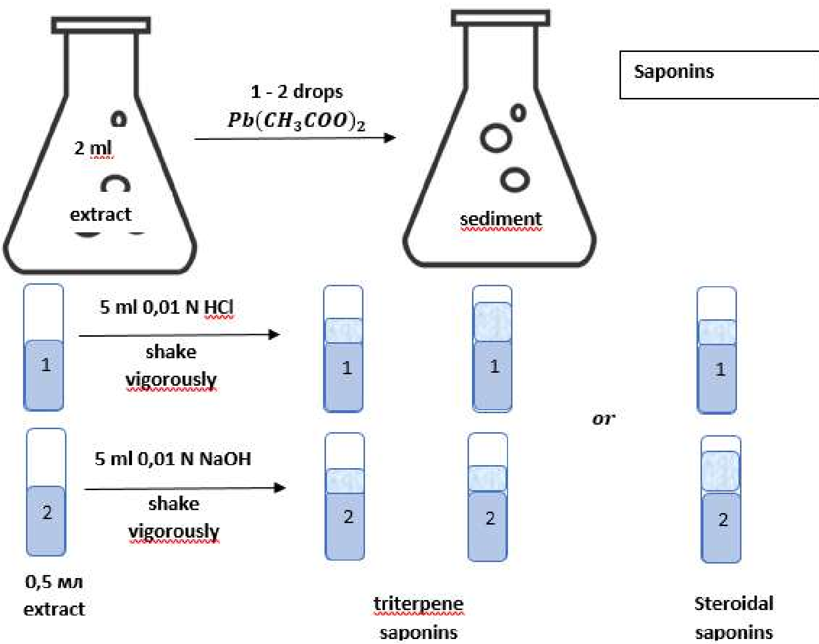

Fig. 27 - Qualitative reactions for the determination of saponins in the extract

2.2.13.5 Definition of alkaloids

To determine alkaloids in the extract, a reaction was carried out with a tannin solution (Fig. 28).

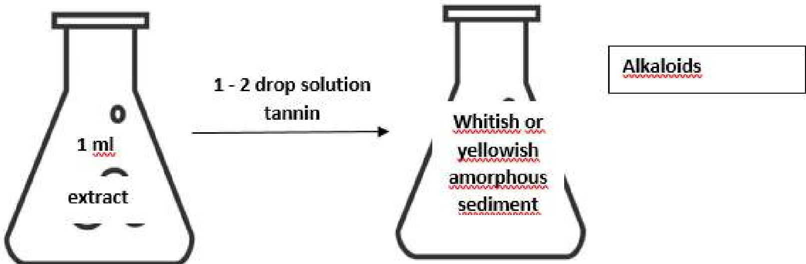

Fig. 28 - Qualitative reaction for determination of alkaloids in the extract

2.2.13.6 Determination of tannins

To determine tannins in the extract, the following reactions were used: reaction with a gelatin solution, reaction with potassium dichromate (Fig. 29), reaction with iron (III) salts, reaction with sodium nitrite (Fig. 30).

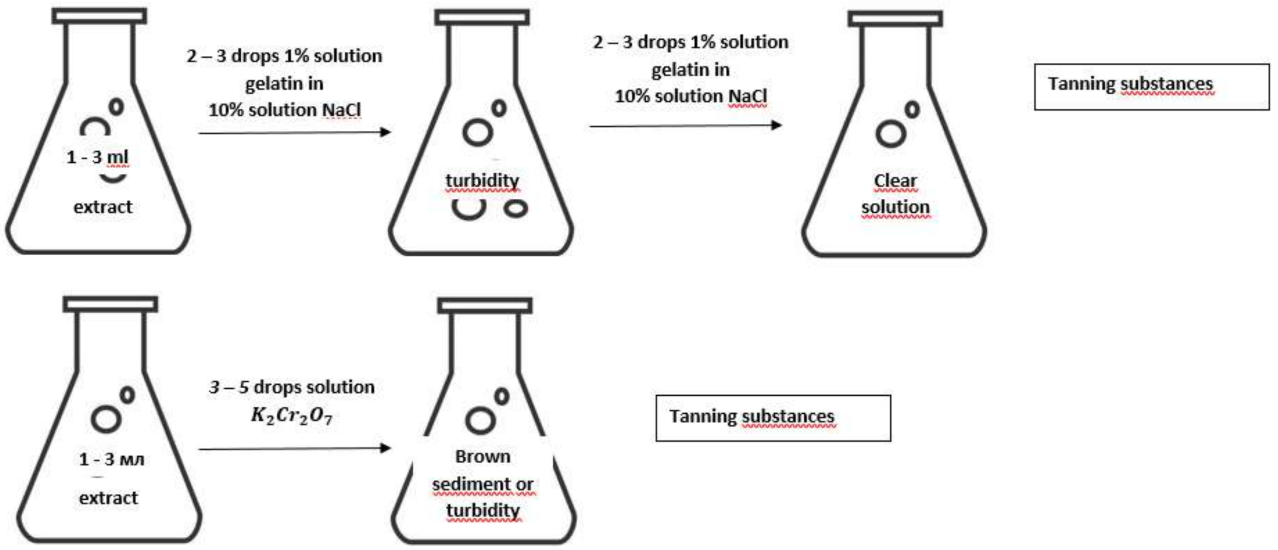

Fig. 29 - Qualitative reactions (reaction with gelatin solution, reaction with potassium dichromate) for determining tannins in the extract

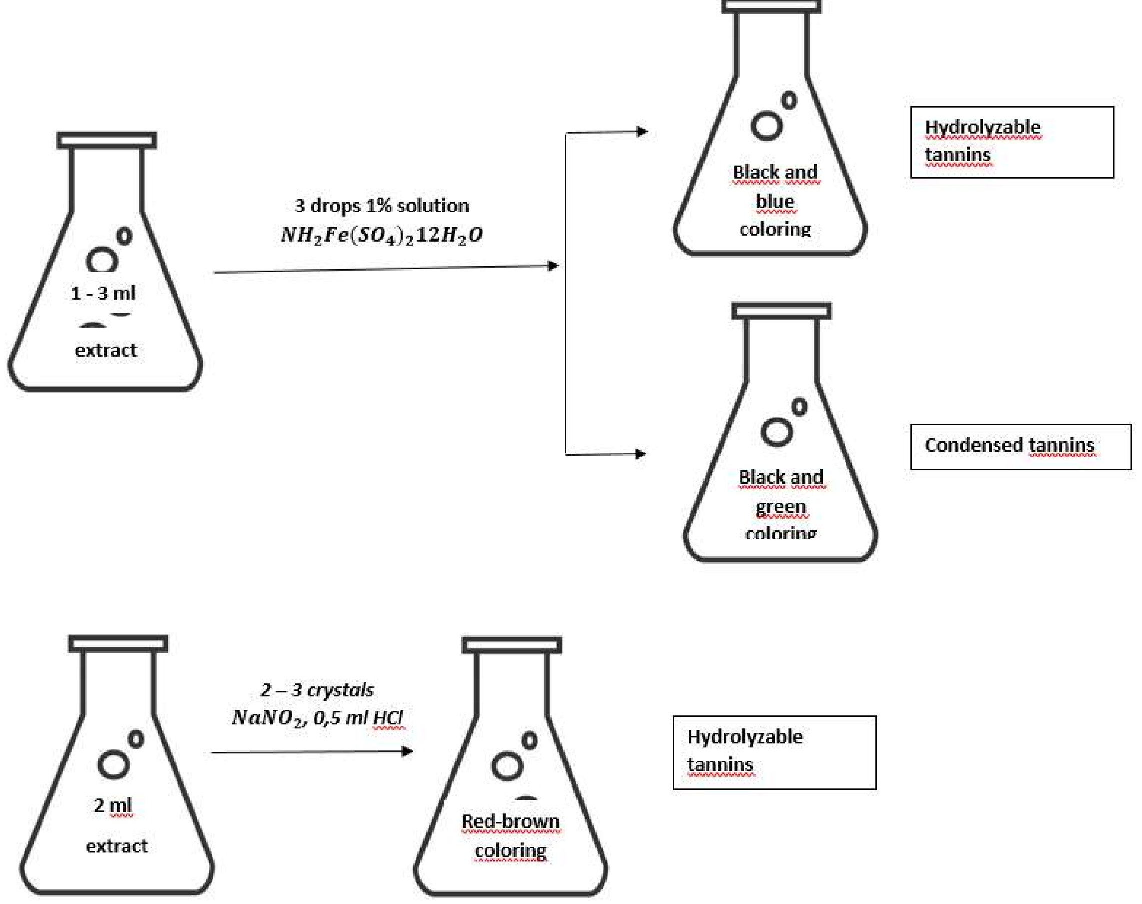

Fig. 30 - Qualitative reactions (reaction with iron (III) salts, reaction with sodium nitrite) for determining tannins in the extract

2.2.14 Statistical data processing

Statistical data processing was performed in Excel. The reliability of differences between samples was assessed using the parametric method for independent samples, determining the Student's t-test. Ten measurements were used for each study option. The calculated t-statistic value was 2.1, which is less than the critical value for this number of observations (degree of freedom 4) and the significance level ($p < 0.05$). The results were significant, the differences between samples were reliable at the reliability level ($p < 0.05$).

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1 Justification for the choice of methods for extracting BAS from plant materials

Dynamic and static extraction methods are used for flavonoid extraction.

Dynamic methods include percolation. This method involves passing an extractant flow through the raw material at a given speed. The process is accelerated by constantly creating a difference in concentrations. In some cases, this allows extracts with a high content of BAS to be obtained, so this method was used to study the extraction of flavonoids from Sophora Japanese fruits under dynamic conditions.

Statistical methods include maceration. This method involves infusing the raw material in an extractant until dynamic equilibrium is established. Maceration is the simplest extraction method with a low degree of process variability, so it was chosen when assessing various factors affecting the process.

The maceration method is often combined with heating in a boiling water bath in order to increase the speed and completeness of flavonoid extraction, in connection with which this method was also used when assessing factors affecting the process.

3.2 Qualitative determination of BAS in the extract from the Sophora Japanese fruits

Plant materials have a complex composition, including various BAS, such as flavonoids, alkaloids, terpenes, phenolic compounds and others. Each plant contains a unique set of chemical compounds, which can vary depending on many factors, including the plant species, growing conditions, climate, soil and processing methods [82].

The dried plant cell wall consists of the main components of the cell wall, which can be partially changed or damaged during the drying process. These include:

- ***Cellulose:*** This is the main polymer that makes up the cell wall of plant cells. Cellulose provides rigidity and strength to the cell wall.
- ***Hemicellulose:*** This is the second most common polymer in the cell wall, which strengthens it and is involved in the formation of intercellular bonds.
- ***Pectin:*** This is a group of polysaccharides that is usually found in the spaces between cellulose and hemicellulose. Pectin has the ability to attract water, which may play a role in retaining moisture in the plant cell.
- ***Lignins:*** These are complex polymers that provide rigidity and stability to the cell wall. Lignins can be particularly strong and resistant to various external factors.
- ***Proteins:*** The cell wall can also contain proteins that perform various functions, such as protection against pests or participation in signaling processes.
- ***Lipids:*** Some lipids, such as waxes, can be partially present in the cell wall and play a role in its hydrophobicity and protection.

Due to this complexity of the composition of plant material, the choice of the extraction method and the optimal process conditions play a decisive role in obtaining an extract with a high concentration of the desired components. Each extraction method has its own advantages and limitations, and the choice of the optimal method depends on the goals and requirements for the final product.

Therefore, it is important to carefully select the extraction method and conduct preliminary studies of the composition of plant materials in order to achieve maximum efficiency and preserve the BAS in the resulting extract.

3.2.1 Determination of flavonoids

As a result of the identification of flavonoids using qualitative reactions (section 2.2.5.1), the following results were obtained:

1. Cyanidin test. When reduced with magnesium in the presence of hydrochloric acid, a weak orange coloration was formed in a test tube with the extract from the fruits of Sophora japonica. Therefore, the extracts from the fruits of Sophora japonica contain flavonols, flavonones or flavones.
2. Reaction with aluminum chloride. A weak lemon-yellow coloration was formed in a test tube with the extract from the fruits of Sophora japonica. Therefore, these extracts contain flavonoids with two oxygroups in the positions C_3 and C_5.
3. Boric acid reaction (Wilson reaction). A bright yellow coloration with yellow-green fluorescence occurred in a test tube with the extract from the fruits of Sophora japonica; upon further addition of the corresponding organic acids, the coloration and fluorescence disappeared. These phenomena indicate the presence of 3- and 5-hydroxy flavones and 3- and 5-hydroxy flavonols.
4. Reaction with ammonia. In a test tube with an extract from the fruits of Sophora japonica, a weak yellow coloration occurred, which turned orange when heated. These phenomena indicate the presence of flavones, flavonols or flavonones.
5. Reaction with iron trichloride. In a test tube with an extract from the fruits of Sophora japonica, a brown-green coloration occurred, which indicates the presence of 3- and 5-hydroxy flavonoids in these extracts.

Thin-layer chromatography for identification of flavonoids in the extract

On the plate with the standard solution of the rutin sample, the extract from the fruits of Sophora japonica after chromatography, a yellow-brown spot of weak color was found on the chromatography line of the rutin standard, the same spot (in size, position and color) was found on the chromatography line of the solution of Sophora japonica.

The Rf coefficient was calculated as the ratio of the velocity of the substance front to the velocity of the solvent front (the distance traveled by the substance to the distance traveled by the solvent). It was 0.6 for the standard sample of rutin and for the obtained extract.

This confirms the presence of flavonoids in the extract from the Sophora Japanese fruits.

3.2.2 Determination of simple phenols

As a result of identification of simple phenols using qualitative reactions (section 2.2.5.2), the following results were obtained:

1. When interacting with ferrous sulfate in the extracts from the fruits of Sophora japonica, a reddish-violet coloration is formed, then a dark-violet coloration, upon standing, a dark-violet precipitate is formed. Therefore, the extract from the Sophora Japanese fruits contains simple phenols.

2. In the reaction with ferric ammonium alum, the extract from the Sophora Japanese fruits gave a dark blue coloration, which indicates the presence of hydrolyzable simple phenols in it.

3.2.3 Determination of polysaccharides

When identifying polysaccharides using qualitative reactions (section 2.2.5.3), the following results were obtained:

1. When interacting with ethyl alcohol in the extract from the Sophora Japanese fruits, mucus is precipitated from the aqueous extract. Flaky clots appear, which eventually precipitate when left to stand - polysaccharides.

2. Reaction with an alkali solution. The extract from the Sophora Japanese fruits in a reaction with sodium hydroxide acquires a lemon-yellow color. Therefore, the extract from the Sophora Japanese fruits contains polysaccharides.

3. Reaction with hydrochloric acid. In a reaction with concentrated hydrochloric acid, the extract from the Sophora Japanese fruits acquires a yellowish-green color. After adding 95% ethanol, the mucus coagulates into a porous sediment. Therefore, the extract from the Sophora Japanese fruits contains polysaccharides.

3.2.4 Determination of saponins

The following results were obtained when identifying saponins using qualitative reactions (section 2.2.5.4):

1. When lead acetate is applied to the extract from Sophora Japanese fruits, saponins are precipitated. Therefore, the extract contains saponins, with triterpene saponins precipitated by medium lead acetate, and steroidal saponins by basic lead acetate.

2. When the extract from Sophora Japanese fruits reacted with hydrochloric acid and alkali, foam was formed, approximately equal in volume and stability in both environments. This indicates the presence of triterpene saponins in the extract.

3.2.5 Determination of alkaloids

The reaction with tannin (section 2.2.5.5) in the extract from the Sophora Japanese fruits resulted in the formation of a whitish amorphous precipitate. Therefore, it contains alkaloids

3.2.6 Determination of tanning substances

The following results were obtained when identifying tannins using qualitative reactions (section 2.2.5.6):

1. No precipitate or turbidity was formed with the gelatin solution. Therefore, the extract from the Japanese pagoda tree fruits contains tannins.

2. The reaction with potassium dichromate in the test tubes with the extract from the Japanese pagoda tree fruits resulted in the formation of a turbid solution. Therefore, the extract from the Japanese pagoda tree fruits contains tannins.

3. When adding a solution of ferric ammonium alum to the test tube with the extract from the Japanese pagoda tree fruits, a black-green color appeared. Therefore, the extract from the fruits of Sophora japonica contains condensed tannins.

4. In the reaction with sodium nitrite in an acidic medium in test tubes with extracts from the fruits of Sophora japonica, no changes occurred. Therefore, the extract from the fruits of Sophora japonica does not contain hydrolyzable tannins.

3.3 Quantitative determination of flavonoids in the extract from Sophora Japanese fruits

Sophora Japanese fruits contain a large amount of flavonoids and are therefore considered valuable medicinal plant raw materials. Thus, the target components in the obtained extract were flavonoids. Since the largest amount of them is rutin, the qualitative determination of the sum of flavonoids was carried out according to this standard.

The first stage was the complexation reaction of solutions of the standard sample of rutin of various concentrations with aluminum chloride (III), then absorption spectra were constructed (Fig. 31) and the analytical wavelength was determined - 410 nm.

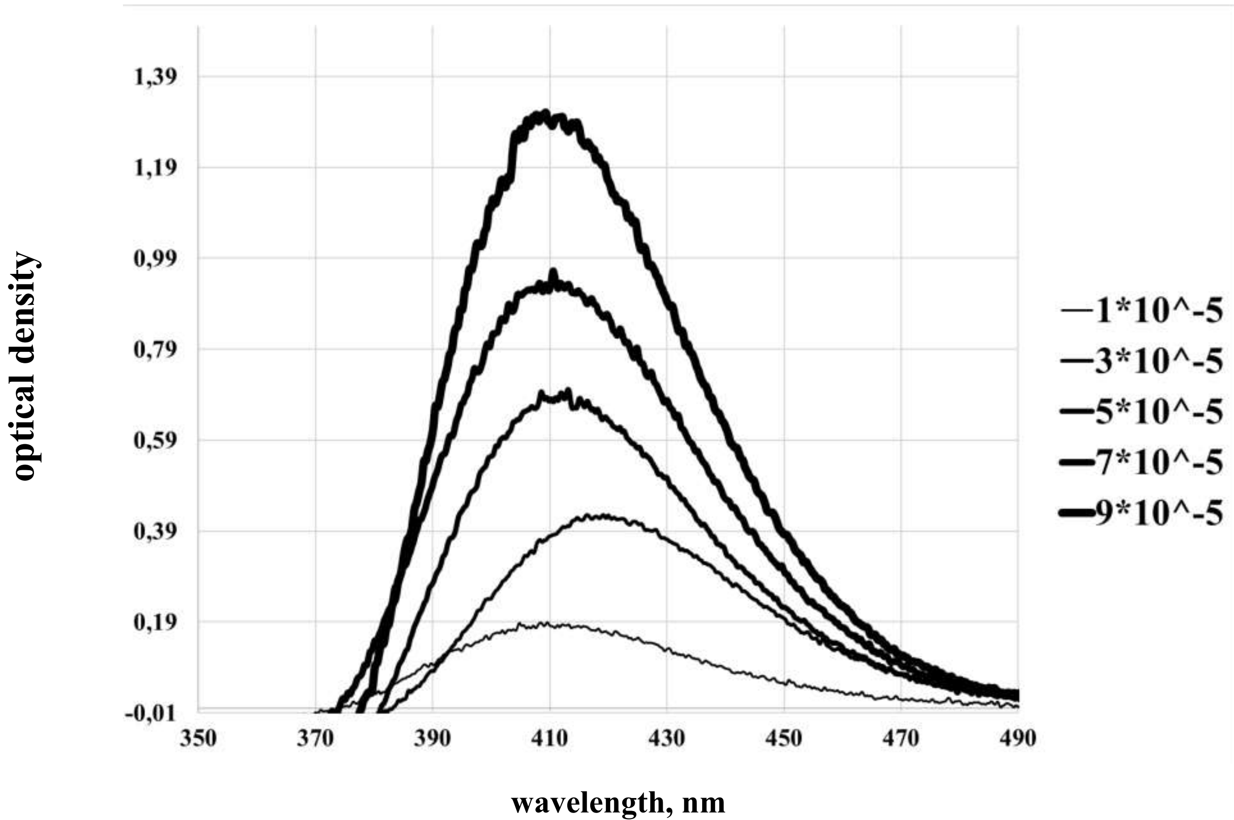

Fig. 31 - Absorption spectra of standard solutions of rutin of different concentrations ($1 \cdot 10^{-5}$ mol/l, $3 \cdot 10^{-5}$ mol/l, $5 \cdot 10^{-5}$ mol/l, $7 \cdot 10^{-5}$ mol/l, $9 \cdot 10^{-5}$ mol/l)

At this wavelength, the optical density of the studied complexes was determined on a photocolorimeter and a calibration graph was constructed for further determination of flavonoids in the extract (Fig. 32) [110].

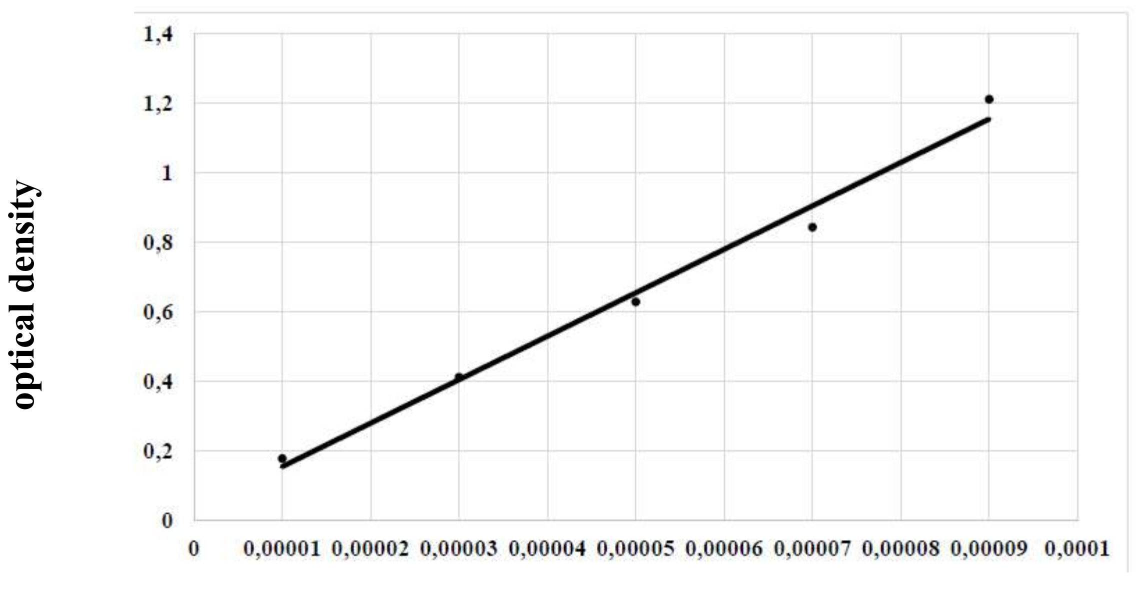

Fig. 32 - Calibration graph (dependence of optical density on rutin concentration)

3.4 Multiple extraction

In order to study the completeness of flavonoid extraction from Japanese pagoda tree fruits, multiple extraction was carried out using the percolation method (section 2.2.2.2). Purified water and 50% alcohol were used as extractants.

The total yield of flavonoids, or the content of flavonoids in the raw material, when using these extractants is approximately the same (within the error limits) (Fig. 33, 34). At the first stage (during the first extraction), the greatest extraction of flavonoids occurs with two extractants, since the difference in concentrations inside the plant cell framework and in the pure extractant is the greatest. At subsequent stages, the concentration of flavonoids inside the dried plant material decreases, and accordingly, the difference in concentrations and the yield of flavonoids decrease. At the first stage of extraction in water, a higher yield occurs than in alcohol, then a sharp decrease in the extractable substances occurs. In alcohol, a more uniform extraction occurs. This is explained by the fact that alcohol first affects the cell framework in such a way that it contracts and the pores are clogged and the diffusion of substances is hindered; at further stages, the cell framework (cellulose, lignin, and other components) is destroyed during hydrolysis. In water, hydrolysis occurs faster and more complete extraction of flavonoids occurs already at the first stage. Thus, the maximum content of flavonoids in the extract from the fruits of Sophora japonica can reach 16 - 18 mg / ml. Consequently, the content of flavonoids in the fruits of Sophora japonica is within 60-70 mg per 1 g of raw material. Extraction in a mixture of surfactants and in the alkaline region gives a yield of flavonoids close to the maximum value.

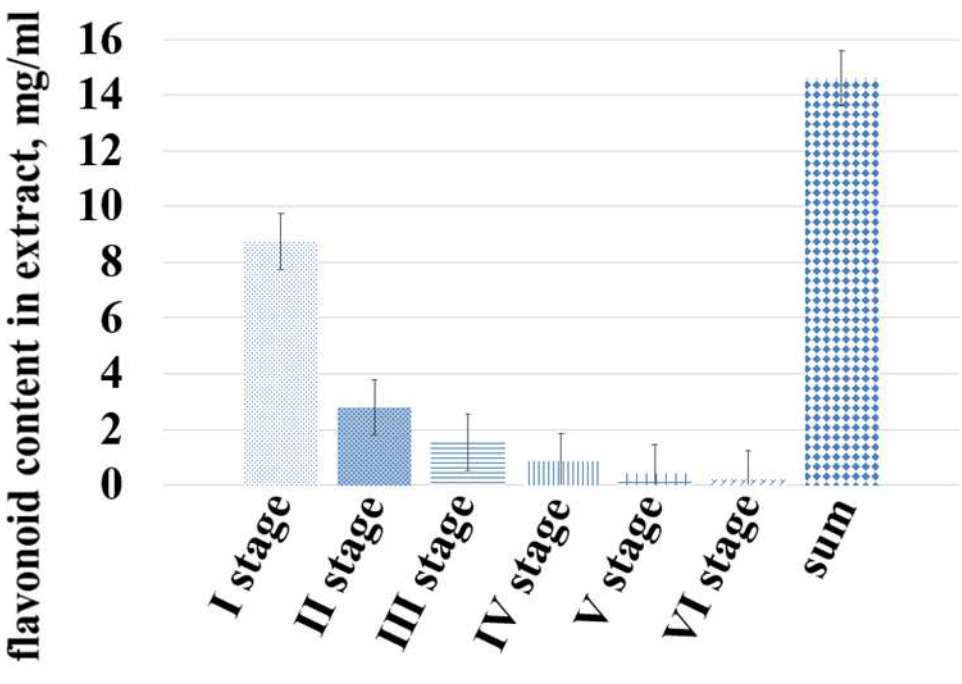

Fig. 33 - Dependence of the flavonoid content in the extract on the extraction stage (with water extraction) and the total flavonoid content in the raw material

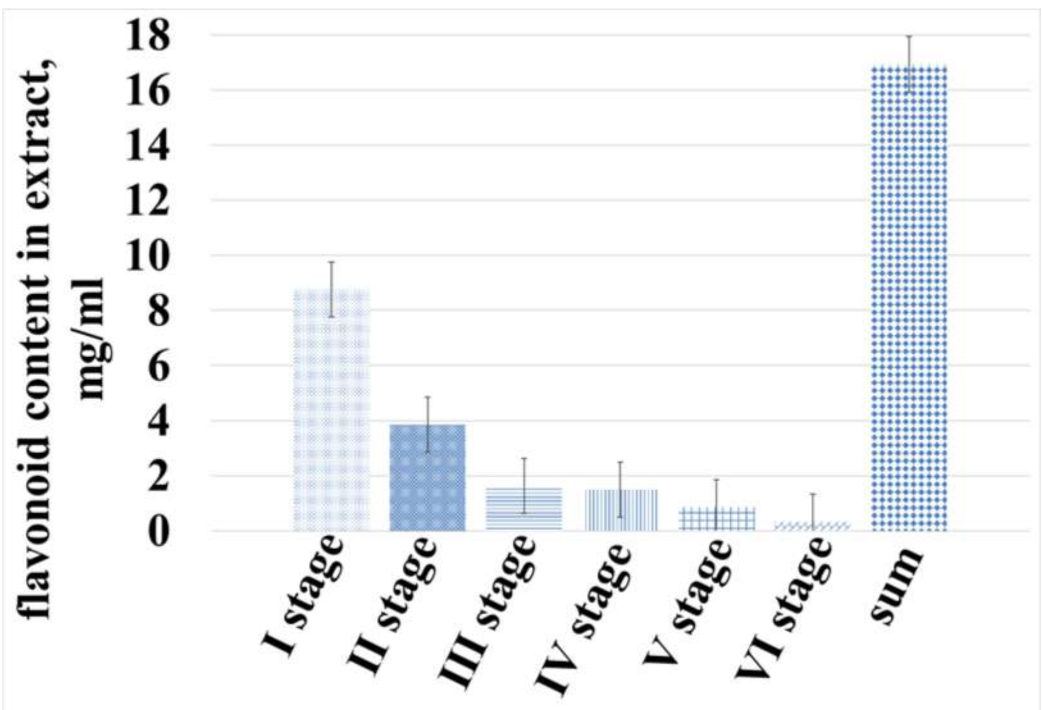

Fig. 34 - Dependence of the flavonoid content in the extract on the extraction stage (with extraction with 50% alcohol) and the total flavonoid content in the raw material

3.5 Influence of physicochemical factors on the process of flavonoid extraction from Sophora Japanese fruits

The process of BAS extraction from plant materials can be influenced by various physicochemical factors, including:

- extraction method;
- temperature;
- type of extractant and its concentration;
- surfactant content in the extractant;
- pH of the medium;
- degree of grinding of plant materials (particle size);
- parameters of swelling of plant materials in the extractant;
- MW- and US- exposure;
- freezing of plant materials.

The degree of influence of various factors is individual for each type of raw material and depends on its composition, therefore, studying these phenomena is important for increasing the speed and completeness of extraction.

3.5.1 Effect of the extraction method and alcohol concentration in the aqueous-alcoholic extractant on the extraction of flavonoids from Sophora Japanese fruits

Extracts from Japanese pagoda tree fruits with different alcohol contents in the extractant (aqueous-alcoholic solution) were obtained by the percolation method (section 2.2.3.2) and extraction by maceration with heating (section 2.2.3.3). After that, the quantitative content of flavonoids was determined.

In the process of extraction by the percolation method, the content of flavonoids in the aqueous extract predominates and gradually decreases with an increase in the alcohol concentration; a sharp decrease in the yield of flavonoids is observed during extraction with 95% alcohol (Fig. 35).

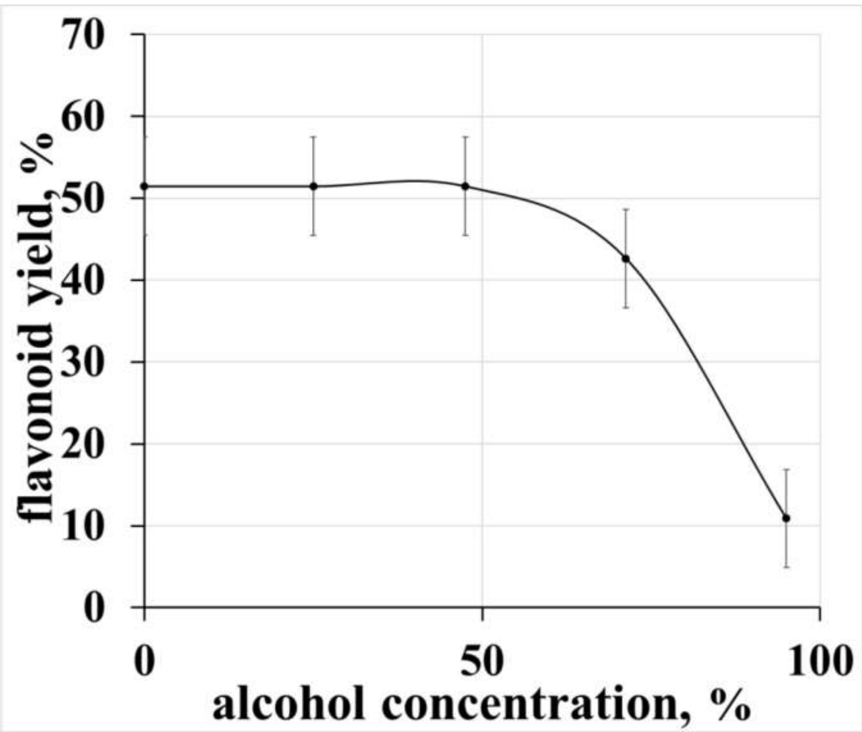

Fig. 35 - Dependence of the yield of total flavonoids on the concentration of alcohol during extraction by the percolation method

During extraction by the maceration method with heating, with an increase in the alcohol content in the water-alcohol solution, the yield of extracted flavonoids increased, the maximum yield is observed at an alcohol content of 50%, then a smooth decline occurs (Fig. 36).

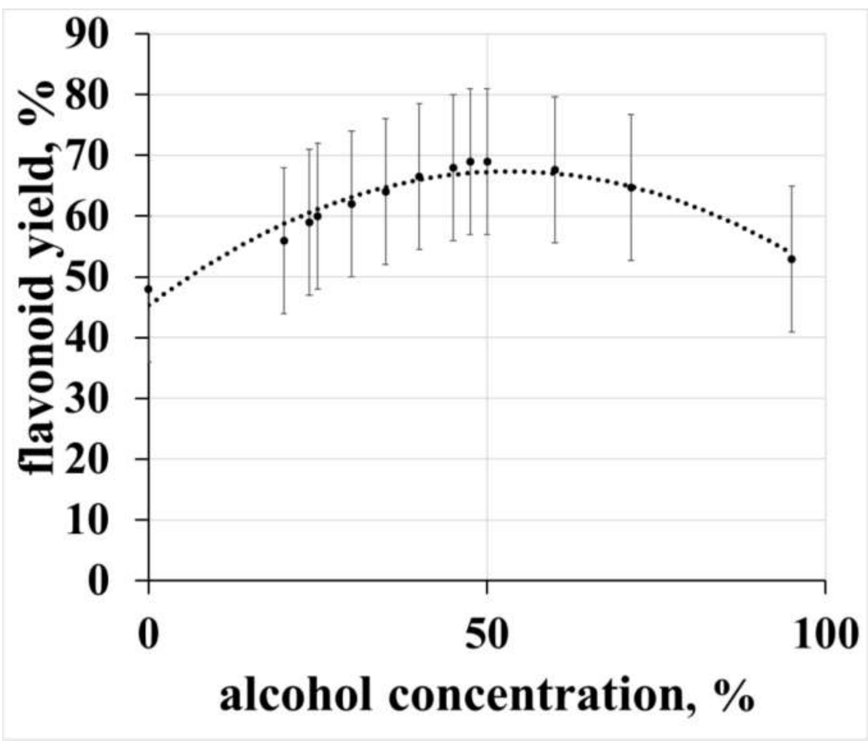

Fig. 36 - Dependence of the yield of total flavonoids on the concentration of alcohol during extraction by maceration with heating

The increase in the yield of flavonoids with an increase in the alcohol content in the solution is explained by the fact that alcohol has surface activity due to the amphiphilic nature of the molecule, as a result of which the surface tension decreases and the contact surface increases during extraction. A further decrease in the yield of flavonoids with a decrease in the alcohol content in the solution is explained by the fact that alcohol promotes the precipitation of mucus, proteins and some other biologically active substances.

Thus, during extraction in ethyl alcohol of various concentrations without heating and with heating, different dependencies were obtained. In both cases, the yield during extraction with water is close to 8 mg / ml. With an increase in the concentration of alcohol in the extractant during extraction by the percolation method, the yield of flavonoids gradually decreases, and when using pure alcohol, it decreases sharply. During extraction by the maceration method with heating, the yield gradually increases and then decreases, the maximum yield corresponds to an ethyl alcohol concentration of 50%.

Ethyl alcohol, on the one hand, takes away the remaining water from the particles of dried plant material, as a result of which they are even more compressed, which complicates the approach of the extractant into the cell (this effect prevails in the percolation process). On the other hand, ethyl alcohol has surface activity, which improves the process of spreading the extractant over the crushed fruits of Japanese pagoda tree. With an increase in temperature, the contact of the solvent with the fruits improves due to a decrease in the viscosity of the alcohol and an acceleration of the diffusion of substances into the extractant, and the destruction of strong bonds of the cell membrane also occurs, which reduces the

effect of cell wrinkling. As noted earlier, aglycone forms of flavonoids also dissolve better in ethyl alcohol, and glycoside forms in water. When exposed to temperatures, it is possible to improve the dissolution of two forms of flavonoids, and the ratio of alcohol and water 1:1 is optimal for dissolving two forms under these conditions, as a result of which their extraction increases.

3.5.2 Extraction in propylene glycol

For the extraction of flavonoids from plant raw materials, not only traditional water-ethanol solutions are used, but also other combinations of organic substances with water. Despite the high viscosity (56.0 mPa*s), the use of propylene glycol is used in the extraction of flavonoids [44, 111].

Extraction in propylene glycol was carried out by maceration with heating (p. 2.2.3.3). The content of flavonoids in the propylene glycol extract exceeds the content of these substances obtained when using water as an extractant, but is lower compared to the extract obtained using 50% ethyl alcohol (Table 5).

Table 5 - Content of flavonoids in the extract using various extractants

Extraction agent	Water	Ethyl alcohol 50%	Propylene glycol
Flavonoid content in the extract, mg/ml	8,30	13,39	9,15

Thus, the obtained results prove that propylene glycol as an extractant gives way to a water-alcohol mixture in the extraction of flavonoids from Japanese pagoda tree fruits. This is consistent with the literature data [111].

3.5.3 Effect of pH on the extraction of flavonoids from Sophora Japanese fruits

The extracts obtained using extractants with different pH were obtained by the percolation method (section 2.2.3.2). In an acidic medium, the yield of flavonoids decreases sharply, and in an alkaline medium, it increases (Fig. 37). However, when the extractant is alkalized above 7.9 ± 0.2, the pH of the extract remains within the pH of 6.62.

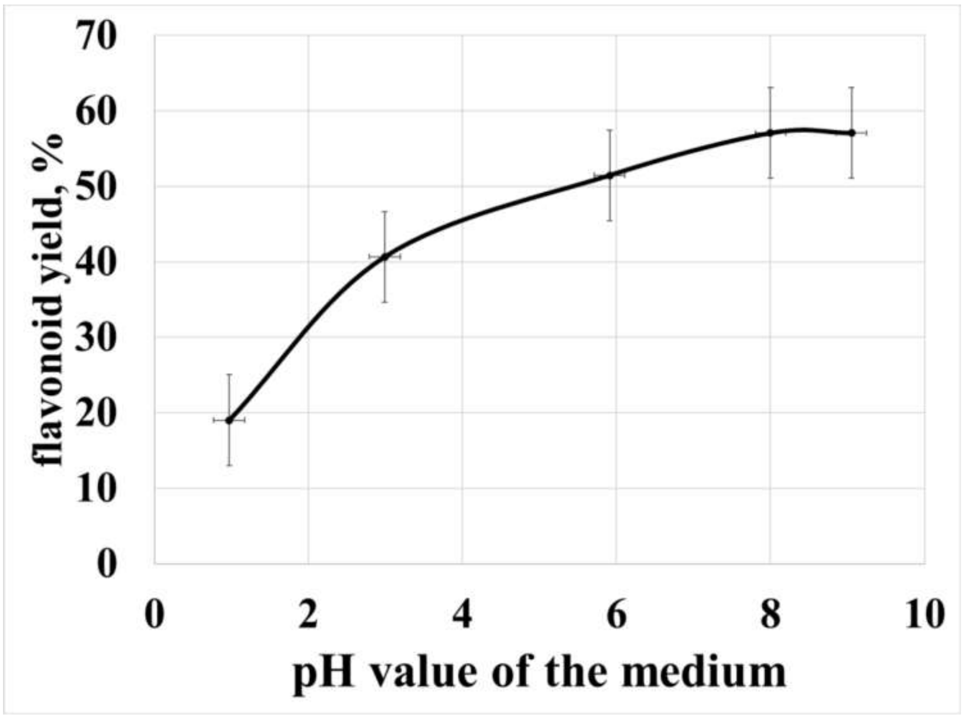

Fig. 37 - Dependence of the yield of the total amount of flavonoids on the pH of the medium (extraction by percolation)

Extraction studies were conducted in the alkaline region at an extractant pH of 9.1 under heating (p. 2.2.3.3). The yield of flavonoids in this case increased significantly (Fig. 38). With an increase in the concentration of OH^{-}, the dissolution of flavonoids in aqueous solutions improves and their diffusion into the extractant is facilitated, and due to the destruction of the framework of the dried plant cell (consisting of cellulose, lignin, etc.) under the influence of temperature, the desorption of substances is facilitated.

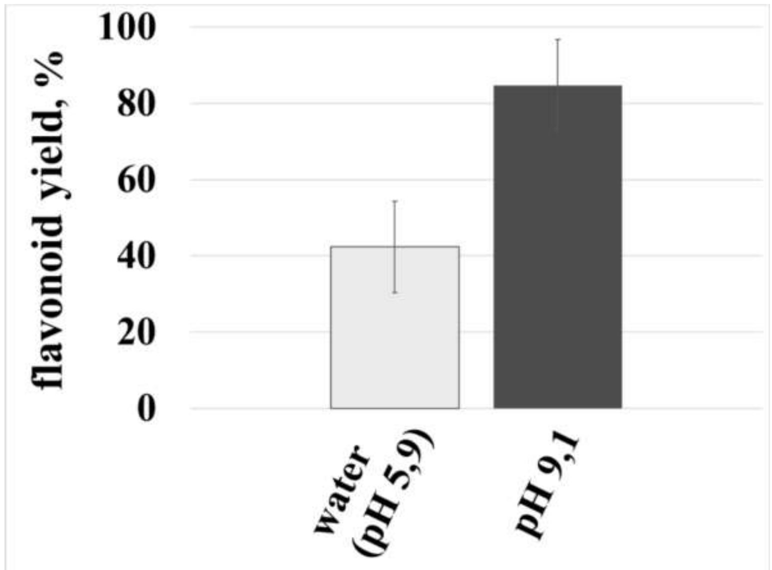

Fig. 38 - Dependence of the yield of the total amount of flavonoids on the pH of the medium (extraction with heating)

3.5.4 Effect of US- and MW- exposure on the extraction of flavonoids

The dependence of the yield of flavonoids during extraction from Sophora Japanese fruits by the maceration method (p. 2.2.2.1) after exposure to MW is shown in Fig. 39, and under ultrasound exposure - in Fig. 40.

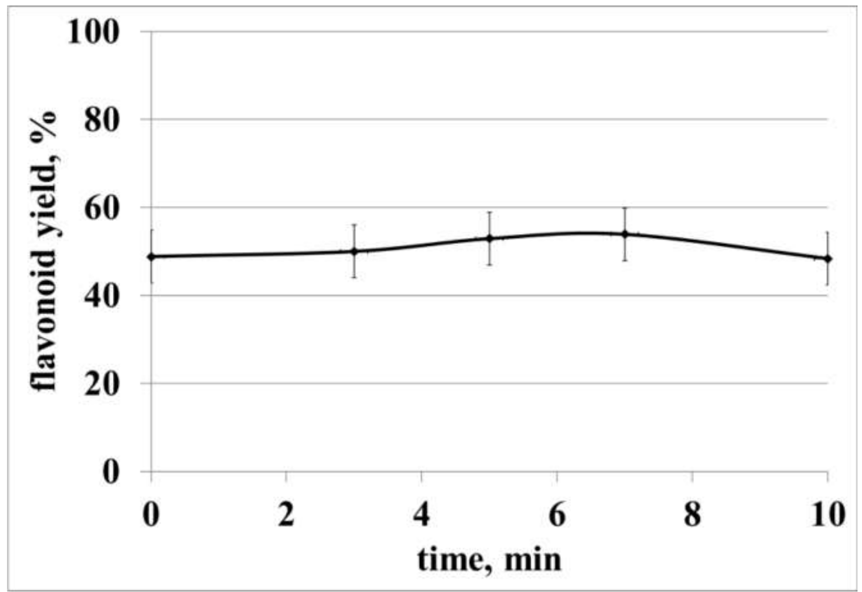

Fig. 39 - Dependence of flavonoid content in the extract on the time of MW exposure (extraction by maceration)

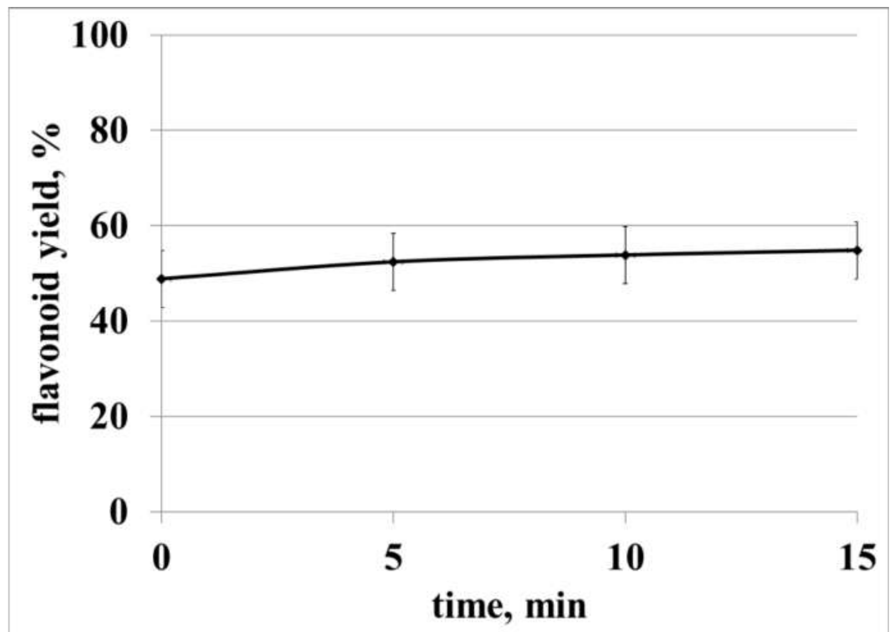

Fig. 40 - Dependence of flavonoid content in the extract on the time of US- exposure (extraction by maceration method)

With increasing time of exposure to MW- radiation, as well as with increasing time of exposure to US- radiation, the yield of flavonoids from Sophora Japanese fruits during extraction by the

maceration method (without heating) remains virtually unchanged. Probably, at a given power and frequency.

MW exposure does not cause sufficient destruction of the shell framework of the plant material.

The dependence of the yield of flavonoids during extraction from Sophora Japanese fruits during heating (p. 2.2.2.3) after exposure to MW radiation is shown in Fig. 41, and during ultrasound exposure - in Fig. 42.

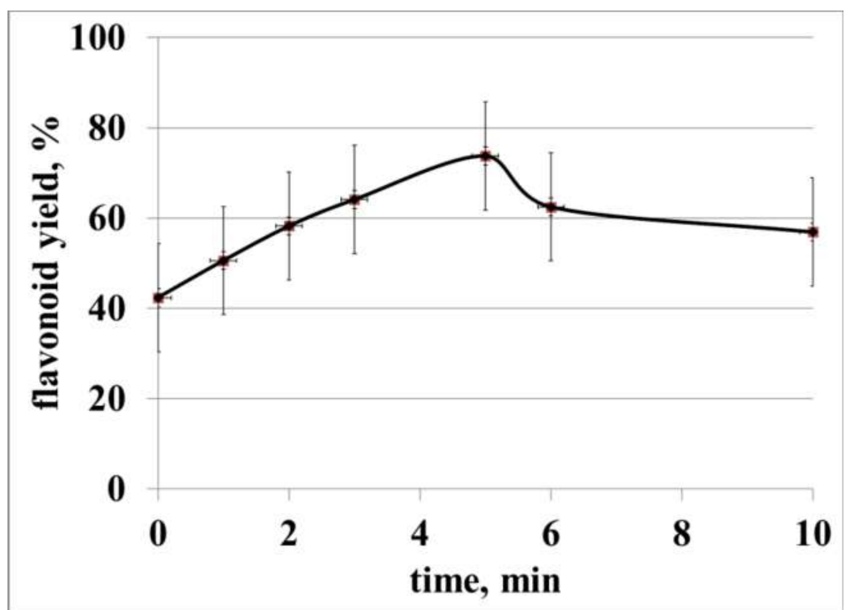

Fig. 41 - Dependence of flavonoid content in the extract on the time of MW- exposure (extraction with heating)

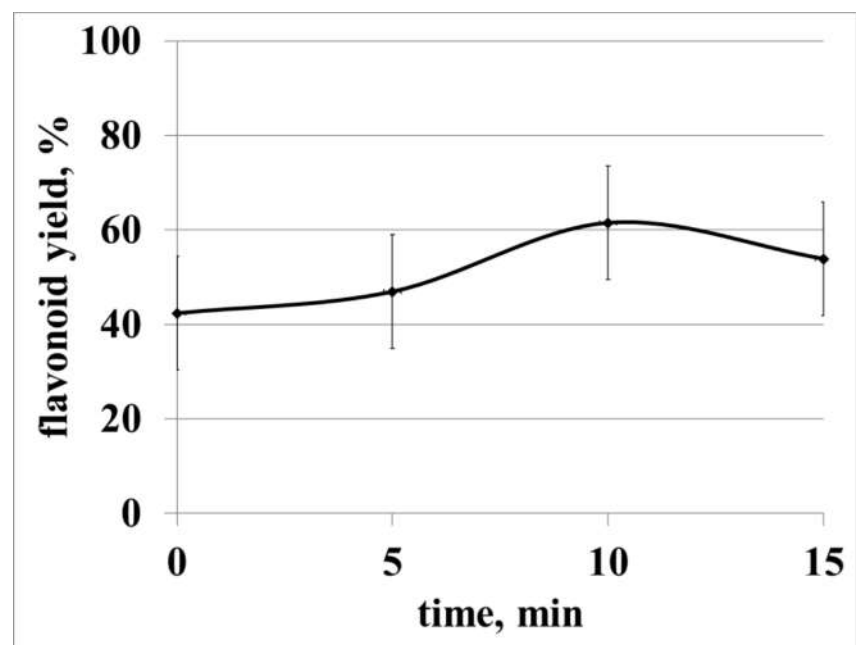

Fig. 42 - Dependence of the flavonoid content in the extract on the time of US- exposure (extraction with heating)

During extraction with heating and preliminary MW exposure (with a power of 900 W and a frequency of 2450 MHz), the rate of flavonoid extraction increases. This occurs due to an increase in the rotational component of water during MW radiation [23]. During the interaction of the extractant with the shell, its destruction begins (hydrolysis of cellulose and lignin), and continues with further heating of the plant cell. As a result, the desorption of flavonoids is facilitated.

An increase in the yield of flavonoids after US- exposure (with a frequency of 35 kHz and an intensity of 0.5 - 1.0 W $/cm^2$) occurs due to the destruction of the framework of the plant shell, due to the turbulent flow of the extractant, the collapse of cavitation bubbles and the rapid heating of the raw material. Another factor increasing the yield of flavonoids may be the improvement of their dissolution in the extractant after MW- and US- exposure. However, with prolonged exposure to temperature, flavonoids are destroyed during both MW and ultrasound treatment - the yield decreases.

The optimal time for microwave exposure (with a power of 900 W and a frequency of 2450 MHz) was determined to be 5 minutes and for US- exposure (with a frequency of 35 kHz and an intensity of 0.5 - 1.0 W $/cm^2$) - 10 minutes. At the same time, with MW- and ultrasound exposure, the yield of flavonoids increases during extraction with heating compared to extraction without preliminary irradiation. If extraction is carried out without heating under similar US- or MW- exposure, an insignificant increase in the yield of flavonoids in the extract is observed in the first case and no changes in the second.

3.5.5 Effect of freezing on flavonoid extraction

Extraction after the freeze/thaw cycle of the swollen sample of raw material was carried out with heating (p. 2.2.2.10). The extraction of flavonoids changed within the error limits (Fig. 43). Thus, freezing of raw material does not affect the destruction of the dried framework of the plant cell.

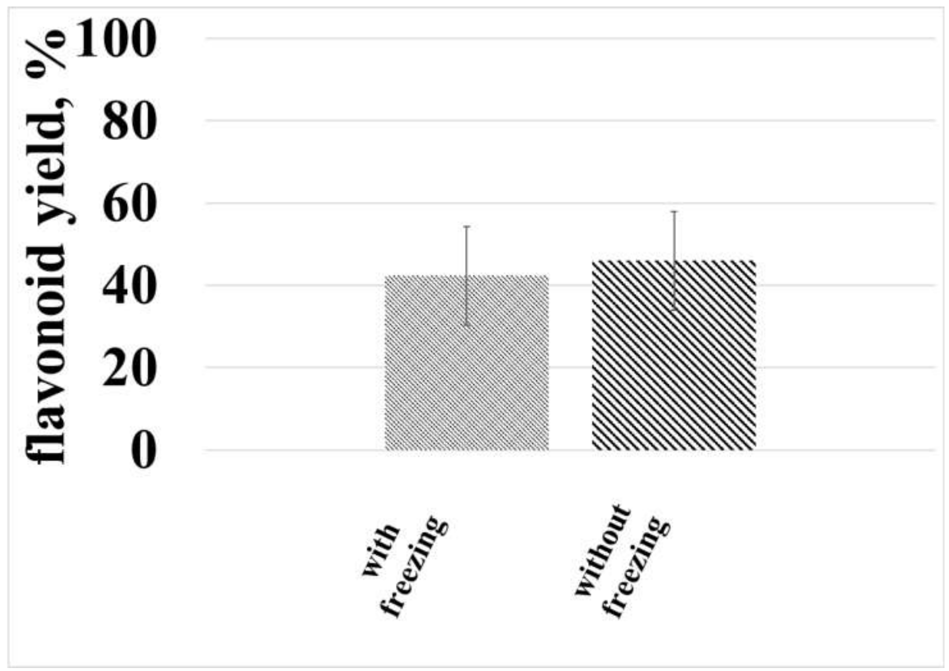

Fig. 43 - Dependence of flavonoid content in the extract after a freeze/thaw cycle of raw materials (extraction with heating)

3.6 Effect of colloidal properties of dispersions of Sophora Japanese fruits particles on the process of flavonoid extraction

3.6.1 Effect of the particle size of Sophora Japanese fruits on flavonoid extraction

Figure 44 shows the dependence of the rutin content in the extracts on the particle size. The extracts were obtained by the percolation method (p. 2.2.3.2). 50% ethyl alcohol was used as an extractant, as the most effective extractant for extracting flavonoids from Sophora Japanese fruits. The flavonoid content in the extract increased with increasing particle size, but having reached a size of 0.1-0.2 cm, it decreased and reached a plateau at a particle size of 0.2-1.0 cm.

On the filter after extraction, with large grinding (particle size less than 0.05 cm), a large amount of gel-like sediment remained, which did not pass through the filter. Filtration of the extract obtained during extraction from raw materials with a particle size of 0.05 to 1.0 cm occurred faster. The residue on the filter was minimal. This may indicate a small release of ballast and gel-like substances into the raw material, which hinder the release of the target components - flavonoids. Despite the fact that, according to the law of diffusion, the extraction rate increases with an increase in the contact surface of the extractant with the particles, excessive grinding of plant materials entails an increase in the number of torn dried cells, and this leads to the washing out of mucus, pectins and other HMVs from them. As a result, pores become clogged, which hinders the process of penetration of the extractant into the cell and diffusion of the target components into the extract, and the filtration process is also hindere [113].

Thus, it was determined that the highest yield of flavonoids is achieved with a particle size of 0.1 – 0.2 cm.

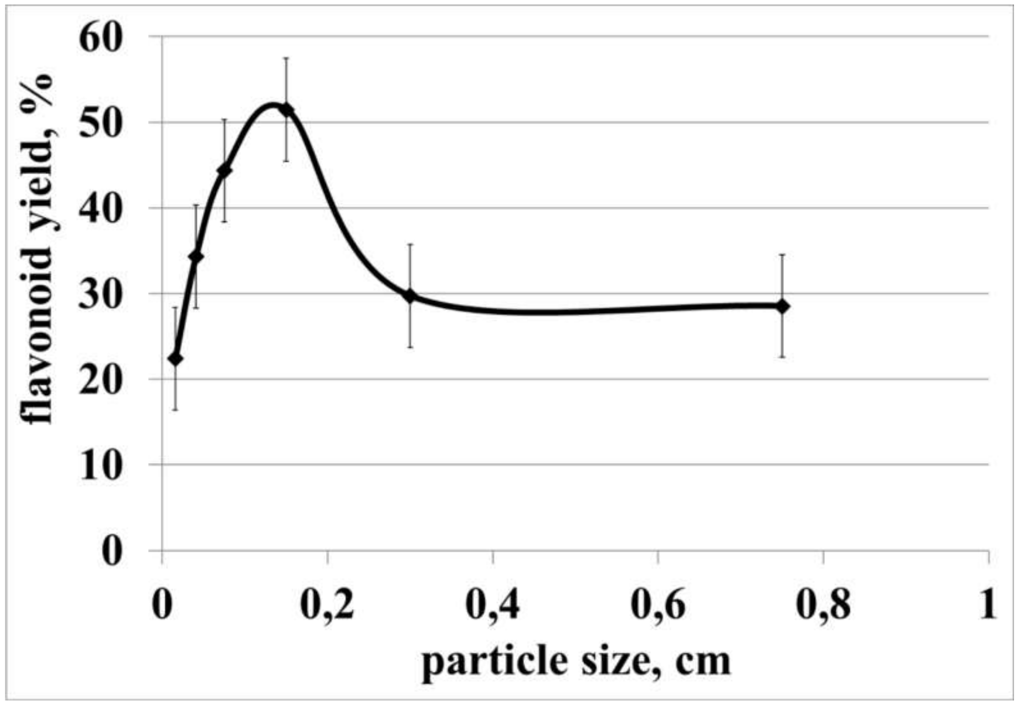

Fig. 44 - Dependence of the flavonoid content in the extract on the particle size of Sophora Japanese fruits

Table 6 shows images of crushed raw materials of various sizes.

Table 6 - Particles of Sophora Japanese fruits of various sizes

Particle size, cm	**Without magnification**	**Under microscope 1.5x15x9x0.20**
Less than 0,035		
0,035 – 0,05		

Continuation table 6 - Particles of Sophora Japanese fruits of various sizes

Particle size, cm	**Without magnification**	**Under microscope 1.5x15x9x0.20**
0,05 – 0,1		
0,1 – 0,2		
0,2 - 0,5		
0,5 – 1,0		

3.6.2 Determination of the point of zero charge and the isoelectric point

During the study of the electrokinetic properties of aqueous dispersions of Sophora Japanese fruits particles, a study was conducted of the effect of the contact time of Sophora Japanese fruits particles with an HCl solution on the ζ-potential (Fig. 45) and pH of the solutions (Fig. 46).

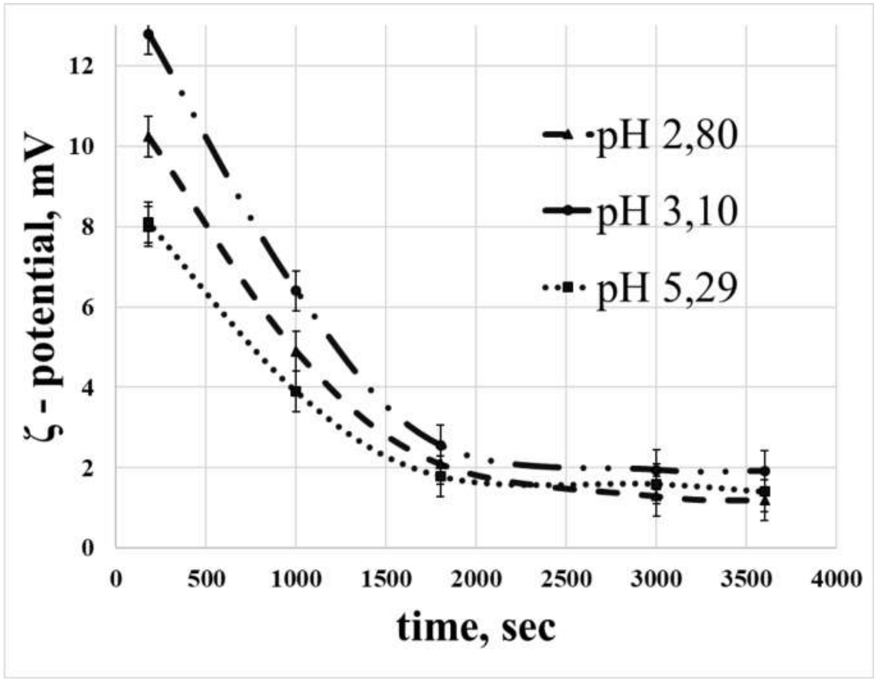

Fig. 45 - Dependence of ζ-potential on the contact time of Sophora particles and solvent at pH (2.80; 3.90; 5.29)

In the first 30 minutes, the electrokinetic potential decreases, and then equilibrium is reached and the process of diffusion of substances from the dispersion into the solution ceases. Thus, this time is optimal for the extraction of BAS from Japanese pagoda tree fruits.

The effect of the extractant pH on the ζ-potential of the Japanese pagoda tree fruit dispersion is shown in Fig. 46. The measurement was carried out 30 minutes after obtaining the extract [112].

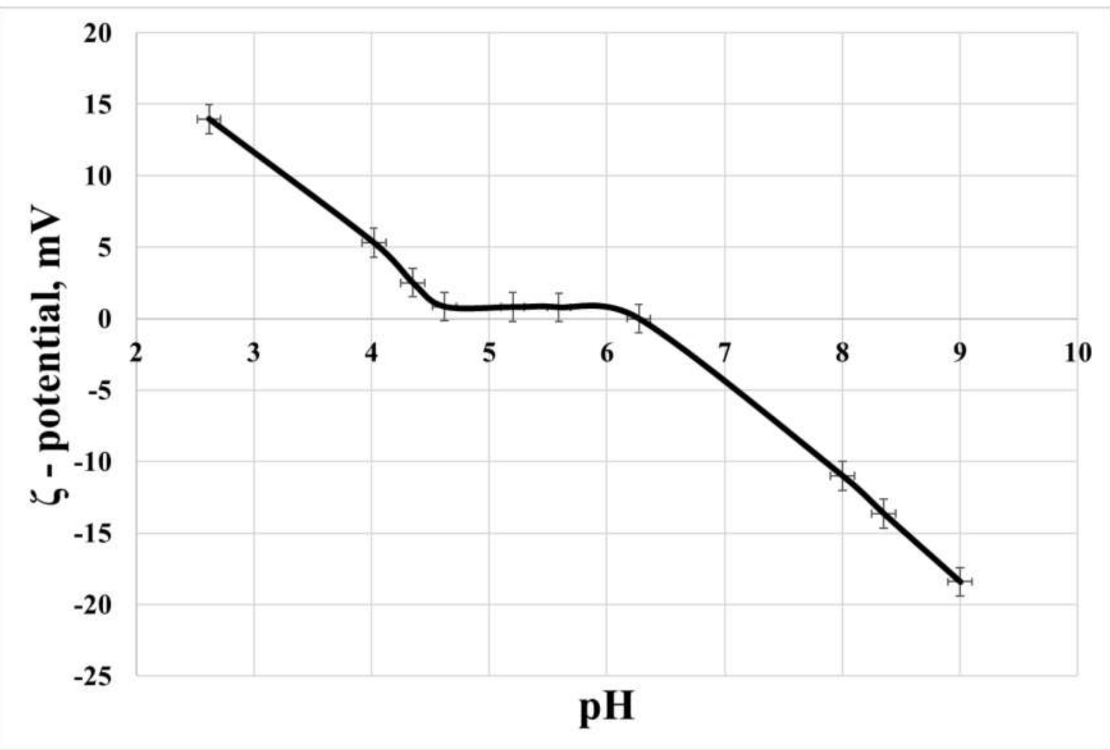

Fig. 46 - Dependence of the ζ-potential of Sophora particles on the pH of the medium

In the acidic region (at pH <6), the particles of the pagoda tree fruits are positively charged; with an increase in pH to the alkaline region, the ζ-potential value decreases. Due to the adsorption and desorption of H^{+}+ and OH^{-} - ions, a charge is formed on the active centers of the pagoda tree particles. The active centers of the particles of Japanese pagoda tree fruits include: amino, carboxyl groups, etc., which are components of proteins, carbohydrates, lipids, nucleic and organic acids of plant cells [114, 115, 116, 117]. The positive charge of particles in the acidic region is due to the sorption of H^+ ions, the negative charge in the alkaline region is due to the sorption of OH^{-}. Within the pH range of ~ 6, the ζ-potential is zero, this value corresponds to the isoelectric point (pH_{iep}). The presence of a plateau on the ζ – pH curve in the pH range of 4-6 is determined by the complex composition of the plant material and the presence of active centers of various natures on the surface of the particles.

The potentiometric titration curves of the dispersion of Japanese pagoda tree, the extract from the fruits and the background solution are shown in Figure 47.

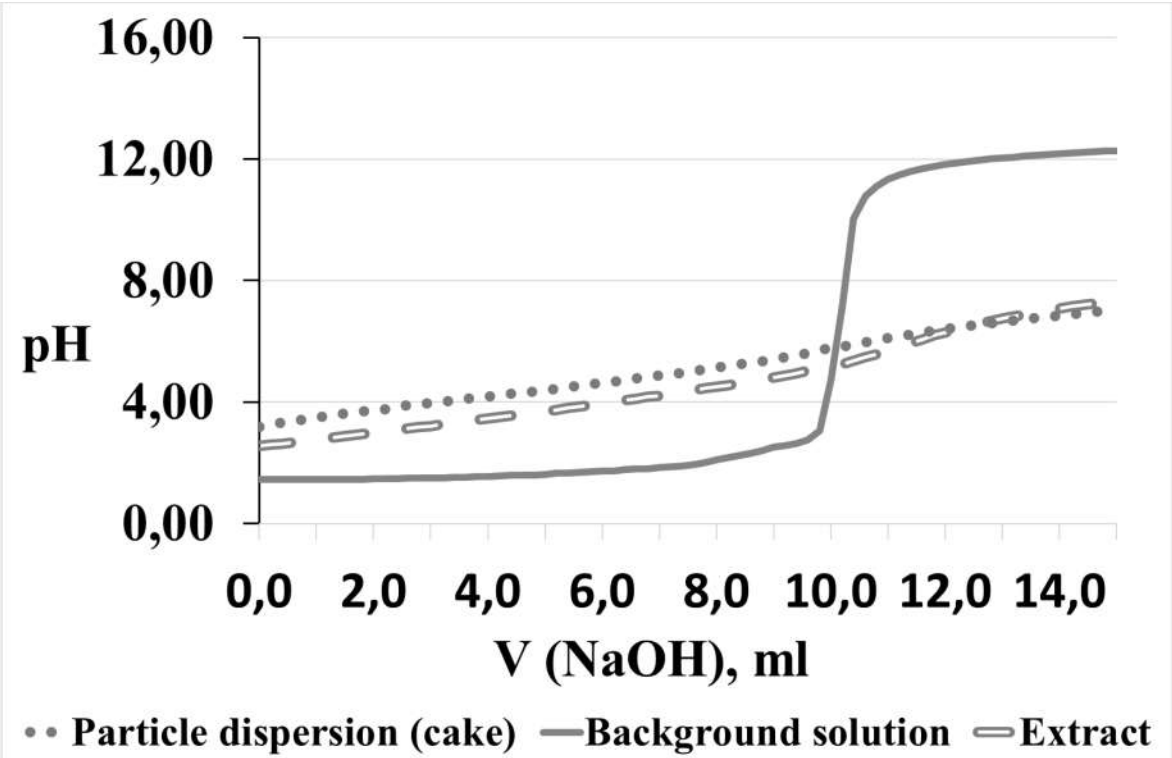

Fig. 47 - Potentiometric titration curves of the aqueous dispersion of Sophora, extract and background solution

Similar results were obtained during titration of the obtained extract, as well as during titration of the particles remaining after filtration (cake). At pH 5.8 in the case of cake titration and at pH 5.2 in the case of the extract, the curves intersect with the original curve (the curve of titration of acid with alkali). These values correspond to the point of zero charge (pH_{pzch}), that is, there is an equality of the number of adsorbed ions H^+ and OH^-. This value is close to the value of pH_{iep}, which confirms the absence of specific sorption of other ions [118].

During the extraction process, biologically active substances pass into the extract, which determine the value of pH_{pzch}. The solid dispersion remaining after the extraction process contains biologically active substances, as evidenced by the similar course of the titration curve of the extract and the solid extract.

The difference in the values of pH_{pzch} of the solid dispersion and the extract indicates the presence of stronger bonds than simple electrostatic interaction between the functional groups of the BAS and the particles of Sophora.

When studying the effect of pH of the medium, it was determined that when the raw material swells in water, during the extraction with water before and after filtration, the pH of the extract changes insignificantly. When extracting with acid with a pH of 2.99, during swelling, the pH of the extract only slightly drops compared to the aqueous extract and increases slightly after filtration. At a pH of 0.97, the changes are more significant. The pH value during swelling increases compared to the original medium,

and then decreases by 2 units. In the alkaline region, the pH is more stable and comparable to the pH of the aqueous extract (Table 7).

The data indicate that in an acidic environment, sorption of H^+ -ions on raw material particles occurs, due to which the pH value increases compared to the initially set value, then during the extraction process, the pH transitions to a more acidic region.

In the alkaline region, sorption of OH^--ions occurs, due to which the pH decreases to a value characteristic of the extraction pH value (Table 3.5.1). Also, sorption of OH^--ions is visually observed during the interaction of raw materials with an alkaline solution - the solution turns black.

In the alkaline pH region, the value of the electrokinetic potential of Sophora Japanese fruits particles increases in absolute value due to an increase in the sorption of OH^-- ions both on the surface of the particles and on the active centers of flavonoid molecules, which enhances the transition of flavonoids to the diffusion layer of the DEL and their desorption from the PSY particles. Comparison of the yield of flavonoids in the pH_{iep} region and in the alkaline region at high values of |ζ| - potential shows a two-fold increase in the yield of flavonoids (Figure 3.8 Dependence of the yield of the total amount of flavonoids on the pH of the medium).

Table 7 - pH values of the extract before filtration and extraction

water pH=5,91	
Extract before filtration	5,55
Extract	5,59
pH=2,99	
Extract before filtration	5,38
Extract	5,62
pH=0,97	
Extract before filtration	1,45
Extract	1,53
pH=7,90 - 8,10	
Extract before filtration	5.52
Extract	5.62
pH=9,00 - 9,10	
Extract before filtration	5.68
Extract	5.63

3.6.3 *Swelling parameters*

Swelling is an important part of the extraction of BAS from plant raw materials. At this stage, the processes of wetting and partial dissolution of the components of the plant cell membrane, desorption

of BAS occur. This process determines the further diffusion of the components into the extractant. Therefore, the study of the factors affecting the parameters of this process is very important for the entire extraction process.

The calculation of the degree of swelling of crushed Japanese pagoda tree fruits was carried out under various conditions described below.

In all cases, graphs of the dependence of the degree of swelling on the time of the process were constructed. The degree of swelling reached a maximum value, after which it did not increase. This corresponds to limited swelling of the plant cell due to the fact that the polymer of which the plant cell consists is a spatial network that prevents the separation of macromolecules from each other and their transition into solution, as well as the content of high-molecular substances (HMS) in it [30, 33]. Hemicelluloses and lignin, which are part of the cell membrane, have a branched and cross-linked structure, the free space of the polymer framework can be actively filled with water upon contact. The maximum swelling degree corresponds to the swelling limit of the cell and is an important characteristic for assessing the swelling process. The maximum degree (α_{max}) and swelling constant (K) are presented in Table 8.

Таблица 8 - Характеристики набухания плодов софоры японской в разных экстрагентах

extractant	**ethyl alcohol 50% water**	**water (distilled)**			**water (distilled)**		**NaCl solution 0,001 M**	**NaI solution 0,001 M**	**NaCNS solution 0,001 M**	**Laureth-2 solution**	**Sodium lauryl sarcosinate solution**	**Cocamidopropylene glycol-dimonium chloride phosphate solution**	**water (distilled)**	**water (distilled**
					Acidified pH= 3.56	**Alkaline pH= 7.94**								
Temperature/ add. onditions	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	20 ºC	**60 ºC**	20 ºC / **US-action**
particle size, cm	0,1 – 0,2	**0,05 – 0,1**	**0,1 – 0,2**	**0,2 – 0,5**	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2	0,1 – 0,2
α_{max}	0.11±0.01	0,17±0.01	0.26 ±0.01	0,60±0.01	0,24±0.01	0,17±0.01	0,24±0,01	0,30±0,01	0,26±0,01	0,41±0,01	0,19±0,01	0,22±0,01	0,39±0,01	0,33±0,01
K, c^{-1} c^{-1}	0.05	0,11	0,07	0,014	0,06	0,1	0,08	0,11	0,08	0,04	0,07	0,09	0,04	0,12

3.6.3.1 Effect of the nature of the extractant on the swelling process of Sophora Japanese fruits

The swelling parameters of Sophora Japanese fruits in various extractants have been determined.

When studying the swelling of Sophora Japanese fruits in 50% ethyl alcohol, it was noted that its addition reduces the maximum swelling degree by more than two times (Fig. 48). Water molecules are more polar than alcohol (the solvating capacity of alcohol is lower), as a result of which the swelling process of the plant cell worsens and the swelling rate constant in an ethyl alcohol solution is lower than in water. Swelling of the raw material in propylene glycol is practically absent - no change in volume was observed.

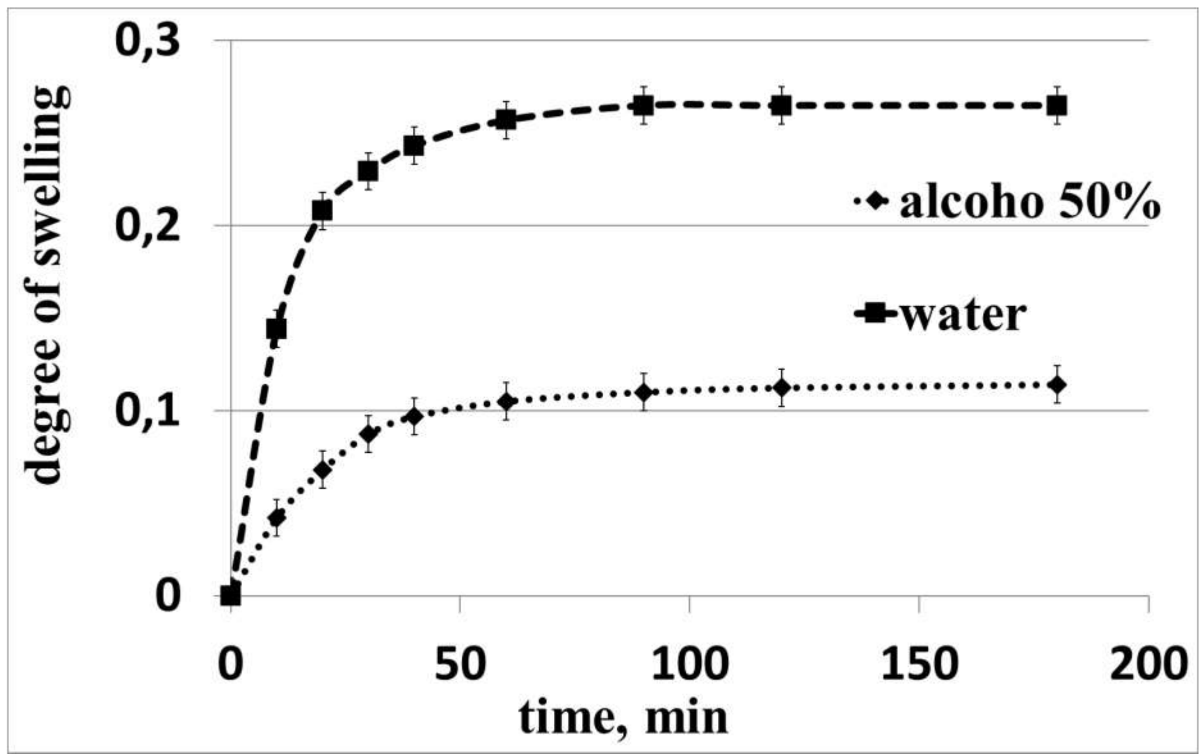

Fig. 48 - Dependence of the swelling degree of Sophora Japanese fruits particles in water and 50% ethyl alcohol on time

3.6.3.2 Effect of the particle size of Sophora Japanese fruits on swelling parameters

The swelling degree curves of the particles of Sophora Japanese fruits of different sizes are shown in Figure 49.

When they swell, the maximum swelling degree of particles of 0.05 - 0.1 cm is the smallest among the cases under consideration and is achieved after 60 minutes, as in the case of a particle size of 0.1 - 0.2 cm. This occurs due to excessive grinding of Sophora Japanese fruits, due to which ballast substances (such as mucus) are released into the extract, which can increase the viscosity of the extractant, clog the pores of the cell, which disrupts the process of extraction of the target components (flavonoids).

With particle sizes from 0.2 cm to 0.5 cm, there was a constant increase in the degree of swelling over a long period of time (as evidenced by the swelling constant (Table 8) due to less destruction of the

cell framework and a decrease in the area of contact of the liquid with the particles of Japanese pagoda tree fruits.

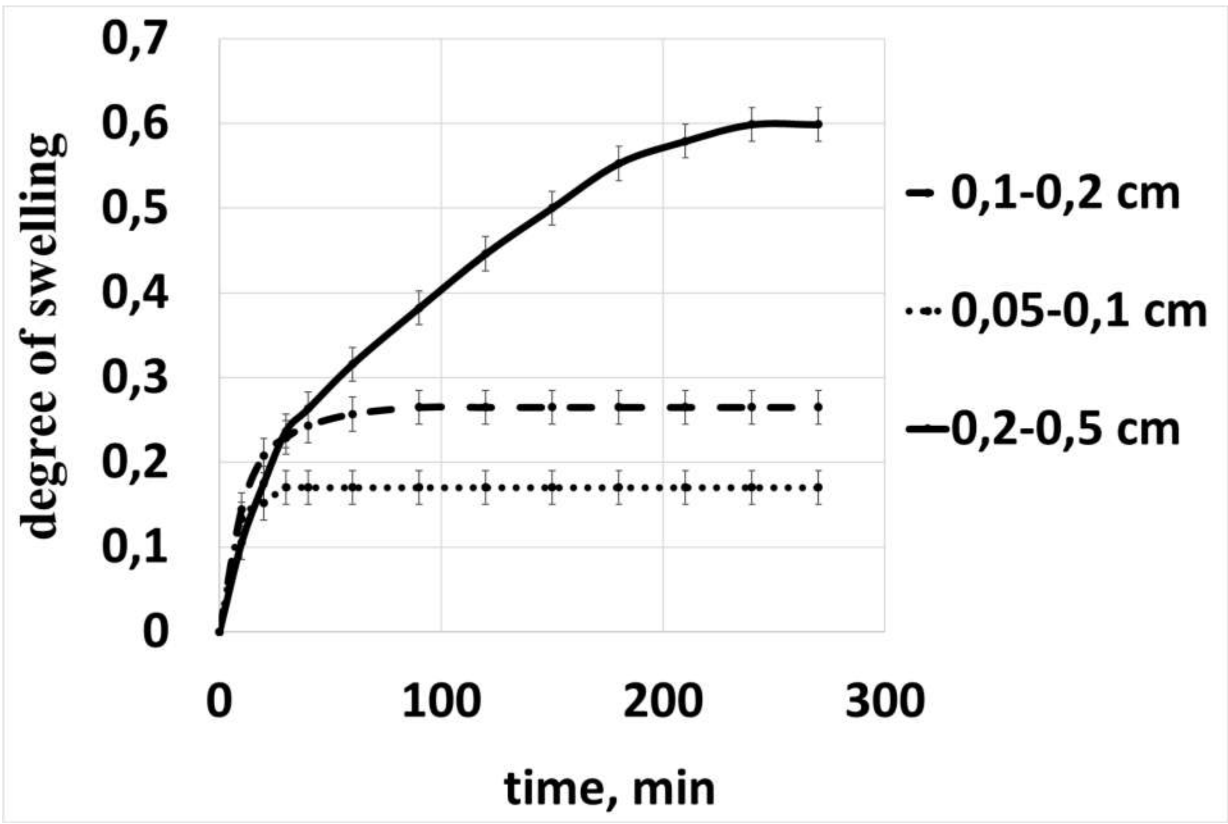

Fig. 49 - Dependence of the degree of swelling of Sophora Japanese fruits on time with particle sizes from 0.05 to 0.5 cm

3.6.3.3 Effect of pH of the extractant on swelling parameters of Sophora Japanese fruits

In water, at a pH of 5.7, the greatest swelling of Sophora Japanese fruits particles occurs; pH deviation into the acidic and alkaline regions did not lead to an increase in the maximum degree of swelling (Fig. 50) [119].

The zero charge point of aqueous dispersions of pagoda tree was determined to be 5.8, and the isoelectric point was 6.0 [22].

Usually, in HMW solutions, swelling in the isoelectric point region is the smallest, which is not observed in our work. This is due to the fact that the particles of pagoda tree fruits have a cellular structure and do not fold into a globule as in HMW solutions. Also, as a result of exposure to an aggressive environment (acidic and alkaline), the internal bonds in the polymer matrix are destroyed and the swelling process worsens [19].

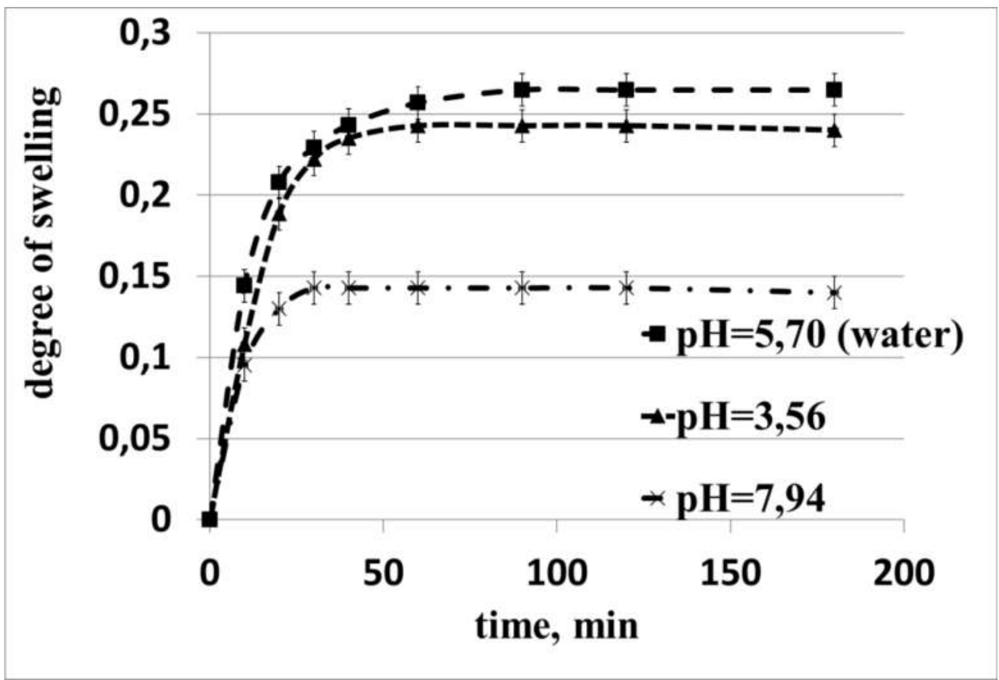

Fig. 50 - Dependence of the degree of swelling of Japanese pagoda tree fruit particles on time at different pH

3.6.3.4 Effect of Electrolytes on the Swelling Parameters of Sophora Japanese fruits

Figure 51 shows the swelling degree versus time for sodium chloride, iodide, and thiocyanate solutions. The effect of electrolytes was studied at a salt concentration of 0.001 M.

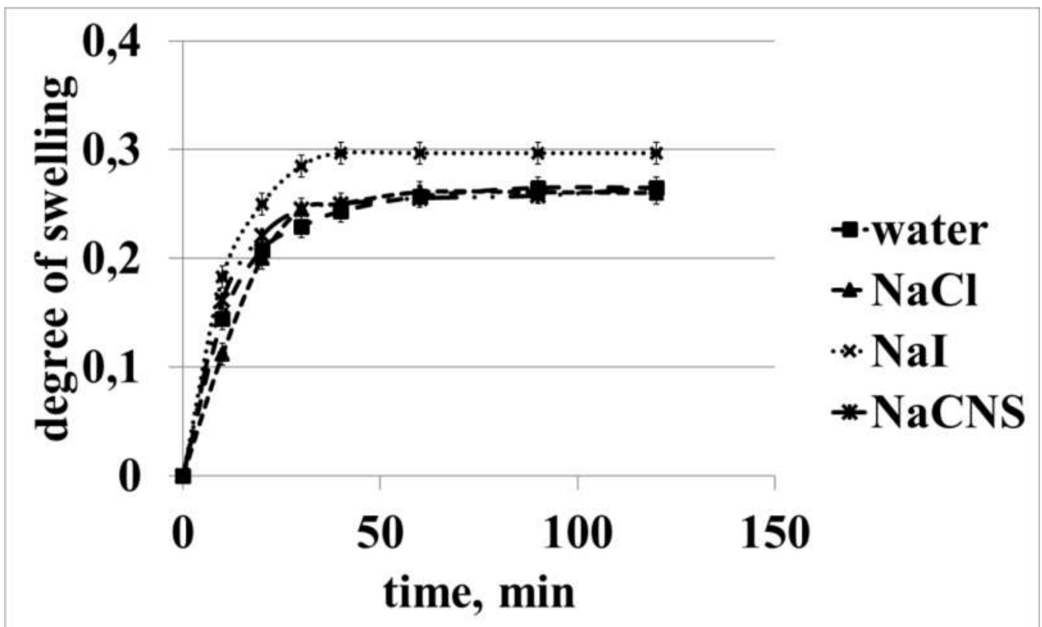

Fig. 51 - Dependence of the swelling degree of Sophora Japanese fruits in an electrolyte solution on time

The greatest effect on the swelling of Sophora Japanese fruits is observed in sodium iodide solutions. In this case, α_{max} is greatest at the maximum swelling rate. When using chloride and thiocyanate, the changes in α_{max} and the swelling rate constant were insignificant (Table 9) [120].

3.6.3.5 Effect of surfactants on swelling parameters of Sophora Japanese fruits

Important characteristics of surfactants are CMC and surfactant type. Working concentrations of surfactants were selected for the study so that they were not high to avoid irritating effects on humans and possible destruction of raw materials, but at the same time were above the CMC. The data are presented in Table 3.6.3.5.1.

Table 9 - Characteristics of individual surfactants and a mixture of surfactants

Designa-tion	Designation	Surfactant name	Mole fractions in surfactant mixture	CMC, mol/l	selected concentration, mol/l
laureth-2	nonionic	laureth-2	-	0,00018	0,004
sodium lauryl sarcosinate	anionic	sodium lauryl sarcosinate	-	0,0028	0,004
miramistin	cationic	miramistin	-	0,00005 [121]	0,005
CGDChPh	amphoteric	Cocamidopropylene glycol dimonium chloride phosphate	-	-	0,004

Then the swelling process in solutions of surfactants of various types (nonionic, anionic, cationic, amphoteric) was studied; the maximum degree of swelling of the extracts was determined (Fig. 52, Table 9).

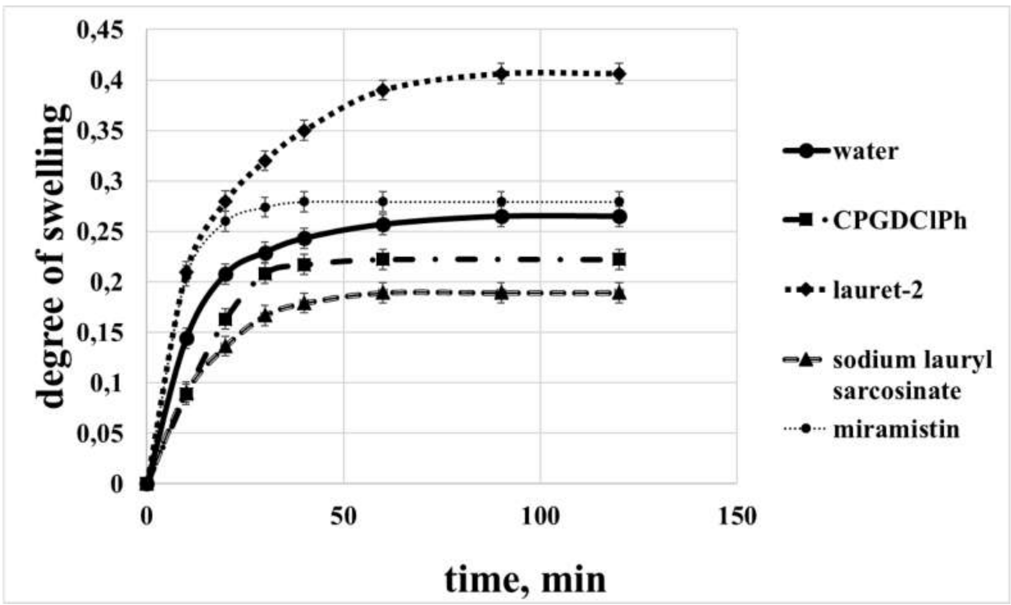

Fig. 52 - Dependence of the degree of swelling of Sophora Japanese fruits in a surfactant solution on time

At a concentration below the CMC, no effect on the swelling of the raw material was noted, since in this case there are not enough molecules in the solution to line up the required amount at the phase boundary to improve wettability, and in this case, micelles are not formed, which would contribute to

better solubilization of flavonoids. Therefore, all studies of Sophora fruit swelling in surfactants were assessed at a concentration above the CMC. When swelling particles of Sophora japonica fruits in a solution of a non-ionic surfactant (laureth-2), α_{max} and the swelling constant are significantly higher than when swelling in water (8, Fig. 52). The amphoteric and anionic surfactants did not have a significant effect on the swelling of the studied object (α_max remained practically unchanged, and the swelling time decreased to 60 minutes (Table 8), which may be due to the presence of a long hydrophobic part in laureth-2, as well as easier destruction of the bond between the hydrophobic region and the hydrophilic (hydroxy group).

3.6.3.6 Effect of temperature on swelling parameters of Sophora Japanese fruits

The degree of swelling of Sophora Japanese fruits particles increases with increasing temperature (Fig. 3.6.3.6.1), this is explained by both an increase in the rate of diffusion of solvent molecules and an increase in the destruction of the cell membrane with increasing temperature [19], facilitating the penetration of solvent molecules into the depth of the pagoda tree particles. Maximum swelling of pagoda tree particles at 20 ˚C is achieved within 60 - 80 minutes, and at 60 ˚C after 120 minutes (Fig. 53). As noted above, at the initial moment of time during heating, the swelling rate increases mainly due to an increase in the diffusion rate of solvent molecules. Longer swelling at 60 ˚C occurs due to the destruction of strong bonds of the plant cell, as well as a decrease in the viscosity of the extractant, which contributes to better spreadability and contact of the plant cell with the liquid, as well as better dissolution of substances at temperature. This process occurs during heating much more strongly and longer than at 20 ˚C, which is in good agreement with the low swelling rate constant at 60 ˚C (Table 8) [122, 123].

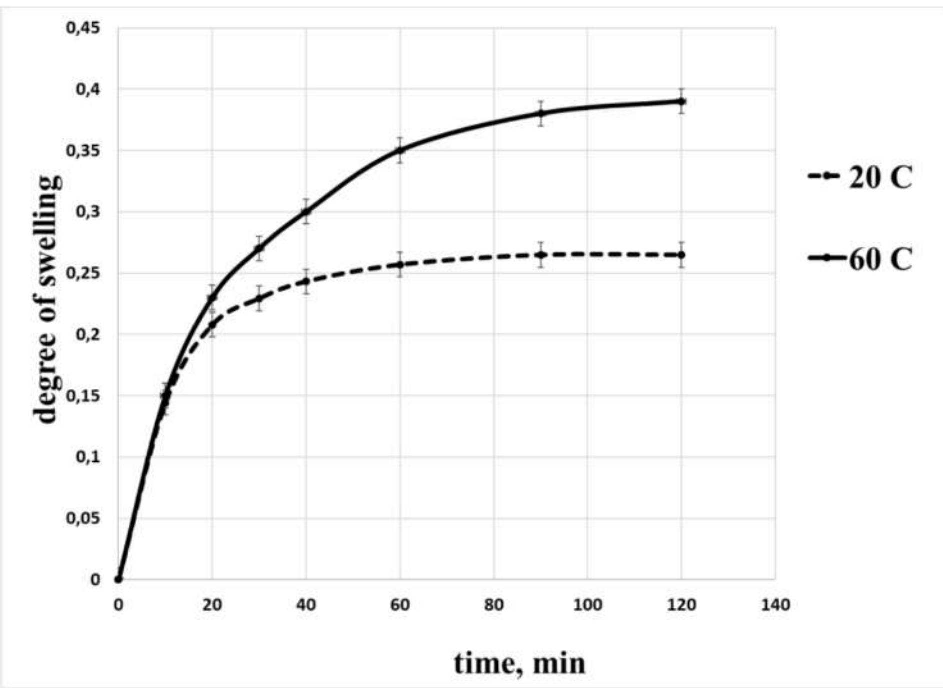

Fig. 53 - Dependence of the degree of swelling of Sophora Japanese fruits particles at a temperature of 60 ˚C and 20 ˚C on time

3.6.3.7 The effect of ultrasound on the swelling parameters of Sophora Japanese fruits

The use of ultrasound helps to increase α_max, which is achieved in a shorter time (40 minutes), as evidenced by the high swelling constant (Table 8, Fig. 54). This occurs due to the destruction of strong bonds of the cell framework.

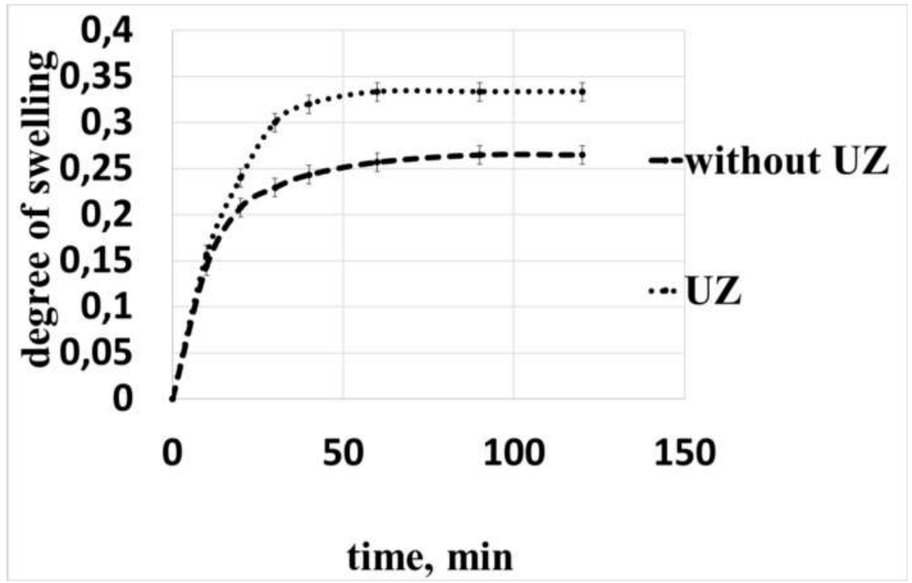

Fig. 54 - Dependence of the degree of swelling of Sophora Japanese fruits particles with and without the influence of ultrasound

3.6.4 Study of flavonoid desorption from aqueous and aqueous-alcoholic dispersions of Sophora Japanese fruits

The desorption process was studied as follows: 10 ml of extractant (distilled water or 50% ethanol solution) were added to 5 g of crushed Sophora fruits. The mixture was left for 30 minutes. During this time, swelling of the Sophora particles occurred and desorption of flavonoids from the swollen particles into the dispersion medium began. Then the dispersion of swollen Sophora particles was placed in a percolator and after 5 minutes the first portion of the filtrate was collected and the content of flavonoids in the filtrate was determined, 10 ml of solvent were added. This procedure was carried out until flavonoids were practically absent in the filtrate. Fig. 55 shows the dependences of desorption (D) of flavonoids on time for dispersions of Sophora Japanese particles in water and in a 50% aqueous-alcoholic solution.

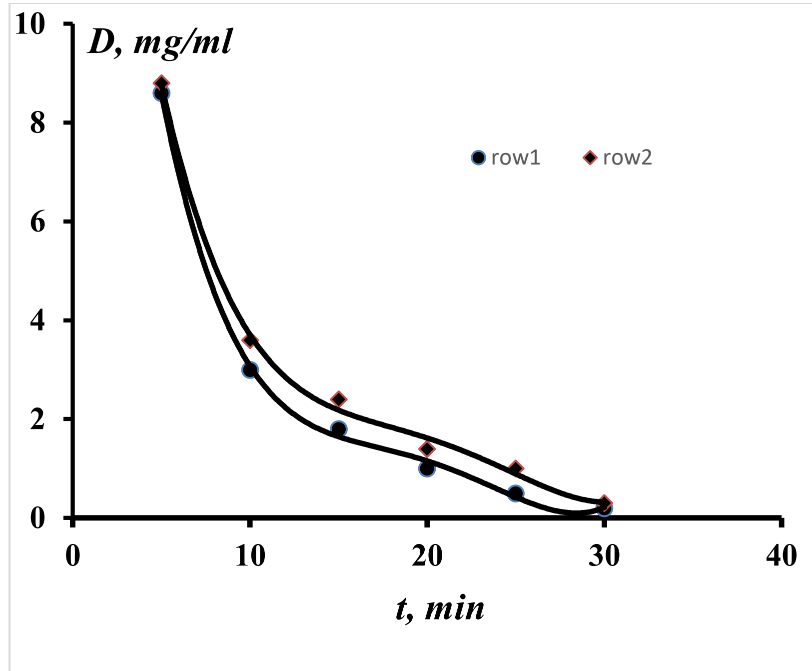

Fig. 55 - Dependence of the amount of desorbed flavonoids from dispersions of Japanese pagoda tree particles on the desorption time: row 1 - dispersions in water; row 2 - in a water-alcohol solution

From Fig. 55 it is evident that desorption in aqueous-alcoholic solutions is higher than in water, which is consistent with the increase in the solubility of flavonoids in aqueous-alcoholic solutions.

The kinetics of desorption corresponds to first-order reactions - the reaction rate is directly proportional to the concentration, and the graph of the dependence of the logarithm of concentration on time is linear [91] is presented in Fig. 56.

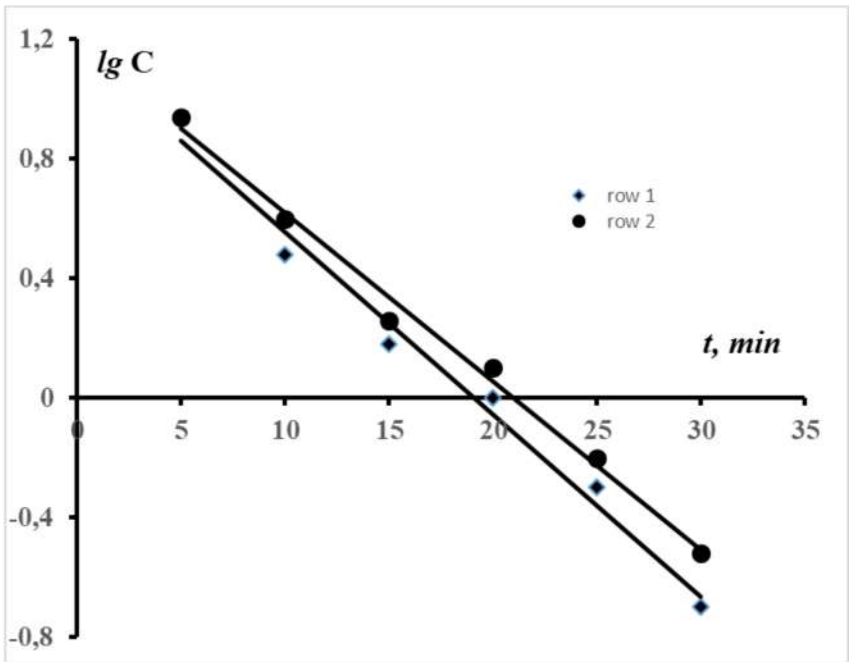

Fig. 56 - Dependence *lgC - t of flavonoid desorption* from dispersions of Japanese pagoda tree particles: row 1 - solvent distilled water, row 2 - 50% ethanol solution

The rate constant (K) for first-order reactions is calculated using equation 32 [86].

$$K = \frac{1}{t}\ln\frac{c_0}{c} \qquad (32)$$

where c_0 - the initial concentration of the reagent, C is the concentration of the reagent corresponding to time t.

The values of the rate constants of the desorption process in distilled water and in a 50% ethanol solution are 0.108 and 0.104 (min^{-1}), respectively. It is evident that the rate constants in different solvents differ insignificantly.

Fig. 57 shows the dependences of the degree of flavonoid extraction on the type of solvent nature.

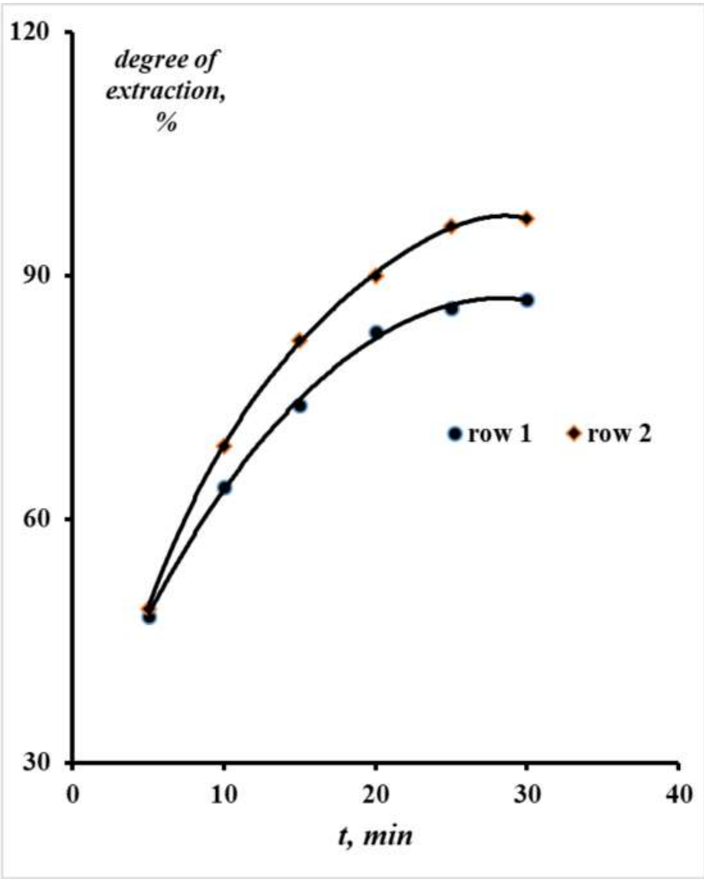

Fig. 57 - Dependence of the degree of flavonoid extraction on the nature of solvents: row 1 - distilled water, row 2 - 50% aqueous ethanol solution

From Fig. 57 it is evident that the degree of extraction in water-alcohol solutions is higher than in water, which is consistent with the results of the extraction study.

3.6.5 The influence of surfactants on the process of flavonoid extraction from Sophora Japanese fruits

The effect of surfactants on the process of flavonoid extraction from Japanese Sophora fruits was studied using ionogenic, non-ionogenic surfactants and their mixtures. To select the optimal concentrations of surfactants, their CMCs were determined using various methods (surface tension,

conductometry and photometry). The extract was prepared by maceration with heating (section 2.2.3.3). Table 10 presents the CMC values for individual surfactants, and Table 3.6.5.1 for surfactant mixtures. For surfactant mixtures, concentrations were calculated in mole fractions [124].

Table 10 - Characteristics of individual surfactants and surfactant mixtures

<table>
<tr><th>Designa-tion</th><th>Designation</th><th>Surfactant name</th><th>Mole fractions in surfactant mixture</th><th>CMC, mol/l</th><th>selected concentration, mol/l</th></tr>
<tr><td rowspan="2">Mixture №1</td><td>non-ionic</td><td>laureth-2</td><td>0,8</td><td rowspan="2">0,00025</td><td rowspan="2">0,0008</td></tr>
<tr><td>anionic</td><td>sodium lauryl sarcosinate</td><td>0,2</td></tr>
<tr><td rowspan="2">Mixture №2</td><td>non-ionic</td><td>laureth-2</td><td>0,2</td><td rowspan="2">0,0008</td><td rowspan="2">0,01</td></tr>
<tr><td>anionic</td><td>sodium lauryl sarcosinate</td><td>0,8</td></tr>
<tr><td rowspan="2">Mixture №3</td><td>non-ionic</td><td>laureth-2</td><td>0,2</td><td rowspan="2">0,0032</td><td rowspan="2">0,004</td></tr>
<tr><td>cationic</td><td>miramistin</td><td>0,8</td></tr>
<tr><td rowspan="2">Mixture №4</td><td>non-ionic</td><td>laureth-2</td><td>0,2</td><td rowspan="2">0,000056</td><td rowspan="2">0,00008</td></tr>
<tr><td>amphoteric</td><td>Cocamido-propylene glycol-dimonium chloridephosphate</td><td>0,8</td></tr>
</table>

Figure 58 shows the results of extraction in aqueous solutions of surfactants of various natures and their mixtures. The extracts were obtained by heating (section 2.3.3.3). From Fig. 3.4.3.1 it is evident that addition of ionic surfactants does not affect content of flavonoids in the extract, and zwitterionic surfactant – cocamidopropylene glycol dimonium chloride phosphate by 28% and nonionic laureth-2 by 55% increase the yield of flavonoids. Nonionic and zwitterionic surfactants have higher surface activity and significantly reduce interfacial tension at the cell – surfactant solution boundary, promoting stronger destruction of plant cells and thus facilitating extraction of flavonoids. Extraction of flavonoids increases more significantly when using surfactant mixture. Mixtures have higher surface activity, are better adsorbed at the interface than individual surfactants, form larger micelles, which increases solubilization of flavonoids [20, 21, 51, 125, 126, 127]. The mechanism of flavonoid extraction from plant materials in the presence of surfactants is in good agreement with Rebinder's theory [128, 129, 130, 131], who considered the mechanism of contaminant removal using surfactants from a physicochemical point of view as adsorption displacement accompanied by wetting, emulsification and solubilization [132, 133, 134, 135].

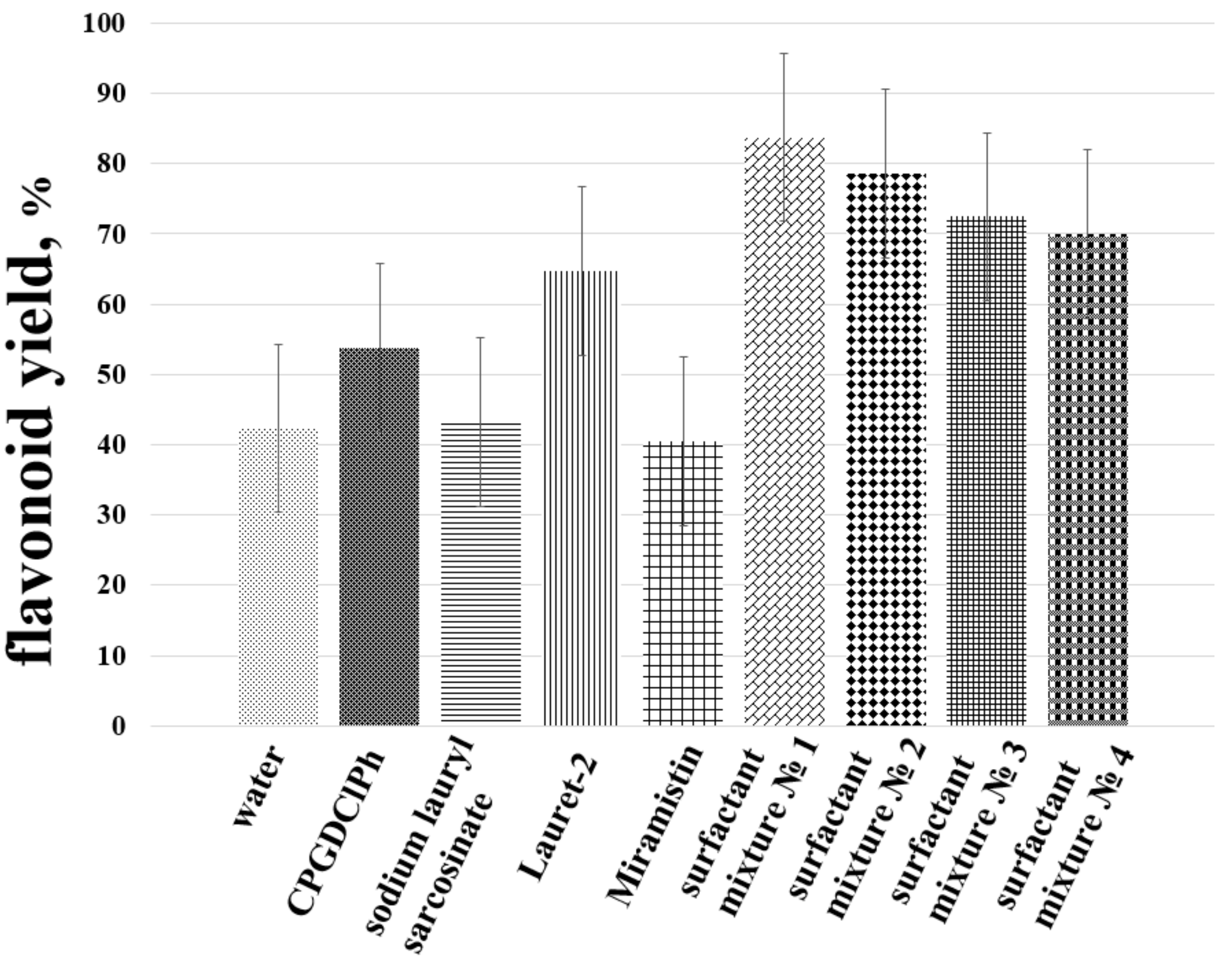

Fig. 58 - Flavonoid content in the extract obtained by percolation using surfactants

3.7 Determination of antioxidant activity of the extract

One of the most important characteristics of drugs is antioxidant activity (AOA). The biological activity of natural substances in combination with antioxidant properties determines the importance of creating new medicinal and cosmetic products physiologically compatible with the human body, obtained from plant materials. The importance of antioxidants is determined by their ability to protect the human body from oxidative damage by suppressing and stabilizing free-radical oxidation processes [136]. In this work, AOA was determined by a potentiometric method [137]. The AOA value was estimated by comparing the AOA of the extract from the fruits of Sophora japonica with a standard substance, which was ascorbic acid.

Measurement of AOA of BAS solutions by a potentiometric method is based on changing the potential of oxidation-reduction electrodes. Electrodes Fe^{+3}/Fe^{+2} were used as oxidation-reduction electrodes [138].

The electromotive force (EMF) of a galvanic cell corresponds to the potential difference between the positive electrode and the negative electr [139]. In our case, the equation for calculating the EMF has the following form (33):

$$E = \varphi_{fe(+3)/Fe(+2)} - \varphi^{0}_{Ag,AgCl/Cl(-)} \quad (33)$$

where E - EMF of the galvanic cell, $\varphi_{fe(+3)/Fe(+2)}$ potential of the oxidation-reduction electrode, $\varphi^{0}_{Ag,AgCl/Cl(-)}$ - the potential of the reference electrode, in this case a standard silver chloride electrode was used as the reference electrode

$$\varphi^{0}_{Ag,AgCl/Cl(-)} = 0{,}200 \text{ B}.$$

The potential of the indicator electrode is described by the equation (34):

$$\varphi_{fe(+3)/Fe(+2)} = \varphi^{0}_{Fe(+3)/Fe(+2)} + \frac{RT}{zF} ln \frac{C_{Fe(+3)}}{C_{Fe(+2)}} \quad (34)$$

where $\varphi^{0}_{Fe(+3)/Fe(+2)}$=0,771B - standard redox electrode Fe^{+3}/Fe^{+2}.

The measurements were carried out in dilute solutions with C~0.001 mol/l, which allows using an activity coefficient equal to 1. The concentration $C_{Fe(+3)}$ - initial concentration of iron (III) n the solution, $C_{Fe(+2)}$ - the initial concentration of iron (II), and $C_x$characterizes the amount of iron that is reduced or oxidized in the oxidation–reduction process on the electrode.

Substituting the values of the electrode potentials in the standard state and the change in the concentrations of iron (III) and (II) in the oxidation–reduction process, we obtain the final equation (35):

$$E = 0{,}771 + 2{,}3\frac{RT}{zF} lg \frac{C_{Fe(+3)-C_x}}{C_{Fe(+2)}+C_x} - 0{,}200 \quad (35)$$

Next, the EMF was measured: for a solution of a mixture of iron (III) iron (II); for a solution of a mixture of iron (III) iron (II) with the addition of an extract from Sophora fruits with a concentration of 0.001 mol/l; for a solution of a mixture of iron (III) iron (II) with the addition of ascorbic acid with the same concentration as the extract - 0.001 mol/l.

Then, C_x was calculated for the extract from Sophora fruits and for ascorbic acid, and the AOA of the extract from Sophora fruits was estimated in comparison with a standard solution of ascorbic acid, which was taken as 100% (36):

$$\text{AOA} = \frac{C_x(extract)}{C_x(ascorbic\ acid)} \cdot 100\% \quad (36)$$

Table 11 shows the results of determining the AOA of the Sophora Japanese fruit extract using the potentiometric method.

Table 11 - Results of determining the AOA of the Sophora Japanese fruit extract using the potentiometric method

Component	C, mol/l	$E,\ B$	C_x, mol/l	AOA, %
$Fe_2(SO_4)_3$	0,001	0,487	0.00011	-
$FeSO_4$	0,001			-
Ascorbic acid	0,001	0,466	0,00021	100
Extract from Sophora fruits	0,001	0,436	0,00041	195

During the experiment, it was found that at a concentration of 0.001 mol/l of flavonoids in the aqueous extract of Sophora Japanese fruit, the AOA of flavonoids is 2 times higher than the AOA of ascorbic acid, which is consistent with the data of the work of Zverev Ya.F. [140, 141]. In the work of Zverev Ya.F., the AOA of individual flavonoids (rutin, quercetin) is 3-5 times higher than the AOA of ascorbic acid, which is possibly due to other conditions for measuring the AOA.

RESULTS

The dissertation determines the influence of colloidal factors and physicochemical conditions on the extraction of target components - flavonoids from Sophora Japanese fruits.

1. The optimal particle sizes of Sophora Japanese fruits for flavonoid extraction were established - 0.1 - 0.2 cm. Particle sizes less than 0.1 cm complicate the extraction of target components due to the formation of mucus and filtration difficulties; particle sizes greater than 0.2 cm are less effective due to a decrease in the contact surface of the extractant with the particles.

2. The significance of plant material swelling on the flavonoid extraction process was assessed. Factors influencing the swelling process of SJF were determined:

- it was shown for the first time that in the region of the isoelectric point of the Sophora Japanese fruits particle surface, there is no minimum swelling, unlike in HMC solutions. This is due to the fact that the particles have a cellular structure and do not fold into a globule, as in HMC solutions;
- the greatest degree of swelling occurs for particles with a size of 0.1 - 0.2 cm, since with a decrease in the particle size, the viscosity of the extractant increases and the diffusion of the solvent in the cell decreases;
- the greatest degree of swelling was noted in a solution of a non-ionic surfactant (Laureth-2). Ionic surfactants are characterized by stronger hydration, which not only does not contribute to swelling, but can lead to the opposite process - dehydration of the particles;
- an increase in temperature significantly increases the degree of swelling of the Sophora Japanese fruits due to an increase in the rate of diffusion of the solvent and more active destruction of plant cells;

- US- radiation contributes to an increase in the degree of swelling of the Sophora Japanese fruits due to greater destruction of the plant cell membrane.

3. The effect of various surfactants and their mixtures on the extraction of flavonoids from SJF was estimated. The highest yield of flavonoids was noted in the presence of a nonionic surfactant. Cationic and anionic surfactants did not affect the degree of flavonoid extraction from Sophora Japanese fruits. Nonionic and zwitterionic surfactants and their mixtures possessed higher surface activity and significantly reduced the interfacial tension at the cell-surfactant solution boundary, promoting intensive destruction of plant cells and thereby facilitating the extraction of flavonoids.

4. It was determined that in the alkaline pH region the value of the electrokinetic potential of SJF particles increases in absolute value due to the increase in the sorption of OH^- - ions both on the surface of the particles and on the active centers of the flavonoid molecules, which enhances their transition to the diffusion layer of the electrical double layer and desorption from the Sophora Japanese fruits particles. Comparison of the yield of flavonoids in the $\text{pH}_{isoelectric\ point}$ and in the alkaline region at high values of the |ζ|-potential shows a twofold increase in the yield of flavonoids.

5. It has been determined that the effect of ultrasound and microwave radiation when using the static extraction method and heating significantly increases the degree of flavonoid extraction due to the turbulent flow of the extractant, the collapse of cavitation bubbles and rapid heating of the raw material. Another reason for the increase in the yield of flavonoids is the improvement of their dissolution in the extractant after microwave and ultrasound exposure.

6. The results of the study of flavonoid desorption from plant raw materials allowed us to establish that the desorption kinetics corresponds to first-order reactions. Calculation of the degree of flavonoid extraction based on the results of desorption showed that their yield is higher in water-alcohol solutions than in water, which is associated with an increase in the solubility of flavonoids in water-alcohol solutions and is consistent with the results of the extraction study.

CONCLUSION

The presented results of the studies of the influence of colloidal factors and physicochemical conditions on the process of flavonoid extraction from Japanese pagoda tree fruits made it possible to determine the optimal parameters for obtaining extracts with a high content of these biologically active substances. The proposed recommendations contribute to the extraction of the maximum possible amount of flavonoids in this type of plant material.

LIST OF ABBREVIATIONS

BAS:	biologically active substances;
MW:	ultra-high-frequency radiation;
EDL:	electrical double layer
US:	ultrasonic radiation;
Surfactants:	surface-active substances;
AS:	anionic surfactants;
CS:	cationic surfactants;
HMS:	high-molecular substances;
DMSO:	dimethyl sulfoxide;
PEO:	polyethylene oxide;
PG:	propylene glycol;
TLC:	thin-layer chromatography;
UV-spectrophotometry:	spectrophotometry in the ultraviolet region;
pH_{pzch} :	pH of the point of zero charge;
pH_{iep} :	pH of the isoelectric point;
AO :	antioxidant activity.

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Appendix A

Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Catechin (flavan-3-ol)	HO, O, OH, OH, OH, OH	Colorless compounds, easily oxidized (acquiring a pink or red color when oxidized), optically active (can be present in the extract in the form of 4 isomers, which have different physical and chemical properties (melting point, rotation angle, etc.), as well as different biological effects).	
Leucoanthocyanidins (flavan-3,4-diols)	HO, O, OH, OH, OH, OH, OH	Colourless compounds, when heated with acids, turn into anthocyanidins and acquire a red colour (cyanidin).	

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Anthocyanins	Cyanidin Delphinidin Malvidin	In an acidic solution they behave as cations (form salts with acids), in an alkaline solution - as anions (form salts with bases), depending on the pH of the medium their color changes.	They are usually found in nature in the form of glycosides - anthocyanins, and the most typical and widespread is cyanidin (3, 5, 7, 3', 4'-pentahydroxyanthocyanidin). Other anthocyanins are also found in plants - delphinidin (3, 5, 7, 3', 4', 5'-hexahydroxyanthocyanidin), malvidin (3, 5, 7, 41-tetrahydroxy- 3', 5'-dimethoxyanthocyanidin)

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Flavanones	Naringenin: R=R_2=H; R_1=OH Eriodictyol: R=H; R_1=R_2=OH Hesperetin: R=H; R_1=OH; R_2=OCH_3 Pinocembrin: R=H Pinostrobin: R=CH_3	The UV spectra of flavanones have one intense absorption maximum at 289 nm and are colorless.	The most common in medicinal plants are pinocembrin, pinostrobin, naringenin, eriodictyol and hesperetin.

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Flavanonols	Pinobanksin: R=R_1=R_2=H Dihydrokaempferol: R=R_1=H; R_2=OH Taxifollin: R=H; R_1=R_2=OH	The UV spectra of flavanols have one intense absorption maximum at 289 nm and are colorless.	The most common in medicinal plants are dihydrokaempferol, pinobanksin, and taxifolin.
Flavones	Chrysin: R=R_1=R_2=H; Апигенин: R=R_2=H; R_1=OH Acacetin: R=R_2=H; R_1=OCH_3 Luteolin: R=H; R_1=R_2=OH	Light yellow, yellow or yellow-green color, UV spectra of flavones have two absorption maxima at 270 nm and at 340-350 nm. 7-O-glycosides of flavones are hydrolyzed under harsh conditions - when heated for 2 hours with 5-10% mineral acids.	The most common flavone aglycones are chrysin, apigenin, acacetin, luteolin, diosmetin, chrysoreol, diuretin and tricin.

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
	Chrysoeriol: R=H; R_1=OH; R_2=OCH_3 Diosmetin: R=H; R_2=OH; R_1=OCH_3 Tricin: R_1=OH; R=R_2=OCH_3		
Flavonols	Galangin: R=R_1=R_2=R_3=H; Kaempferol: R=R_1=R_3=H; R_2=OH Quercetin: R=R_1=H; R_2=R_3=OH Isorhamnetin: R=R_1=H; R_2=OH; R_3=OCH_3 Myricetin: R=H; R_1=R_2=R_3=OH Herbacetin: R_1=R_3=H; R=R_2=OH	Yellow or yellow-green color, for UV spectra of flavonols are characterized by two absorption maxima at 260 nm and at 360-370 nm of the spectrophotometric method. Flavonol-3-glycosides are easily hydrolyzed when heated with weak solutions of mineral acids.	The most common flavonol aglycones are galangin, kaempferol, quercetin, isorhamnetin, myricetin, and herbacetin.

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Isoflavones	HO, O, O, OCH_3 Формононетин		
Chalcones	Api — Glc, O, OH, OH, O HO, OH, OH, Glc — O, O	In an acidic environment, chalcones are converted into the corresponding flavanones.	Typical chalcones are licuraside (the aglycone is isoliquiritigenin) and isosalipurposide.

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Dihydrochalcones	2',6'-hydroxy-4'-methoxy dihydrochalcone: poplar buds		
Aurons	Sulfuretin		
Flavolignans	Silybin	Products of oxidative combination of flavonoids and phenylpropanoids, most often cinnamic alcohols.	

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
Bioflavonoids	Catechin 6',8-dimer Catechin-4,8-dimer	They differ from each other in the different combination of two aglycone molecules, the structure of the combined flavonoids and the nature of the bond.	The most typical biflavonoids are components of tea leaves (catechin dimers), ginkgo biloba (amentoflavone, ginkgetin), and St. John's wort (biapigenin).

Continuation Table A.1 - Physicochemical properties of flavonoids

name	***chemical formula***	***physical and chemical properties***	***note***
	Amentoflavone: R=H Ginkgetin: R=CH_3		